Resident Handbook

Division of Transplantation and Hepatic Pathology
University of Pittsburgh Medical Center
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Anatomic Transplantation Pathology Rotation

Clinical Responsibilities of the Division

The Division of Transplantation Pathology is responsible for pathology support for the Thomas E. Starzl Transplantation Institute. This includes evaluation of primary recipient disease, resected donor organs, and resected allografts. Evaluation of post-transplant biopsies for rejection and other causes of graft dysfunction comprise the main daily workload. This Division also evaluates biopsies of native organs from transplant patients and handles all native liver biopsy specimens. Some native kidney biopsies are also performed in this Division; these are not incorporated into resident rotations.

The Division conducts six separate weekly clinicopathologic conferences to ensure quality control of biopsy results and to keep an open channel of communication between the clinical physicians and transplantation pathologists. In addition, there are two intradivisional quality assurance slide review conferences per week, to ensure agreement among the pathologists in grading rejection and to discuss interesting and/or difficult cases.

Categorization of Specimens and Structure of "Signout"

Specimens that come to the Division for review fall into five categories. They include "Bigs," of which the majority are diseased native organs removed at the time of transplantation; "Quicks," mainly biopsies such as surveillance gastrointestinal biopsies; native liver biopsies; skin biopsies for GVHD; lymph node biopsies to evaluate for PTLD, etc; "Stats," mainly organ allografts biopsies used to monitor rejection; and "Consults" which consist of outside slides submitted for review. The Division also handles a portion of medical kidney biopsies.
The priority ranking the specimens receive, the structure of signout and reporting of the results are designed to best serve the transplant patients and clinical physicians involved in their care. "Stat" specimens receive the highest priority. These biopsies are submitted to Pathology before 11 AM and permanent H&E slides are ready for review by 2:30-3:30 PM the same day. "Quicks" and "Consults" are next in priority, and have a one day or less turnaround whenever possible. "Bigs" receive the next highest priority, and are signed out as expeditiously as possible. Native kidney biopsy results are transmitted to physicians in a provisional manner and signed out as special studies become available.

The staff service responsibilities are divided as follows: One staff pathologist takes weekly responsibility for the Quicks and Stats, and also handles any frozens that occur during the workday. This rotation runs from 7:30 AM until 5 PM, Monday through Friday. A second pathologist takes responsibility for all Bigs and Consults for this time period. This second pathologist covers nightly call during the week, and additionally covers the entire service for the weekend. The services are staggered in the following way: Saturday and Sunday, pathologist A covers everything. Monday through Friday, Pathologist A covers Bigs/Consults/Night call and Pathologist B covers Quicks and Stats. Saturday and Sunday, Pathologist C covers everything. Monday through Friday Pathologist C covers Bigs/Consults/Night call and Pathologist D covers

Quicks and Stats…and so forth. Holidays are treated like any other day of the week or weekend. The turnover times between shifts are 7:30 AM and 5:00 PM.

Resident Responsibilities

The level of resident responsibility depends upon three factors: the level of training, competence, and the desire to assume responsibility. PGY-1 level residents are generally responsible for all "big" cases, including gross evaluation, organization, and review of the slides and finally, signout with the pathologists. The gross processing of cases can usually be accomplished by mid-morning or early afternoon. The resident is expected to sit in on signouts and to participate according to the level of his/her experience. When more than one resident is on rotation, it is the residents' responsibility to divide the workload between them. Residents >PGY-1 may want to assume more responsibility by reviewing "quicks" and "consults" to enhance learning opportunities. A satisfactory division of labor in the past has been for the PGY-1 to assume responsibilities for "bigs"and >PGY-1 to take "quicks" and "consults." The cases are then shared at signout time. Unfortunately, because of the urgency of Stat specimens, it is often not possible for the residents to review the cases before the official signout. The pathologist and resident review the cases together on a daily basis, and the preliminary results are recorded daily in the "Stat Book," immediately outside the signout room. A recent change has been to deliver the "quicks" at 10:30 AM. Depending upon the signout time, this may give the resident an opportunity to review these cases upon delivery. The "big" specimens offer excellent learning opportunities in inflammatory and neoplastic liver disease and cardiovascular pathology. Most renal disease tends to be endstage and native kidneys are often not resected at the time of transplant, in contrast to other organs. "Consult" cases offer excellent review of late posttransplant liver, kidney, and heart pathology, and review of native liver disease.

The resident will be provided with desk space in Transplantation Pathology, and should remain "on-site" during the rotation. If the resident will be away from the Division, it is his/her responsibility to notify the pathologist or secretary of this. This minimizes misplaced slides, reports, requisitions, etc. All slides, typed gross reports with requisitions, special stains, etc. will be delivered to Transplantation Pathology. These should go into the common signout basket and are not delivered to individual mailboxes. It will be the resident's responsibility to organize the cases for which she/he has "taken charge."

Learning Resources

The Division keeps glass slide study sets of liver, kidney, heart, intestine and pancreas transplantation, as well as special topic slide boxes for resident review. These cases may be photographed, but otherwise they are not to leave the Division. Older study slides are in individual binders and are maintained by Ms. Joyce Marcoz. These can be signed out for study from her. In addition we maintain an ongoing collection of slides placed in containers in the signout room and available for resident review. These slides may be photographed but should not be removed from the Division. We are currently transferring these cases to a whole slide digital imaging format for permanent reference via the internet.

Transplantation Pathology on the World-Wide Web

We have put great effort into producing an informative and up-to-date transplant pathology site on the World-Wide Web. This is designed to be a working resource for the practicing pathologist who must deal with transplant-related material. We urge you to take advantage of this site while you are with us. This will benefit you long after you leave the residency program, since you will be able to access it at any time and from any site. The address is http://tpis.upmc.edu. and is currently being transferred to a separate server under the address http://tpis.upmc.com. The grading schemas that appear throughout this handout have been copied from our web pages. You should check the website directly for the most recent versions of these schemas. There is much additional information on line that has not been reproduced for this handout. Remember that this material is copyrighted and cannot be copied for commercial use. You are welcome to use it in lectures and presentations, and we hope that you would give credit to us for this material. Your comments and suggestions for improvements to this site are welcome.

This handout itself contains copies of a number of published papers. We have not obtained copyrights for these, and this handout is strictly for your private use as a member of our Department. We wish you the best of luck in your training with us and in your career as a pathologist. You should always feel free to consult with us or to just stop by to say "Hello."

Weekly Schedule

The following is an "idealized" weekly work schedule. Signout times are variable and should be determined between staff and resident. Also, some signouts may occur in E733 and others may occur in the pathologist's office. Note that residents are not required to attend Sunday signout. Check with the signout pathologist for specific times of signout during a given week, as this schedule may also be modified by other conferences and commitments.
"Conf" refers to the Conference Room in the Division.

Day	Time	Room	Activity
Monday	2:30 – 5:00 p.m.	E733	Quicks, Consults, Bigs signout
Tuesday			
	10:30 – 11:30 a.m.	E733	Quicks signout
	1:00 – 1:30 p.m.	E733	Slide Review Conference
	2:30 – 4:00 p.m.	E733	Stats, Consults, Bigs signout
	3:00 – 4:00 p.m.	Conf	Kidney Transplant Conference
Wednesday			
	10:30 – 11:30 a.m.	E733	Quicks signout
	1:00- 2:00	Conf	Research/Admin Conf.
	2:30 – 4:00 p.m.	E733	Stats, Consults, Bigs signout
	3:00 – 4:00 p.m.	Conf	Liver Tumor Conference
Thursday	10:30 – 11:30 a.m.	E733	Quicks Signout
	1:00-1:30	E733	Slide Review Conference
	2:30 – 4:00 p.m.	E733	Stats, Consults, Bigs signout
	3:00 – 4:00 p.m.	E733	Liver Transplant Conference
Friday	10:00-12:00	Conf	Small Bowel Transplant Conference
	10:30 – 11:30 a.m.	E733	Quicks signout
	2:30 – 5:00 p.m.	E733	Stats, Consults, Bigs signout
Saturday	9:00 – 11:00 a.m.	E733	Bigs, Quicks signout
	2:30 – 3:30 p.m.	E733	Stats signout
Sunday	10:30 – 11:00 a.m.	E733	Quicks signout (Staff only)
	2:30 – 3:30 p.m.	E733	Stats signout (Staff only)

Staff Locations and Telephone Numbers

	Name	Office	Phone	Page	Fax
STAFF	Askren, Linda	E742 MUH	647-2067		647-2084
	Demetris, MD, A. Jake	C903 PUH	647-8375		647-5237
		E-1548 BST	624-6645	2237	624-6654
	Duquesnoy, PhD, Rene	W-1552 BST	624-1075	2344	624-6666
	Marcoz, Joyce	C909 PUH	647-7645		647-5237
	Nalesnik, MD, Michael	E-1549 BST	624-6667	2006	624-6666
	Randhawa, MD, Parmjeet	C903.1 PUH	647-7646	2798	647-5237
	Wu, MD, PhD, Tong	C902 PUH	647-9504	2795	647-5237
	Zeevi, PhD, Adriana	W-1551 BST	624-1073	2024	624-6666
OTHER	Satellite Office	C901 PUH	647-9505		647-5237
	Transplant Signout Room	C909 PUH	647-8381 647-5695 647-9646		647-5237
	Audiovisual/TPIS Office	C900	647-9509 647-8716		647-5237
	Transplant Resident/Fellow Office	C911	647-7641		647-5237

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Historic Landmarks in Clinical Transplantation: Conclusions from the Consensus Conference at the University of California, Los Angeles

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Abstract. The transplantation of organs, cells, and tissues has burgeoned during the last quarter century, with the development of multiple new specialty fields. However, the basic principles that made this possible were established over a three-decade period, beginning during World War II and ending in 1974. At the historical consensus conference held at UCLA in March 1999, 11 early workers in the basic science or clinical practice of transplantation (or both) reached agreement on the most significant contributions of this era that ultimately made transplantation the robust clinical discipline it is today. These discoveries and achievements are summarized here in six tables and annotated with references.

The symposium making up this issue of the *Journal* was held at the University of California, Los Angeles (UCLA) and announced by the Department of Surgery hosts as "a unique and historic meeting at which pioneers of transplantation from around the world will present and discuss landmarks in the advancement of transplantation biology." The participants (in alphabetical order) were: Leslie B. Brent (London), Roy Y. Calne (Cambridge, UK), Jean Dausset (Paris), Robert A. Good (St. Petersburg, FL), Joseph E. Murray (Boston), Norman E. Shumway (Palo Alto), Robert S. Schwartz (Boston), Thomas E. Starzl (Pittsburgh), Paul I. Terasaki (Los Angeles), E. Donnall Thomas (Seattle), Jon J. van Rood (Leiden).

Each of these 11 pioneers provided for publication their reflections about their own unique contributions. The ultimate objective, however, was to reach a consensus by the group on what were the most critical historical discoveries that made transplantation a form of clinical therapy. Carl G. Groth (Stockholm) was invited to be the Chairman for these consensus deliberations and to prepare the executive summary.

Historical landmark status was restricted to contributions made at least a quarter of a century ago. By this time it had been established that rejection of organ allografts could be prevented or reversed with immunosuppressive drugs and that variable donor-specific immunologic tolerance of the graft subsequently developed in many patients. Long-term survival of human recipients of organ and bone marrow allografts had been repeatedly obtained, ensuring continuation of such clinical efforts. A large number of HLA antigens had been discovered, allowing efforts at tissue matching to proceed. The scientific articles annotating this progress are listed in six tables under the following headings: transplantation immunology, bone marrow transplantation, renal transplantation, liver transplantation, heart transplantation, and tissue matching. The material presented in these tables, including the citations, originated from the participants of the symposium.

It should be noted that transplantation could not have proceeded without contemporaneous advances in general and thoracic surgery, medicine, and anesthesia, such as open-heart surgery, renal dialysis, antibiotics, and intensive care technology. The

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Table 1. Transplantation immunology.

Author	Discovery or application	Year published	Reference
Gibson	Defined the immunologic nature of skin allograft rejection in humans, confirmed subsequently with controlled rabbit experiments.	1943	1
Owen	Discovered that bovine dizygotic twins with placental vascular anastomoses (freemartin cattle) were red blood cell chimeras.	1945	2
Burnet	Based on Owen's observations and on studies of lymphocytic choriomeningitis virus by Traub, Burnet, and Fenner postulated "the development of tolerance . . . during embryonic life."	1949	3
Anderson	Demonstrated mutual tolerance to skin grafts by freemartin cattle twins and speculated that "actively acquired tolerance" was responsible.	1951	4
Billingham	Produced actively acquired donor specific tolerance to skin allografts in mice injected during late fetal life with donor hematolymphopoietic cells.	1953	5
Simonsen	Independently demonstrated GVHD in chick embryos (manifested as	1957	6
Billingham	pancytopenia) and mice (runt disease) after intravenous injection of adult spleen cells.	1957	7
Starzl	Reported evidence that human kidney allografts under azathioprine-prednisone induced variable donor specific nonreactivity.	1963	8

GVHD: graft-versus-host disease.

cardiopulmonary resuscitation procedures introduced during the 1950s were particularly influential because they mandated redefinition of death in terms of irreversible brain damage rather than the cessation of heartbeat and respiration. While salvaging countless victims of cardiac or pulmonary arrest, the new methods also resulted in brain-dead corpses on physiologic life support.

In 1966, at a symposium on medical ethics in London, G.P.J. Alexandre described the criteria of brain death that had been used in Belgium and France for discontinuing mechanical ventilation of "heart-beating cadavers." It became possible thereby to remove kidneys and other organs from cadaver donors with an intact circulation. The concept was further elaborated in a Harvard-based ad hoc committee report in 1968 in the *Journal of the American Medical Association.* The impact on transplantation of cadaver organs was immediate and lasting.

Transplantation Immunology

The modern age of transplantation immunology (Table 1) [1–8] began with three seminal observations. First, rejection is a host-versus-graft (HVG) immune reaction. Second, a similar immune reaction [graft-versus-host (GVH)] may occur in reverse and lead to lethal graft-versus-host disease (GVHD). Third, it is possible under well defined experimental conditions to avert rejection as well as GVHD and to induce tolerance of alloantigens, which is strongly associated with the persistence in the recipient of donor leukocyte chimerism.

The next step was the recognition that organ allografts are inherently tolerogenic, a property without which their transplantation with long survival in the recipient would not be possible (Table 1). The tolerance induced by organs usually is manifested only under an umbrella of immunosuppression, but it is not a prerequisite in some animal models, particularly if the allograft is the leukocyte-rich liver (see also Table 4).

The discoveries listed in Table 1 were made piecemeal over a period of 25 years, obscuring the fact that all three of the fundamental phenomena studied by early workers (i.e., HVG, GVH, and acquired tolerance) were involved, but to different degrees, in the "acceptance" of organ allografts and the tolerance induced by allogeneic bone marrow following recipient cytoablation. In 1992 the mechanistic linkage of engraftment after these two kinds of transplantation was established with the discovery of donor leukocyte microchimerism in long-surviving human organ recipients.

The clonal selection theory proposed in 1949 by Burnet and Fenner marked the beginning of a new wave in immunology, from which transplantation is often viewed as a mere stream. Instead, transplantation is a mighty tributary. It fostered research into the mechanisms of the destructive antigraft immune response and the control of this response. From these efforts, directly or indirectly, came the discovery of the function of the lymphocyte (1959–1961) and the role of the thymus in the ontogeny of the immune system (1961); delineation (1958–1963) of the human major histocompatibility complex (MHC); distinction of the T and B lymphocyte subsets (1967–1968); and mainly by study of antiviral immune responses, demonstration of the MHC-restricted nature of the adaptive immune response (1968–1974).

Bone Marrow Transplantation

Bone marrow transplantation (Table 2) [9–22] had its roots in radiobiology and hematology, and it was influenced by clinical studies of certain inherited immune deficiency diseases. Early in these efforts it was learned that engraftment of histoincompatible bone marrow can cause lethal GVHD in a recipient rendered immunologically defenseless by cytoablation, a complication also predicted in recipients with immune deficiency disease. Consequently, the preclinical and clinical development of bone marrow transplantation was delayed until reliable methods of HLA typing and matching became available.

The first completely successful bone marrow transplantations were in children with immune deficiency diseases whose family donors were selected with relatively primitive first-generation tissue-matching techniques. Because of their T cell deficiency, these recipients did not require the cytoablation and postgrafting immunosuppression needed with other indications for bone marrow transplantation. With the use of methotrexate as an immunosuppressant in cytoablated recipients, bone marrow transplantation subsequently was applied with steadily improving results in those with an array of benign and malignant hematolymphopoietic dis-

Table 2. Bone marrow transplantation.

Author	Discovery or application	Year published	Reference
Jacobson	Protection against lethal irradiation by spleen shielding, mistakenly ascribed to humoral factors.	1951	9
Lorenz	Protection against lethal irradiation by injection of bone marrow, mistakenly ascribed to humoral factors.	1951	10
Main	Protection against lethal irradiation in mouse by infusion of bone marrow cells and subsequent acceptance of skin allograft from the marrow donor (tolerance). Recognized analogy to neonatal tolerance.	1955	11
Ford	Proved with cytogenetic techniques that marrow cells of mouse reconstituted with bone marrow after lethal total body irradiation (TBI) were donor origin.	1956	12
Barnes	First attempt to treat leukemia in mice by bone marrow transplantation after lethal TBI.	1957	13
Thomas	First attempts to treat malignancy in human patients by high dose chemotherapy or TBI and an infusion of marrow, showing safety of the infusion and one example of transient engraftment.	1957	14
Thomas	Two children with leukemia given twice the lethal dose of TBI and bone marrow from an identical twin had benign hematologic recovery. Recurrence of leukemia led to the subsequent addition of chemotherapy to TBI.	1959	15
Thomas	First outbred animals (dogs) to be successfully engrafted with allogeneic marrow; conditioning with TBI and treatment after grafting with a short course of methotrexate. Graft rejection, other causes of graft failure, and GVHD described.	1962	16
Mathé	World's first prolonged engraftment of human allogeneic bone marrow; adult recipient with leukemia conditioned with TBI. Died without disease recurrence after 20 months, probably from complications of GVHD.	1963	17
Storb	After developing dog typing sera, achieved survival of most histocompatibility matched, but not of unmatched, recipients of bone marrow from littermate donors. Recipients cytoablated and treated with a short course of postgraft methotrexate.	1968	18
Gatti[a]	After initial illuminating analyses of the inborn errors of lymphocyte development [X-linked agammaglobulinemia, thymic alymphoplasia, and severe combined immunodeficiency disease (SCID)] as experiments of nature, Good suggested a new two-component concept of immunity and performed the world's first completely successful bone marrow transplant in a child with otherwise uniformly lethal X-SCID. A second marrow transplant from the same donor cured a complicating aplastic anemia in this patient, also for the first time.	1968	19
Bach[a]	This was followed by a partially successful allogeneic bone marrow engraftment in a child with Wiskott-Aldrich syndrome.	1968	20
deKoning[a]	Successful allogeneic bone marrow plus thymus engraftment was done subsequently in a child with lymphopenic immune deficiency.	1969	21
Thomas	Review of bone marrow transplantation, including description of first large series of patients with aplastic anemia or leukemia given allogeneic marrow grafts from matched siblings. Problems with GVHD and opportunistic infections defined, with emphasis on the importance of histocompatibility, and discussion of possible use of matched unrelated donors.	1975	22

[a]These three patients did not need myeloablation or postgraft immunosuppression.

eases, other kinds of malignancies, and numerous inborn errors of metabolism.

Kidney Transplantation

Three factors made the kidney a pathfinder organ in transplantation (Table 3) [8, 23–47]. One was the development of dialysis for the treatment of acute, and ultimately chronic, renal failure. The second was the fact that the kidney is a paired organ, ensuring a supply of surgically removed "free kidneys" and, increasingly after 1953, physiologically ideal live donor kidneys. Third, its technical simplicity and the ease with which allograft function could be monitored made kidney transplantation ideal for laboratory and clinical investigation.

By 1974 kidney transplantation had already gone through the four eras shown in Table 3 defined by: no immunosuppression, immunosuppression with total body irradiation (TBI), the first use of drugs to prevent rejection (azathioprine) or reverse it (prednisone), and the introduction of adjunct anti-lymphocyte antibody therapy. Each major improvement in immunosuppression up to 1974 and subsequently permitted goals in kidney transplantation to be reached that were not attainable before.

Thus the transition from no therapy to TBI corresponded with the step from identical to fraternal twin transplantation. The change to azathioprine-based treatment established kidney transplantation as a clinical service from 1963 onward, especially using kidneys from living related donors. Cadaver kidney transplantation burgeoned with the acceptance of brain death during the late

Table 3. Kidney transplantation during four eras.

Author	Discovery or application	Year published	Reference
Preimmunosuppression			
Carrel	Developed vascular anastomotic techniques used for organ transplantation today.	1902	23
Lawler	Surgically excised ("free") kidney allograft transplanted to recipient nephrectomy site. Function controversial.	1950	24
Küss	Free kidneys or kidneys from guillotined donors transplanted with surgical techniques still used today.	1951	25
Michon	First use of living related donor kidney (mother to son): good function before rejection at 3 weeks.	1953	26
Hume	Nine cadaveric or free kidneys transplanted, eight to thigh and one to an orthotopic location. One thigh kidney functioned for 5 months.	1955	27
Murray	First transplantation of identical twin kidney on 12/23/54,	1955	28
Merrill	reported first in abstract [28] and more completely the following year [29]. Later report of first nine cases included description of first posttransplant pregnancy.	1956	29
Total body irradiation			
Murray	Renal allograft from fraternal twin transplanted (1/24/59) to a	1960	30
Merrill	recipient preconditioned with sublethal TBI [30] more fully reported elsewhere [31]. This was the first long survival of an organ allograft, an objective not previously achieved in an animal model.		31
Hamburger	Second successful fraternal twin kidney transplantation using TBI, performed June 1959.	1959	32
Hamburger	Successful transplantations of two living related but nontwin kidney allografts using TBI; secondary steroid administration mentioned.	1962	33
Küss	Eighteen-month survival of two nonrelated kidney allografts using TBI; secondary steroid and 6-mercaptopurine (6-MP) administration noted, without details.	1962	34
Chemical immunosuppression			
Schwartz	Showed in rabbits given bovine serum albumin (BSA) while also being treated with 6-MP that the 6-MP suppressed the antibody response to BSA and rendered the animals tolerant of the foreign protein. The experiments were driven by the hypothesis that the proliferating immunocytes of an expanding antigen-specific clone would be selectively vulnerable to antimetabolite drug therapy.	1959	35
Schwartz	Independently demonstrated a 6-MP dose-related prolongation	1960	36
Meeker	of rabbit skin allograft survival.	1959	37
Calne	Moved from the skin to an organ allograft model and	1960	38
Zukoski	demonstrated (independent from each other) prolongation by 6-MP of canine kidney allograft survival.	1960	39
Calne	Further extensive preclinical studies (in Murray's Boston laboratory) of a report on efficacy in dogs of 6-MP and its analogue azathioprine.	1961	40
Murray	Clinical trials begun with 6-MP and azathioprine.	1962	41
Murray	Report of first 13 patients treated with 6-MP or azathioprine, one of whom reached 1 year with a still functioning but failing kidney allograft on 4/5/63.	1963	42
Starzl	First systematic use of azathioprine and prednisone with long survival of most of kidney allografts.	1963	8
Starzl	Clinical experience summarized with azathioprine/prednisone therapy in recipients of 67 kidney allografts and 6 baboon xenografts.	1964	43
Antibody immunosuppression			
Waksman	Demonstration of anti-lymphocyte serum (ALS) efficacy with skin allograft test model in rats.	1961	44
Woodruff	Showed additive protection of skin allografts in rats using ALS combined with thoracic duct drainage.	1963	45
Monaco	Convincing demonstration of the therapeutic value of ALS in the canine kidney transplant model.	1966	46
Starzl	First clinical trial of anti-lymphocyte globulin (ALG) as an adjunct to azathioprine and prednisone for human kidney transplantation. With the hybridoma technology of Kohler and Milstein (1975) monoclonal antibodies could be raised against discrete immunologic targets. In 1981 anti-CD3 antibody (OKT3) was introduced clinically.	1967	47

Table 4. Liver transplantation.

Author	Discovery or application	Year published	Reference
Preimmunosuppression			
Welch	First mention of hepatic transplantation in the literature, with insertion of an auxiliary liver in unmodified dogs.	1955	48
Moore	Independent studies in Boston and Chicago of liver replacement	1960	49
Starzl	(orthotopic transplantation) in unmodified dogs.	1960	50
Starzl	Transplantation in dogs of multiple abdominal viscera, including liver and intestine, nearly identical to human procedures done three decades later.	1960	51
Immunosuppression era			
Starzl	World's first three attempts at orthotopic liver transplantation in humans (March 1, May 5, and June 24, 1963) with maximum survival of 21 days.	1963	52
Starzl	Discovery that splanchnic venous blood of dogs contained hepatotrophic factor(s), the most important of which was later proved to be insulin; the finding dictated methods of liver allograft revascularization.	1964	53
Starzl	First >1-year survival after liver replacement in any species (here mongrel dogs) with recognition of the liver's unusual ability to induce tolerance under a 3- to 4-month course of azathioprine, or in this canine model after only a few perioperative injections of ALS or ALG [47].	1965	54
Cordier	Observed that liver allografts in untreated pigs frequently were not rejected. This finding of spontaneous tolerance to livers was promptly confirmed by Peacock and Terblanche in Bristol and by Calne in Cambridge.	1966	55
Starzl	First report of prolonged survival of four (of seven) children after orthotopic liver transplantation between July 1967 and March 1968.	1968	56
Calne	Report of first four patients in the Cambridge (England) liver replacement series, including an adult with >4 months survival.	1968	57
Calne	Showed that spontaneous tolerant pig liver recipients also were tolerant to skin and kidney allografts from the same donor.	1969	58
Starzl	Text summarizing experience at the University of Colorado with 25 liver replacements to March 1969 and 8 cases elsewhere.	1969	59
Starzl	Metabolic abnormality of Wilson's disease corrected, first of more than two dozen liver-based inborn errors cured or ameliorated with liver replacement. These liver recipients and patients cured of mesoderm-based inborn errors by bone marrow transplantation were the first examples of effective genetic engineering.	1971	60

1960s and the subsequent establishment of organ procurement agencies, usually associated with clinical immunology laboratories for tissue (HLA) matching. By 1974 renal transplantation had become a government-financed component of health care in most Western countries.

Liver Transplantation

After a failed trial in 1963, liver transplantation was successfully performed in humans in July 1967 (Table 4) [48–60]. Hepatic replacement was initially viewed as too difficult to be technically feasible, particularly in terminally ill patients for whom artificial organ support comparable to renal dialysis was not available. Instead, challenges generated by its surgical difficulty and physiologic complexity made liver transplantation the co-leader after 1963 (with the kidney) or the leader in the development of broadly applicable advances of surgical technique, immunosuppression, and means of multiple organ procurement and preservation.

Despite a high mortality rate during the first year after liver transplantation, nearly two dozen recipients from this early era have been stable for 20 to more than 29 years using immunosuppression with azathioprine, prednisone, and antilymphocyte globulin (ALG). The proof of the liver's unusual tolerogenicity (Tables 1, 4) is that most of these patients have been able to discontinue immunosuppressive therapy without rejecting their grafts.

The ripple effects of liver transplantation included discovery of the first hepatotrophic factors (beginning with insulin) that are involved in hepatic growth control and regeneration. More than two dozen liver-based inborn errors of metabolism have been corrected by liver transplantation, with clarification of disease mechanisms in some.

Heart Transplantation

The landmarks of heart transplantation are summarized in Table 5 [61–69]. Studies of heart transplantation were carried out at Stanford University in dogs and subhuman primates from the late 1950s to 1967. The results justified the decision by this group to proceed clinically, as announced by interview in the November 20, 1967, issue of the *Journal of the American Medical Association.* On December 3, heart replacement was carried out in Cape Town following an extended visit by the South African team leader to Stanford and other American transplant centers. The first South African recipient died from infection after 18 days, but the second patient (January 2, 1968) lived several years. On January 5, 1968,

Table 5. Heart transplantation.

Author	Discovery or application	Year published	Reference
Cass	Described standard current practice of combining the multiple pulmonary venous and venacaval anastomoses into two large atrial anastomoses. No dogs survived the operation.	1959	61
Lower	Independently developed same procedure as Cass/Brock, preserving allografts with immersion hypothermia. Dogs recovered.	1960	62
Lower	Technically successful canine heart-lung transplantation in nonimmunosuppressed dogs with 5-day survival. With long survival the same operation was done under cyclosporine two decades later, first in monkeys and then in humans.	1961	63
Lower	Immersion hypothermia of canine allografts at 2°–4°C adequately preserved dog hearts for 7 hours.	1962	64
Dong	Demonstrated normal heart function and reinervation of cardiac autografts 2 years after transplantation in dogs.	1964	65
Hardy	Transplantation of chimpanzee heart to human recipient. The heart was too small to support the circulation and failed after 2 hours.	1964	66
Lower	First long survival (up to 9 months) of heart allografts in any species (here dogs). Azathioprine-based immunosuppression was guided by electrocardiogram (ECG) voltage changes, especially R-wave diminution.	1965	67
Barnard	Description of the world's first transplantation of a human heart in Cape Town on 12/3/67, with 18 days survival. A second attempt in New York on 12/6/67 failed after 6 hours. A third recipient, operated in Cape Town on 1/2/68, survived for several years.	1967	68
Stinson	The world's fourth human heart transplantation at Stanford on 1/5/68 was successful and inaugurated the long-standing thoracic organ transplant program at that institution.	1970	69

the Stanford program recorded its inaugural human case, which was successful.

Graft survival after heart transplantation using triple-drug immunosuppression (azathioprine, prednisone, ALG) was essentially equivalent to that of cadaver kidney transplantation. As with kidney and liver transplantation, many of the pioneer cardiac recipients enjoyed an excellent quality of life, ensuring prompt acceptance and widespread application of all these operations when better immunosuppression became available.

Tissue Matching

The ABO blood groups, the compatibility of which was later found to be a requirement for transfusion and for bone marrow and organ transplantation, were discovered in 1901 [70]. Similarly, it was necessary to develop methods to type human tissue antigens and then to determine which were compatible or incompatible with those of the donor (Table 6) [70–94]. This was made possible with the discovery in transfused patients, and in women who had been pregnant, of leukoagglutinating and lymphocytotoxic antibodies that recognized alloantigens.

The introduction of computer-assisted search systems allowed delineation of families of antibodies that reacted with individual alloantigens and also made feasible the grouping of alloantigens into the two closely associated series that are now called HLA-A and HLA-B. The demonstration of crossover of the A and B antigens established HLA as a closely linked supergene. After 1964 use of the microcytotoxicity test greatly facilitated the standardization of HLA typing and the search for HLA antigens. The method was adapted for donor-recipient crossmatching and subsequently for the detection of pretransplant sensitization to HLA alloantigens.

HLA matching has been a stringent requirement for bone marrow transplantation (Table 2). For organ transplantation, the lymphocytotoxic crossmatch has been of crucial importance. Although there is clear evidence that the HLA system contains the dominant histocompatibility antigens, it has not been possible to identify which mismatches would result in failure. Nonetheless, HLA-identical sibling kidney allografts provide the highest graft survival rates. These are approached by survival rates of zero HLA-mismatched cadaver kidneys, justifying kidney sharing.

Quarter Century after 1974

The advent of cyclosporine two decades ago was a watershed for both bone marrow [95] and organ [96] transplantation. When the new drug was substituted for azathioprine, allograft survival and the quality of recipient life improved dramatically. In particular, the transplantation of cadaver organs was upgraded from a frequently feasible but unpredictable service to a reliable one. The results of organ transplantation were further enhanced after another decade with the introduction of tacrolimus [97]. Other promising drugs and monoclonal antibody preparations have been introduced more recently or are in various stages of preclinical or clinical evaluation. However, the therapeutic principles have remained essentially the same as were originally developed with azathioprine, prednisone, and ALG.

With more potent immunosuppressive agents, the field of transplantation has expanded continuously over the last 25 years. Heart–lung and lung transplantations were extensions of the heart procedure. Although survival of a lung recipient for 10 months had been accomplished as early as 1969 [98], the first examples of survival exceeding 1 year were not reported for heart–lung transplantation until 1982 [99] and for lung transplantation until 1987 [100]. Efforts at transplantation of abdominal organs expanded from the liver-only to the liver combined with small bowel [101]

Table 6. Tissue matching.

Author	Discovery or application	Year	Reference
Landsteiner	Discovery of ABO blood groups.	1901	70
Gorer	Described single dominant histocompatibility locus (later H-2) in mouse, analogous to the human leukocyte antigen (HLA) system.	1948	71
Dausset	Discovered first HLA antigen (MAC) using antiserum from transfused patients.	1958	72
Van Rood	Independently demonstrated HLA antibodies in pregnant	1958	73
Payne	women.	1958	74
Van Rood	First use of computers to make sense of the complex reactions produced by human antibodies, allowing identification of antigens currently known as HLA-B 4 and 6, as well as leukocyte antigen grouping.	1963	75
Starzl	Hyperacute rejection of ABO-incompatible kidneys (from host isoagglutinins) and rules to prevent it.	1964	76
Terasaki	Description of microcytoxicity test, critical for further development and practical use of HLA typing.	1964	77
Bach	Independently described mixed lymphocyte culture (MLC) test	1964	78
Bain	of histocompatibility.	1964	79
Payne	Defined allelic system now known as HLA-A 1, 2, and 3.	1964	80
Van Rood	Described antigens now known as HLA-B7+B27 and HLA-B8 as part of a closely associated system.	1965	81
Dausset	Proposed single locus for the HLA system, analogous to the mouse H-2 system.	1965	82
Terasaki	Description of hyperacute kidney rejection associated with	1965	83
Kissmeyer-Nielsen	antigraft lymphocytotoxic antibodies and proposed prevention with cytotoxic crossmatch (Terasaki), confirmed and extended the following year with the leukoagglutinin test (Kissmeyer-Nielsen).	1966	84
Terasaki	First prospective trial of HLA matching for donor selection.	1966	85
Van Rood	Proposal that initiated the first international organ exchange organization.	1967	86
Ceppellini	Coined the term "haplotype" to indicate the chromosomal combination of HLA alleles.	1967	87
Amos	Showed that the MLC reaction was detecting the HLA-D locus.	1968	88
Kissmeyer-Nielsen	Described the first crossover between HLA-A and HLA-B, proving that HLA identified a chromosomal region and not a single locus.	1969	89
Dausset	Demonstrated the importance of HLA compatibility for the survival of skin grafts in unmodified human volunteers.	1970	90
Starzl	Long survival frequently achieved at all levels of HLA	1970–1	91
Mickey	mismatch using a living donor and cadaveric kidneys. However, the best function, histologic appearance of allografts, and survival as well as the least dependence on immunosuppression was with zero-HLA mismatched kidney allografts.		92
Terasaki	Identification of presensitized patients at high immunologic risk using the panel reactive antibody (PRA test).	1971	93
Van Leeuwen	Identified the first sera that could be used for HLA-DR typing. This formed the basis on which HLA-DR serology was developed.	1973	94

and to the more complex multiple abdominal visceral grafts [102]; in the end it resulted in successful engraftment of the small bowel alone [103]. Tacrolimus played a crucial role in making the abdominal procedures involving intestine clinically applicable.

Although pancreas transplantation was offered at first only to diabetic patients who also were undergoing kidney transplantation for diabetes-associated end-stage renal disease [104], pancreas transplantation alone has been performed more recently in nonuremic diabetics [105]. The alternative appealing approach of transplanting the isolated islets of Langerhans only was attempted during the 1970s but did not result in success (defined as insulin independence) until 1990 in a patient with postpancreatectomy diabetes [106] and 1991 in a patient with type I diabetes [107]. Success with this procedure still is achieved only in occasional cases.

Résumé

La transplantation d'organes, de cellules et de tissus a littéralement explosée dans ce dernier quart de siècle, avec le développement d'une multitude de nouvelle spécialités. Cependant, les principes de base qui ont rendu ceci possible ont été établi sur trois décennies, commençant pendant la deuxième guerre mondiale et terminant en 1974. Pendant la conférence de consensus historique tenu à l'UCLA du 25 au 27 mars, 1999, 11 chercheurs sur la transplantation travaillant en sciences

fondamentales et/ou en clinique se sont mis d'accord sur les contributions les plus significatives de cette période et ont donné à la discipline de transplantation sa crédibilité présente. Ces découvertes et accomplissements ont été résumés en six tableaux, dotées de 93 références.

Resumen

En los últimos 25 años se ha producido un auténtico renacimiento por lo que a trasplantes de órganos, células y tejidos se refiere, lo que ha propiciado el desarrollo de múltiples áreas nuevas de especialización. Sin embargo, los principios que hicieron posible los trasplantes se establecieron hace más de 3 décadas, ya que las investigaciones al respecto se realizaron en el periodo de tiempo comprendido desde los comienzos de la 2ª Guerra Mundial al final de 1974. En la histórica conferencia de consenso, celebrada en UCLA, del 25 al 27 de marzo de 1999, 11 investigadores pioneros, procedentes tanto de las ciencias básicas como de la clínica y del tratamiento mediante trasplantes, alcanzaron un acuerdo sobre, cuáles fueron los hitos más importantes de este periodo, que permitieron que la técnica de los trasplantes sea hoy una especialidad clínica bien definida y en continua expansión. Estos descubrimientos y realizaciones se resumen en 6 tablas y 93 referencias bibliográficas.

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Trends in Organ Donation and Transplantation in the United States, 1996–2005

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Key words: Allocation, OPTN, organ donation, SRTR, survival rates, waiting list

Introduction

This brief overview of solid organ transplantation in the United States is produced as part of the 2006 *OPTN/SRTR Annual Report*. The *Annual Report* is prepared by the Scientific Registry of Transplant Recipients (SRTR) in collaboration with the Organ Procurement and Transplantation Network (OPTN) under contract with the Health Resources and Services Administration (HRSA). The report gathers a large amount of information on many aspects of solid organ transplantation in one publication, making it a valuable resource for patients, the transplant community, the public and the Federal Government.

The 2006 SRTR Report on the State of Transplantation comprises nine articles devoted to specific topics in solid organ transplantation. Each article was written by a group of experts in the field of transplantation and provides a comprehensive look at the current state of transplantation and trends over the past decade. The text and figures in these articles are based on recent SRTR analyses and the extensive reference tables of the 2006 *Annual Report*, which were prepared by the Arbor Research Collaborative for Health (formerly known as the University Renal Research and Education Association, or URREA), which with the University of Michigan has been the contractor for the SRTR since October 2000. These nine articles and the data tables on which they are based are included in the *Annual Report* and are available online, at the websites of the SRTR and OPTN (www.ustransplant.org and www.optn.org).

Summary Statistics on Organ Transplantation in the United States

As of the end of 2004 there were 153 245 people living with a functioning organ transplant in the United States. This number reflects an increase by about 1.8% over the prior year and a 1.7-fold increase since 1996.

The total number of organs transplanted annually increased from 26 541 in 2004 to 27 527 in 2005, an increase of 986 (4%). The transplanted organs with the highest percentage increases were intestine (31%) and lung (20%), as shown in Table 1. These organs came from 14 488 organ donors in 2005, 335 more donors than in 2004 (2%). The increase in the total number of donors resulted from a substantial increase of 443 (6%) deceased donors and a slight decrease of 108 (2%) living donors. This is the first time in the past decade that a decrease in the number of living donors was observed from one year to the next. A deceased donor usually provides several organs to benefit multiple patients with organ failure. The organ donation and transplantation collaborative initiatives of the Division of Transplantation at HRSA have successfully focused on increasing the number of deceased donors and on the number of organs per donor by working with professionals at organ procurement organizations, donor hospitals and transplant centers (1).

Overall there were approximately 90 000 people registered on organ waiting lists at the end of 2005 (63 814 actively waiting and 26 053 with 'inactive' status), a 5% increase over the number of people waiting for an organ at the end of 2004. The overall percentage of wait-listed patients with inactive status rose from 14% in 1996 to 29% in 2005; percentages vary considerably by organ. The largest increase was in the number of people on the kidney transplant waiting list, increasing by 8% from 57 389 in 2004 to 62 294 in 2005, a net addition of 4905 candidates (Table 2). This large waiting list is in part due to the cumulative effect of the imbalance between supply of organs and demand (need) for organs over past years. The net change in the total number of candidates on the waiting list at year-end from one year to the next provides an indication of the balance between supply and demand during that year. A net growth indicates that the waiting time on average increases, whereas a decline in the number of patients on the waiting list projects a shortening of average waiting times. Figure 1 shows that for 2004–2005, the organ supply fell short of the increasing need not only for kidneys but also to a lesser degree for livers (by 1%, or 117 livers) and pancreata. By contrast, there is good news for other organs, particularly for lungs and hearts, for which organs both the supply and demand increased and the size of the waiting list decreased. From 2004 to 2005, the size of the heart,

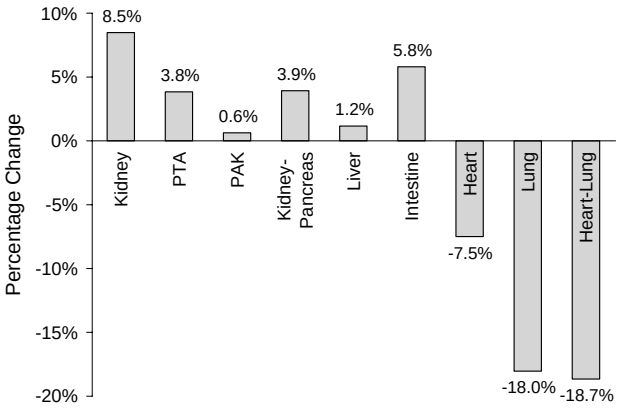
Table 1: Growth in number of transplanted and recovered organs, 2004–2005

Organs transplanted				Organs recovered from deceased donors			
Transplanted organs	2004	2005	Percent change	Recovered organs	2004	2005	Percent change
Total	26 541	27 527	3.7%	All DD organs	25 221	26 910	6.7%
Deceased donor	19 551	20 635	5.5%				
Living donor	6990	6892	−1.4%				
Kidney	15 674	16 072	2.5%	Kidney	12 570	13 313	5.9%
Deceased donor	9027	9509	5.3%				
Living donor	6647	6563	−1.3%				
Pancreas				Pancreas (all)	2010	2034	1.2%
PTA	130	129	−0.8%				
PAK	419	343	−18.1%				
Kidney-pancreas	880	896	1.8%				
Liver	5779	6000	3.8%	Liver	6404	6784	5.9%
Deceased donor	5457	5679	4.1%				
Living donor	322	321	−0.3%				
Intestine	52	68	30.8%	Intestine	168	185	10.1%
Heart	1961	2063	5.2%	Heart	2096	2220	5.9%
Lung	1168	1405	20.3%	Lung	1973	2374	20.3%
Deceased donor	1153	1404	21.8%				
Living donor	15	1	−93.3%				
Heart-lung	37	32	−13.5%	Heart-lung	NA	NA	NA

Source: *2006 OPTN/SRTR Annual Report*, Tables 1.2 and 1.7.

lung and heart-lung waiting lists dropped by 8%, 18% and 19%, respectively. The dramatic changes for lungs can be largely attributed to a new deceased donor lung allocation policy that was implemented in May 2005. The allocation policy was changed from a system based on waiting times to one based on net survival benefit from transplantation and medical urgency (waiting list mortality risk) (2).

Continuing a trend extending back more than 10 years, the transplant candidate population is increasingly older (Figure 2). In 1996, 7% of the overall waiting list candidates were 65 or older; in 2005, that percentage was 13%. The proportion of candidates aged 50–64 rose as well, from 34% in 1996 to 44% in 2005. The percentages of candidates in every age group below 50 years, including pediatric candidates (under 18 years), have dropped over the decade.



Source: 2006 OPTN/ SRTR Annual Report, Table 1.3.

Figure 1: Change in numbers of patients on waiting lists, 2004–2005.

Key outcomes after transplantation include (1) survival of transplant recipients and (2) the function of transplanted grafts. Table 3 displays 1- and 5-year unadjusted patient survival for all transplant recipients by organ, using the most recent cohort for which adequate follow-up exists. The cohort used to compute 1-year survival consists of recipients transplanted in 2003–2004, while the cohort for 5-year survival is based on recipients transplanted in 1999–2004. One-year patient survival rates were highest for kidney and pancreas recipients, ranging from about 95% to 98%; corresponding survival for liver, intestine and heart recipients was approximately 87–91%, about 85% for lung,

Table 2: Waiting list candidates (active and inactive combined), 2004–2005

Organs	End of Year 2004	2005	Percent change
Total	85 610	89 884	5.0%
Kidney	57 389	62 294	8.5%
PTA	502	521	3.8%
PAK	971	977	0.6%
Kidney-pancreas	2 381	2474	3.9%
Liver	16 967	17 168	1.2%
Intestine	191	202	5.8%
Heart	3210	2970	−7.5%
Lung	3828	3139	−18.0%
Heart-lung	171	139	−18.7%

Source: *2006 OPTN/SRTR Annual Report*, Table 1.3.
PTA = pancreas transplant alone; PAK = pancreas after kidney.

Table 3: Unadjusted 1- and 5-year patient survival by organ

Organ transplanted	1-year survival	5-year survival
Kidney		
Deceased donor	94.7%	80.7%
Living donor	98.0%	90.4%
Pancreas alone	94.9%	90.2%
Pancreas after kidney	95.5%	83.6%
Kidney-pancreas	95.1%	85.8%
Liver		
Deceased donor	86.9%	73.4%
Living donor	91.2%	76.8%
Intestine	87.5%	50.2%
Heart	88.1%	73.7%
Lung	84.9%	51.6%
Heart-lung	66.7%	43.6%

Source: *2006 OPTN/SRTR Annual Report*, Table 1.13.

Table 4: Unadjusted 1- and 5-year graft survival by organ

Organ transplanted	1-year survival	5-year survival
Kidney		
Deceased donor	89.5%	67.1%
Living donor	95.1%	80.3%
Pancreas alone	72.8%	53.4%
Pancreas after kidney	78.7%	56.4%
Kidney-pancreas (kidney)	91.8%	76.3%
Kidney-pancreas (pancreas)	85.2%	71.1%
Liver		
Deceased donor	82.4%	67.4%
Living donor	84.0%	68.8%
Intestine	78.5%	40.1%
Heart	87.5%	72.6%
Lung	83.3%	48.9%
Heart-lung	64.1%	41.5%

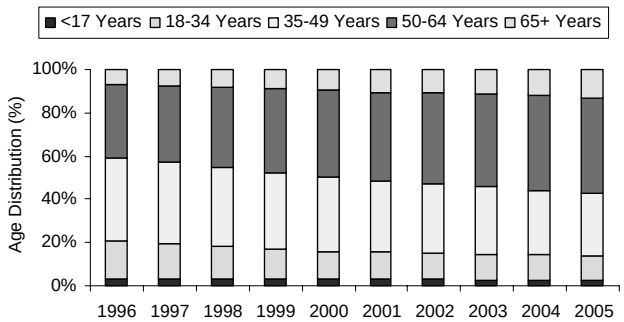
Source: *2006 OPTN/SRTR Annual Report*, Table 1.13.

and lowest for the small number of heart-lung recipients with approximately 67% surviving at 1 year.

Table 4 shows the percentage of transplanted organs that are still functioning (graft survival) 1 and 5 years after transplantation by type of organ. Like patient survival, graft survival is calculated based on the same most recent cohorts for which sufficient follow-up was available. Graft survival rates are lower than corresponding patient survival rates because patients may survive a graft failure by receiving a second transplant or with an alternative therapy, such as dialysis for kidney transplant recipients or insulin therapy for pancreas transplant recipients.

Transplantation at a Glance

The full-page figures at the end of this article (Figures 3–10) offer 'dashboard' views of the state of transplantation for different organs. Sets of summary graphics are included for six organs (kidney, pancreas, liver, intestine, heart and lung) as well as two common multi-organ procedures (simultaneous pancreas-kidney and pancreas after kidney). For this overview, we have omitted separate figures for heart-lung transplants, given the extremely small numbers of these procedures. Below we describe the three graphs shown for each organ.



Source: 2006 OPTN/ SRTR Annual Report, Table 1.3.

Figure 2: Age distribution on waiting lists, all organs, 1996–2005.

Number of transplants and size of active waiting list

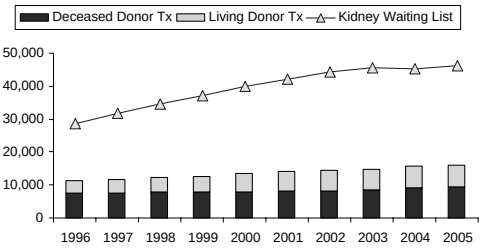
These figures compare, for each of the last 10 years, the size of the active waiting list and the number of transplants performed. The size of the waiting list is a snapshot of the number of candidates active on the waiting list at the end of the year, although additional patients were listed or removed at some time during the year. The number of transplants includes all transplants performed over the year. This difference in ways of counting explains why for some organs (e.g. lung), the number of transplants performed during a certain year may exceed the number of people awaiting that organ on the last day of the same year. Other instances of the narrowing gap between waiting list size and number of transplant reflect changes in allocation policy and wait-listing practices.

Age distribution of recipients and active waiting list

In this overview, we have grouped all pediatric patients (<18 years) together, for ease of viewing. The *OPTN/SRTR Annual Report* data tables (and the accompanying text analyzing them) break this group out into several age groups: <1 year, 1–5 years, 6–10 years and 11–17 years. See 'Pediatric Transplantation in the United States, 1996–2005', an accompanying article in this report, for details (3). Here we have included only the data for 1996 and 2005; additional detail may be found in the organ-specific articles of this report (4–6).

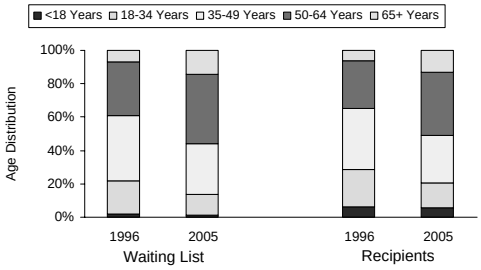
Unadjusted patient and graft survival

These overview figures show survival of the transplanted organ (graft) and survival of transplant recipients (patient) at various time points following transplantation: 3 months, 1 year, 3 years and 5 years. The figures are based on information about the most recent cohorts possible that still allow sufficient follow-up time for data collection and ascertainment.



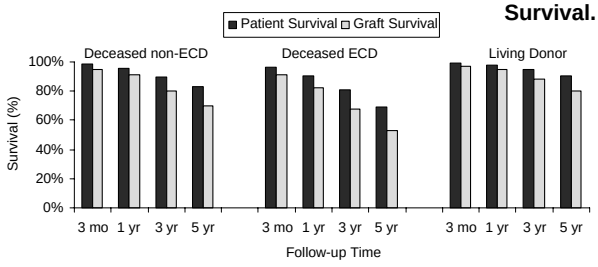
Number of Transplants and Size of Active Waiting List.

There is a very large gap between the number of patients waiting for a transplant and the number receiving a transplant. This gap has been widening, which means that the waiting times from listing to transplant continue to increase. Living donor transplants had increased until 2004 while deceased donor transplants increased gradually to 2005. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 5.1a.



Age Distribution of Recipients and Active Waiting List.

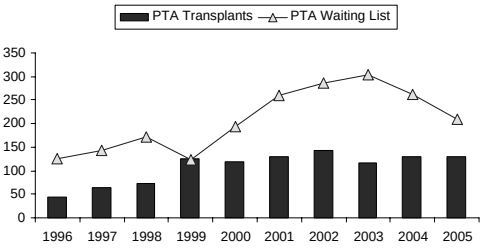
During the past decade the age distribution of candidates on the waiting list has changed such that older candidates now make up a much larger fraction of patients actively awaiting an organ. The same pattern is observed for transplant recipients except that ages <35 years show a greater representation than on the waiting list. Source: *2006 OPTN/SRTR Annual Report*, Tables5.1a, 5.4a, 5.4b, 5.4c.



Unadjusted Patient and Graft Survival.

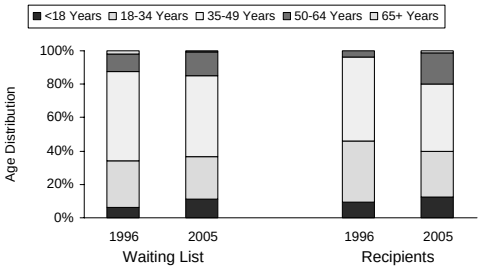
Patient survival in recent years has been improving. Five-year patient survival percentages based on transplants during 1999-2004 are clearly higher for living donors (90%) than for standard donor deceased donors (83%) and lowest among deceased donors (69%). Graft survival is lower since patients may live on dialysis or receive another transplant after graft failure. Source: *2006 OPTN/SRTR Annual Report*, Tables 5.10a, 5.10b, 5.10c, 5.14a, 5.14b, 5.14c.

Figure 3: Kidney transplantation at a glance.



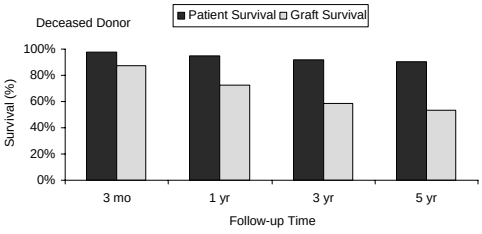
Number of Transplants and Size of Active Waiting List.

The number of patients on the waiting list for a pancreas transplant alone has decreased since 2003, which resulted in a narrowinggap between the number of patients waiting for a pancreas transplant alone (PTA) and the number receiving one.However, this gap was still present in 2005. The number of PTA per year has been stable in recent years. Source: *2006 OPTN/SRTR Annual Report*,Tables 1.7, 6.1a.



Age Distribution of Recipients and Active Waiting List.

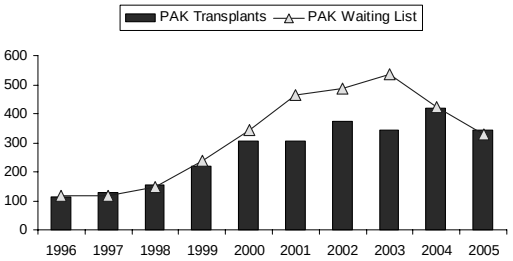
For PTA, more pediatric candidates were wait-listed and received a transplant in 2005 than in 1996. At the same time, the fraction of recipients over age 50 has grown. Pediatric diabetic patients rarely have kidney failure before age 18, but they are candidates for PTA. Source: *2006 OPTN/SRTR Annual Report*, Tables 6.1a, 6.4.



Unadjusted Patient and Graft Survival.

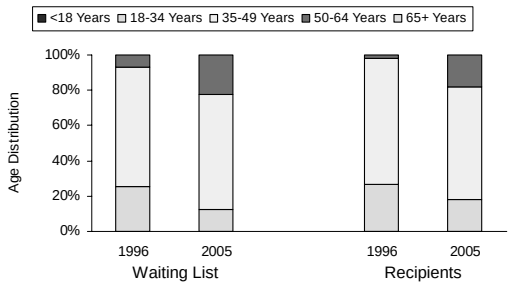
For PTA transplants, patient survival in recent years has been excellent; such patients do not usually have advanced kidney failure. The five-year patient survival is 90%. Graft survival is considerably lower since patients may live after graft failure through treatment with insulin. Source: *2006 OPTN/SRTR Annual Report*, Tables 6.10, 6.14.

Figure 4: Pancreas transplantation alone at a glance.



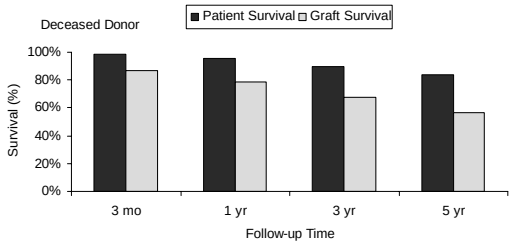
Number of Transplants and Size of Active Waiting List.

As with PTA, the number of patients on the waiting list for a PAK transplant has decreased since 2003. The gap between candidates and recipients decreased too. The number receiving a transplant matched the number of candidates at the end of 2004 and 2005. The number of PAK transplants has decreased in 2005 from its highest level of the decade in 2004. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 7.1a.



Age Distribution of Recipients and Active Waiting List.

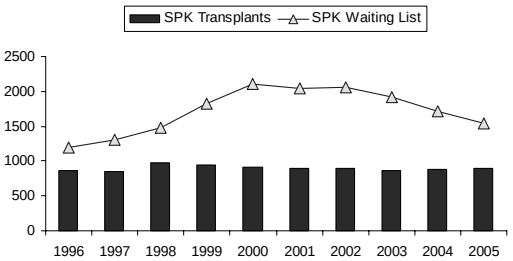
For PAK, more patients over 50 were wait-listed and received a transplant in 2005 than in 1996. At the same, time fewer candidates and recipients were in the age group of 18-34. Since recipients are mostly type 1 diabetics, the ages below 18 and above 65 are virtually unrepresented. Recipients include transplants from both living and deceased donors. Source: *2006 OPTN/SRTR Annual Report*, Tables 7.1a, 7.4.



Unadjusted Patient and Graft Survival.

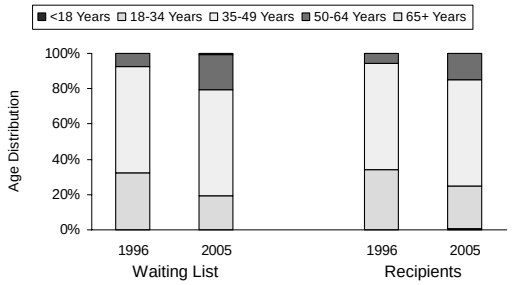
For PAK transplants, patient survival has been similar to that seen for simultaneous kidney-pancreas transplant recipients. Five-year patient survival is 84%. Graft survival is considerably lower since patients may live after graft failure through treatment with insulin. Source: *2006 OPTN/SRTR Annual Report*, Tables 7.10, 7.14.

Figure 5: Pancreas after kidney transplantation at a glance.



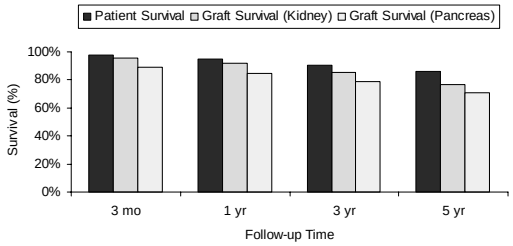
Number of Transplants and Size of Active Waiting List.

SPK accounts for the large majority of all pancreas transplants and has been stable over the last decade. The gap between the number of patients waiting for a transplant and the number receiving a transplant has been large, but has substantially decreased since 2000. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 8.1a.



Age Distribution of Recipients and Active Waiting List.

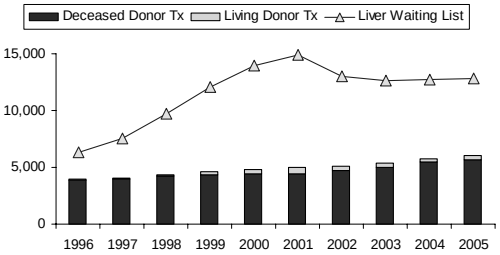
For SPK transplantation, a greater fraction of patients over age 50 were wait-listed and received a transplant in 2005 than in 1996. At the same time, fewer candidates and recipients were in the 18-34 age group. Since recipients are mostly type 1 diabetics, the ages below 18 and above 65 are virtually unrepresented. Recipients include transplants from both living and deceased donors. Source: *2006 OPTN/SRTR Annual Report*, Tables 8.1a, 8.4.



Unadjusted Patient and Graft Survival.

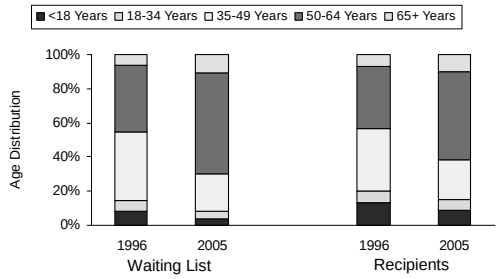
Patient survival has improved for SPK recipients in recent years. All SPK transplants are from deceased donors and their five-year patient survival is 86%. Graft survival is lower since patients may live after graft failure through treatment with insulin. Source: *2006 OPTN/SRTR Annual Report*, Tables 8.10, 8.14.

Figure 6: Simultaneous pancreas-kidney transplantation at a glance.



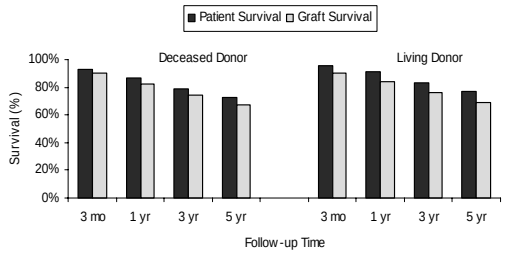
Number of Transplants and Size of Active Waiting List.

The large increase in the number of patients awaiting a liver transplant has stabilized since 2002, when the MELD/PELD system began. The number receiving a deceased donor liver transplant has gradually increased, more steeply in 2004 and 2005. The gap between the numbers of candidates and recipients has been slowly shrinking since 2002. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 9.1a, 9.1b.



Age Distribution of Recipients and Active Waiting List.

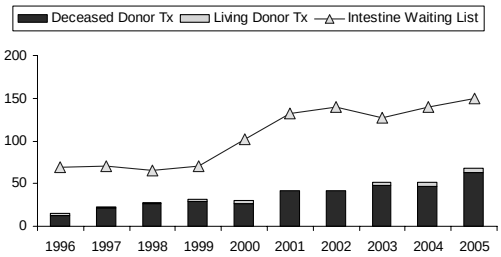
The numbers of candidates and recipients age 35-49 have remained fairly constant over the decade, but the age groupís proportion by both measures has declined. Recipients include transplants from both living and deceased donors. Source: *2006 OPTN/SRTR Annual Report*, Tables 9.1a, 9.4a, 9.4b.



Unadjusted Patient and Graft Survival.

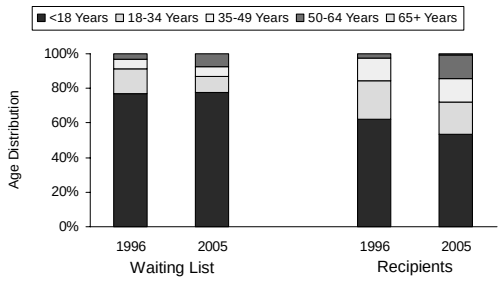
Patient survival in recent years has been improving for both deceased donors and living donors, with 73% and 77% of patients, respectively, alive five years following transplantation. Graft survival is lower since patients may live after graft failure through repeat liver transplantation. Source: *2006 OPTN/SRTR Annual Report*, Tables 9.10a, 9.10b, 9.14a, and 9.14b.

Figure 7: Liver transplantation at a glance.



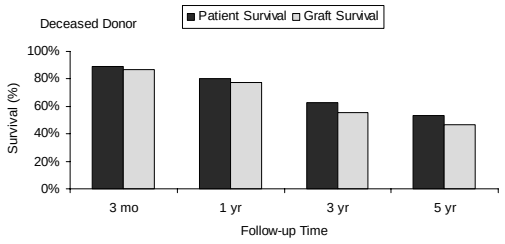
Number of Transplants and Size of Active Waiting List.

The numbers of patients on the intestine waiting list and the number receiving a transplant both approximately doubled between 1996 and 2005. The difference between the number of candidates and transplant recipients has been increasing through the second half of the decade. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 10.1a.



Age Distribution of Recipients and Active Waiting List.

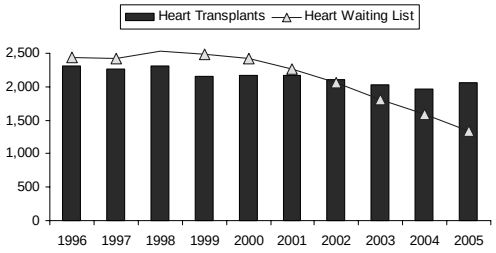
About 75% of intestine candidates have been in the pediatric age group. The small group of candidates in the age group >50 years doubled during the decade. Adult recipients make up a greater portion of recipients than candidates. The age group older than 50 shows a large increase during the decade in both categories. Source: *2006 OPTN/SRTR Annual Report*, Tables 10.1a, 10.4.



Unadjusted Patient and Graft Survival.

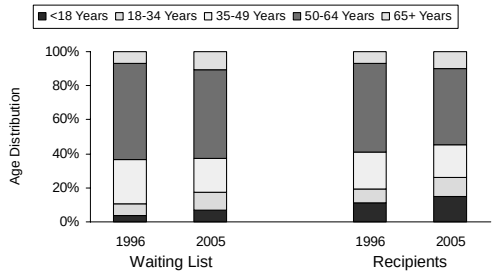
One-year patient survival reached 80% in 2005; however, longer-term survival at 5 years is 53%. Graft survival is lower, as patients may survive graft failure through parenteral alimentation or retransplantation. Source: *2006 OPTN/SRTR Annual Report*, Tables 10.10, 10.14.

Figure 8: Intestine transplantation at a glance.



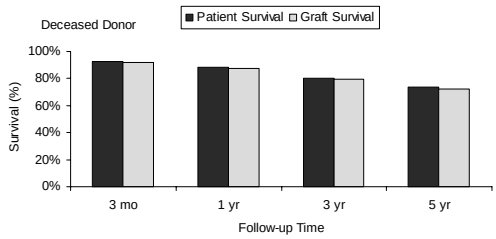
Number of Transplants and Size of Active Waiting List.

The number of heart transplants increased in 2005 after several years of gradual reduction. The number of patients awaiting a heart has decreased steeply since 2000, likely reflecting improvements in medical therapy. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 11.1a.



Age Distribution of Recipients and Active Waiting List.

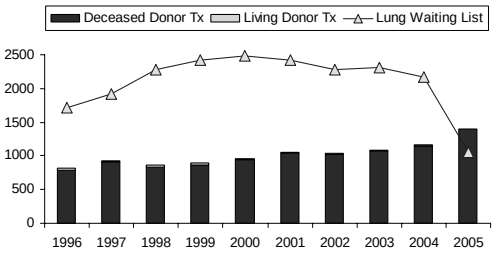
Trends in the age distribution of wait-listed candidates show that the ages below 34 and above 65 years have increased, while ages 35-64 years are less represented. The pattern of trends for transplant recipients is similar although the ages below 35 years have larger percentages than on the waiting list. Source: *2006 OPTN/SRTR Annual Report*, Tables 11.1a, 11.4.



Unadjusted Patient and Graft Survival.

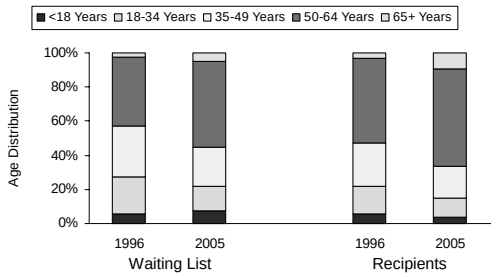
Patient survival has been improving in recent years for heart recipients. At one and five years following heart transplantation 88% and 74% of patients, respectively, are alive. Graft survival is very similar because very few patients may receive a second heart transplant after graft failure. Source: *2006 OPTN/SRTR Annual Report*, Tables 11.10, 11.14.

Figure 9: Heart transplantation at a glance.



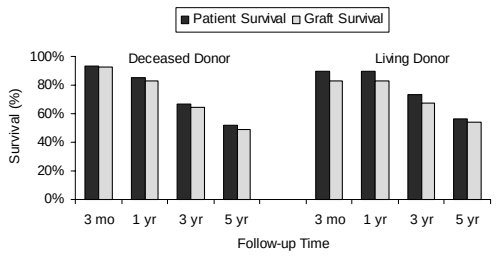
Number of Transplants and Size of Active Waiting List.

The number of lung transplants has increased steeply in the last year. The number of patients awaiting a transplant dropped steeply in 2005 after a stable pattern during the prior seven years. This sharp reduction is largely attributable to changes in allocation policy, which now considers urgency and benefit rather than time spent waiting. Source: *2006 OPTN/SRTR Annual Report*, Tables 1.7, 12.1a.



Age Distribution of Recipients and Active Waiting List.

The lung waiting list shows a mixed trend in age distribution, with increasing percentages of candidates who are pediatric or older than 50. Candidates 18-49 years old show a corresponding reduction in the percentage of the waiting list. The pattern for transplant recipients shows similarly a strong increase for ages above 50 years, but the decrease in percentages for younger ages includes pediatric ages. Recipients include transplants from both living and deceased donors. Source: *2006 OPTN/SRTR Annual Report*, Tables 12.1a, 12.4a, 12.4b.



Unadjusted Patient and Graft Survival.

Patient survival has been improving in recent years for both deceased and living donor lung transplant recipients. At one year following deceased donor and living donor lung transplantation 85% and 89% of patients, respectively, were alive. With the recent good results at one year, it is hoped that longer term patient survival will improve. Graft survival is very similar because very few patients may live after graft failure through a second lung transplant. Source: *2006 OPTN/SRTR Annual Report*, Tables 12.10a, 12.10b, 12.14a, 12.14b.

Figure 10: Lung transplantation at a glance.

Articles in the *2006 SRTR Report on the State of Transplantation*

The graphics above give a quick view of the major trends addressed and analyzed in each of the organ-specific articles of this report. Articles on kidney and pancreas (4), liver and intestine (5) and heart and lung (6) provide detailed trends in donation, waiting time, allocation, post-transplant outcomes and the demographics of both candidates and recipients. Additionally, these articles supplement the reporting of 10-year trends with updates on recent changes in allocation policy, immunosuppression, clinical practice and other areas relevant to the transplantation of different organ types.

In this year's report, the three organ-specific articles are preceded by a review of trends in organ donation and utilization (7) including recent efforts to increase the number of donors, and an article devoted to the particular outcomes and policy concerns of pediatric transplantation (3).

This year's report concludes with three 'special-focus' articles that look closely at issues of recent interest to the transplant community. An article on organ acceptance rates (8) examines what happens when transplant centers turn down kidneys offered by an organ procurement organization, as low acceptance rates may contribute to inefficiency in organ distribution. An article on geographic variability in access to kidney transplantation (9) examines rates in wait-listing, receiving a living donor kidney transplant, and receiving a deceased donor kidney transplant after being placed on the waiting list, identifying wide disparities in access across the United States. Finally, an article on repeat transplantation (10) focuses on the growing trend of same-organ retransplantation and its effects on the transplant community as a whole and on individual recipients, who are more likely to have inferior outcomes following retransplantation. These articles all include special analyses performed by the SRTR and touch on topics that are timely and have implications for policy and clinical practice.

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This study was approved by HRSA's SRTR project officer. HRSA has determined that this study satisfies the criteria for the IRB exemption described in the 'Public Benefit and Service Program' provisions of 45 CFR 46.101(b)(5) and HRSA Circular 03.

Note on sources: The articles in this report are based on the reference tables in the *2006 OPTN/SRTR Annual Report*, which are not included in this publication. Many relevant data appear in the figures and tables included here; other tables from the *Annual Report* that serve as the basis for this article include the following: Tables 1.1–1.4, 1.7, 1.13, 1.14, 5.1a, 5.10a–c, 5.14a–c, 5.4a–c, 6.1a, 6.10, 6.14, 6.4, 7.1a, 7.10, 7.14, 7.4, 8.1a, 8.10, 8.14, 8.4, 9.1a–b, 9.10a–b, 9.14a–b, 9.4a–b, 10.1a, 10.10, 10.14, 10.4, 11.1a, 11.10, 11.14, 11.4, 12.1a, 12.10a–b, 12.14a–b and 12.4a–b. All of these tables may be found online at: http://www.ustransplant.org.

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Perspectives in Organ Preservation

Mark-Hugo J. Maathuis, Henri G. D. Leuvenink, and Rutger J. Ploeg

Maintaining organ viability after donation until transplantation is critically important for optimal graft function and survival. To date, static cold storage is the most widely used form of preservation in every day clinical practice. Although simple and effective, it is questionable whether this method is able to prevent deterioration of organ quality in the present era with increasing numbers of organs retrieved from older, more marginal, and even non-heart-beating donors. This review describes principles involved in effective preservation and focuses on some basic components and methods of abdominal organ preservation in clinical and experimental transplantation. Concepts and developments to reduce ischemia related injury are discussed, including hypothermic machine perfusion. Despite the fact that hypothermic machine perfusion might be superior to static cold storage preservation, organs are still exposed to hypothermia induced damage. Therefore, recently some groups have pointed at the beneficial effects of normothermic machine perfusion as a new perspective in organ preservation and transplantation.

Keywords: Transplantation, Ischemia-reperfusion, Preservation, Hypothermic machine perfusion, Normothermic machine perfusion.

(*Transplantation* 2007;83: 1289–1298)

Despite better insights in surgical technique, immunosuppressive agents, and treatment of postoperative complications, 5- and 10-year results in organ transplantation have only moderately improved in the past decades (*1*). One explanation for this slightly disappointing fact is that more experience has led to an increased acceptance of older and more complex recipient candidates. Another reason is the fact that due to the persistent shortage, criteria for inclusion of deceased donors have been extended. Organs are nowadays more often retrieved from older, more marginal, and sometimes non-heart-beating (NHB) donors than 10 years ago. Between 1988 and 1995, the United Network of Organ Sharing (UNOS) registered a 170% increase in the number of deceased donors more than 50 years of age (*2*, *3*). The use of older donor kidneys, livers, and pancreata has resulted in a decrease in graft function and survival compared to grafts retrieved from young donors (*4–7*). Marginal and NHB donor organs suffer from additional warm ischemic injury. As a result these organs have higher primary nonfunction (PNF) and delayed graft function (DGF) rates compared to heart-beating deceased donors (*8–11*).

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Maintaining organ viability during preservation is an important prerequisite for successful outcome after transplantation. With the current practice to accept older and more injured donor organs, improvement of preservation techniques has now become a must. To date, most centers use static cold storage (CS) to preserve organs. This preservation method, however, was developed in an era with younger donors with good-quality organs (*12*). With the introduction of extended donor criteria the limitations of CS have probably been reached.

This review aims to describe a number of principles and pathophysiological mechanisms as well as current techniques in abdominal organ preservation.

Organ Preservation by Static Cold Storage

Currently, CS is the preferred organ preservation method in most centers. Simple cold storage starts with a rapid vascular washout to allow cooling of the organ, removal of blood components, and equilibrate the CS solution with the tissue (*13*, *14*).

Hypothermia

The principle of CS preservation is based on suppression of metabolism and catabolic enzymes by hypothermia (4°C). Metabolic rate is halved with each 10°C drop in temperature resulting in a remaining 10–12% metabolism at 4°C (*15*). Already in the early 1960s, it was shown that cooling by itself was able to improve preservation of small bowel, kidney, and liver: the so-called temperature effect (*16–18*). To further extend cold ischemic time (CIT) and counteract the detrimental side effects of the required hypothermia, special preservation solutions are necessary: the solution effect (*19*). Cell swelling, acidosis, and the production of radical oxygen spe-

cies (ROS) upon reperfusion are important side effects of hypothermia. To reduce these undesirable effects CS solutions include a number of specific compounds (*20*, *21*). The composition of several preservation solutions is illustrated in Table 1.

Cell Swelling

A very prominent alteration in the cellular structure during hypothermia is the formation of edema (*22*). The responsible mechanism is an impaired activity of Na^+/K^+ ATPase. As a result, sodium is no longer extruded but passively enters the cell. This creates a hyperosmolar intracellular environment and subsequently an influx of water. To prevent cell swelling, impermeants and colloids are added to preservation solutions.

Effective impermeants are saccharides and nonsaccharide anions. Molecular weight (MW) determines the effectiveness of saccharides to prevent cell swelling, with larger saccharides being more effective (*23–25*).

Glucose (MW 180) is a monosaccharide and was used in early CS solutions such as EuroCollins solution. When it became evident that glucose passes the cell membrane and becomes a source of lactate in an anaerobic environment, it was no longer considered as an effective impermeant (*26*). The slightly larger monosaccharide mannitol (MW 182) is not a source of lactate since it is not metabolisable and will not enter the cell through

TABLE 1. Composition of organ preservation solutions

	EC (76)	HOC (15)	PBS (28)	UW (80)	HTK (86)	CEL (90)	IGL-1 (35)
Colloids (g/L)							
HES	—	—	—	50	—	—	—
PEG-35	—	—	—	—	—	—	1
Impermeants (mM)							
Citrate	—	80	—	—	—	—	—
Glucose	195	—	—	—	—	—	—
Histidine	—	—	—	—	198	30	—
Lactobionate	—	—	—	100	—	80	100
Mannitol	—	185	—	—	38	60	—
Raffinose	—	—	—	30	—	—	30
Sucrose	—	—	140	—	—	—	—
Buffers (mM)							
Citrate	—	80	—	—	—	—	—
Histidine	—	—	—	—	198	30	—
K_2HPO_4	15	—	—	—	—	—	—
KH_2PO_4	43	—	—	25	—	—	25
$NaHCO_3$	10	—	—	—	—	—	—
NaH_2PO_4	—	—	13	—	—	—	—
Na_2HPO_4	—	—	56	—	—	—	—
Electrolytes (mM)							
Calcium	—	—	—	—	0.0015	0.25	0.5
Chloride	15	—	—	20	32	42	—
Magnesium	—	—	—	—	4	13	—
Magnesium sulphate	—	40	—	5	—	—	5
Potassium	115	79	—	120	9	15	25
Sodium	10	84	125	25	15	100	120
ROS scavengers (mM)							
Allopurinol	—	—	—	1	—	—	1
Glutathione	—	—	—	3	—	3	3
Mannitol	—	185	—	—	38	60	—
Tryptophan	—	—	—	—	2	—	—
Additives (mM)							
Adenosine	—	—	—	5	—	—	5
Glutamic acid	—	—	—	—	—	20	—
Ketoglutarate	—	—	—	—	1	—	—

EC, EuroCollins; HOC, hypertonic citrate/Marshalls solution; PBS, phosphate-buffered sucrose; UW, University of Wisconsin cold storage solution; CEL, Celsior; HTK, histidine-tryptophan-ketoglutarate; IGL-1, Institut George Lopez; HES, hydroxyethyl starch; PEG-35, polyethylene glycol with an average MW of 35 kDa; ROS, reactive oxygen species.

facilitated transport. In addition, mannitol has a beneficial effect as a scavenger of reactive oxygen species, and was therefore added in Marshalls, Bretschneider's histidine-tryptophan-ketoglutarate (HTK), and Celsior solutions. Sucrose (MW 342) is a disaccharide and is used in the renal preservation solution phosphate-buffered sucrose (*27*, *28*). Raffinose (MW 504) is the largest one and a trisaccharide. It was added as an impermeant in the University of Wisconsin (UW) CS solution (UW-CSS) developed by Belzer and Southard.

Nonsaccharide impermeants such as negatively charged gluconate, citrate, and lactobionate limit cell swelling by electrochemical forces. Effectiveness of these anions is determined by molecular weight as well as charge. Although hypertonic citrate (HOC) contains citrate, both UW-CSS and Celsior use the anion lactobionate.

As impermeants are predominantly effective at the level of cell membranes and the interstitial compartment, colloids are used for the intravascular compartment. These macromolecules are retained in the vascular spaces and act by imparting colloid osmotic pressure. Colloids were originally added to hypothermic machine preservation solutions (MPS) to prevent tissue edema due to hydrostatic pressure. Belzer and his group first used cryoprecipitated plasma, then albumin, and finally diafiltrated hydroxyethyl starch (HES) as they aimed at developing one solution suitable for both CS and hypothermic machine perfusion (HMP). The feasibility of HES as a colloid in UW-CSS has been extensively debated. HES prevents interstitial edema but also increases viscosity (*29*, *30*). For short preservation times, addition of a colloid has been doubted, although some organs such as the pancreas appeared to be more susceptible to edema when HES is omitted (*31*). Analyzing the effect of HES on red blood cells (RBCs), several authors have shown an increased RBC aggregability in both human and rat whole blood when large molecular sized HES is present (*30*, *32*). This effect could partially explain the frequently slower washout of blood and initially patchy reperfusion of organs when UW-CSS is used in clinical practice (*33*).

The HES controversy initiated a search for other colloids, such as dextran and polyethylene-glycol (PEG) (*34–36*). In this respect, UW-PEG preserved rat livers have shown lower transaminase levels, higher bile flow, and higher urea synthesis rate after transplantation (*37*). Several experimental studies have now confirmed the efficacy of PEG for liver as well as for kidney, pancreas, and small bowel preservation (*38–41*).

In contrast to UW-CSS, both HTK and Celsior do not contain a colloid. In a prospective study with short CIT, both solutions showed equal efficacy compared to UW-CSS for the preservation of kidney and liver grafts (*42*). With prolongation of preservation times beyond 24 hours, the presence of a colloid does appear to be important to maintain organ viability (*43*).

Energy and Acidosis

At a temperature of 0–4°C, cold storage results in a rapid depletion of cellular adenosine triphosphate (ATP). Within 4 hours, nearly 95% of ATP has disappeared with a shift to adenosine monophosphate as the predominant nucleotide. During CS, anaerobic metabolization of 1 mol glucose, however, only yields 2 mol ATP versus a maximum of 38 mol in aerobic glycolysis. Moreover, two lactic acid molecules are formed leading to acidosis (*13*, *44*).

The contribution of acidosis to ischemic injury is pH dependent. Severe acidosis activates phospholipases and proteases causing lysosomal damage and eventually cell death (*45*). Mild acidosis (pH 6.9–7.0), however, has been suggested to have a protective effect by inhibiting phosphofructokinase as the rate-limiting step in glycolysis (*45*, *46*). Adequate control of pH is therefore an important function of preservation solutions. UW-CSS uses phosphate as a buffer, while Celsior and HTK use histidine. Of those two solutions, HTK has the highest buffering capacity due to a high concentration of histidine (*21*).

Reactive Oxygen Species

Reactive oxygen species (ROS) are widely recognized as important mediators of postreperfusion induced organ injury (*47*). CS per se, however, has also been shown to promote ROS production, probably due to mitochondrial damage (*48*, *49*). An extensively studied generator of ROS is xanthine oxidase, which simultaneously produces hydrogen peroxide (H_2O_2) and the superoxide anion (O_2^-) (*50*, *51*). The subsequent reduction of H_2O_2, catalyzed by iron, leads to hydroxyl radical formation (·OH). Free or chelatable iron is not only a catalyst of ROS formation but also contributes directly to hypothermia induced injury by mediating mitochondrial damage and induction of apoptosis (*52–54*). ROS react rapidly with other molecules which will result in severe damage to lipids, nucleic acids, and proteins (*55*, *56*). The subsequent cell death mechanism appears to be ATP dependent. ATP is required for the execution of the apoptotic cell death program whereas complete ATP depletion will lead to necrosis (*57*, *58*). As free radical–mediated injury during preservation is strongly correlated with the absence of immediate and reduced long-term kidney function (*56*), preservation solutions aim to counteract ROS mediated injury during preservation and especially at time of reperfusion.

In UW-CSS, the compounds allopurinol and glutathione (GSH) were included to prevent formation of ROS. Allopurinol inhibits xanthine oxidase, which improved kidney preservation, whereas liver or pancreas preservation remain almost unaffected (*59*).

GSH is a tripeptide that is oxidized to glutathione disulphide together with converting peroxides. Experimental studies have shown the importance of GSH in an isolated perfused rabbit liver model. In the absence of GSH, more lactate dehydrogenase (LDH) was released into the perfusate (*60*), which was confirmed in the canine kidney transplant model. Subsequent studies have shown that GSH is especially important in long-term liver preservation (*61*).

GSH is also used in Celsior solution, whereas in HTK tryptophan might protect the organs against ROS-mediated damage. The antioxidative effects of tryptophan are controversial. Tryptophan can act as an antioxidant through its oxidative metabolites in the kyunerine pathway, such as 5-hydroxy-tryptophan (*62*). On the other hand, tryptophan can be pro-oxidant as well by presenting low molecular weight iron in a redox cycling event (*63*, *64*). In a cultured rat hepatocyte experiment, the amount of thiobarbituric acid reactive substances (TBARS), as a marker for ROS mediated injury, was measured. After 24 hours of preservation, TBARS were

significantly higher in HTK-preserved hepatocytes compared to UW-CSS, suggesting a superior antioxidant capacity of UW-CSS (*65*).

Electrolyte Composition

During the pioneering years in organ preservation a high potassium/low sodium ratio of the solution (intracellular type) was assumed necessary to prevent cell swelling. It was hypothesized that due to the inactivity of Na^+/K^+ ATPase during hypothermia, an intracellular sodium/potassium ratio in the extracellular fluid compartment would prevent sodium and chloride from entering the cell (*66*). Balancing extracellular sodium ions and intracellular protein anions creates the so-called Donnan equilibrium, which prevents edema formation (*24*). Intracellular type solutions such as UW-CSS were long considered to be pivotal for preservation of cell viability (*66*, *67*). Recent work, however, has suggested equal or improved results of extracellular type solutions with a low potassium/high sodium ratio, such as Celsior and HTK (*68–73*). This clearly demonstrates that sodium/potassium ratios as such do not play a central role in preservation. Also, a low potassium content will facilitate the washout of blood during organ procurement as no potassium induced vasospasm will occur (*70*, *74*).

In summary, essential components of effective preservation solutions are impermeants or colloids, an adequate buffering capacity, and anti-oxidants. In the next section, the clinical merits of some prominent preservation solutions for abdominal organs will be discussed.

Current Cold Storage Solutions

The first static CS preservation solution was developed by G.M. Collins in 1969 (*75*), which was modified by the Eurotransplant Foundation in 1976 by eliminating magnesium (Table 1) (*76*). EuroCollins (EC) solution was a simple and cheap intracellular type preservation solution. Phosphate was used for pH buffering and glucose served as the osmotic agent. In the late 1970s, an Australian group developed a HOC solution, which is also known as Marshalls solution. This solution was effective for 72 hours of canine kidney preservation and is still in clinical use (*77*). Another simple solution is phosphate-buffered sucrose, developed by Coffey and Andrews in the early 1980s. Phosphate-buffered sucrose was shown to be effective in kidney preservation confirming the hypothesis that high concentrations of impermeant saccharides suppress hypothermic cell swelling (*27*, *78*). When UW-CSS became available, a randomized clinical trial comparing EuroCollins with UW-CSS in kidney preservation showed that DGF was significantly lower in the UW-CSS group (23% vs. 33%). Also, 1-year graft survival was found to be significantly higher in the UW-CSS group. As a result of this study, EC was no longer the preferred solution for clinical abdominal organ preservation in Europe (Fig. 1A).

University of Wisconsin Solution

Continuous and systematic research by Belzer and Southard led to the development of the University of Wisconsin Solution in 1987. Metabolic inert substrates such as lactobionate and raffinose served as osmotic agents. HES was used as a colloid. Scavengers (glutathione, allopurinol) and an ATP precursor (adenosine) were added to the solution. Today, UW-CSS is still considered the gold standard preservation solution for kidney, liver, pancreas, and small bowel (Fig. 1) (*29*, *79–85*).

Histidine-Tryptophan-Ketoglutarate Solution

HTK solution was initially introduced as a cardioplegic solution in open-heart surgery by Bretschneider in the 1970s but was also tested in kidney, liver, and pancreas transplantation (*86*). The basic design of the solution consists of histidine, a very potent buffer, combined with two amino acids. Tryptophan serves as membrane stabilizer while ketoglutarate acts as substrate for anaerobic metabolism during preservation. HTK has a low viscosity and, to achieve complete tissue equilibration, high volumes (~15 l) have to be rinsed through the organs at low flow rates. A multicenter randomized prospective trial comparing UW-CSS versus HTK in kidney preservation showed equal results in terms of incidence of DGF (33% vs. 33%) (*79*). For prolonged cold storage times (>24 hours) little data is available. One single center study reported a twofold increase in incidence of DGF after HTK kidney preservation compared to UW-CSS when CIT was longer than 24 hours (*87*). The opposite was shown in another study with a DGF rate of 16% after HTK preservation versus 56% after UW-CSS (*88*). Direct comparison of these conflicting findings, however, is impossible due to a different definition of DGF in both studies.

In liver preservation, it has been suggested that HTK could be advantageous due to its low potassium concentration. Therefore, the need to flush out the potassium-rich

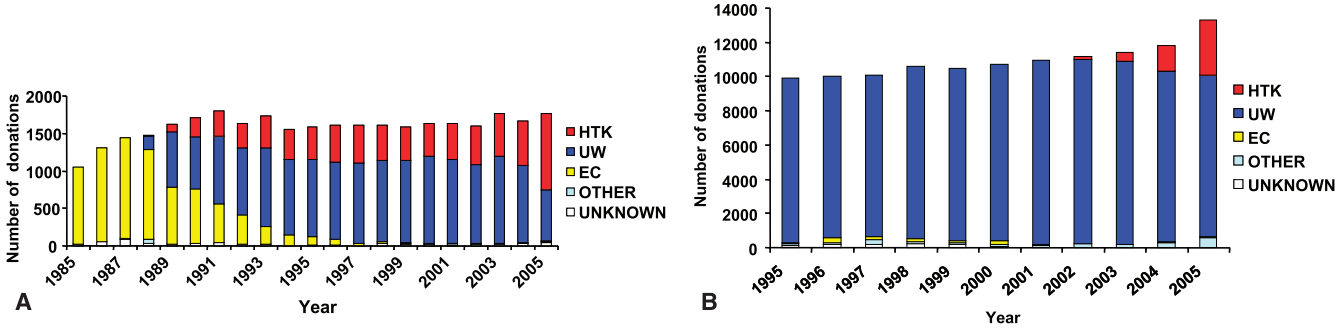

FIGURE 1. (A) Use of cold storage solutions in Eurotransplant region in deceased donors from 1985–2005 (based on Eurotransplant data of October 2006). (B) Use of cold storage solution in the United States in deceased donors from 1995–2005 (based on Organ Procurement and Transplantation Network data of October 2006).

UW-CSS from the organ prior to reperfusion would be limited. Although patient numbers were relatively small and cold ischemic times short, two studies using HTK in liver preservation showed equality of HTK and UW-CSS for short-term preservation (*81*, *89*). Despite the lack of a proper randomized and controlled trial, HTK is currently used by many centers as a preservation solution for all abdominal organs retrieved for transplantation (Fig. 1) (*88*).

Celsior Solution

Celsior is an extracellular type preservation solution developed in 1994 for CS preservation of cardiac grafts (*90*). This solution, however, proved to be effective in preserving abdominal organs as well (*42*, *83*, *84*). It combines the inert osmotic agent philosophy of UW-CSS with the strong buffering capacity of HTK. Reduced glutathione is added as a free-radical scavenger. Currently, it has been successfully used in clinical heart, lung, liver, pancreas, kidney, and small bowel preservation (*91*, *92*). The likelihood whether Celsior will eventually replace UW-CSS may depend on the results of a sufficiently powered multicenter trial.

New Solutions

The increasing awareness that ischemia/reperfusion injury does determine a significant part of posttransplant outcome has stimulated research in the field of preservation injury and the development of new preservation solutions. A relatively new preservation solution developed at the University of Amsterdam is Polysol. Its composition is based on the fact that metabolism is still present at 4°C. Polysol has been tested both as an experimental CS solution and as HMP solution (*93*, *94*). It is a classic preservation solution enriched with amino acids, vitamins, and antioxidants (*95*). Many components in Polysol, however, have not yet been evaluated separately. In experimental liver preservation studies, superiority over HTK was seen in CS preservation of steatotic livers showing improved functional parameters, such as oxygen consumption, bile production, and damage markers (*93*). Transplantation data in experimental and clinical preservation are now required to demonstrate the efficacy of Polysol. Based on its "metabolic support" design, however, beneficial effects of Polysol can be expected.

Another new and now clinically available preservation solution is IGL-1 (Institut George Lopez), developed by the Lyon group in France. IGL-1 builds on the heritage of both UW-CSS and Celsior (*35*, *93*, *96*). It combines the extracellular composition of Celsior with the colloidal support of UW-CSS using polyethylene glycol (PEG) instead of HES. In a porcine kidney autotransplantation model with IGL-1, PEG was found to limit influx of macrophages by approximately 50% (*97*). Polymers, such as PEG, spontaneously bind to cell and tissues surfaces and sterically stabilize the underlying surface from interactions with other components. The main advantage of this "immunocamouflage" is that it directly modifies inherent immunogenicity of donor tissue (*98*, *99*). PEG does not exert any aggregating effects on RBCs and in combination with the extracellular composition of IGL-1, washout of blood during the donor operation should be superior to UW-CSS (*30*, *32*, *100*).

Both rat and porcine transplantation studies of liver and kidney have shown encouraging results in terms of organ function after transplantation following preservation with IGL-1 (*41*, *69*, *101*). The first preliminary clinical results in renal transplantation with IGL-1 demonstrated a reduction in DGF compared to kidneys preserved with UW-CSS (5.7% vs. 13.8%, respectively). Also, less apoptosis was seen in IGL-1 preserved kidneys (*102*). Until now, however, patient numbers have been too small to draw clinically relevant conclusions and a randomized controlled multicenter study will have to confirm the initial results. Given its extracellular composition and the beneficial effects of PEG, IGL-1 could be considered a promising successor to UW-CSS.

Despite the fact that CS preservation methods have facilitated many transplant programs all over the world, it appears that the increasing challenge to maintain viability in extended-criteria donor organs is touching the limits of CS preservation. Even with beneficial additives and enriched compositions, static CS, at best, slows down ischemic damage. Furthermore, pretransplant viability testing is limited and preservation time is still counted in hours rather than in days. To further improve organ viability, a more dynamic preservation method is needed to better fulfil the metabolic demands of damaged organs. Therefore, many groups have recently switched gears and are revisiting the possibilities of hypothermic machine perfusion (HMP) (*103–105*).

Preservation by Hypothermic Machine Perfusion

In the early 1970s, hypothermic machine perfusion (HMP) was used by many centers in the United States and Europe to preserve kidneys, allowing transportation to a transplant center (*106–109*). Although modern HMP systems are smaller, lighter, and more sophisticated than the original machine used by Belzer and coworkers, the principles of HMP have not changed.

Machine perfusion generates a controlled continuous or pulsatile recirculating flow of preservation solution at 0–4°C. This continuous flow allows complete perfusion of the organ promoting a thorough washout of blood and subsequent tissue equilibration with the preservation solution. Beneficial effects claimed on behalf of machine perfusion are a low incidence of DGF, the possibility of real-time viability assessment, the ability to provide metabolic support during perfusion, and the potential to add pharmacologic agents to the perfusate.

In kidney preservation, both in animal experiments and in historical controlled retrospective clinical studies, HMP has been demonstrated to provide better early graft function compared to CS (*110*, *111*). In addition, when kidneys retrieved from extended-criteria, marginal, or NHB donors were analyzed, HMP was found to be superior to CS (*112–116*). Unfortunately, in most studies no prospective randomization was performed and patient numbers were not large enough to allow extrapolation of the results. Recently, Wight et al. reported an excellent meta-analysis based on aggregated results of the current literature concerning HMP versus CS, clearly demonstrating a 20% reduction in DGF with HMP (*117*). DGF reflects a compilation of accumulated risk factors and depends on the presence or absence of independent donor, preservation, and recipient characteristics (*29*). Possibly, some of the detrimental effects caused by these risk factors can be reduced with HMP. The occurrence of DGF requires continuation of dialysis and is associated with

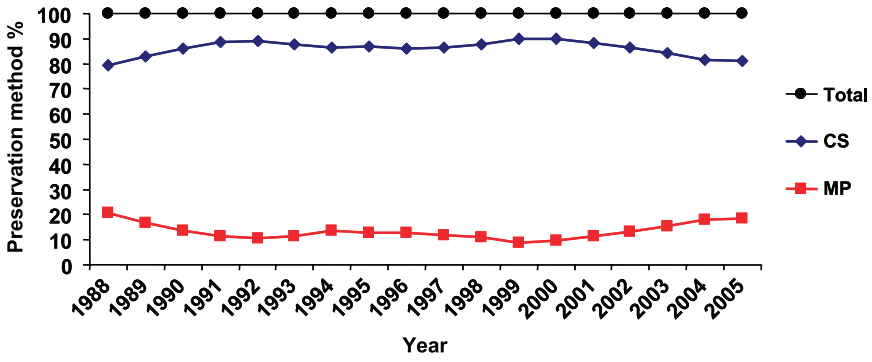

FIGURE 2. Relative amount of renal machine (MP) and cold storage preservation (CS) in the United States from 1988–2005 (based on Organ Procurement and Transplantation Network data of October 2006).

an increased incidence of acute rejection and inferior long-term outcome (*118*, *119*). While individual studies suggest potential benefits of HMP such as reduced DGF rates, less acute rejection, and improved short- and long-term function, no comparative study of these modalities has been performed under strict conditions (*120*). For this reason, recently a European multicenter prospective randomized clinical trial has been conducted in the Netherlands, Belgium, and Germany comparing HMP versus CS in a consecutive series of more than 300 donors and 600 kidney transplants (*121*).

Most experience with HMP concerns the kidney (Fig. 2). Only scarce experimental data exist in experimental liver transplantation by the groups of Belzer, Slapak, and Brettschneider (*122–124*). Several strategies regarding perfusion of portal vein and/or hepatic artery have been applied. In 1986, D'Alessandro, and later Pienaar, from the Madison group managed to successfully transplant canine livers after 72 hours HMP (*125*, *126*). Clinical application of HMP in liver transplantation, however, has been limited to recent pioneering work of Guarrera et al. (*103*).

Overall, both experimental and clinical data suggest that HMP improves kidney and liver preservation. Modern, portable, and stand-alone HMP systems for kidney preservation are now available, allowing user-friendly transportation within an international organ sharing system. Therefore, a broader clinical application of HMP should be considered to reduce the impressively high DGF rate of 60–85% in NHB kidneys and possibly reduce the Achilles heel in liver transplantation: ischemic type biliary lesions (*127–130*).

New Approaches in Organ Preservation

Apart from HMP, several other concepts have been developed to allow expansion of the donor pool. During the past decades, not only age but also the type of organ donors has changed. The cause of death has shifted from a relative healthy donor with cerebral trauma to older patients suffering from cerebral hemorrhage. As a result, average donor organ quality has decreased and the task to at least maintain the quality of the graft before transplantation has become much more important.

A rather unusual but attractive technique to resuscitate damaged kidneys and livers is the perfusion of gas through the vasculature. This concept was initially described by Bunzl in 1954 and named "persufflation" by Isselhard in 1972. It consists of retrograde venous application of humidified pure oxygen (O_2) at 13–18 mmHg during CS (*131*, *132*). Renal persufflation preservation has been applied clinically in a small pilot study including 10 paired kidneys. Although numbers were small, persufflated kidneys did show improved initial function compared to CS (*133*). Its application in liver preservation was extensively studied by the group of Minor. In several experiments, they showed that gaseous oxygenation during CS was highly effective in improving liver graft viability (*134–136*). Using this method, survival after 45 minutes of warm ischemia in a NHB liver transplant model was 100%, compared to 0% in the CS group (*136*).

Another, more static, way to deliver O_2 to CS grafts is the dual-layer perfluorocarbon technique. Perfluorocarbons (PFC) are hydrocarbons in which most of the hydrogen atoms have been replaced with fluorine. The attractive property of PFC is a very high capacity for dissolving O_2. PFC liquids can store 20–25 times greater amounts of O_2 than water or blood. In addition, the very low O_2 binding constant of PFC allows a more effective release of O_2 in tissue than hemoglobin does. These properties make PFC-based solutions interesting for organ preservation (*137*).

PFC was first used in organ preservation as a component of the two-layer method (TLM) (*138*). The TLM is comprised of UW-CSS and oxygenated PFC for pancreas preservation. During preservation by TLM, canine pancreas grafts continuously generated ATP up to 96 hours (*139*). In animal models, TLM appeared to be useful not only for pancreas but also for small bowel preservation (*140*). In the clinical setting, however, TLM remains controversial because it did not improve whole pancreas transplantation (*141*). Furthermore, there is debate about its effects on islet isolation. Although some small clinical trials have reported beneficial effects, the largest and most recent survey did not demonstrate superiority over UW-CSS in the field of human islet isolation (*142–144*).

The latter alternative approaches all have in common that they take advantage of the beneficial effect of O_2 during hypothermic preservation. Improving the energy status of organs during preservation leads to earlier recovery, especially in ischemically damaged organs. Whereas O_2 supports metabolism, various other gaseous compounds that act on signal transduction have also proven their efficacy to improve graft viability in the experimental setting. Donor pretreatment with carbon monoxide (CO) at low concentrations in a rat small intestine transplant model reduced pro-inflammatory interleukins and improved survival to 100% compared to 58% in air-treated controls (*145*). Similarly, in rat liver transplants, exposure of the recipient to CO-suppressed induction of tumor necrosis factor-α, inducible nitric oxide synthase,

and intercellular adhesion molecule-1. Liver grafts showed improved liver function and less neutrophil infiltration after CO exposure (*146*). Nitric oxide (NO), the radical produced from L-arginine by the enzyme NO synthase (NOS), is a potent vasodilatator that inhibits platelet and neutrophil aggregation and adhesion (*147*, *148*). This effect is potentially beneficial for preservation. Vasodilatation will improve organ washout during procurement, whereas the immunological effects of NO may limit reperfusion damage. Adding NO during cold ischemia improved small bowel viability in both rat and pig autotransplantation models (*149*). In addition, topical exposure of rat kidneys with NO significantly reduced the effects of 60 minutes of warm ischemia (*150*).

These experiments suggest that exposure of the graft to CO and/or NO during preservation might induce a protective effect before reperfusion. HMP devices could thus enable administration of these compounds, either via an oxygenator as a gas or by pharmacological donors in the solution.

Outlook

As often before in transplant history, major improvements in preservation will probably be derived from new philosophies instead of adaptations of current strategies. Ideally, good preservation should facilitate the use of marginal and older organs and provide real-time viability assessment before transplantation. Normothermic (37°C) or subnormothermic (25–32°C) perfusion is becoming popular as a preservation alternative that may indeed achieve these goals (*151*). In canine kidney transplantation after 120 minutes of warm ischemia, 18 hours of normothermic perfusion allowed eventual recovery of normal renal function, whereas primary nonfunction occurred in all kidneys preserved for 18 hours with static CS (*152*). Raising the temperature during preservation provides more adequate ways to test and optimize graft viability and allows elimination of hypothermia induced injury (*13*, *153*).

Normothermic perfusion of the abdominal organs using a cardiopulmonary bypass system followed by CS has already been applied in human kidney transplantation. This so-called normothermic recirculation protocol showed significant improvements in a group of 44 NHB kidneys. PNF and DGF rates were 0% and 12.5%, respectively, compared to 22.5% and 55% for conventional preservation techniques. Despite the fact that this study was retrospective and included patients over a 12-year period, it suggests a potential benefit for clinical application of normothermic techniques (*154*).

In liver preservation, normothermic perfusion of porcine livers subjected to 60 minutes of warm ischemia resulted in functioning liver grafts, whereas the animals transplanted with CS livers all died. Normothermic perfused livers demonstrated stable metabolic function with adequate production of coagulation factors, hyaluronic acid clearance, glucose metabolism, and significantly lower transaminases compared to CS grafts (*151*, *155*, *156*).

The voluminous perfusion setup, necessity of continuous monitoring during perfusion, and technical complexity, however, have limited clinical application of normothermic machine perfusion (NMP) so far (*153*, *157*). To introduce NMP as a feasible option in clinical practice a combination of techniques has to be used. After an initial period of conventional hypothermic preservation, allowing transportation to a specialized facility, NMP can be started. In kidney preservation it has been shown that HMP with intermittent NMP improves graft survival of canine kidneys after 30 min warm ischemia (*158*). The liver, however, is more vulnerable. Recently, initial NMP for 24 hours was compared to 4 hours of CS followed by 20 hours of NMP in a porcine NHB model with 60 minutes of WIT. The latter combination, however, was ineffective as the benefits of NMP were lost due to the short CS period (*159*).

Overall, NMP offers several advantages over conventional preservation techniques. Therefore, the development of a portable and easy-to-handle stand-alone device is crucial for the introduction of NMP into day-to-day practice for kidney and liver preservation.

CONCLUSION

Organ preservation has always been crucial for transplant outcome, but will become even more important in the present era with increasing numbers of older, more marginal, and NHB donors. Although CS has proven its efficacy in the past, it seems that the limitations of this technique have been reached. To maintain organ viability, more efforts are necessary to reduce ischemia/reperfusion injury and initiate repair. Awaiting the results of several clinical trials, hypothermic machine perfusion, or even normothermic machine perfusion may be (re)introduced in clinical preservation in general or for special categories of donor organs.

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Transplant Tolerance: Converging on a Moving Target

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Enthusiasm for tolerance induction has been tempered by the realization that it is more difficult to achieve clinically than was predicted by experimental models. Unlike the view that the immune response to an allograft is ordered and thus predictable, we view alloimmunity as highly plastic and molded by previous and ongoing experiences with allogeneic and environmental antigens. This implies that an individual's response to an allograft changes over time and that responses of seemingly similar individuals may vary greatly. This variability highlights the need to develop assays for monitoring the recipient immune response as well as individualized methods for therapeutic immune modulation.

Keywords: Tolerance, Transplantation, Immunosuppression, Regulation, Deletion.

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The Target: Transplantation Tolerance

The ability to consistently induce robust, sustained, donor-specific tolerance would offer many benefits to transplant recipients. However, although the theoretical basis for acquired tolerance was established in the mid-20th century, clinical success has been rare and unpredictable. Advances in our understanding of the mechanisms that mediate rejection and tolerance increasingly allow the induction of tolerance in many experimental models. This, together with a rapidly expanding armamentarium of more selective biologic immunosuppressants, has rekindled interest in transitioning tolerance to the clinic.

Balancing this enthusiasm is the realization that achieving tolerance has proven to be significantly more difficult in humans than in animals. Together with the improving outcome of clinical transplantation, this has caused some to question the need for transplantation tolerance. Nevertheless, chronic immunosuppression remains associated with expense, risks of infections and malignancies, and drug-specific toxicities including hypertension, glucose intolerance, and hypercholesterolemia. These unwanted consequences of chronic immunosuppression result in a 5–10 fold increase in the all-cause mortality of transplant recipients relative to the general population independent of the effects of rejection (*1*). The nephrotoxicity of calcineurin inhibitors also affects recipients of renal and nonrenal allografts and contributes to the increasingly common development of chronic allograft nephropathy and end-stage renal disease in recipients of extrarenal organs (*2*). Lastly, recent data suggest that, unlike immediate outcomes, long-term outcomes of transplantation may not be improving (*3*). Viewed as a whole, these problems continue to fuel enthusiasm for tolerance.

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How is the Target Moving?

Barriers to Attaining Transplantation Tolerance

Several features of the immune system conspire against the development of transplantation tolerance. First, the innate immune system is designed to detect threats to homeostasis. The act of transplantation itself causes injury which in turn triggers responses by multiple components of the innate immune system. Two models have been proposed to describe how innate immune responses are initiated. The danger model hypothesizes that cellular components of the innate immune system respond to tissue injury by producing soluble mediators that perpetuate the inflammatory state and promote the maturation of adaptive immune responses (*4*). The pattern recognition receptor model postulates that highly conserved molecules expressed on the surface of injured cells engage receptors expressed by cells comprising the innate immune system (e.g., toll-like receptors) thereby triggering innate immunity (*5*).

A second factor that likely poses a barrier to tolerance is the unusually large proportion of the T-cell repertoire capable of recognizing alloantigens. Although it has been estimated that fewer than one in 100,000 T cells recognize a given nominal antigen, it has been reported that 7% of the T cell repertoire undergoes proliferation in response to alloantigens (*6*). This large clone size may mediate an early, aggressive immune response that irreversibly damages a transplanted organ before potentially protective responses can develop. Recognition of alloantigens can occur via two distinct pathways. The first and dominant process begins upon organ reperfusion when passenger leukocytes or APCs migrate to recipient secondary lymphoid organs where direct donor antigen recognition occurs (*7, 8*). It is now clear that naïve re-

sponses are critically dependent on secondary lymphoid organs and rejection is greatly attenuated in their absence (*9, 10*). As donor APCs are unlikely to be replenished, the direct response would be predicted to dissipate over time. At later time points, the recognition of alloantigens is believed to be primarily via the indirect pathway of antigen presentation (*11*). Indirect presentation refers to the recognition of donor peptides presented by recipient MHC molecules and APCs. The magnitude of the indirect response is significantly less than that of the direct response, possibly reflecting the lower clone size of T cells capable of recognizing indirectly presented antigens. It has been suggested that the indirect pathway plays a particularly important role in chronic rejection (*12*). Distinguishing between these two methods of alloantigen recognition may have therapeutic implications. For example, costimulation blockade has been reported to inhibit T cell priming via the indirect, but not direct, pathway (*13*).

The development of immunologic memory poses a third potential impediment to the development of transplantation tolerance. Memory cells differ from naïve cells in that they have higher functional avidity, lower activation thresholds, the ability to rapidly engage effector functions, and the potential to circulate widely through peripheral tissues. By virtue of these properties, allospecific memory cells represent a clear threat to transplanted organs. Until recently, many have viewed one's initial encounter with an alloantigen as a naïve response. However, the growing recognition that alloimmunity is strongly influenced by heterologous responses to previously encountered antigens suggests that even first exposures to alloantigen provoke responses by crossreactive memory cells (*14, 15*). Thus, memory cells may be important mediators of allograft damage even in "naïve" recipients. Heterologous immunity is likely an important factor contributing to the different success rates of tolerance regimens in clinical transplantation versus experimental models that typically use young, specific-pathogen-free animals.

Homeostatic proliferation following therapies that cause massive T-cell depletion may represent a fourth barrier to tolerance (*16*). T cells from lymphopenic hosts undergo extensive proliferation. The phenotype and function of the re-emerging T cells is similar to that of memory T cells. Furthermore, memory T cells have been reported to be relatively resistant to depletion (*17*). Thus, following massive T-cell depletion, recipients may be selectively repopulated by homeostatically proliferating memory or memory-like T cells that are resistant to maneuvers that typically inhibit naïve responses. Taken together, these four factors produce barriers that vary not only between similar individuals, but also vary over time in the same individual. Thus, the route to tolerance is highly variable.

Mechanisms of Tolerance

Based upon our current approach to organ transplantation, tolerance appears an infrequent occurrence that requires the appropriately timed disruption of numerous immune mechanisms. However, tolerance is the default response to a multitude of self and environmental antigens, and many of the mechanisms that maintain self-tolerance may also be capable of promoting allograft tolerance. Tolerance mechanisms can be broadly classified as either central or peripheral. Central tolerance refers to the deletion within the thymus of T cells whose affinity for self antigens is inappropriately high and thus likely to result in autoimmunity. The tolerance displayed by neonatal mice to a transplanted organ demonstrates the robustness of these central mechanisms. Similarly, central deletion is an important mechanism promoting tolerance to organ allografts in mice displaying mixed hematopoietic chimerism following bone marrow transplantation (*18*).

In addition to central deletion, a number of mechanisms operating in the periphery have been reported to contribute to the tolerant state. Peripheral mechanisms for maintaining tolerance include ignorance (*9*), anergy (*19*), regulation or suppression (*20–22*), and apoptosis or peripheral deletion (*23, 24*).

Ignorance as a mechanism mediating tolerance was demonstrated by Lakkis et al. who showed that naïve mice lacking secondary lymphoid organs accepted skin or heart allografts indefinitely (*9*). Impaired rejection in this model resulted from the failure of T cells to be primed when they encountered donor antigens outside of lymphoid organs. The findings that in some settings recipient T cells can be primed by alloantigens encountered within the allograft (*25*) and that memory T cells can be reactivated after encountering antigen outside of secondary lymphoid organs (*26*) suggest that the role of this mechanism may be limited.

Active, antigen-specific suppression of immune responses was first reported in the 1970s (*27,28*). Though interest in this phenomenon waned, it was rekindled by the demonstration that $CD4^+CD25^+$ T cells from rats bearing long-term surviving cardiac allografts could transfer tolerance to untreated recipients (*29*). A growing body of experimental and clinical evidence suggests a role for regulatory T cells in the induction and maintenance of tolerance (reviewed in *21*). While $CD4^+CD25^+$ cells constitute the most widely recognized phenotype of regulatory cells, cells of other lineages including NK1.1, $C8^+CD28^-$, and $CD3^+CD4^-CD8^-$ cells may display regulatory properties (*30–32*). Other markers of regulatory T cells may include CD45RB, GITR, CTLA4, CD103, and FOXP3 (*33–41*).

At least three mechanisms appear to be important for tolerance mediated by regulatory T cells. Preclinical and clinical evidence suggests that T_R1, T_H3, and NKT cells mediate regulatory effects at least in part via production of the cytokines IL-10 and TGFβ (*33, 42–45*). The function of $CD4^+CD25^+$ T reg also appears to be affected by the expression of the cell surface molecules GITR (*36, 37*) and CTLA4 (*42*) which may contribute to the contact dependent effects of regulatory cells (reviewed in *21,22*). Finally, anergic $CD4^+$ T cells may mediate their regulatory effects by inhibiting the maturation and function of dendritic cells (*46*).

Although regulation has been extensively studied in experimental systems, far less is known about its role in clinical tolerance. Salama et al. reported that $CD25^+$ regulatory cells developed as early as three months after renal transplantation and persisted for years (*47*). Furthermore, evidence of regulation in vitro as detected by ELISPOT analysis was more commonly observed in rejection-free recipients than those who had experienced rejection. Analysis of a limited number of operationally tolerant transplant recipients using the transvivo DTH assay has also suggested a role for regulation that

was dependent upon the production of TGFβ and/or IL-10 and the expression of CTLA4 (*48, 49*).

Although transplant tolerance may develop as a result of spontaneous regulatory mechanisms, clinical application of regulation is likely to require interventions that purposely produce regulatory cells. Many of these approaches (i.e., DST and in vitro generation of T reg) depend upon exposure to donor antigen, and are facilitated by the demonstration that recipient T cells need not be tolerized to each foreign antigen expressed by the transplanted organ. Rather, linked, dominant suppression has been described (*50–52*). In rodent and large animal models, the expression of one MHC molecule recognized by regulatory T cells effectively suppresses the response to other mismatched MHC molecules. A final consideration pertinent to the clinical application of regulatory mechanisms is the possible effect of currently used immunosuppressive agents on T regs. Although still controversial, some agents such as calcineurin inhibitors may inhibit the development of tolerance by preventing the activation or function of regulatory T cells (*53*).

In addition to ignorance and regulation, peripheral deletion of alloreactive T cells may contribute to tolerance. This can occur in the setting of chronic alloantigen stimulation or when alloantigen is encountered under suboptimal conditions for T-cell activation. Clonal exhaustion has been reported after liver transplantation as an example of how chronic stimulation may induce peripheral T cell deletion (*54*). Alternatively, the blockade of costimulatory signals such as CD28 or CD154 at the time T cells encounter alloantigens has been reported to result in incomplete T-cell activation followed by anergy and apoptosis (*55*). Several groups have now demonstrated that peripheral deletion mediated by apoptosis is an important event contributing to long-term allograft acceptance (*23, 24*). Apoptosis of T cells can be mediated either by cell surface death receptors or cytokine withdrawal. The death receptors mediating apoptosis are comprised of TNF receptor superfamily members that have a cytoplasmic death domain that binds cell-signaling proteins such as TRADD, FADD, FLICE, and caspase-3 leading to apoptosis. Cytokines promoting T-cell survival include the common γ chain cytokines IL-2, IL-4, IL-7, and IL-15. Their absence predisposes cells to active, apoptotic death.

Moving Targets

From this review, it should be obvious that there are multiple barriers to tolerance and multiple mechanisms that, in the correct setting, may promote tolerance. For example, both genetic factors such as CCR5 gene polymorphisms (*56*) and acquired factors such as the development of allo-reactive memory populations will differ between recipients and fundamentally affect the nature of the immune response to transplanted organs. It is also likely that the organ transplanted affects the nature of the immune response and the probability of developing tolerance (*47, 57*). How these multiple variables interact will determine the outcome of transplantation. The multiplicity of possible interactions explains our current inability to predict the outcome of transplantation in seemingly similar recipients.

In addition to variations between recipients in anti- and pro-tolerogenic factors, it is increasingly accepted that tolerance may be dependent upon the sequential development of more than one mechanism (*58,59*). For example, profound T-cell depletion eliminates the large population of cells with direct alloantigen specificity. However, other protolerant mechanisms are required to maintain tolerance as recipient T cells re-emerge. This is exemplified by the finding that tolerance in NOD and IL-$2^{-/-}$ recipients treated with sirolimus, an agonist IL-2 and antagonist IL-15-related fusion protein was associated with the early deletion of alloreactive T cells followed by the development of $CD4^+CD25^+$ regulatory T cells (*60*). Deletion of donor-reactive T cells has also been shown to occur in recipients rendered tolerant to organ allografts following bone marrow transplantation (*61*). However, even in this model it may not be accurate to ascribe the development of donor-specific tolerance entirely to deletion. Tolerance developing after the infusion of donor bone marrow in mice treated with busulfan and short-term blockade of CD28 and CD154 is initially dependent upon $CD4^+$ T cells (*62*). Although tolerance is eventually associated with deletion of donor-reactive T cells, this process requires months for completion, implying that mechanisms other than depletion are responsible for early graft acceptance. Using the trans-vivo DTH assay, we have recently demonstrated the existence of regulatory cells that produce IL-10 and/or TGFβ at early time points following transplantation (*63*). Interestingly, these regulatory cells were not detected at later time points, suggesting that they may be deleted together with alloaggressive recipient T cells. Thus, the mechanisms which induce tolerance may be distinct from those that maintain tolerance. Finally, mechanisms that protect organ allografts at one point in time may be harmful at later times. For instance, treatment of mice with an anti-CD4 antibody or gallium nitrate allows the long-term acceptance of heart allografts. This effect is mediated by regulatory cells that produce TGFβ (*64*). However, with time, heart grafts from anti-CD4 and gallium nitrate treated recipients develop chronic rejection, which has been postulated to be the result of the profibrotic effects of TGFβ (*65*).

How Can We Converge on the Target?

The Impact of Early Tolerance Studies

Our current concept of transplantation tolerance remains heavily influenced by the original studies of acquired tolerance (*66*). These studies predicted that tolerance should be achievable following a brief intervention that fundamentally changes the immune system. Ideally, this intervention would be initiated at the time of transplantation and result in permanent, antigen-specific organ acceptance. However, it is important to recall that even in the landmark study of Billingham et al., only two of the five recipients were tolerant (two developed acute rejection and one developed chronic rejection). Nevertheless, the view that tolerance could be acutely induced has been reinforced by experiments demonstrating that prolonged, if not indefinite, allograft survival could be achieved following brief treatments with agents such as CTLA4-Ig, anti-CD154, or anti-CD4 (*65, 67*).

Moving Transplantation Tolerance to the Clinic

For some time, nonhuman primate (NHP) models have been used to evaluate the safety and efficacy of the most promising tolerogenic regimens developed in rodent models prior to their use in humans. However, when compared to the

results obtained using rodents, virtually all tolerogenic strategies have proven far less effective in NHPs (*68*). This may be the result of the homogeneity of rodent models (i.e., uniform age, environmental exposure, and genetic background) and the reductionist design of studies aimed at determining the role of a specific pathway/molecule. Attempts to closely replicate these approaches in large animal preclinical or clinical transplantation have been largely unsuccessful, likely due to the more diverse environmental exposure and genetic background of primate transplant recipients.

One of the more promising approaches tested in NHPs is brief therapy with an antibody against the CD3 molecule coupled to a diphtheria-derived immunotoxin (*69*) that mediates profound but reversible T cell depletion. Approximately 40% of the treated animals develop operational tolerance following pretransplant T-cell depletion, as indicated by the absence of rejection and the presence of donor-specific hyporesponsiveness following T cell repopulation. This approach was made more reproducible by the addition of deoxyspergualin, a polyamine antibiotic that inhibits APC function (*70*). Based on this approach, a number of groups have undertaken clinical trials utilizing early recipient T-cell depletion. Several investigators have used alemtuzumab (an anti-CD52 monoclonal antibody) to induce profound T-cell depletion. Despite achieving depletion that is equivalent to that obtained using anti-CD3-immunotoxin with respect to kinetics, magnitude, and effectiveness within the secondary lymphoid tissues, treatment with alemtuzumab alone or in combination with deoxyspergualin is not sufficient to induce tolerance in adult humans (*71, 72*). This experience has led other groups to combine the T-cell depletion with other agents such as sirolimus (*73*). Although this approach remains promising, initial studies have shown a significant incidence of acute rejection (*73*) and an unusually high incidence of antibody-mediated rejection. The failure of these T-cell-centric approaches suggests that other components of the immune system, such as B cells, NK cell, or monocytes, may need to be specifically targeted to achieve tolerance.

Chimerism and Tolerance

Combined nonmyeloablative bone marrow and solid organ transplantation has been shown to induce robust tolerance in rodents (*18, 62*). In humans, successful bone marrow transplantation allows the acceptance of subsequent organ allografts from the same donor in the absence of immunosuppression (*74*). These observations form the basis for clinical trials, sponsored by the Immune Tolerance Network, of combined bone marrow and kidney transplantation. Using a nonmyeloablative conditioning regimen (cyclophosphamide, thymic irradiation, and antithymocyte globulin) and a short course of cyclosporine, two patients have been reported to display functional tolerance and sustained antitumor responses at 2 and 4 years following combined bone marrow and kidney transplantation from the same donor (*75*). To date, this approach has proven less effective for non-HLA identical donor and recipient pairs. The effectiveness of this approach may be in large part related to its ability to target multiple mechanisms of rejection and tolerance. There is experimental evidence to suggest that these types of regimens control alloreactive recipient T cells by central deletion and peripheral mechanisms including anergy, deletion, and regulation (*18, 76, 77*). Despite targeting multiple mechanisms, combined bone marrow/solid organ transplantation does not invariably prevent the development of chronic allograft vasculopathy (*78, 79*). This suggests that in addition to inhibiting multiple mechanisms that promote acute rejection, tolerogenic strategies will also need to target a discreet group of alloimmune responses that cause chronic allograft injury, or alternatively promote protective responses that suppress injurious responses.

CONCLUSION

A New Mind-Set Towards Tolerance Induction

Fifty years after the initial description of acquired transplant tolerance, the occurrence of tolerance in clinical transplantation remains largely accidental and unpredictable. However, the argument in favor of continued efforts to design, test, and implement tolerogenic regimens in transplantation remains as compelling today as at any time in the past. The heterogeneous nature of clinical transplantation together with the redundancy and plasticity of the immune system conspire against the acquisition of tolerance, and suggest that when tolerance does develop, it may be impermanent. In this review, we propose that successful tolerogenic strategies will need to inhibit multiple destructive immune responses while promoting immune responses that protect the transplanted organ. Furthermore, a single such regimen might not prove to be uniformly effective as the targeted mechanisms may vary significantly between recipients and within a given recipient over time. The clinical observation that transplantation tolerance may be lost, often after several years or even decades of drug-free allograft acceptance, supports the notion that the immune response of tolerant patients mutates over time. Thus, the timing, as well as the nature, of tolerance-promoting interventions may need to be varied. Consequently, as we converge on tolerance, we are not focusing on one static target, but many moving targets. If long-term allograft acceptance requires a series of discreet immunologic responses or nonresponses, it seems unlikely that a single, brief intervention will result in long-term allograft acceptance. Rather, a longer period of treatment sequentially targeting developing immune responses as they occur may afford the greatest likelihood of achieving drug-free allograft acceptance. If we are to apply such an approach to transplantation, we will need to develop a battery of monitoring techniques that are capable of quantifying the balance of destructive and protective mechanisms following transplantation.

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2005 Update of Banff 97 Diagnostic Categories for Renal Allograft Biopsies

1. Normal

2. Antibody mediated rejection-due to documented anti-donor antibody ('suspicious for' if antibody not demonstrated); may coincide with categories 3-6

a. Acute antibody-mediated rejection

Type (Grade)	Histopathological Findings
I	ATN-like; C4d positive, minimal inflammation
II	Capillary margination and/or thromboses, C4d positive
III	Arterial v3 changes, C4d positive

b. Chronic active antibody-mediated rejection

Grade	Histopathologic Findings
---	Glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries; C4d positive

3. Borderline Changes:"Suspicious" for acute T-cell-mediated rejection

Grade	Histopathological Findings
"Suspicious"	This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2 or t3 with i0 or i1) although the i2 t2 threshold for rejection diagnosis is not met (may coincide with categories 2, 5 and 6)

4. T-cell mediated rejection (may coincide with categories 2, 5 and 6)

a. Acute T-cell-mediated rejection

Type (Grade)	Histopathological Findings
IA	Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)
IB	Cases with significant interstitial infiltration (> 25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)
IIA	Cases with mild to moderate intimal arteritis (v1)
IIB	Cases with severe intimal arteritis comprising > 25% of the lumenal area (v2)
III	Cases with "transmural" arteritis and/or fibrinoid change and necrosis of medial smooth muscle cells with accompanying

	lymphocytic inflammation (v3)

b. Chronic active T-cell-mediated rejection	
Type	**Histopathological Findings**
---	"Chronic allograft arteriopathy" (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)

5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology	
Grade	**Histopathological Findings**
	Note: Grades I, II and III may include nonspecific vascular and glomerular sclerosis, but severity is graded by tubulointerstitial features
Grade I (mild)	Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)
Grade II (moderate)	Moderate interstitial fibrosis and tubular atrophy (26-50% of cortical area)
Grade III (severe)	Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)

6. Other: Changes not considered to be due to rejection	
Diagnosis	**Histopathological (and other) features**
Chronic hypertension	Arterial/fibrointimal thickening with reduplication of elastica, usually with small artery and arteriolar hyaline changes
Calcineurin toxicity	Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of hypertension or diabetes. Tubular cell injury with isometric vacuolization
Chronic obstruction	Marked tubular dilatation. Large Tamm-Horsfall protein casts with extravasation into interstitium, and/or lymphatics
Bacterial pyelonephritis	Intratubular and peritubular neutrophils, lymphoid follicle formation
Viral infection	Viral inclusions on histology and immunohistology and/or electron microscopy

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Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

Specimen Adequacy and Lesion Scoring (Banff '97)

Specimen Adequacy (a necessary prerequisite for numeric coding)	
Unsatisfactory	Less than 7 glomeruli & no arteries
Marginal	7 glomeruli with one artery
Adequate	10 or more glomeruli with at least two arteries
Minimum Sampling	
7 slides	3 H&E, 3 PAS or silver stains, and 1 trichrome, section thickness 3-4 microns.

Quantitative Criteria for Tubulitis ("t") Score (assumes minimum sampling)	
t0	No mononuclear cells in tubules
t1	Foci with 1 to 4 cells/tubular cross section or 10 tubular cells
t2	Foci with 5 to 10 cells/tubular cross section
t3	Foci with >10 cells/tubular cross section, or the presence of at least two areas of tubular basement membrane destruction accompanied by i2/i3 inflammation and t2 tubulitis elsewhere in the biopsy.

Quantitative Criteria for Mononuclear Cell Interstitial Inflammation ("i")	
i0	No or trivial interstitial inflammation (<10% of unscarred parenchyma)
i1	10 to 25% of parenchyma inflamed cells
i2	26 to 50% of parenchyma inflamed
i3	>50% of parenchyma inflamed
Indicate presence of remarkable numbers (>10% of total cells) of eosinophils, polys, or plasma cells (specify which) with an asterisk on i	

Quantitative Criteria for the Early Type of Allograft Glomerulitis ("g")	
g0	No glomerulitis
g1	Glomerulitis in <25% of glomeruli
g2	Segmental or global glomerulitis in about 25 to 75% of glomeruli
g3	Glomerulitis (mostly global) in >75% glomeruli

Quantitative Criteria for Arteriolar Hyaline Thickening ("ah")	
ah0	No PAS-positive hyaline thickening
ah1	Mild-to-moderate PAS-positive hyaline thickening in at least one arteriole
ah2	Moderate-to-severe PAS-positive hyaline thickening in more than one arteriole
ah3	Severe PAS-positive hyaline thickening in many arterioles

Indicate arteriolitis (significance unknown) by an asterisk on ah

Quantitative Criteria for Intimal Arteritis ("v")	
v0	No arteritis
v1	Mild-to-moderate intimal arteritis in at least one arterial cross section
v2	Severe intimal arteritis with at least 25% luminal area lost in at least one arterial cross section
v3	Arterial fibrinoid change and/or transmural arteritis with medial smooth muscle necrosis with lymphocytic inflammation
Note number of arteries present and number affected. Indicate infarction and/or interstitial hemorrhage by an asterisk (with any level v score)	

Quantitative Criteria for Allograft Glomerulopathy ("cg")	
cg0	No glomerulopathy, double contours in <10% of peripheral capillary loops in most severely affected glomerulus
cg1	Double contours affecting up to 25% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
cg2	Double contours affecting 26 to 50% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
cg3	Double contours affecting more than 50% of peripheral capillary loops in the most affected of nonsclerotic glomeruli
Note number of glomeruli and percentage sclerotic	

Quantitative Criteria for Interstitial Fibrosis ("ci")	
ci0	Interstitial fibrosis tissue in up to 5% of cortical area
ci1	Mild- Interstitial fibrosis tissue in 6 to 25% of cortical area
ci2	Moderate- interstitial fibrosis of 26 to 50% of cortical area
ci3	Severe interstitial fibrosis of >50% of cortical area

Quantitative Criteria for Tubular Atrophy ("ct")	
ct0	No tubular atrophy
ct1	Tubular atrophy in up to 25% of the area of cortical tubules
ct2	Tubular atrophy involving 26 to 50% of the area of cortical tubules
ct3	Tubular atrophy of >50% of the area of cortical tubules

Quantitative Criteria for Fibrous Intimal Thickening ("cv")	
cv0	No chronic vascular changes
cv1	Vascular narrowing of up to 25% lumenal area by fibrointimal thickening of arteries ± breach of internal elastic lamina or presence of foam cells or occasional mononuclear cells*
cv2	Increased severity of changes described above with 26 to 50% narrowing of vascular lumenal area*
cv3	Severe vascular changes with >50% narrowing of vascular lumenal area*
* in most severely affected vessel. Note if lesions characteristic of chronic rejection (elastica breaks, inflammatory cells in fibrosis, formation of neointima) are seen	

Quantitative Criteria for Mesangial Matrix Increase ("mm")*	
mm0	No mesangial matrix increase
mm1	Up to 25% of nonsclerotic glomeruli affected (at least moderate matrix increase)
mm2	26-50% of nonsclerotic glomeruli affected (at least moderate matrix increase)
mm3	>50% of nonsclerotic glomeruli affected (at least moderate matrix increase)

***** The threshold criterion for the moderately increased "mm" is the expanded mesangial interspace between adjacent capillaries. If the width of the interspace exceeds two mesangial cells on the average in at least two glomerular lobules the "mm" is moderately increased

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[FRAMES] [NO FRAMES]

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Meeting Report

Banff '05 Meeting Report: Differential Diagnosis of Chronic Allograft Injury and Elimination of Chronic Allograft Nephropathy ('CAN')

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The 8th Banff Conference on Allograft Pathology was held in Edmonton, Canada, 15–21 July 2005. Major outcomes included the elimination of the non-specific term 'chronic allograft nephropathy' (CAN) from the Banff classification for kidney allograft pathology, and the recognition of the entity of chronic antibody-mediated rejection. Participation of B cells in allograft rejection and genomics markers of rejection were also major subjects addressed by the conference.

Key words: Banff classification, central slide review, scoring

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The 8th Banff Conference on Allograft Pathology was held in Edmonton, Canada from 15 to 21 July 2005. A large group of clinicians, pathologists, and researchers met in plenary and specialty sessions and participated in several active consensus discussions. A summary of major topics and results of consensus discussions are provided in this manuscript.

Allograft Fibrosis and Atrophy Revisited

A major topic discussed at the 8th Banff Conference was the elimination of the term 'chronic allograft nephropathy' or CAN from the Banff schema for diagnosis and grading of renal allograft rejection (1,2). Originally coined fifteen years ago in 1991 as a more generic alternative to the then popular and misleading term 'chronic rejection,' acceptance of 'CAN' did succeed in reversing the misconception that all late scarring of the graft was due to alloimmune injury/rejection. However, there are now over 550 PubMed citations using the term, many fostering the misconception that 'CAN' is a specific disease rather than just another term for non-specific parenchymal scarring. In this consensus report are outlined targeted alterations in the Banff schema replacing 'CAN' as a diagnostic term. The rationale for this update of the Banff schema is the misusage of 'CAN' as a generic term for all causes of chronic renal allograft dysfunction with fibrosis that inhibits the accurate diagnosis and appropriate therapy. In order to treat something, first you would need a definitive diagnosis, which is not artificial but rather specifies the underlying disease process(es). Thus there is an emerging need for an appropriate classification of chronic allograft injury. On the other hand, with the burgeoning recent literature, the role of alloantibody in chronic renal allograft deterioration and the corresponding morphological changes are increasingly recognized, making the identification of an antibody-mediated

component of chronic rejection reaction possible. The second part of the revisions on the Banff schema reflects the outlined pathological criteria for chronic antibody-mediated rejection (AMR) in kidney allografts which emerged from a consensus process after in-depth discussions at the 2005 Banff meeting.

Chronic Alloimmune Injury/Rejection versus Non-Immune Injury

Use of the non-specific term 'CAN' has tended to undermine recognition of morphological features enabling diagnosis of specific causes of chronic graft dysfunction. For example, many allograft recipients are hypertensive, which can lead to chronic allograft injury with fibrosis; pathological changes recognizable in the allograft include arterial fibrointimal thickening with duplication of internal elastica (fibroelastosis), arteriolar and small artery hyalinosis, glomerulosclerosis, interstitial fibrosis and tubular atrophy (IF/TA) (3). Chronic calcineurin inhibitor toxicity produces hyaline arteriolar changes, sometimes with peripheral hyaline nodules, and IF/TA either in 'striped' ischemic or diffuse form (4–6). Co-incident thrombotic microangiopathy and/or isometric vacuolization of tubular cells suggests ongoing toxic injury (7,8). Chronic obstruction in or extrinsic to the ureter can lead to IF/TA with relative glomerular sparing: dilated tubules, atubular glomeruli and intratubular Tamm–Horsfall protein casts with extravasation into the interstitium are pathological features suggestive of obstruction, which can be recognized in the allograft (9). Chronic polyomavirus infection can lead to IF/TA with chronic inflammation—intranuclear viral inclusions, highlighted on immunostaining for the SV40 large T antigen, are diagnostic of infection, though they may be sparse or even absent in very late fibrotic stages of polyoma virus nephropathy (10). Many recurrent and de novo glomerular or vascular diseases can also lead to glomerulosclerosis and IF/TA, both early and late post-transplant. In addition, de novo diabetic changes are becoming more common in allografts. All of these specific causes of IF/TA can and should be recognized by the pathologist (Table 1).

Table 1: Morphology of specific chronic diseases

Causes of IF/TA (non-rejection)	
Etiology	Morphology
Chronic hypertension	Arterial/fibrointimal thickening with reduplication of elastica, usually with small artery and arteriolar hyaline changes.
CNI[1] toxicity	Arteriolar hyalinosis with peripheral hyaline nodules and/or progressive increase in the absence of hypertension or diabetes. Tubular cell injury with isometric vacuolization.
Chronic obstruction	Marked tubular dilation. Large Tamm–Horsfall protein casts with extravasation into interstitium, and/or lymphatics.
Bacterial pyelonephritis	Intratubular and peritubular neutrophils, lymphoid follicle formation.
Viral infection	Viral inclusions on histology and immunohistology and/or electron microscopy.

[1] CNI, calcineurin inhibitor toxicity.

In addition, chronic alloimmune injury is an important cause of IF/TA in the graft. The Banff schema already mandates recognition and notation of morphological features of 'true' chronic rejection. Arterial and capillary changes have been emphasized as discriminating features (1). Recent data on alloantibodies and C4d in chronically failing renal allografts indicates a pathogenic role of humoral immunity in a subset of patients with chronic allograft dysfunction. There is strong evidence that anti-HLA antibodies participate in chronic rejection and previous studies have associated circulating anti-HLA antibodies with chronic vascular damage and late graft failure (11–13). In a large prospective trial, HLA antibodies were detected in 20.9% of 2278 renal allograft recipients, and graft failure at 1 year occurred more frequently in patients who developed de novo alloantibodies than in those who did not (8.6% vs. 3%) (14). De novo production of donor HLA-specific antibodies was shown in 51% of 112 renal transplant recipients with graft failure compared with 2% of 123 stable controls and the presence of alloantibodies predicted the subsequent development of chronic allograft rejection and graft loss (13). However, the majority of patients with anti-donor HLA antibodies do not demonstrate a progressive loss of transplant function within the follow-up periods. It is possible that the accumulation of antibody-mediated injury takes a longer time, or that only certain classes of anti-donor antibodies can mediate chronic injury or that cellular regulatory mechanisms are in play that counteract the injury mechanisms. Alternatively, the presence of anti-donor antibodies may not be sufficient to mediate the full spectrum of allograft injury without the concomitant activity of cell-mediated allograft immunity.

Recent reports have described morphologic features of chronic rejection in association with capillary-endothelial C4d deposits and concomitant circulating anti-donor antibodies (15–20). Mauiyyedi et al. (15) demonstrated deposition of C4d in peritubular capillaries (PTC) in 61% of 38 chronic rejection cases with chronic transplant glomerulopathy (TG) and/or 'chronic allograft arteriopathy' (arterial intimal fibrosis with intimal mononuclear inflammatory cell and/or foam cell infiltration) and most of the C4d positive chronic rejection cases had antidonor HLA antibody (88%). Regele et al. (16) detected C4d deposits in PTC in 34% of 213 renal allograft recipients with chronic allograft dysfunction. PTC C4d deposition was strongly associated with TG (53% of positive vs. 14% of negative biopsies) and severe PTC basement membrane multilayering (PTCBMML) (15 of 21 in positive vs. 3 of 22 in negative cases). Furthermore, C4d deposits in PTC preceded the development of

TG in follow-up biopsies. Vongwiwatana et al. (18) reported C4d deposition in PTC in 25% of 24 patients with TG but none with recurrent IgA nephropathy. PTCBMML was significantly increased in TG. Thus, the authors suggested that the association of TG with PTCBMML and C4d in PTC indicates a generalized disorder of the graft microcirculation and its basement membrane due to AMR in at least some cases. Sijpkens et al. (19) identified TG in 18 (1.6%) of 1111 kidney transplants with at least 6 months of graft function, and found C4d deposits in the glomerular capillary walls in 10/11 biopsies with TG. PTC C4d deposits were demonstrated in 4 and anti-HLA antibodies in 3 of the 10 biopsies with glomerular C4d deposits, suggesting that some of the glomerular staining was non-specific. Smavatkul et al. (21) reported increased graft loss over a 2-year period in patients with biopsy-proven graft fibrosis that were C4d positive (60%) compared to those that were negative (30%), and found TG and macrophage infiltrates as predictors of graft failure in grafts that were C4d positive.

The Diagnostic Triad of Late or Chronic Antibody-Mediated Rejection

Based on this accumulated literature, at the 2005 Banff meeting criteria for identification of late or chronic AMR were discussed and defined. The diagnostic criteria of late/chronic AMR include the following: (1) *morphological features* including TG (duplication or 'double contours' in glomerular basement membranes, Banff score cg1–3, see Figure 1) and/or PTCBMML (see Figure 2) and/or IF/TA with or without PTC loss, and/or fibrous intimal thickening in arteries without duplication of the internal elastica; (2) diffuse *C4d* deposition in PTC and (3) the presence of *donor specific antibody* (DSA) (Table 2). Diffuse C4d positivity has been defined as bright linear staining along PTC involving over half of sampled capillaries (2). The term 'late or chronic' means a slow but active process extending over some time (22). Indeed, the presence of C4d itself provides the best in situ evidence for an active humoral immunologic process (22,23). Other morphologic features that may accompany late AMR are aggregation of mononuclear inflammatory cells in PTC (16) (see Figure 3), transplant glomerulitis (19) (see Figure 4), and a plasma cell infiltrate in the interstitium (24). As with acute AMR, if only C4d deposits (with no DSA) or DSA (with no C4d) is present, with documented morphologic capillary changes, a diagnosis of 'suggestive of chronic AMR' can be made, although activity is more difficult to assess in the absence of C4d.

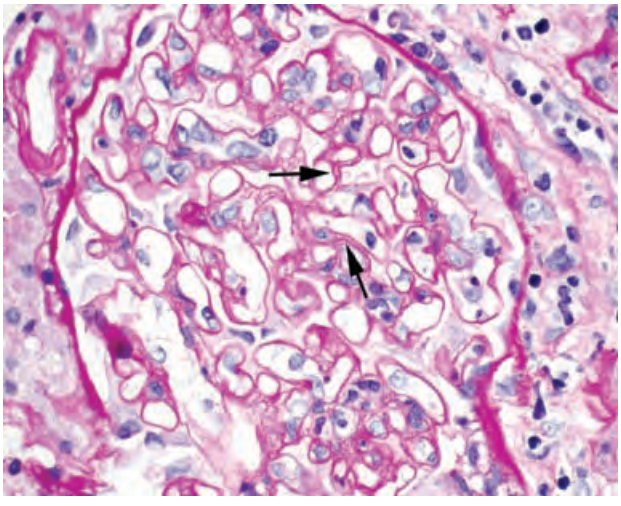

Figure 1: Chronic transplant glomerulopathy with numerous double contours (arrows) in glomerular basement membranes (PAS, original magnification ×600).

Figure 2: Marked splitting and multilayering in peritubular capillary basement membranes (arrows) in a renal allograft biopsy (uranyl acetate-lead citrate, original magnification ×5000).

Endothelial cells are thought to be the predominant target of antibody mediated injury (22,23). It has been suggested that the binding of complement-fixing alloantibody to endothelium induces tissue injury and acute rejection through the lysis of endothelial cells, coagulation (endothelial cell activation), complement activation and subsequent recruitment of macrophages and neutrophils. Recently, late/chronic AMR has been proposed as a partial accommodation (resistance of a graft to alloantibody-mediated injury) state which might be sufficient to prevent cell lysis through incomplete inhibition of complement but insufficient to prevent smoldering endothelial cell injury and activation (23). Dr. Jeffrey Platt emphasized accommodation as a possible contributor to chronic rejection in his presentation at the Banff meeting. Indeed, it has been shown that nucleated cells exposed to sublytic doses of the complement membrane attack complex become resistant to lytic complement doses (25). Dr. Platt suggested that accommodation may allow the allograft to survive long enough to acquire chronic rejection. Further studies are needed to determine whether true accommodation occurs, or whether

Table 2: Banff 97 diagnostic categories for renal allograft biopsies—Banff'05 update

1. Normal
2. Antibody-mediated rejection
Due to documented anti-donor antibody ('suspicious for' if antibody not demonstrated); (may coincide with categories 3–6)

Acute antibody-mediated rejection

Type (grade)

I. ATN-like – C4d+, minimal inflammation

II. Capillary-margination and/or thromboses, C4d+

III. Arterial – v3, C4d+

Chronic active antibody-mediated rejection[1]

Glomerular double contours and/or peritubular capillary basement membrane multilayering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries, C4d+
3. Borderline changes: 'suspicious' for acute T-cell-mediated rejection
This category is used when no intimal arteritis is present, but there are foci of tubulitis (t1, t2 or t3 with i0 or i1) although the i2 t2 threshold for rejection diagnosis is not met (may coincide with categories 2, 5 and 6)
4. T-cell-mediated rejection[1] (may coincide with categories 2, 5 and 6)

Acute T-cell-mediated rejection

Type (grade)

IA. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of moderate tubulitis (t2)

IB. Cases with significant interstitial infiltration (>25% of parenchyma affected, i2 or i3) and foci of severe tubulitis (t3)

IIA. Cases with mild to moderate intimal arteritis (v1)

IIB. Cases with severe intimal arteritis comprising >25% of the luminal area (v2)

III. Cases with 'transmural' arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic inflammation (v3)

Chronic active T-cell-mediated rejection[1]

'Chronic allograft arteriopathy' (arterial intimal fibrosis with mononuclear cell infiltration in fibrosis, formation of neo-intima)
5. Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology[1]

Grade

I. Mild interstitial fibrosis and tubular atrophy (<25% of cortical area)

II. Moderate interstitial fibrosis and tubular atrophy (26–50% of cortical area)

III. Severe interstitial fibrosis and tubular atrophy/loss (>50% of cortical area)
(may include non-specific vascular and glomerular sclerosis, but severity graded by tubulointerstitial features)
6. Other: Changes not considered to be due to rejection-acute and/or chronic (the diagnoses given in Table I); may coincide with categories 2–5

[1]Indicates changes in the updated Banff'05 schema.

the presence of alloantibody and complement in the absence of classical histological changes simply reflects subtle allograft injury over a long time frame (26).

TG and PTCBMML tend to occur concomitantly, and both lesions show basement membrane thickening and multilayering, which are regarded as markers of past or recent

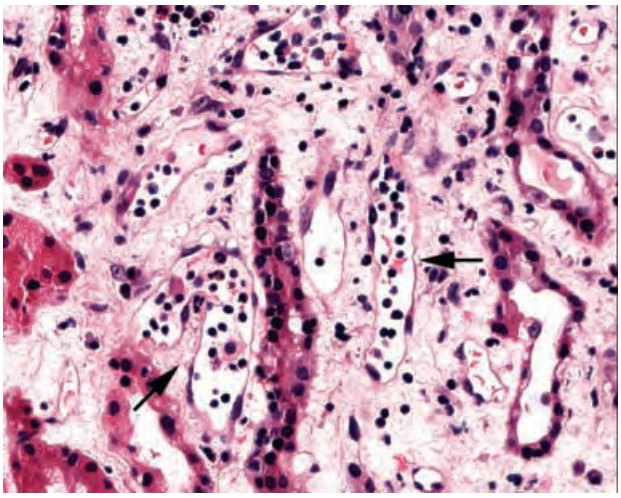

Figure 3: Aggregates of mononuclear inflammatory cells in dilated peritubular capillaries, scored as ptc3 (Hematoxylin and Eosin, original magnification ×265).

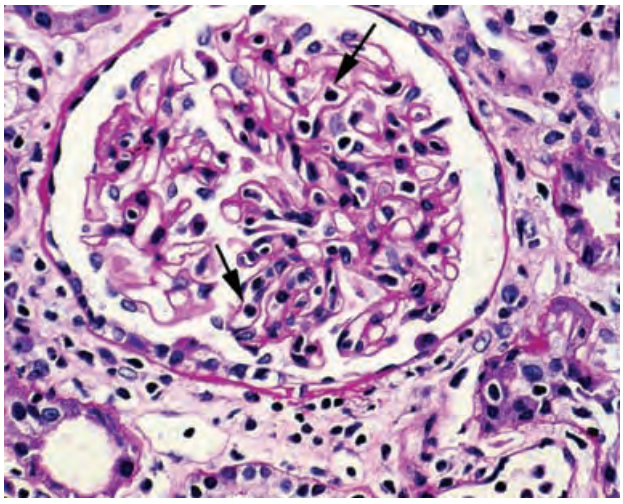

Figure 4: Transplant glomerulitis with infiltrating mononuclear inflammatory cells (arrows) within capillary loops (PAS, original magnification ×400).

endothelial cell injury and repair (15,17–22,27–32). Initially, Monga et al. (28,29) described splitting and multilayering of PTC basement membranes in renal allografts in association with TG. Ivanyi et al. (30) have reported moderate (5–6 layers) and severe (≥7 layers) PTCBMML in 16% and 12% of allograft biopsies and in 21% and 38% of failed transplant nephrectomy specimens with chronic rejection, respectively. Recently, Regele et al. (16) associated endothelial C4d deposition with TG, PTCBMML and accumulation of mononuclear inflammatory cells in PTC. Similarly, Mauiyyedi et al. (33) correlated marked PTCBMML with the presence of C4d in PTCs; they found 4.7 ± 1.8 layers in C4d+ cases versus 1.9 ± 1.2 layers in those that were C4d−. Thus the association of C4d deposition and alloantibody with TG and PTCBMML in some cases suggests AMR as a pathogenesis in at least a subset of patients. On the other hand, the precise definition of PTCBMML is critical when comparing studies describing associations with PTCBMML. For instance, Drachenberg et al. (34) showed that TG was mostly associated with severe PTCBMML (more than 6 layers), whereas lesser degrees of these changes (mostly 2–3 layers) were observed in transplants with other types of glomerulopathies and in native kidneys with various types of immune complex glomerulonephritis, diabetes, and hypertension. A representative picture of marked PTCBMML is shown in Figure 2.

The thickening and lamination of PTC basement membranes might be appreciated on periodic acid-Schiff or silver stains at least in advanced cases (15) and sometimes in Toluidine blue stained EM thick sections but would not allow one to define the severity of lesion, that is, count the layers. The question of whether electron microscopy should be routinely done on every or some subset of renal allograft biopsies remains open and should be addressed at the next Banff meeting along with the feedback from transplant physicians.

The pathogenesis of C4d negative TG and PTCBMML is unclear. In contradiction with the previous observations, three recent studies found no significant correlation between TG and C4d deposition in PTC (35–37) or in glomerular capillaries (36). Akalin et al. (36) showed glomerular infiltration by CXCR3+ ICOS+ activated T cells in grafts with TG/CAN, but not in CAN alone, suggesting an ongoing effector T-cell response to glomerular antigens can result in TG. At the 2005 Banff meeting, Dr. Colvin suggested possible causes of TG that is not associated with C4d staining: (1) technical/sampling error in AMR (e.g. PTC may disappear with allograft fibrosis); (2) residual injury from prior episodes of AMR; (3) T-cell-mediated TG or (4) non-alloimmune causes of TG (such as thrombotic microangiopathy). PTCBMML also appears to be a non-specific regenerative response to various types of injury both in transplants and native kidneys, including obstructive uropathy, thrombotic microangiopathy, analgesic nephropathy, various types of glomerulonephritis and radiation nephritis (30,32,34). Thus, definitive diagnosis of 'chronic' AMR requires a combination of morphologic changes (e.g. TG and/or PTCBMML and/or IF/TA and/or chronic arterial changes), with positive C4d immunostaining, and demonstration of DSA.

Category 5 in the Banff classification now includes only those cases for which no specific etiologic features can be defined (see Table 2). Quantitation of these changes is based on the percentage of cortex involved by IF/TA. Another change in the updated schema is the replacement of 'cellular rejection' with 'T-cell-mediated rejection'. Cellular rejection is associated with a primarily T-cell infiltrate, although the other inflammatory cells including

Table 3: Changes from Banff '97 and '01 diagnostic categories

Category 2. Antibody-mediated rejection now includes 2 subcategories:

Acute antibody-mediated rejection

Chronic active antibody-mediated rejection

Category 3. Borderline changes: 'suspicious' for acute T-cell-mediated rejection

This category is used when no intimal arteritis is present, but there are foci of mild tubulitis (t1) and at least i1. It is now defined more clearly that t2, t3 with i0 or i1 is also under the borderline category.

Category 4. Acute/active cellular rejection is now replaced with T-cell-mediated rejection and includes two subcategories:

Acute T-cell-mediated rejection

Chronic active T-cell-mediated rejection

Category 5. Chronic/sclerosing allograft nephropathy 'CAN' is now replaced with:

Interstitial fibrosis and tubular atrophy, no evidence of any specific etiology

Category 6. Other, changes not considered to be due to rejection-acute and/or chronic. The specific diagnoses responsible for chronic allograft injury, given in Table 1, are represented under category 6.

macrophages/monocytes, B cells, NK cells and plasma cells could also present in the graft and might contribute to the alloimmune response. However, we think that the more definitive term 'T-cell mediated' should be regarded to be similar to the antibody-mediated category as indicating the immunological component that is specifically recognizing the alloantigens. It should also be emphasized that both rejection types have cellular participation (macrophages/monocytes, etc.). Thus the term of 'cellular rejection' is now replaced with 'T-cell mediated rejection' as category #4 with subcategories of 'acute T-cell mediated rejection' and 'chronic active T-cell mediated rejection'. Major changes from the previous Banff schema are summarized in Table 3.

The Pathology of Antibody-Mediated Rejection

Complement deposition as a mediator and/or marker for AMR was discussed in the context of kidney, liver and heart allografts. Method standardization and guidelines for interpretation of complement staining were provided by Drs. Collins and Colvin, summarized elsewhere (38). In the kidney, PTC staining appears quite specific for alloantibody using either monoclonal antibody with immunofluorescence detection on frozen tissue, or polyclonal antibody with immunoperoxidase (IP) detection on paraffin sections; the former, however, is more sensitive (39). Glomerular capillary staining may be a marker for alloantibody effects using polyclonal antibody and IP staining in paraffin embedded tissue (19), but it can also be caused by immune complex deposition in glomeruli. At this time the clinical significance of C4d deposition in a graft with normal histology is unknown. In contrast to patients with anti-HLA antibody, diffuse PTC staining for C4d is commonly detected in well-functioning allografts in patients with anti-A or -B blood group antibodies, without histological evidence of injury (40). The complexity of control of the complement cascade, and resistance to injury with possible arrest of the cascade as a marker for 'accommodation' were emphasized (41). However, recipients with positive cross-match (HLA-incompatible) were recently shown to have increased risk for TG one year after transplantation in comparison to ABO-incompatible and conventional allografts (22% vs. 13% vs. 8%, respectively), and prior AMR appeared as an independent determinant for development of TG (42).

Capillary margination of inflammatory cells is an important histological marker of AMR in kidney and heart allografts, and acute capillaritis in lung allografts may be an equivalent process. Marginating neutrophils are more specific for AMR (43), but both neutrophils and mononuclear cells/monocytes have been associated with PTC C4d staining (16,44). Aggregation of mononuclear cells in PTC is shown in Figure 3. Given the importance of PTC

Table 4: The proposal of quantitative criteria for peritubular capillary margination of inflammatory cells ('ptc') score[1]

ptc0—no significant cortical peritubular inflammatory changes
ptc1—cortical peritubular capillary with 3–4 luminal inflammatory cells
ptc2—cortical peritubular capillary with 5–10 luminal inflammatory cells
ptc3—cortical peritubular capillary with >10 luminal inflammatory cells

[1]Use asterisk (*) to indicate only mononuclear cells and absence of neutrophils.

margination of inflammatory cells as a histological feature of AMR, Ian Gibson proposed a scoring method for quantitation ('ptc' score) at the Banff 2003 conference and reviewed this at the 2005 conference. The proposal focuses on the most severely involved PTCs, in analogy to other inflammatory rejection features such as tubulitis (Table 4). The number of luminal inflammatory cells includes all types (neutrophil, monocyte/macrophage and lymphocyte), with an asterisk (*) used to indicate only mononuclear cells and absence of neutrophils. The extent of the PTC inflammation in the biopsy should be documented, either as focal (<50% of cortical area) or diffuse (>50% of cortical area). The presence of associated PTC dilatation may also be noted. Areas affected by acute pyelonephritis or necrosis, and subcapsular cortex with non-specific inflammation should not be scored. Inflammatory cells within PTC must be distinguished from interstitial inflammation by careful examination of basement membrane stains (PAS, silver). Inflammatory cells within veins and medullary vasa recta should not be scored.

Several groups represented at the Banff 2005 conference indicated that they are using this scoring system. It is particularly applicable to comparison of sequential biopsies from the same graft, for example, in assessing responses to rejection treatments, as well as for documenting biopsy features in clinical trials. Some provisional reports using the peritubular capillaritis scoring system have been published (45,46), confirming its applicability, and showing high 'ptc' scores associated with AMR, and that lower 'ptc' scores can be associated with progressive chronic graft injury (46). It must be emphasized that the 'ptc' score alone does not equate with any specific diagnosis, and ongoing reproducibility and diagnostic studies are required, but the 'ptc' score helps to direct the pathologist to careful examination of the PTC.

B Cells in the Renal Allograft

The role of B cells in allograft rejection and ischemic injury was also highlighted at the 2005 Banff conference. Memory B cells and long-lived plasma cells in bone marrow may persist for years. Initial B-cell activation leads to the formation of short-lived plasma cells that provide the first burst of antibody. Long-term antibody responses, however, are maintained by non-dividing, long-lived plasma cells that produce high-affinity antibody. It should be noted that the B cells or plasma cells reside in lymphoid compartments during AMR and antibodies enter the graft as the effector molecules of humoral immunity (47). 'Lymphoid neogenesis' has been described in renal allografts with prominent lymphoid aggregates (48), though not all lymphoid aggregates are associated with acute rejection (AR) (49). In other contexts (e.g. rheumatoid arthritis, SLE), such aggregates can locally secrete tissue-specific pathogenic antibodies. B cell tolerance may also be possible, as reviewed by Dr. Cascalho (50).

The presence of molecular markers associated with B cells has also been identified in a subset of clinical cases of AR; immunostaining of allograft biopsy tissue confirmed significant numbers of B cells in the inflammatory infiltrates (51). The presence of B cells/markers was associated with worse outcome in this series. However, the frequency of B cell infiltrates in allografts in either AR or non-specific injury has not been extensively studied, nor has the association of allograft B cell infiltrates and AMR/presence of DSA. Recent interest in B cells in allografts has been spurred by the availability of anti-B cell therapies such as rituximab. A few centers have begun to routinely perform immunohistochemistry for B cells in allograft biopsies that have inflammatory infiltrates, for quantitative assessment and pattern of localization. B cell-rich infiltrates should be denoted with an asterisk on the 'i' score in the Banff scoring system. In the short term, these observations could guide therapy for those cases of AR that are B cell-rich and resistant to standard immunosuppression. However, evidence is lacking at this point whether anti-B cell therapy can reverse a resistant episode of B cell-rich rejection. In the long term, detection of B cell markers will provide important data in regard to incidence of significant B cell infiltrates, effects of same on response to therapy, clinical correlates and effect on outcome.

Genomics Markers in Solid Organ Transplantation

Molecular approaches and techniques were the subject of a pre-meeting symposium as well as sessions during the 2005 Banff conference. Techniques discussed included gene expression profiling using high density and DNA microarrays, transcriptome (gene chips) or quantitative PCR, metabolomics and proteomics. The importance of a 'biological' approach was emphasized, correlating gene expression array data with RT-PCR and Western immunoblotting and other proteomic technologies that can validate the actual levels of differentially expressed proteins as well as their post-translational modifications, such as phosphorylation that determine activation and molecular network signaling. It was considered equally important to cross-validate the expression levels of both gene transcripts and proteins, and with the biopsy pathology and clinical data to derive the fullest possible picture. Potential applications of array-based data include definition of disease mechanisms, identification of targets for pharmacological intervention, calibration of indicator systems for drug development, revision of new end points for trials, and development of new diagnostic and monitoring systems that could be applied to blood, fluids (urine, bile) or tissue specimens. The importance of using these strategies to focus on 'real' clinical issues was emphasized, with cluster analysis to identify clinically relevant genetic information.

An ultimate aim is to develop a genomics supported 'Banff classification' for diagnosis and grading of rejection and

other processes in allografts. The potential pitfalls in using genomics markers exclusively for differential diagnosis of acute and chronic dysfunction in allografts were discussed, based on the burgeoning literature in this area (52). Studies are often based on small cohorts of patients and may not include individuals with allergic drug reactions, systemic or intragraft infections or other inflammatory processes. Therapeutic regimens may alter findings and correlations, as has been shown for steroid treatment (53). Many studies of molecular markers for AR have not addressed discrimination between subtypes of AR (tubulointerstitial vs. vascular, cell- vs. antibody-mediated). Sarwal reported that C4d-positive cases of AR fall in each of the 3 AR categories defined by gene profiling. In addition, no obvious differential gene expression could be defined in cases of allograft fibrosis due to different causes (51). At the molecular level, the predominant signatures for gene expression may reflect the predominant pathological mechanisms in the biopsied tissue, for example, fibrosis or inflammation. If this is the case, then a specific cause may be difficult to connect directly to a particular molecular profile without additional data including clinical and histopathological data.

Identification of a few relevant diagnostic markers may be more useful and reasonable for diagnostic application in the near future, particularly, since array data often need to be markedly pruned in order to provide discrimination between patient groups (54). Currently molecular screening of blood and urine represent a promising alternative to invasive biopsy procedures for surveillance to detect early AR, but do not provide enough discriminatory power. At the present time, the assays are not statistically robust enough for clinical guidance. At least for the foreseeable future, the biopsy remains the 'gold standard' for definitive allograft assessment, though exciting alternatives are on the horizon.

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OVERVIEW

ROLE OF DONOR KIDNEY BIOPSIES IN RENAL TRANSPLANTATION

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Recent years have seen an increasing use of marginal donors to expand the organ pool available for renal transplantation (*1–5*). Donors considered in the marginal category include those with age >55 years, hypertension, diabetes mellitus, acute tubular necrosis, disseminated intravascular coagulation, prolonged cold ischemia time, and non-heart-beating donors. Patient and graft outcome obtained with such suboptimal donors has been comparable to that obtained with ideal donors in some studies (*6*, *7*), but significantly worse in others (*2*, *8–10*). These variable results presumably reflect the use of organs with different degrees of functional reserve. It has been estimated that donor factors can account for 35–64% of the variation in recipient serum creatinine and creatinine clearance after transplantation (*11*, *12*). Hence, it stands to reason that demonstration of satisfactory donor kidney function before accepting an organ for transplantation would improve both short- and long-term graft function.

Donor assessment should begin with a review of clinical data, but in cases of traumatic death adequate prior medical records are not always available. Some centers have used an arbitrary age cut off to exclude donors with senile arterionephrosclerosis. However, this is not entirely a satisfactory approach, because of individual variability in the rate at which kidney tissue ages. Thus, the percentage of sclerotic glomeruli in human kidneys varies between 0.2–16.7% at age 55 years and 1.5–23.0% at age 75 years (*13*). Data from a study conducted at the University of Pittsburgh indicated that 17/30 (57%) donors aged 60–75 years had 0–10% glomeruli sclerotic: clearly a decision to reject these donors based on age alone would have been inappropriate (*3*). Conversely, mild histologic abnormalities can be present in younger individuals much more commonly than is generally appreciated. Arteriolar hyalinosis has been reported in 25- to 34-year-old subjects, and considered to be a marker for early onset atherosclerotic disease (*14*).

Laboratory evaluation of donor renal function is important, and should include urine examination as well as blood chemistry. It should be kept in mind that mild proteinuria can occur secondary to glomerular or tubular ischemia reflecting agonal changes occurring before death. Acceptable cut off values for proteinuria used by different centers range between 0.5–3.0 g/24 hr (*15*, *16*). Blood urea and serum creatinine are readily available parameters for the assessment of renal function, but can rise significantly due to conditions such as renal hypoperfusion and acute tubular necrosis, which do not per se contraindicate transplantation. Creatinine clearance has also been used for screening of donors, and is superior to serum creatinine in that it is not affected by donor age, muscle mass, or obesity, However, clear-cut guidelines on the use of creatinine clearance as a criterion for donor selection have not yet been developed. Some authors have suggested a donor creatinine clearance measurement >60–70 ml/min for accepting marginal organs for single kidney transplantation (*15*, *17*). In contrast, others investigators have recommended double kidney transplantation when the donor creatinine clearance is less than 90–100 ml/min (*18*). Allograft function cannot be simply predicted by evaluating the donor creatinine clearance, because of multiple post-transplant variables such as acute tubular necrosis, antibody or cell-mediated rejection, and calcineurin inhibitor nephrotoxicity. Another confounding factor is the occurrence of compensatory renal parenchymal hypertrophy, when the donor nephron mass is insufficient to meet the metabolic needs of the recipient. Compensatory changes have been shown to result in an approximately 20% rise in estimated creatinine clearance in the allograft kidney within 4–6 months of transplantation (*16*).

The remainder of this review will focus on the role that a pretransplant biopsy can play in helping to define the structural integrity and functional reserve of a donor kidney under consideration of transplantation. A biopsy should be considered mandatory when the donor in question is in the marginal category. At Pittsburgh, we have set an arbitrary cut-off age of 55 years, beyond which all donors are biopsied to evaluate the severity of senile arterionephrosclerosis. A strong case can be made to include a pretransplantation or postperfusion biopsy in the routine work up of all donors, irrespective of age and clinical setting. This would provide baseline anatomic data with which future biopsies can be compared. Preexisting lesions such as capillary thrombosis, arteriolosclerosis, glomerulosclerosis, and interstitial fibrosis can be recorded, so that the occurrence of the same lesions in posttransplantation biopsies is not misconstrued as evidence of calcineurin inhibitor nephrotoxicity or chronic allograft

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nephropathy. Lack of knowledge about the extent of preexisting changes in a donor kidney complicates the interpretation of posttransplant biopsies.

Biopsy techniques vary from institution to institution. I prefer a generous wedge biopsy about 1-cm long and 0.5-cm deep. This suggested size ensures that at least half the cortical depth is available for evaluation, and minimizes erroneous conclusions due to superficial subcapsular scarring secondary to senile arteriosclerosis. Some centers prefer that both a wedge and a needle biopsy be performed to provide assurance that the deep cortex has been adequately sampled. A needle biopsy alone may not permit reliable assessment of the extent of glomerulosclerosis due to limited sampling. One study has suggested that sample adequacy be defined by the presence of a minimum of 25 glomeruli (*19*). This contention was based on the observation that a statistically significant relationship between percent glomerulosclerosis and graft loss was observed only if biopsies with more than 25 glomeruli were analyzed. In another study, the relationship between percent glomerulosclerosis and graft function was found to hold irrespective of the number of glomeruli present at biopsy (*2*). However, in that study this relationship was lost on multivariate analysis if a correction was made for donor age. This led the authors to state that if the donor age is known, data on glomerulosclerosis do not add any additional vital information. However, as pointed out earlier, age-associated changes in the human kidney are extremely variable and can not be predicted without a biopsy. Additionally, a biopsy can detect the presence of previously undocumented chronic diseases such as hypertensive or diabetic nephropathy, and chronic tubulointerstitial nephritis.

Urgent histological processing of donor biopsies is needed, when the decision to use the donor kidney is contingent on the morphologic findings. Because prolonged cold ischemia can adversely affect long-term graft function, the biopsies need to be interpreted as soon as possible. Rapid processing protocols can allow permanent sections to be available for reading within 2 hr. Consistently providing this level of service, however, necessitates that both a histotechnologist and an anatomic pathologist to be on call round the clock. As an alternative, a frozen section service with only a pathologist being on continuous call can be offered. Frozen section morphology is adequate to recognize sclerotic glomeruli, advanced interstitial fibrosis, and arteriosclerosis. However, freezing artifacts can lead to interstitial widening, which can be confused with fibrosis, if one does not insist on demonstrating a definite collagenous matrix. Retraction of tubular epithelium from the basement membranes makes it difficult to recognize tubular atrophy. Frozen sections are also not reliable for assessment of mesangial cellularity, glomerular capillary wall thickening, and diabetic lesions such as small capsular drop lesions or early Kimmelstein-Wilson nodules. Gross thrombosis can be recognized at frozen section, but small fibrin thrombi in the capillaries are more difficult to evaluate.

Interpretation of a kidney biopsy from a donor with senile arterionephrosclerosis or other chronic renal disease calls for a semiquantitative evaluation of the degree of glomerulosclerosis, arteriosclerosis, and interstitial fibrosis present. The use of Banff criteria for grading chronic allograft nephropathy is suggested to ensure center to center uniformity in this assessment (*20*). If most of the glomeruli are patent, and there is only mild arteriosclerosis and interstitial fibrosis present, the donor kidney is suitable for use. However, the extent of acceptable chronic changes within the donor kidney has not yet been rigorously defined. A widely accepted empiric rule is that kidneys with more than 20% sclerotic glomeruli not be used (*8*). At Pittsburgh, surgeons are also hesitant to use any kidney with more than mild interstitial fibrosis (more than 25% of cortical area affected) or mild arteriosclerosis (more than 25% luminal occlusion). Glomerular, interstitial, and vascular lesions in any given biopsy are frequently proportional to each other, even though this interrelationship is somewhat imperfect (*21*). Hence, we have taken the approach that moderate or severe changes in any of the major anatomic compartments in a donor kidney should contraindicate transplantation. Recently, it has been shown that the maximal planar area of the nonsclerotic glomeruli is also a predictor of long-term graft function (*22*).

Several investigators have studied interobserver variability in grading morphologic changes in donor biopsies. Pokorna et al. reported moderate to good reproducibility with calculated weighted kappa scores of 0.66 for percent glomerulosclerosis, 0.78 for interstitial fibrosis, and 0.83 for arteriolar hyalinosis (*2*). Wang et al. addressed this issue by (1) comparing histological changes in paired baseline biopsies from the same donor, and (2) comparing baseline donor biopsies with sequential posttransplant biopsies from the same recipient (*19*). Using linear regression analysis, the precision of estimating percent glomerulosclerosis in paired biopsies was good only if analysis was restricted to biopsies with more than 14 glomeruli ($r=0.83$ for paired biopsies and $r=0.56$ for sequential biopsies). The κ statistic for arteriolar hyalinosis was 0.55 for paired biopsies and 0.38 for sequential biopsies. Discrepancies in grading arteriolar hyalinosis were found in 10% of paired biopsies and 20–30% of sequential biopsies. Sund et al. reported poor reproducibility in the grading of arteriosclerosis and arteriolar hyalinosis in sequential biopsies, based on calculated kappa scores of 0.046 and 0.122, respectively (*22*). These disappointing results presumably reflect variation in the distribution and severity of vascular lesions in this patient population. The lesions were more pronounced in the pretransplant biopsy compared to the posttransplant biopsy. It was suggested that this difference resulted from a propensity of vascular lesions to affect deeper vessels, which are more likely to be sampled when a biopsy gun is pointed directly at the surface of a donor kidney. In support of their contention, the authors pointed out that the cortico-medullary junction was indeed more often sampled in biopsies obtained before transplantation.

Several studies have validated the clinical utility of donor biopsies by formal statistical analysis. Seron et al. examined postperfusion biopsies, and showed a correlation between interstitial fibrosis and serum creatinine measured 12 months posttransplant (*9*). Leunissen et al. showed a correlation between a histological chronicity score obtained at postperfusion biopsy and creatinine clearance measurement performed 3 months later (*23*). Lehtonen et al. found the chronic allograft damage index in a donor biopsy to predict long-term graft function (*24*). Gaber et al. showed that postperfusion biopsies with >20% glomerulosclerosis ($n=8$) were associated with an 88% incidence of delayed graft function (7/8 grafts), 38% graft loss (3/8 grafts), and a mean serum creatinine of 2.6 ± 0.1 mg/dl at 6 months (*8*). Based on this

data, it was suggested that kidneys with >20% glomerulosclerosis not be used for transplantation. However, this conclusion was derived from a study group of only eight patients with an unusually high percent glomerulosclerosis (mean 39±6%). The control group of patients used for comparison had significantly lower glomerulosclerosis (8±1%). Pokorna et al. described a 3-year graft survival of 74.7% in 67 patients with 20.0–47.6% glomerulosclerosis, but 11% of these recipients had primary non-graft function, and a mean 1-year glomerular filtration rate of 41.4 ml/min (*2*). Several studies have demonstrated a relationship between donor arteriosclerosis and posttransplant function (*25–27*). Hyaline changes in the smaller arteriolar sized vessels also correlate with 1 year serum creatinine (*28*) and rate of graft failure (*19*).

The reason why interstitial fibrosis, glomerulosclerosis, arteriolar hyalinosis, or arteriosclerosis have variably been identified as the critical parameter in different studies is probably the result of patient selection and methodological considerations. For example, in one study where donor glomerulosclerosis, but not interstitial fibrosis, was found to predict graft function, cases with >55% and <55% interstitial fibrosis were compared with regards to the incidence of satisfactory graft function defined simply as a patient being alive without maintenance dialysis (*19*). In a second study, interstitial fibrosis was found to be predictive, if biopsies showing no interstitial fibrosis were compared with those showing any level of interstitial fibrosis, and graft function was assessed by calculated creatinine clearance (*3*). Failure to detect the effect of interstitial fibrosis in some studies may also partly reflect the patchy nature of this lesion, which in turn, may be due to the patchy nature of arteriosclerosis and arteriolar hyalinosis in the kidney.

In contrast to the literature discussed above, one can also find studies that fail to find any correlation between donor biopsy findings and posttransplantation graft function (*7*, *22*, *29–32*). This is surprising given the intuitively expected relationship between anatomic architecture and physiological function in the kidney. Closer analysis of many of these studies reveals methodological problems such as (1) small numbers of patients, (2) insufficient histological detail for critical evaluation, (3) studies limited to biopsies with only mild histological changes, (4) lack of correction for variables such as prolonged cold ischemia or acute rejection, and (5) use of only crude patient or graft survival rates in evaluating outcome (*1*, *7*). In some clinical settings, the expected effect of donor histology can probably be overshadowed by other confounding clinical variables. Thus, many surgeons prefer to give kidneys from older donors to older recipients, who have a weaker immune system. This may result in lower rejection and reasonable graft survival, despite changes of senile arterionephrosclerosis in the donor organ. One study has suggested that improvements in medical care have now reduced the importance of donor age as a critical factor in renal transplantation (*33*).

The preceding discussion has focused primarily on donor biopsies performed for old age, hypertension, or donor diabetes mellitus. Clinical concern about pretransplant ischemic injury is another relatively common reason for requesting a donor biopsy. Predisposing factors for such injury include a history of donor hypotension, use of pressors during donor medical management, prolonged cold/warm ischemia time, a non-heart-beating donor, and chronic parenchymal or vascular disease in the donor. Acute tubular necrosis, the histological counterpart of ischemic injury, is difficult to evaluate on frozen section, except in cases with frank coagulative necrosis or infarction. Even with ideal permanent section morphology, correlations between clinical renal dysfunction and histological acute tubular necrosis are imperfect. Solez et al. could not demonstrate any correlation between histological severity of acute tubular necrosis and duration of oliguric acute renal failure in the native (nontransplanted) kidney (*34*, *35*). Lehtonen et al. found that chronic changes in the donor biopsy did not correlate with immediate posttransplant graft function (*24*). A similar lack of correlation has been observed with donor vascular disease (*27*). However, others have reported that histological scoring for acute tubular necrosis (*2*, *26*, *36*) or apoptosis (*37*) predicts delayed graft function. There is evidence that prolonged cold ischemia and delayed graft function predisposes to vascular rejection in kidneys derived from older donors (*35*). Whether delayed graft function adversely affects long-term graft survival independently of rejection is controversial (*36*).

Donor biopsies performed in the setting of disseminated intravascular coagulation need to be evaluated for the extent of microvascular injury. Organs with diffuse and extensive glomerular thrombosis should be discarded. However, the presence of scattered capillary thrombi present in a minority of glomeruli does not necessarily contraindicate transplantation. When the donor serum creatinine is normal or marginally elevated, successful transplantation has been reported. Isolated fibrin thrombi can apparently be dissolved by an intact fibrinolytic system (*31*, *37*), although this may result in a transient microangiopathic hemolytic anemia in a few instances (*38*). Mate kidneys recipients from the same coagulopathic donor can have different graft outcomes due to variations in pre- and posttransplant factors (*39*).

Occasionally, pretransplant or postperfusion biopsies show changes consistent with glomerulonephritis, and allow the glomerular disease in the allograft kidney to be traced back to the organ donor. The risk of this scenario is probably the highest for IgA nephropathy, a disease with high prevalence in some geographic regions. Based on isolated case reports in the literature, it would appear that mild glomerular changes in a donor biopsy can probably be ignored. Thus, it has been documented that modest donor-derived IgA deposits do not cause significant graft dysfunction, and can spontaneously resolve with time (*40*, *41*). Similar observations have been made regarding donor-derived postinfectious glomerulonephritis, membranoproliferative glomerulonephritis type I and lupus nephritis (*42–44*). Focal segmental sclerosis attributable to donor disease has been shown not to progress in the posttransplantation period (*31*).

The final indication for a donor kidney biopsy is the presence of a grossly visible nodule noticed during harvesting of the organ. When histological examination shows a benign cyst, leiomyoma or angiomyolipoma, it is safe to proceed with transplantation. However, finding a small epithelial neoplasm can generate dilemmas that may be difficult to resolve, particularly when a high grade carcinoma is not demonstrated. The distinction between a so-called renal adenoma and a small low grade renal cell carcinoma is arbitrary, and traditionally based on the size of the lesion, although it is now increasingly recognized that lesions of any size can metastasize. If the donor lesion is small (less than 0.5 cm) and

completely excised, the risk of residual or recurrent carcinoma in the recipient is probably extremely small. Dr. Israel Penn has reported six cases, where wide excision of the donor nodule led to an uneventful course documented by up to 186 months of posttransplantation follow up (*45-48*). The rare occurrence of posttransplant renal allograft carcinoma, despite the estimated 7–25% incidence (based on routine autopsy data) of small renal cell neoplasms in donor kidneys, also suggests that the use of such kidneys might be reasonable, at least in the context of informed recipient consent. Nonetheless, this is a controversial issue, and some transplant centers may not accept organs with small epithelial neoplasms.

In summary, a kidney biopsy is essential in the clinical work-up of marginal donors who are being evaluated for renal transplantation. In fact, it should be the standard of care to obtain a baseline biopsy from all kidneys before implantation, irrespective of the donor's medical history. Such a practice can consistently document premature arterionephrosclerosis and other clinically unsuspected renal disease in the donor. Lack of knowledge about the extent of preexisting changes in a donor kidney complicates the diagnosis of chronic allograft nephropathy and drug induced hyalinosis in posttransplant biopsies. While examining donor biopsies, an effort should be made to grade the severity of glomerulosclerosis, interstitial fibrosis, arteriosclerosis, and arteriolar hyalinosis present. Review of available evidence suggests that donor organs with <20% glomerulosclerosis and mild interstitial fibrosis or arteriosclerosis give clinically acceptable results. If the biopsy changes are more pronounced, the prospect of implanting a suboptimal organ with reduced graft life has to be weighed against the alternate option of continuing to support the patient by dialysis. Double kidney transplantation can also be considered in the latter situation (*16*).

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Minireview

BK Virus Infection in Transplant Recipients: An Overview and Update

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BK virus infection after kidney transplantation has been a subject of great interest in the past decade. This article traces the discovery of BK virus and the subsequent development of our knowledge about this emerging pathogen. The pathobiology of the virus is summarized with particular reference to epidemiology, interactions with host cell receptors, cell entry, cytoplasmic trafficking and targeting of the viral genome to the nucleus. This is followed by a discussion of clinical features, laboratory monitoring and therapeutic strategies. Finally, we present potential cellular mechanisms that explain the basis of virus-mediated damage to the human kidney.

Key words: BK virus, epidemiology, JC virus, kidney, pathobiology, pathogenesis, polyomavirus, renal, SV40 virus, transplantation

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Virology

Polyomavirus BK virus (BKV) is a double-stranded DNA virus with a 5-kb genome. It has been classified in the Polyomaviridae family, which includes JC virus (JCV), a well known cause of progressive multifocal leukoencephalopathy, and the simian virus SV40 (1). The BKV genome comprises the non-coding control region (NCCR), the early-coding region coding for the small and large T antigens, and the late-coding region coding for the viral capsid proteins (VP1, VP2 and VP3) and agnoprotein. The NCCR contains (a) the origin of replication (ori) and (b) the regulatory regions containing enhancer elements that can alter viral transcription. T antigen binds to tumor suppressor proteins Rb and p53 and initiates the cell cycle in host cells. VP1, VP2 and VP3 are structural proteins that make up the viral capsid. The VP1 gene displays considerable genetic heterogeneity, and this genetic variation has led to recognition of viral genotypes I, II, III and IV. Agnoprotein plays a role in several cellular processes, including cell cycle progression, DNA repair, viral capsid assembly and virion release from cell.

Historical Aspects

BKV was first isolated in 1970 from a Sudanese kidney transplantation recipient with a ureteric stricture. Epidemiological studies showed that up to 90% of some human populations become exposed to BKV by adulthood (1). After kidney transplantation, 10–60% of patients were noted to excrete virus in the urine. However, viruria was typically asymptomatic or associated with only transient graft dysfunction, though occasionally, virus-induced tissue damage was noted at allograft nephrectomy or at autopsy. A new era in the study of BKV began when BKV nephropathy (BKVN) was diagnosed by a needle biopsy in a renal transplant recipient suspected of having acute rejection. This case was diagnosed in 1993 at Pittsburgh and published in 1996. In the following years, additional cases were reported from kidney transplant centers worldwide (2–5). It is commonly believed that this epidemic of BKVN in the 1990s is the result of potent immunosuppressive drugs such as tacrolimus, mycophenolate mofetil and sirolimus.

Mode of Transmission

Given that polyomavirus is latent in the kidney, it is not surprising that the donor kidney itself appears to be an important source of infection in transplant recipients. Donor seropositivity has been implicated in development of BK viruria, viremia or BKVN in pediatric and adult transplant recipients (6–9). The mode of viral transmission in the general population is incompletely understood, but multiple routes of infection are likely involved (1). Thus, BKV DNA has been amplified from 0–40% of urine samples, and 1% of nasopharyngeal aspirates obtained from infants with respiratory infections. The possibility of feco-oral transmission has been recently raised by the demonstration of viral DNA in urban sewage. Blood, semen, genital tissues and normal skin biopsies have also been shown to contain BKV. Transplacental transmission of polyomaviruses from mother to fetus has been recorded.

Cell Entry and Intracellular Trafficking

BKV interactions with host cellular receptors have been the subject of only limited investigations. The primary receptor binding determinant on BKV is the VP1 protein. The host cell receptor for BKV appears to be an N-linked glycoprotein, in which GT1b and GD1b have been identified as component gangliosides (10). Both these gangliosides have an α-(2-8) linked di-sialic acid-motif as a common feature. An α-(2-3) sialic acid linkage has also been shown to be important (10,11). Despite considerable homology at the genetic level, BKV differs from other polyomaviruses with regard to the chemical nature of its receptor. Thus, the JCV receptor is an N-linked glycoprotein containing terminal α-(2-3)- and α-(2-6)-linked sialic acids. The mouse polyomavirus binds to receptors containing α-(2-3)-linked sialic acid N-glycoproteins as well as α4β1 integrins. SV40 VP1 interacts with major histocompatibility class I proteins and O-linked glycan molecules.

The mode of BKV entry into the cell and routes of intracellular trafficking are currently being clarified. Electron microscopic observations on human biopsy material show that BKV entry into host cells is similar to SV40, and mediated by non-clathrin coated vesicles resembling caveolae. In contrast, JCV enters the cell by clathrin-dependent endocytosis. The mechanisms of endocytosis and intra-cellular trafficking utilized by BKV have not been investigated in detail. However, it has been established that the route from cell membrane to the nucleus includes the endoplasmic reticulum and microtubules (12,13). There may also be participation of the Golgi apparatus, and other cytoskeletal elements such as actin, and microfilaments, as has been shown for other members of the polyomavirus family. The mechanism by which polyomavirus traverses the nuclear envelope to enter the nucleus is only partially understood. VP2 and VP3 contain a nuclear transport signal that may facilitate nuclear targeting of the viral mini-chromosome. Nucleoporin, a protein associated with the nuclear pore complex, has also been implicated. The uncoating process of polyomaviruses has been stated to occur after the virions have entered the cell nuclei, but it has been shown for SV40 virus that some disassembly can occur in the endoplasmic reticulum.

Risk Factors for Infection

Conflicting information has been reported on risk factors for BK infection in transplant recipients (9,14–16). Risk factors may be donor, recipient, transplant or virus related. Reported donor-related factors include deceased-donor versus living-donor transplant, the presence of active BKV or cytomegalovirus (CMV) infection, donor seropositivity and the absence of HLA-C7. Reported recipient-related risk factors include older age, male gender, Caucasian race, diabetes mellitus, CMV infection, prior renal tubule injury, recipient seronegativity and the absence of HLA-C7. Risk factors associated with transplantation include procurement injury, cold-ischemia time, delayed graft function, immunosuppression, especially with maintenance tacrolimus, mycophenolate mofetil or sirolimus, or treatment of acute rejection with lymphocyte depleting agents or steroids, drug-toxicity, and increased number of HLA mismatches. Viral-related factors include variants in VP1 and sequence alterations in the NCCR. Most of these risk factors are unavoidable, unable to be modified or their risk contribution has not been consistently shown from study to study, perhaps because the type and intensity of immunosuppression may override any individual or combination of risk factors. Thus, the type and degree of immunosuppression is the most modifiable factor. A randomized prospective trial of 200 kidney transplant recipients showed that the incidence of BK viruria or viremia was not increased with thymoglobulin induction as compared to no induction, use of tacrolimus as compared to cyclosporine or the use of mycophenolate mofetil as compared to azathioprine (15). However, using detection of BK viruria or viremia as a surrogate for the intensity of immunosuppression, the combination of tacrolimus and mycophenolate or cyclosporine and azathioprine were the most potent, and the combination of cyclosporine and mycophenolate the least potent for the development of BK viruria or viremia. An interventional strategy with discontinuation of the antimetabolite upon detection of viremia was used and no BKVN was observed. Thus, it appears that it may not be the type but rather the intensity of immunosuppression that is the greatest risk factor for BK infection and thus BKVN.

Systematic clinical observations of human subjects undergoing polyomavirus seroconversion have not been reported, and the role of BKV antibodies remains unclear. An increasing anti-BK antibody titer has been seen to develop with a decrease of immunosuppression and treatment of BKVN (17). However, high-titer anti-BK antibody in the donor has been associated with an increased likelihood of development of BK viruria and viremia in the recipient (18).

Clinical Features

The most frequent symptom associated with BKV infection is an upper respiratory infection. Sporadic reports of acute cystitis, with or without hematuria, are also reported. After primary infection has resolved, the virus enters a latent phase. It appears that viral latency can be maintained in a number of different sites, particularly the urogenital tract (kidneys, urinary bladder, prostate, cervix and vulva, as well as testes, prostate and seminiferous tubules, as detected in semen) and hematolymphoid tissues (peripheral blood mononuclear cells, tonsils). Reactivation of latent virus has been reported in old age, pregnancy and diabetes mellitus, and immunosuppression associated with congenital immunodeficiency, organ transplantation or HIV infection. The most striking feature of BK infection in kidney

transplant recipients is the lack of fever, malaise, myalgias, leukopenia, anemia, thrombocytopenia or other symptoms or signs typical of viral infection, despite viral loads exceeding a billion copies/mL in the urine or 100 000 copies/mL in the blood (15,16). BKVN presents with renal dysfunction without other clinical signs or symptoms.

Laboratory Diagnosis and Monitoring

Laboratory monitoring strategies for BKV are still evolving. Quantitative nucleic acid-based viral load assay of urine or blood are becoming widely used for BKV screening (15,19–22). Detectable virus in the blood is more predictive of BKVN than viruria alone. Some medical centers prefer urine cytology as the primary screening technique (23,24). While urinary 'decoy cells' have excellent sensitivity for the detection of overt BKVN, polymerase chain reaction (PCR) is four times more sensitive than urine cytology for monitoring asymptomatic viruria (25). Additionally, PCR provides a more objective estimate of true viral load, and can distinguish BK viruria from JC viruria. JCV excretion in the urine is usually insignificant, although very rare cases of JCV-associated interstitial nephritis are on record. Decoy cells are not stable, whereas DNA is, and PCR may be used for monitoring of patients at a distance from the transplant center. The relative costs of PCR versus cytology are a center-dependent variable. Laboratory screening for BKV should certainly be done for any unexplained rise in serum creatinine. In addition, it is very desirable to monitor patients periodically, and one potential monitoring strategy is shown in Figure 1. The cost-effectiveness of screening has been formally evaluated in only one study to date (26). In this investigation, it was determined that routine BKV screening becomes cost-effective only if the incidence of BKVN in a transplant program exceeds 2.1%. The cost of screening was found to be substantially offset by the savings related to reductions in immunosuppression following diagnosis of BKVN. No anti-viral agents were administered. The assumptions made in this study regarding the incidence of BKVN, cost of testing, management strategy and risk of graft loss may not be applicable to all medical centers in the United States.

Definitive diagnosis of BKVN requires a biopsy and demonstration of BKV inclusions in tubular epithelial or Bowman's capsular epithelial cells. Viral infection is accompanied by varying degrees of inflammatory cell infiltrates, tubular atrophy and fibrosis. The cytopathic effect seen by light microscopy is typical, but not pathognomonic for BKVN. Confirmatory immunohistochemistry or *in situ* hybridization studies are usually performed using antibodies against specific for BKV proteins or probes complementary to viral DNA (Figure 2). Electron microscopy can be used to demonstrate unenveloped, viral particles, approximately 40 nm in diameter. Since BKVN can be focal in distribution, ideally two biopsy cores should be examined. The availability of medullary parenchyma increases the diagnostic sensitivity. Negative biopsy results cannot rule out BKVN with certainty, and a diagnosis of 'presumptive BKVN' can be made if there is renal allograft dysfunction associated with BK viremia.

Biopsy findings have been shown to have prognostic significance and three histological patterns of BKVN have been proposed: (a) BKVN A: mild viral cytopathic changes, with little or no inflammatory infiltrates or fibrosis, (b) BKVN B: mild to moderate viral cytopathic changes with significant

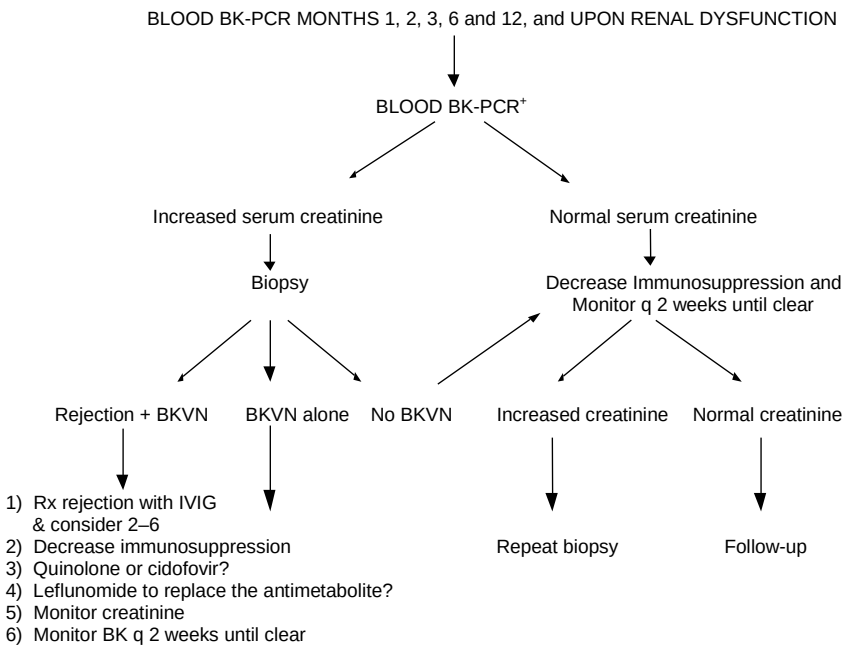

Figure 1: BK monitoring algorithm.

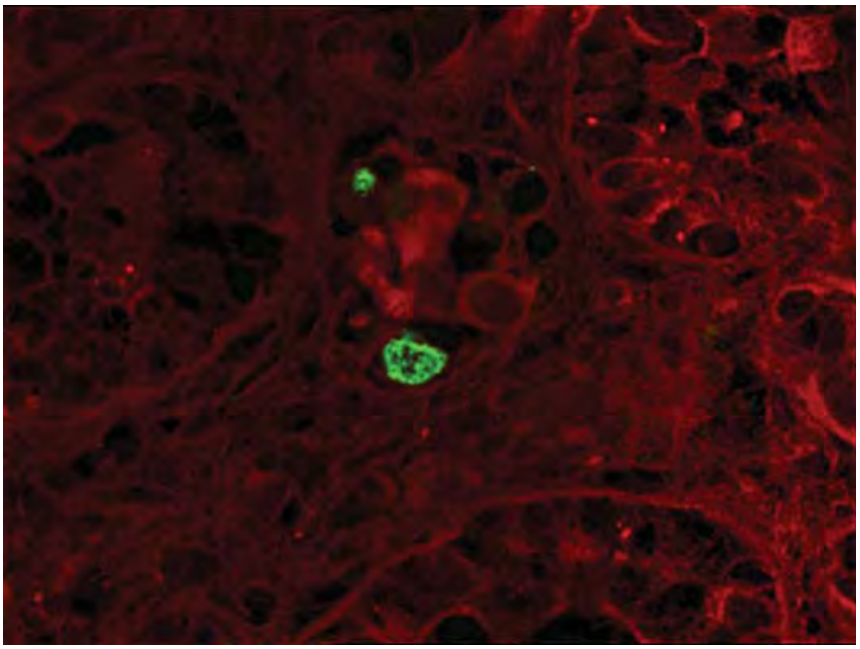

Figure 2: BK virus replication in the nucleus of a renal tubular epithelial cell, as demonstrated by a fluorescein-labeled antibody directed against VP1, a viral capsid protein that is synthesized late in the lytic life cycle.

inflammatory infiltrates, but limited fibrosis (≤ ci1+) and (c) BKVN C: prominent tubular atrophy and interstitial fibrosis, with usually sparse cytopathic changes, and variable inflammatory infiltrates. BKVN A carries the best prognosis, while BKVN C is associated with the worst long-term outcome (27).

Inflammatory cell infiltrates and tubulitis in biopsies with BKVN may represent an immune response to the infection or concurrent allograft rejection. A definitive diagnosis of rejection concurrent with viral nephropathy should only be made if there is endarteritis, fibrinoid arterial necrosis, glomerulitis or accumulation of the complement degradation product C4d along peritubular capillaries.

Pathogenesis

The pathogenesis of tissue damage in polyomavirus infected tissues is a subject of considerable interest. Transition from latent to lytic infection in the human kidney is likely initiated by ischemic, calcineurin inhibitor, or rejection-associated injury. This would explain, in part why most cases of BKVN occur in the allograft kidney, although disease in the native organ has been recorded. Using DNA microarray analysis of allograft kidney biopsies, it has been shown that BKVN is associated with up-regulation of several major groups of mRNAs, including CD8, Interferon-γ, CXCR3 and perforin. It is notable that these molecules are also up-regulated in acute cellular rejection, and this illustrates why the differential diagnosis between viral nephropathy and acute cellular rejection is problematic (28). Additionally, there is up-regulation of molecules associated with graft fibrosis, including matrix collagens, TGF-β, MMP2, MMP9 and markers of epithelial-mesenchymal transformation. The latter finding attests to the role of viral infection in promoting chronic allograft nephropathy.

Treatment

The treatment of BKVN is unsatisfactory, since no uniformly effective anti-viral drugs are currently available. Prevention of BKVN may be a better strategy than treatment of established disease. One large study of patients with prospective monitoring of urine and blood, and preemptive withdrawal of the anti-metabolite upon development of viremia, showed that this strategy resulted in clearance of viremia and viruria, and appeared to prevent progression to BKVN without increasing the risk of acute rejection (15). Another smaller prospective study showed that viremia and viruria could resolve or decrease over time with standard reductions in immunosuppression, without preemptive withdrawal of any component of the immunosuppressive regimen (16). Reducing the intensity of maintenance, immunosuppression currently represents the primary treatment of well established BK nephropathy. However, in patients with progressive graft dysfunction not responding to this maneuver, anti-viral treatment should be considered. Protocols and success rates are heterogeneous, with graft loss ranging from <10% to >80% (14). Anti-viral agents used with anecdotal success include cidofovir, leflunomide, quinolone antibiotics and intravenous immunoglobulin (14,29–32). The efficacy of these strategies is unclear, because reduction of immunosuppression has been used along with all of the strategies. Additionally, an *in vitro* study has shown that the 50%

effective concentration (EC50) for either leflunomide (39.7 μg/mL) or cidofovir (36.3 μg/mL) is higher than what may be achieved clinically with conventional dosing (33). Blood leflunomide levels above 40 μg/mL in the context of other reductions of immunosuppression have been associated with viral clearance or decrease in BK-viral load, but the pharmacokinetics of leflunomide are unpredictable, and even patients on 60 mg/day may fail to achieve 40 μg/mL (30,34). For cidofovir, the EC50 is much higher than peak plasma concentrations that are typically achieved with the low-dose (0.25–1.0 mg/kg) treatment regimens described in most publications related to BKVN (33). Esterification of cidofovir with hexadecyloxypropyl, octadecyloxyethyl or oleyloxyethyl groups results in up to 3-log lowering of EC_{50} and markedly increased selectivity index *in vitro*. Oral bioavailability and reduced nephrotoxicity are additional potential advantages of these derivatives over unmodified cidofovir (35). A cautiously conducted controlled clinical trial of these compounds in the management of BKVN appears to be warranted. Re-transplantation is safe and usually not complicated by recurrent BKV infection in the recipient. If allograft nephrectomy is performed, preemptive re-transplantation may be performed even during the phase of active viremia (36).

In conclusion, there is increasing recognition of BKV infections after kidney transplantation. Improved techniques of clinical monitoring and preemptive adjustment of immunosuppression have led to a reduction in the incidence of overt viral nephropathy. However, in patients who do develop BKV-induced allograft injury, we do not have reliable anti-viral drugs available at this time. The impact of long-term low-grade viruria or viremia on the development of chronic allograft nephropathy requires further study.

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Minireview

Recurrent Glomerulonephritis After Kidney Transplantation

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Thirty to fifty percent of kidney transplant recipients have glomerular diseases as the underlying causes of end-stage renal failure. While recurrence of glomerulonephritis is an important cause of late renal allograft failure, the risk factors for recurrence are largely unknown or imprecise and prediction remains difficult. Recurrent disease usually presents with similar manifestations as the native disease. With regard to treatment of recurrent glomerular disease in the renal allograft, plasma exchange may be effective in reducing proteinuria in patients with early recurrence of focal and segmental glomerulosclerosis, but immunosuppressive therapy is generally ineffective in the prevention or treatment of recurrent disease. General supportive measures including strict blood pressure control and inhibition or blockade of the renninangiotensin pathway are helpful in retarding the rate of deterioration in renal allograft function. Despite the risk of recurrence, kidney transplantation following primary glomerulonephritides enjoys graft and patient survival rates comparable to other causes of end-stage renal failure. With a few exceptions, living related renal transplantation is not contraindicated in view of the favorable outcome and the donor shortage. This review discusses commonly encountered recurrent glomerulonephritides, with special emphasis on the influence of post-transplant prophylactic immunosuppression and emerging treatments.

Key words: Glomerulonephritis, recurrence, renal transplant, treatment

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Introduction

Glomerulonephritis is the underlying cause of end-stage renal failure in 30–50% of kidney transplant recipients (1). These patients are at risk of the recurrence of their original diseases. Recurrent glomerulonephritis was previously considered to be a minor contributor to graft loss. Introduction of newer immunosuppressive agents have reduced graft loss directly by decreasing the incidence of acute rejection and indirectly through the consequent reduction of chronic allograft nephropathy (1,2). With the prolongation of graft survival, the effect of recurrent disease on graft outcome assumes increasing importance. Studies on recurrent disease are difficult since not all patients have undergone native kidney biopsy and most centers perform graft biopsies only when there are abnormal clinical or laboratory features. The reported incidence of recurrent disease is thus influenced by prevailing clinical practice and could over- or underestimate the true occurrence. In this regard, it may be impossible to differentiate between *de novo* and recurrent disease. Accurate dissection of the contribution by recurrent disease toward graft dysfunction is also difficult in view of the often concomitant histological features of chronic allograft nephropathy or chronic nephrotoxicity due to calcineurin inhibitors. Many a time, full evaluation of biopsy specimen with combination of light microscopy, immunofluorescence and immunohistochemical studies and electron microscopy is needed to delineate different pathologies that coexist in the same patient. Despite these difficulties, there is accumulating evidence that recurrent glomerulonephritis is an important cause of graft loss in the long-term follow-up of renal allograft recipients (1,3,4).

The latest registry study, reported by Briganti et al. on 1505 patients with both native and graft biopsies, showed that graft loss due to recurrent glomerulonephritis was the third most frequent cause for graft loss 10 years after kidney transplantation. The risk of graft loss from recurrence increased with the years of follow-up, from 0.6% at first postoperative year to 8.4% at the 10th year (1). The recurrence rate, clinical course and impact on graft survival vary between different types of glomerulonephritis. This review aims to provide updated knowledge on recurrent renal diseases after kidney transplantation, focusing on recent findings with new post-transplant immunosuppressive regimens and treatment.

Immunoglobulin A Nephropathy

Immunoglobulin A Nephropathy (IgAN) is the most common type of glomerulonephritis worldwide and is the primary cause of renal failure in 20% of kidney transplant recipients. The pathogenetic mechanisms are complex and incompletely understood. It is likely to be related to the aberrant synthesis of abnormally *O*-glycosylated IgA1 in

Table 1: Recurrence rate of IgA nephropathy and risk of recurrence and graft loss from recurrent IgAN in relation to the donor type

				Recurrence rate[1]		Graft loss due to recurrence	
	Follow-up duration (mean) (months)	No. of allografts Total	(R/NR)	No. (%) Total	(R/NR)	No. (%) Total	(R/NR)
Berger et al. 1984 (6)	>24	32	(13/19)	17(53.1%)[2]	(9/8)	0	
Bachman et al. 1986 (9)	20 ± 13	13	(6/7)	6(46.2%)	(5/1)	1 (7.6%)	(1/0)
Odum et al. 1994 (7)	3–183	51	–	17(33.3%)	–	5 (9.8%)	–
Hartung et al. 1995 (10)	45.9 ± 10	128	–	47(36.7%)	–	9 (7.0%)	–
Kesser et al. 1996[3] (11)	68.1 ± 37.2	84	(3/25)[5]	13(15.5%)	(2/11)[5]	4 (4.8%)	–
Frohnert et al. 1997 (12)	78 (3–156)[6]	53	(41/12)	10(19%)	(8/2)	3 (5.7%)	(2/1)
Ohmacht et al. 1997[4] (13)	54 (7–127)	61	–	20(29.9%)	–	10 (16.4%)	–
Bumgardner et al. 1998 (14)	61 ± 37	61	(18/43)	18(29.5%)	(6/12)	7 (11.5%)	(4/3)
Freese et al. 1999 (15)	67 (11–159)[6]	104	(47/57)	13(12.5%)	(11/2)	6 (5.8%)	–
Kim et al. 2001 (16)	2–164	90	(60/30)	19(21.1%)	(13/6)	2 (2.2%)	–
Wang et al. 2001 (17)	52 (18–155)[6]	48	(17/31)	14(29.2%)	(6/8)	4 (8.3%)	(3/1)
Ponticelli et al. 2001 (18)	70.4 ± 50.5	106	(21/85)	37(35%)	(9/25)	4 (3.8%)	–
Andresdottir et al. 2001 (19)	67.2 ± 54	79	–	17(21.5%)	–	1 (1.3%)	–
Briganti et al. 2002 (1)	12–120	532	–	–	–	15 (2.8%)	–
Choy et al. 2003 (20)	100.0 ± 5.8	75	(32/43)	14(18.7%)	(9/5)	3 (4.0%)	(1/2)
Moriyama 2005 (21)	67.8 ± 19.9	49	(44/5)	13(26.5%)	(12/1)	5 (10%)	(5/0)

RD = related donor, NRD = nonrelated donor.
Recurrent rate for RD = 29.8%; NRD = 22.7%. Breslow–Day test of Homogeneity of odds ratio: chi-square = 10.29, df = 10, p = 0.416. Mantel–Haenszel estimate of Common odds ratio: 2.14 (95% CI = 1.42, 3.23; p < 0.001).
Percentage of graft loss from RD = 34.8%; NRD = 24.1%. Breslow–Day test of Homogeneity of odds ratio: chi-square = 7.37, df = 5, p = 0.194. Mantel–Haenszel estimate of Common odds ratio: 1.95 (95% CI = 0.64, 5.97; p = 0.243).
(%) = Percentage was calculated from number of graft loss due to recurrent IgAN/total number of patients with primary IgAN.
[1]Recurrence rate in patients with clinical symptoms of proteinuria/hematuria/renal impairment.
[2]Recurrence rate in patients with histological changes but clinically asymptomatic.
[3]Included 13 patients who suffered from underlying Henoch-Schonlein purpura.
[4]Included 4 patients who suffered from underlying Henoch-Schonlein purpura.
[5]Only 28 allografts had information with respect to the donor type.
[6]Median.

patients with IgAN. Mesangial deposition of polymeric IgA1 with abnormal *O*-glycosylation initiates glomerular inflammation and injury with progressive loss of renal function (5).

Recurrent IgAN is common after transplantation. Great variation in the incidence of recurrence has been reported because of difference in duration of follow-up and biopsy policy of different transplant centers (Table 1). Most centers performed renal biopsy only when patients presented with clinical symptoms of proteinuria, hematuria or decline in renal function. This would potentially underestimate the rate of recurrence as patients who were clinically asymptomatic but with histological changes in the graft kidneys would remain undiagnosed. For centers where routine protocol biopsies were being carried out in all transplant recipients, histological recurrence with mesengial IgA deposits and mesangial hypercellularity had been reported in 50–60% of patients (6,7). Recurrence rate reported for patients with renal biopsies for clinical symptoms ranged from 13–50% (9–21) (Table 1). Clinical manifestations are similar to primary IgAN and include microscopic hematuria, proteinuria and slow decline in renal function. Clinical course of recurrent IgAN had been reported to be benign initially (6,8). However, with increasing long-term data, it is apparent that recurrent disease is not as benign as had been reported previously (7,9–18,20,21). Graft loss from recurrence with histological features of diffuse mesangial proliferative expansion and glomerular sclerosis were reported between 1.3% and 16% (1,7,9–21) (Table 1). The estimated 10-year incidence of graft loss due to recurrence was 9.7% (CI = 4.7–19.5%) from the latest registry report containing the largest number of IgAN patients (1).

It is interesting to note that renal allograft survival for the first 5 years post-transplant is better in patients with primary IgAN compared to other primary diseases (8,11,19,20). The proposed mechanism included increased occurrence of allo-reactive IgA anti-HLA antibodies which may block the deleterious effect of IgG and IgM antibodies on the graft, and the immunological dysfunction of patients with IgAN (8). Despite the better graft survival of IgAN patients for the early post-transplant period, graft survival becomes comparable and might be worse than patients with other underlying renal diseases when data with follow-up beyond 10 years becomes available (16,18,20), suggesting other factors including recurrent disease contributing to graft loss becomes more apparent with long-term follow-up. No single parameter including age, gender, race, HLA

typing, pre-transplant course or biochemical characteristic of serum IgA can predict recurrence.

The relationship between the risk of recurrence and the donor type remains controversial. Some studies had reported a higher risk of disease recurrence in related donors (6,9,11,15,17), while others reported no added risk (12,14,16). Pooling all available data from literature that contained information on graft recurrence (6,9,11,12,14–18,20,21) and graft loss (9,12,14,17,20,21) in relation to donor type and estimate the risk by Mantel–Haenszel estimate of common odds ratio showed a higher risk of disease recurrence among transplant recipients with related donors (common odds ratio 2.14, $p < 0.001$), but the risk of graft loss was not increased (common odds ratio 1.95, $p = 0.24$) (Table 1). Whether this apparent paradox could be due to insufficient follow-up remains to be investigated. Given the fact that the graft survival of patients with primary IgAN is excellent for the first decade post-transplant, it is inappropriate to refrain from living related donor transplantation even though there may be a slight risk of recurrence. In contrast, familial IgAN should be rigorously excluded in potential living related donors since familial IgAN is associated with high risk of development of renal failure in affected members (22). Moriyama et al. reported higher risks of recurrence and graft loss in patients with latent IgA deposition from donor kidneys (majority were living related donors) (21). Whether such latent IgA deposition or the load of immune deposits might be detrimental to graft survival remain speculative.

The situation is quite different for patients with prior graft loss due to recurrent IgAN because the risk of recurrence in the second transplant (20–100%) is much increased (13–15,18). Ohmacht et al. reported a graft loss rate of 60% in their patients with a follow-up duration of 21–51 months (13) while two other series reported good graft function despite of recurrence in their patients up to 92 months of follow-up (14,18). In this regard, living donor transplant should be discouraged if recurrence and graft failure occur within few years after first transplant. However, such a transplantation is not a problem if their first graft functions beyond 10 years post-transplantation.

There is no effective therapy for the prevention or treatment of recurrent IgAN. Calcineurin inhibitors, in the presence or absence of induction therapy, do not influence the recurrent risk. Despite initial enthusiasm, newer immunosuppressive drugs are ineffective in preventing recurrence. Anecdotal reports that mycophenolate mofetil might have averted progression to allograft failure in recurrent IgAN are not substantiated by recent studies (18,23). Data on sirolimus are limited. Development of IgAN with nephrotic range of proteinuria had been reported in two transplant recipients after conversion from a calcineurin inhibitor-based immunosuppression to sirolimus (24). Steroid free or rapid steroid withdrawal regimen does not seem to affect the recurrent risk (25). The effect of fish oil in recurrent IgAN has not been systematically examined. Angiotensin converting enzyme inhibitor and angiotensin receptor blocker are commonly used for reduction of proteinuria and preservation of renal function in patients with recurrence as in IgAN of native kidneys (26,27).

Henoch-Schönlein purpura (HSP) has been regarded by many as the systemic variant of IgAN. Renal manifestation of HSP is indistinguishable from IgAN. Currently available data suggest that the recurrence rate after transplantation in patients with HSP is similar to that of IgAN (13,19,28).

Focal and Segmental Glomerulosclerosis

Focal and segmental glomerulosclerosis (FSGS) is a histological diagnosis that encompasses not only the idiopathic form (primary FSGS) but also a variety of secondary causes including glomerular hyperfiltration, toxic injury or viral infection leading to similar sclerotic lesions, recurrence risk of which depends on the underlying disorder. Primary FSGS has a recurrence rate of 20–50% after kidney transplantation leading to graft failure in 13–20% of patient in 10 years after kidney transplantation (1,4). Clinical manifestations of recurrent FSGS include early onset of massive proteinuria, usually within first year post-transplant, hypertension and graft dysfunction.

The pathogenesis of recurrent FSGS is unclear. A circulating permeability factor which increases the glomerular permeability to albumin and is removable by plasmapheresis or immunoadsorption therapy has long been suspected to play an important role. Savin et al. developed an *in vitro* bioassay for the permeability factor (29), and had shown that patients with high permeability factor activity in pretransplant sera were more likely to develop recurrence (29,30). However, recent data suggest that the absence or loss of an inhibitor of a normally present factor in plasma rather than the addition of a circulating factor could be the underlying cause for the glomerular permeability alteration (31,32). Further complicating the picture is the recognition of the pivotal role of the podocyte in the pathogenesis of proteinuria in various glomerulopathies. Acquired or inherited defect in the slit—diaphragm proteins (podocin [NPHS2], nephrin [NPHS1], α-actinin 4 and CD2AP) on the glomerular basement membrane have been reported in 15% of patients with primary FSGS (33,34). Recurrence which would not be expected in the genotypically normal donor kidneys have been reported in recipients with mutations of podocin, more so for the heterozygous than the homozygous mutations (34). This suggested that etiology of recurrent FSGS is likely multifactorial involving interaction between genetic and extra-renal mechanisms (putative permeability factor).

Risk factors for recurrence include younger age, rapid progression of original disease with development of end-stage renal failure within 3 years, mesangial hypercellularity of

native kidney, Caucasian race and a history of previous graft failure due to recurrence (4,35,36). Earlier reports have suggested a higher risk of graft loss with related donors (36) but recent reports showed that the risk of graft loss was similar between living donors and deceased donors (1,37). Patients who have recurrence of FSGS in the first year after transplantation with rapid loss of their graft are at a very high risk (>80%) of having recurrence and graft dysfunction in subsequent grafts (38). In this regard, living donor transplant should be avoided in patients who have lost their first graft in a rapid fashion.

Early institution of plasmapheresis is important as the effectiveness of treatment decreases with the increased number of sclerosed glomeruli. Relapse after cessation of plasmapheresis can be prevented or reversed by chronic plasmapheresis or concurrent treatment with cyclosporine or cyclophosphamide (29,30,35). Improved long-term renal outcome is observed in patients who achieve remission following treatment for early recurrence (35). Preemptive perioperative plasmapheresis for 2–8 sessions starting 1 week before operation for living donor transplant or immediately post-transplant for deceased donor transplant had been reported to reduce recurrence in children (39) and high-risk patients (40). The role of preemptive plasmapheresis in prevention of recurrence in high-risk group still awaits confirmation by larger clinical trial. There is a recent case report of complete resolution of proteinuria with rituximab (which was used to treat his post-transplant lymphoproliferative disease) in a patient who developed severe recurrent FSGS 2 weeks post-transplant with persistent proteinuria despite of prolong courses of plasmapheresis (41). The response of resistant proteinuria in this patient to the anti-CD20 antibody might shed some light in management of patients who failed to respond to conventional treatment although efficacy and long-term safety need further evaluation with prospective trial.

Newer immunosuppressive agents such as sirolimus have increasingly been used to replace calcineurin inhibitors to avoid calcineurin inhibitors associated nephrotoxicity and to treat chronic allograft nephropathy. However, a number of case reports have reported the development of *de novo* or recurrent FSGS when cyclosporine was replaced with sirolimus, with subsequent improvement after switching back to cyclosporine (24,42,43). The beneficial effect in this regard seemed specific to cyclosporine (43). Paradoxically, sirolimus had been reported in a recent study that 12 out of 21 patients with steroid resistant FSGS achieved complete or partial remission of their proteinuria after 6 months of therapy (44). In view of the accentuation of glomerular damage due to the proinflammatory effects of sirolimus and its derivatives in animal models (45), caution still need to be exercised when sirolimus is used in patients with underlying FSGS. Early steroid withdrawal did not lead to an increase in recurrent FSGS or graft loss from recurrent disease, although long-term data are still awaited (25,46). Data from small series have implicated increased recurrent FSGS with antilymphocytic antibodies (47) and anti-IL2 receptor antibodies (48).

Membranoproliferative (Mesangiocapillary) Glomerulonephritis

Secondary causes of membranoproliferative (mesangiocapillary) glomerulonephritis (MPGN) (type I) include infections such as viral hepatitis B or C and systemic diseases. Treatment of these underlying causes may thus reduce the risk of recurrence. Recurrent disease should also be differentiated from *de novo* MPGN which occurs as part of the histological changes in patients with chronic transplant nephropathy.

Both type I (with mesangial and subendothelial deposits) and type II (dense deposit disease) primary MPGN have high rates of recurrence after transplantation. Type I MPGN recurs in 20–50% of patients. Clinical manifestations include proteinuria and deterioration of renal function. Risk factors for recurrence include HLA-B8DR3, living related donors and previous graft loss from recurrence (49). The overall incidence of allograft loss at 10 years due to recurrence is around 15% (1). The risk of graft loss from recurrence in a second graft in patients who have experienced a recurrence in the first graft is as high as 80% (49).

Recurrent disease is much more frequent in type II disease, and up to 80–100% of patients are affected. These patients usually present with nonnephrotic range proteinuria within the first year posttransplant and slowly declining renal function. There is no correlation between complement level and recurrence risk. Graft loss due to recurrence occur in 15–30% of patients after 5 years (50). Type III MPGN (with both subepithelial and subendotherial deposits) has been considered as a variant of type I disease, and there are few data regarding its recurrence after kidney transplantation.

A recent report has suggested that the severity of histological abnormalities in the native kidney (interstitial fibrosis, crescent formation and mesangial proliferation) rather than the type of MPGN is related to recurrence risk. Nevertheless, type II MPGN usually has more aggressive glomerular changes and thus a higher risk of recurrence, and poorer prognosis (51). No effective therapy is available for prevention or treatment of recurrent MPGN.

Membranous Nephropathy

Secondary causes of membranous nephropathy (MN) including viral infections and malignancy should be screened. Treatment of these underlying causes may reduce the risk of recurrence in secondary MN. Idiopathic MN recurs in 10–30% of patients after kidney transplantation. Recurrent disease should also be differentiated from *de novo* MN,

which is the most common *de novo* glomerulopathy in renal allografts. The clinical presentation of recurrent disease is characterized by nephrotic range proteinuria. The mean onset time is approximately 10 months post-transplant as compared with the more insidious and later onset of symptoms in *de novo* MN, an entity thought to be related to chronic rejection (52,53). Recent demonstration of antibodies against 'neutral endopeptidase', a protein expressed on the human podocyte cell membrane, causing severe membranous glomerulonephritis in a fetus, suggested that 'neutral endopeptidase' probably plays a significant role in the pathogenesis of the membranous glomerulonephropathy (54). No risk factor for recurrence has been identified. The initial concerns with regard to the risk of recurrence with living related donors, presence of HLA-DR3 in the recipient, and the aggressiveness of native disease have not been substantiated (53). Graft failure from recurrence occurs in 10–15% of patients after 10 years (1). Cyclosporine and mycophenolate mofetil which have been used in treatment of primary MN do not prevent or change the course of recurrent disease (53). There is also no report to suggest therapeutic advantage of tacrolimus or cyclophosphamide over cyclosporine.

Antineutrophil Cytoplasmic Antibody-Associated Glomerulonephritis (Pauci-Immune Cresentic Glomerulonephritis)

Despite better recognition and improved treatment of antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis, a proportion of patients still progress to end-stage renal failure. Recurrence in patients with Wegener's granulomatosis (WG), microscopic polyangiitis (MPAN) and idiopathic necrotizing crescentic glomerulonephritis (CGN) have been reported. Nachman et al. pooled data from 127 patients and reported that 17% of patients had recurrence of vasculitis after 4–89 months of follow-up. Three-fifths of them had renal manifestation and two patients lost their grafts due to recurrence (55). A more recent study by Briganti reported a 10-year incidence of allograft loss of 7.7% in patients with pauci-immune cresentic glomerulonephritis (1).

Pre-transplantation disease course, cANCA or pANCA specificity, disease subtype (WG, MPAN or CGN), ANCA titer (in the absence of clinically active disease) at the time of transplantation, duration of follow-up or donor type do not predict recurrence (55). It is advisable to defer kidney transplantation until the disease is inactive (55). Patients with renal relapses generally showed good response to cyclophosphamide (55–57). For patients with cellular crescents on renal biopsies and high ANCA titer, favorable outcome with combination therapy comprising cyclophosphamide, plasmapheresis with or without intravenous immunoglobulin had been reported (57,58).

Systemic Lupus Erythematosis

Although the prognosis of lupus nephritis has improved over the past few decades, lupus nephritis remains an important cause of end-stage renal failure. Histological recurrence has been reported in up to 30% (59) of transplant recipients. Clinically significant recurrent disease occurs in 2–9% (2,60). With the higher morbidity and poorer general condition of patients during active disease, most centers would postpone renal transplantation until the disease become quiescent for at least 6–9 months (60,61). The duration of dialysis before transplantation and serological status in the absence of clinically active disease do not predict recurrence (59–61). There are anecdotal reports on the efficacy of mycophenolate mofetil in recurrent lupus nephritis (62,63). Graft loss due to recurrent lupus nephritis is uncommon, occurring in 2–4% (59–61). Long-term patient and graft survival are similar to kidney allograft recipients with other underlying diseases (59–61).

Antiglomerular Basement Membrane Disease

Histological recurrence had been reported in up to 50% of patients when kidney transplantation was performed while circulating antiglomerular basement membrane disease (anti-GBM) antibodies were still present (64). With the current practice of deferring transplantation until the disease become quiescent and circulating anti-GBM antibody levels become undetectable for at least 12 months, clinical recurrence is rare and consisted of isolated case reports only (1,3). Good treatment response had been reported in one patient who developed recurrence with positive anti-GBM antibody and cresentic glomerulonephritis treated with pulse steroid, plasmapheresis and cyclophosamide (65) while another patient responded to treatment with immunoadsorption and cyclophosamide (57).

Conclusions

With improving long-term renal allograft survival, recurrent disease has increased prominence as a significant contributor to late graft loss. Knowledge on the risk factors for recurrence, onset time and impact on graft function is prerequisite to informed decisions (Table 2). There are minimal data on the risk of recurrent disease with new immunosuppressive agents, although anecdotal observations caution cyclosporine and/or corticosteroid withdrawal in patients with a history of FSGS, and animal data suggest that it is pertinent to examine the impact of sirolimus on recurrent glomerular diseases. Apart from plasmaphresis for patients with recurrent FSGS, there is no consensus on strategies to prevent or treat recurrent glomerular disease in the kidney allograft. It is important to emphasize that the majority of patients with primary glomerulonephritis as the underlying cause of renal failure enjoy excellent graft and patient

Table 2: Risk of recurrence and graft loss and treatment strategies for different types of glomerulonephritis

	Clinically relevant[1] recurrent risk[2]	Risk of graft loss due to recurrence 5–10 years post-transplant[2]	Prevention/treatment strategies
IgAN	13–46%	2–16%	ACEI and/or ARB for patients with proteinuria ± renal impairment due to recurrent IgAN (26,27)
FSGS	20–50%	13–20%	Avoid living donors for patients with history of rapid graft loss from recurrence (38) Preemptive perioperative plasmapheresis (PP) for 2 weeks for patients with high risk of recurrence (39,40) Chronic PP with or without cyclophosphamide or cyclosporine for patients with relapse after initial course of PP (29,30,35) ? Avoid omission of calcineurin inhibitors in sirolimus based immunosuppressive regimen (24,42,43) ? Avoid induction therapy (47,48)
MPGN			
Type I	20–25%	~15%	No effective preventive or treatment measures
Type II	80–100%	15–30%	Exclude secondary causes
Membranous nephropathy	10–30%	10–15%	No effective preventive or treatment measures Exclude secondary causes
ANCA-associated glomerulonephritis	~17%	6–8%	Defer transplant till disease inactive (55) Cyclophosamide for recurrence (55,56) Combine therapy with PP, cyclophosphamide ± intravenous immunoglobulin for recurrence with high titer of ANCA and cellular crescents in renal biopsies (57,58)
SLE	2–9%	2–4%	Defer transplant till disease inactive (60,61) Consider mycophenolate mofetil for recurrence (62,63)
Anti-GBM	Rare	Rare	Defer transplant till disease inactive Combine therapy with PP/immunoabsorption and cyclophosphamide for recurrence with high anti-GBM titer and cellular crescents in renal biopsies (57,65)

[1] Clinical relevant refer to patients with clinical symptoms of proteinuria/hematuria/renal impairment.
[2] % of transplanted patients.

survival. Also, in spite of the controversy over the risk of recurrence with certain types of glomerulonephritis when the source of allografts is from living donors, the graft survival is largely comparable to patients with other causes of end-stage renal failure. Thus, living related kidney donation can still be encouraged in carefully selected patients and donors. Caution should be exercised in patients with previous rapid graft loss due to recurrent disease in view of the markedly increased risk with subsequent transplants. Research toward identification of biological or immunological markers for individual glomerulonephritis should provide tools to better identify and prevent recurrence.

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Grading of Acute Liver Allograft Rejection

Global assessment of rejection grade made on a review of the biopsy and after the diagnosis of rejection has been established.

Global Assessment*	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see reference below)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

* Verbal description of mild, moderate or severe acute rejection could also be labeled as Grade I,II and III, respectively.

Reference Anonymous. Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document. *Hepatology* 1997;25(3):658-63.

Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

[FRAMES] [NO FRAMES]

REJECTION ACTIVITY INDEX (RAI)

Criteria which can be used to score liver allograft biopsies with acute rejection, as defined by the World Gastroenterology Consensus Document.

Category	Criteria	Score
Portal Inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile Duct Inflammation Damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1

Venous Endothelial Inflammation	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3
Total RAI Score = _/9		
Reference Anonymous. Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document. *Hepatology* 1997;25(3):658-63.		

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[FRAMES] [NO FRAMES]

Histologic Features of Early and Late Chronic Liver Allograft Rejection

Structure	Early CR	Late CR
Small bile ducts (<60 um)	Degenerative changes involving a majority of ducts (eosinophilic transformation of the cytoplasm; increased N:C ratio; nuclear hyperchromasia; uneven nuclear spacing; ducts only partially lined by biliary epithelial cells) Bile duct loss <50% of portal tracts	Degenerative changes in remaining bile ducts Loss in >=50% of portal tracts
Terminal hepatic venules and zone 3 hepatocytes	Intimal/lumenal inflammation Lytic zone 3 necrosis and inflammation Mild perivenular fibrosis	Focal obliteration Variable inflammation Severe (bridging) fibrosis
Portal tract hepatic arterioles	Occasional loss involving <25% of portal tracts	Loss involving >25% of portal tracts
Other	So-called "transition" hepatitis with spotty necrosis of hepatocytes	Sinusoidal foam cell accumulation; marked cholestasis
Large perihilar hepatic artery branches	Intimal inflammation, focal foam cell deposition without lumenal compromise	Lumenal narrowing by subintimal foam cells Fibrointimal proliferation
Large perihilar bile ducts	Inflammation damage and focal foam cell deposition	Mural fibrosis

Reference

- Demetris A, Adams D, Bellamy C, Blakolmer K, Clouston A, Dhillon AP, Fung J, Gouw A, Gustafsson B, Haga H, Harrison D, Hart J, Hubscher S, Jaffe R, Khettry U, Lassman C, Lewin K, Martinez O, Nakazawa Y, Neil D, Pappo O, Parizhskaya M, Randhawa P, Rasoul-Rockenschaub S, Reinholt F, Reynes M, Robert M, Tsamandas A, Wanless I, Wiesner R, Wernerson A, Wrba F, Wyatt J, Yamabe H. : Update of the International Banff Schema for Liver Allograft Rejection: Working Recommendations for the Histopathologic Staging and Reporting of Chronic Rejection *Hepatology 31(3):792-799, 2000*

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Modified HAI Grading: Necroinflammatory Scores

Periportal or Periseptal Interface Hepatitis (piecemeal necrosis) (A)	Score	Confluent Necrosis (B)	Score	Focal (spotty) Lytic Necrosis, Apoptosis, and Focal Inflammation* (C)	Score	Portal Inflammation (D)	Score
Absent	0	Absent	0	Absent	0	None	0
Mild (focal, few portal areas)	1	Focal confluent necrosis	1	One focus or less per 10x objective	1	Mild, some or all portal areas	1
Mild/moderate (focal, most portal areas)	2	Zone 3 necrosis in some areas	2	Two to four foci per 10x objective	2	Moderate, some or all portal areas	2
Moderate (continuous around <50% of tracts or septa)	3	Zone 3 necrosis in most areas	3	Five to ten foci per 10x objective	3	Moderate/marked, all portal areas	3
Severe (continuous around >50% of tracts or septa)	4	Zone 3 necrosis + occasional portal-central (P-C) bridging	4	More than ten foci per 10x objective	4	Marked, all portal areas	4
		Zone 3 necrosis + multiple P-C bridging	5				
		Panacinar or multiacinar necrosis	6				

Total Modified HAI = __/18

References

1. Ishak K, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-699.
2. Knodell RG, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1(5):431-5

*****Does not include diffuse sinusoidal infiltration by inflammatory cells.

Additional features which should be noted but not scored:	*Immunohistochemical findings*
❍ Bile-duct inflammation and damage ❍ Lymphoid follicles ❍ Steatosis, mild moderate or marked ❍ Hepatocellular dysplasia, large- or small-cell ❍ Adenomatous hyperplasia ❍ Iron or copper overload ❍ Intracellular inclusions (eg. PAS-positive globules, Mallory bodies)	❍ Information on viral antigens, lymphocyte subsets or other features, when available, should be recorded and may be semi-quantitatively expressed

Modified Staging: architectural changes, fibrosis and cirrhosis*

Change	Score
No fibrosis	**0**
Fibrous expansion of some portal areas, with or without short fibrous septa	**1**
Fibrous expansion of most portal areas, with or without short fibrous septa	**2**
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	**3**
Fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)]	**4**
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	**5**
Cirrhosis, probable or definite	**6**

References

1. Ishak K, et al. Histological grading and staging of chronic hepatitis. *J Hepatol* 1995;22:696-699.
2. Knodell RG, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. *Hepatology* 1981;1(5):431-5

*****_Additional features which should be noted but not scored:_ Intra-acinar fibrosis, perivenular ('chicken wire' fibrosis) and phlebosclerosis of terminal hepatic venules.

Click here to see guide to score interpretation.

Autoimmune Hepatitis: Revised Scoring System (1999)

(International Autoimmune Hepatitis Group, J. Hepatology 31: 929-938, 1999)

Feature	-5	-4	-3	-2	-1	0	+1	+2	+3
Sex						Male		Female	
Alk phos:ALT or Alk phos:AST (note 1)				>3		1.5-3.0		<1.5	
Serum globulins or IgG above normal						<1x normal	1-1.5x normal	1.5-2x normal	>2x normal
ANA, SMA, or LKM1 (note 2)						<1:40	1:40	1:80	>1:80
AMA		Positive				Negative			
Hepatitis viral markers (note 3)			Positive						Negative
Drug history (note 4)		Yes					No		
Average alcohol intake				> 60 gm/day				<25 gm/day	
Histology	Absence of all of the following: interface hepatitis, lympho-plasmacytic infiltrate, and liver cell rosettes		Biliary changes (note 5) or other defined changes (note 6) (-3 each)				Predominantly lympho-plasmacytic infiltrate, liver cell rosettes (1 each)		Interface hepatitis
Other autoimmune disease (note 7)						Absent		Present	

Seropositivity for other defined autoantibodies (note 8)								Present	
HLA DR3 or DR4 (note 9)						Absent	Present		
Response to therapy (note 10)								Complete	Relapse

Interpretation of scores: An aggregate score greater than 15 prior to therapy constitutes a definite diagnosis of AIH. A score of 10-15 is interpreted as probable AIH. A score greater than 17 following therapy is considered positive, and a score of 12-17 after therapy is considered probable, for the diagnosis of AIH.

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Note 1	The ratio refers to the degree of elevation above upper normal limits (UNL) of these enzymes, i.e., (IU/L alk phos/UNL alk phos)/(IU/L ALT/UNL ALT)	return
Note 2	As determined by indirect immunofluorescence on rodent tissues or, for ANA, on HEp-2 cells. Lower titers, esp. of LKM-1, are significant in children and should be scored at least +1	return
Note 3	SCore for markers of hepatitis A, B, and C viruses (i.e., positive or negative for IgM anti-HAV, HBsAg, IgM anti-HBc, anti-HCV and HCV-RNA). If a viral etiology is suspected despite seronegativity for these markers, tests for other potentially hepatotropic viruses such as CMV and EBV may be relevant.	return
Note 4	History of recent or current use of known or suspected hepatotoxic drugs.	return
Note 5	"Biliary changes" refers to bile duct changes typical of PBC or PSC, ie granulomatous cholangitis or severe concentric periductal fibrosis, with ductopenia, established in an adequate biopsy specimen, and/or a substantial periportal ductular reaction, so-called marginal bile duct proliferation with a cholangiolitis, with copper/copper-associated protein accumulation.	return
Note 6	Any other prominent feature or combination of features suggestive of a different etiology	return
Note 7	Score for history of any other autoimmune disorder(s) in patient or first-degree relatives.	return
Note 8	The additional points should be allocated only in patients seronegative for ANA, SMA, and LKM-1. Other "defined" autoantibodies include pANCA, anti-LC1, anti-SLA, anti-ASGPR, anti-LP, and anti-sulfatide.	return
Note 9	The additional points should be allocated only in patients seronegative for ANA, SMA, and LKM-1. HLA DR3 and DR4 are mainly of relevance to North European, Caucasoid, and Japanese populations. One point may be allocated for other Class II antigens for which there is published evidence of their association with AIH in other populations.	return

Note 10	Assessment of response to therapy is shown in the Table and may be made at any time. Points should be added to those accrued for features at initial presentation.	return

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Definitions of Response to Therapy (AIH Scoring System 1999)

(International Autoimmune Hepatitis Group, J. Hepatology 31: 929-938, 1999)

Response	Definition		
Complete	Either or both of the following: marked improvement of symptoms and return of serum ALT or AST, bilirubin and immunoglobulin values completely to normal within 1 year and sustained for at least a further 6 months on maintenance therapy, or a liver biopsy specimen at some time during this period showing at most minimal activity.	or	Either or both of the following: marked improvement of symptoms together with at least 50% improvement of all liver test results during the first month of treatment with AST or ALT levels continuing to fall to less than twice the upper normal limit within 6 months during any reductions toward maintenance therapy, or a liver biopsy within 1 year showing only minimal activity.
Relapse	Either or both of the following: an increase in serum AST or ALT levels of greater than twice the upper normal limit or a liver biopsy showing active disease, with or without reappearance of symptoms, after a "complete" response as defined above.	or	Reappearance of symptoms of sufficient severity to require increased (or reintroduction of) immunosuppression, accompanied by any increase in serum AST or ALT levels, after a "complete" response as defined above.

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Reference

- Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, Chapman RW, Cooksley WGE, Czaja AJ, Desmet VJ, Donaldson PT, Eddleston ALWF, Fainboim L, Heathcote J, Homberg J-C, Hoofnagle JH, Kakumu S, Krawitt EL, Mackay IR, MacSween RNM, Maddrey WC, Manns MP, McFarlane IG, Meyer zum Büschenfelde K-H, Mieli-Vergani G, Nakanuma Y, Nishioka M, Penner E, Porta G, Portmann BC, Reed WD, Rodes J, Schalm SW, Scheuer PJ, Schrumpf E, Seki T, Toda G, Tsuji T, Tygstrup N, Vergani D, Zeniya M. International Autoimmune Hepatitis Group Report: Review of criteria for diagnosis of autoimmune hepatitis. *J Hepatology 1999; 31:929-938.*

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Histological Scoring System for Nonalcoholic Fatty Liver Disease

Components of NAFLD Activity Score (NAS) and Fibrosis Staging

Nonalcoholic Steatohepatitis Clinical Research Network

NAS Components (see scoring interpretation)			
Item	**Score**	**Extent**	**Definition and Comment**
Steatosis	**0**	**<5%**	**Refers to amount of surface area involved by steatosis as evaluated on low to medium power examination; minimal steatosis (<5%) receives a score of 0 to avoid giving excess weight to biopsies with very little fatty change**
	1	**5-33%**	
	2	**>33-66%**	
	3	**>66%**	
Lobular Inflammation	**0**	**No foci**	**Acidophil bodies are not included in this assessment, nor is portal inflammation**
	1	**<2 foci/200x**	
	2	**2-4 foci/200x**	
	3	**>4 foci/200x**	
Hepatocyte Ballooning	**0**	**None**	
	1	**Few balloon cells**	**The term "few" means rare but definite ballooned hepatocytes as well as cases that are diagnostically borderline**
	2	**Many cells/prominent ballooning**	**Most cases with prominent ballooning also had Mallory's hyalin, but Mallory's hyaline is not scored separately for the NAS**
Fibrosis Stage (Evaluated separately from NAS)			
	0	**None**	
	1	**Perisinusoidal or periportal**	
	1A	**Mild, zone 3, perisinusoidal**	"delicate" fibrosis

Fibrosis	**1B**	**Moderate, zone 3, perisinusoidal**	"dense" fibrosis
	1C	**Portal/periportal**	This category is included to accommodate cases with portal and/or peri portal fibrosis without accompanying pericellular/perisinusoidal fibrosis
	2	**Perisinusoidal and portal/periportal**	
	3	**Bridging fibrosis**	
	4	**Cirrhosis**	

Total NAS score represents the sum of scores for steatosis, lobular inflammation, and ballooning, and ranges from 0-8. Diagnosis of NASH (or, alternatively, fatty liver not diagnostic of NASH) should be made first, then NAS is used to grade activity. In the reference study, NAS scores of 0-2 occurred in cases largely considered not diagnostic of NASH, scores of 3-4 were evenly divided among those considered not diagnostic, borderline, or positive for NASH. Scores of 5-8 occurred in cases that were largely considered diagnostic of NASH

Reference: Kleiner D.E., Brunt E.M., Van Natta M., Behlinh C., Contos M.J., Cummings O.W., Ferrell L.D., Liu Y.-C., Torbenson M.S., Unalp-Arida A., Yeh M., McCullough A.J., Sanyal A.J. for the Nonalcoholic Steatohepatitis Clinical Research Network. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 41:1313-1321, 2005.

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Banff Schema for Grading Liver Allograft Rejection: An International Consensus Document

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A panel of recognized experts in liver transplantation pathology, hepatology, and surgery was convened for the purpose of developing a consensus document for the grading of acute liver allograft rejection that is scientifically correct, simple, and reproducible and clinically useful. Over a period of 6 months pertinent issues were discussed via electronic communication media and a consensus conference was held in Banff, Canada in the summer of 1995. Based on previously published data and the combined experience of the group, the panel agreed on a common nomenclature and a set of histopathological criteria for the grading of acute liver allograft rejection, and a preferred method of reporting. Adoption of this internationally accepted, common grading system by scientific journals will minimize the problems associated with the use of multiple different local systems. Modifications of this working document to incorporate chronic rejection are expected in the future. (HEPATOLOGY 1997;25:658-663.)

The success of hepatic transplantation has resulted in its widespread use for treatment of many patients with endstage liver disease; it is currently offered by more than 100 centers worldwide. One-year survival rates range from 70% to 90%; and long-term survival of 50% to 60% of patients is not uncommon.[1] Therefore, an increasing number of physicians, including pathologists, many of whom have no specific training in transplantation biology, will become involved in the care of organ allograft recipients.

Despite the good short-term and acceptable long-term survival after hepatic transplantation, the morbidity associated with long-term immunosuppression is significant and rejection remains a persistent, but usually manageable, problem. Clinical research to improve patient survival and lessen morbidity is, therefore, inherent to the clinical practice of hepatic transplantation. Because patient follow-up and successful application of developments could be simplified by a common scale of recognizing, naming, and grading the severity of acute liver allograft rejection, members of an international consensus panel recently agreed upon a common nomenclature and set of definitions.[2] The group next agreed to create an internationally acceptable grading system, which has already been developed for kidney,[3] heart,[4] and lung.[5] At the Third Banff Conference on Allograft Pathology, a group of specialists in liver transplantation from North America, Europe, and Asia met for this purpose.

DEFINITION OF ACUTE REJECTION

In general, organ allograft rejection can be defined as, "an immunological reaction to the presence of a foreign tissue or organ, which has the potential to result in graft dysfunction and failure."[2] This report is specifically concerned with acute rejection, recently defined by the international consensus document on terminology for hepatic allograft rejection[2] as, "inflammation of the allograft, elicited by a genetic disparity between the donor and recipient, primarily affecting interlobular bile ducts and vascular endothelia, including portal veins and hepatic venules and occasionally the hepatic artery and its branches."[2] Early rejection, cellular rejection, nonductopenic rejection, rejection without duct loss, and reversible rejection are synonyms for acute rejection that appear in the literature, but their use is discouraged. The general clinical, laboratory, and histopathological abnormalities listed below were derived from the international consensus document.[2]

CLINICAL AND LABORATORY FINDINGS

Viewed from a biological perspective, any recipient's immune system will likely be perturbed after transplantation, resulting in immune activation.[2] However, viewed from a clinical perspective, because of baseline immunosuppressive therapy only some recipients manifest clinical symptoms of allograft recognition with, in the case of liver transplantation, liver biochemical abnormalities (most often), or frank hepatic dysfunction.[2] Therefore, it is important to distinguish between "biological" and "clinically relevant" rejection. The latter may require additional immunosuppressive treatment, although the distinction is not always achievable and treatment philosophies differ at various centers. This is particularly true for hepatic allografts, which are widely acknowledged to be unique. They are more resistant than others to humoral rejection, and are accepted without immunosuppressive therapy in some small and large experimental animal species. Of potential importance for human transplantation is the observation that in all animals in which a liver allograft is eventually accepted without drugs, the allograft undergoes a transient acute rejection crisis.[6-9] Thus, it should be understood that the histopathological diagnosis of acute rejection may not automatically signal that treatment is indicated, particularly if it is low grade. Adoption of a standardized histopathological grading system possibly could help determine if, and at what point, the histopathological severity of rejection can predict the need for, and success of antirejection

Abbreviations: RFH, Royal Free Hospital; RAI, rejection activity index.

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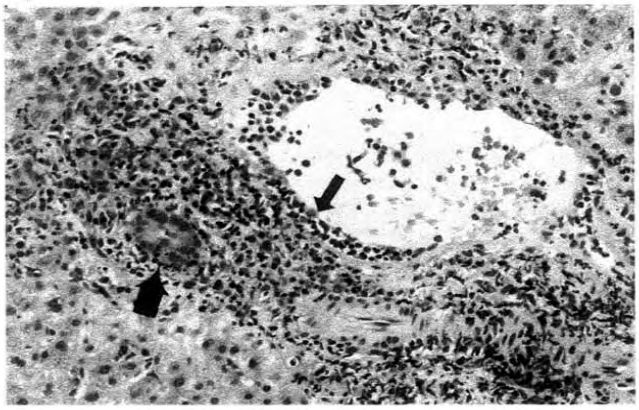

FIG. 1. Grading of acute rejection assumes the diagnosis has already been established: this portal tract shows all three of the typical histopathological features, two of which are required to make the diagnosis. There is: 1) a portal inflammatory infiltrate containing blastic lymphocytes and eosinophils; 2) subendothelial localization of the inflammatory cells in a portal vein branch (small arrow), and 3) inflammation and damage of small bile ducts (large arrow). If the subendothelial inflammation similar to this was present in most or all of the portal and/or hepatic venules, an RAI score of 2 for venous endothelial inflammation would be assigned.

therapy (see "Clinicopathological Correlation and Treatment of Acute Rejection").

When clinically apparent, acute rejection is usually first recognized between 5 and 30 days after transplantation. Earlier or later presentations can be seen in patients that receive less than therapeutic baseline immunosuppression. The clinical findings in early phases of mild acute rejection are often absent, although in late or severe cases, clinical findings include fever as well as swelling, cyanosis, and tenderness of the allograft. Bile often becomes pale in color and the flow is decreased. Occasionally, ascites develops because of liver swelling with increased intrahepatic pressure.[2]

Liver dysfunction, when present, usually manifests as concomitant nonselective elevations of the results of some or all of the standard liver injury tests, including total bilirubin, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, and alkaline phosphatase.[2] Leukocytosis and eosinophilia are also frequently present. Unfortunately, all clinical and laboratory findings lack sensitivity or specificity. The diagnosis is considered on clinical grounds and confirmed by examination of a core needle biopsy specimen. Some centers find that fine-needle aspirates of the allograft are useful adjunct.

HISTOPATHOLOGIC FINDINGS

Most investigators have observed similar histopathological findings associated with acute rejection.[10-22] Core needle biopsy specimens may show the following: 1) mixed but predominantly mononuclear portal inflammation, containing blastic (activated) lymphocytes, neutrophils, and frequently eosinophils; 2) bile duct inflammation/damage; and 3) subendothelial inflammation of portal veins or terminal hepatic venules.[2] At least two of these three features are required for a histopathological diagnosis of acute rejection (Fig. 1). Biochemical evidence of liver damage manifests as increased results of tests for liver injury, usually elevation of serum γ-glutamyl transpeptidase and alkaline phosphatase activities, are also frequently present. The diagnosis is strengthened if > 50% of the ducts are damaged or if unequivocal endotheliitis of portal vein branches or terminal hepatic venules can be identified. Occasional cases show mild mononuclear inflammation of the perivenular regions with only focal portal tract changes. Additional findings such as ductopenia, spill-over/piecemeal necrosis, eosinophilia, lobular inflammation, perivenular necrosis, arteritis, and inflammatory bridging, have been used in some systems for histopathological grading (see below).

Treatment of acute rejection with additional immunosuppression before a biopsy specimen is obtained may make the histopathological diagnosis more difficult, because of subsequent loss of the subendothelial infiltration of veins and of eosinophils, and a relative decrease in the number of mononuclear inflammatory cells.

GRADING OF ACUTE LIVER ALLOGRAFT REJECTION (CRITIQUE OF CURRENTLY POPULAR SYSTEMS)

The panel reviewed each system and agreed that the consensus scheme should fulfill the following criteria: scientific correctness, clinical relevance, simplicity, and reproducibility. They also recognized the need for flexibility and future modifications and therefore proposed a working formulation format for the current document.

The grading system used in Pittsburgh[23] is derived from those developed for kidney allograft.[24] It is based on the concept that serious injury from rejection is related to vascular compromise and ischemia, which can morphologically manifest as inflammatory or necrotizing arteritis and/or parenchymal necrosis and hemorrhage. The grading system developed in Minnesota by Snover et al.[19] is more specific to the liver and is based on a combination of an estimate of the severity of the inflammation and the presence and severity of damage or loss of key structures targeted for injury, such as the arterial vasculature or bile ducts. The above two systems have the advantage of simplicity[19, 23] and rely on pathophysiological concepts validated in renal transplantation. Prognostic significance has been shown at a single center.[19] Unfortunately, some of the features used in these schemes to define severe rejection are rarely found, poorly reproducible, or present so frequently in nonrejection complications that their usefulness in grading scheme is limited.[25] For example, while inflammatory or necrotizing arteritis[19, 23] represents a serious injury to the allograft, reproducibly identifying it in core needle biopsies is problematic.[25] In contrast, ballooning of perivenular hepatocytes[19] is frequently present in nonrejection graft syndromes and may not imply serious graft injury from an immunological insult. Bile duct loss, which has also been used to identify severe acute rejection more accurately reflects chronic rejection and possibly, a stage rather than a grade of rejection.

Kemnitz et al.[20,26] have devised a scheme similar to those mentioned above. However, increased emphasis is placed on precise numerical estimates of lobular injury, such as the percentage of necrosis, which may be difficult to reproduce and may not necessarily reflect rejection-related injury. Moreover, none of the systems was tested for reproducibility.

The European grading system for acute liver allograft rejection, developed by Hubscher and Dousset et al. at Birmingham[27-29], is based on a semiquantitative analysis of the diagnostic triad of Snover et al.[18] In this system, portal inflammation, bile duct damage, and venous endothelial inflammation are each graded semiquantitatively on a scale of 0 (absent) to 3 (severe). The individual scores are then added to produce an overall rejection score of 0 to 9, which is then converted to a rejection grade as follows: 0 to 2 = no rejection, 3 = borderline (consistent with), 4 to 5 = mild, 6 to 7 = moderate, and 8 to 9 = severe acute rejection. This system offers the attractive feature of quantifying the necro-inflammatory activity, as has recently become popular in the reporting and follow-up of patients with chronic hepatitis.[30-33] It also shows a good correlation between histological severity and clinical biochemical signs of graft dysfunction.[29] However, no obvious prognostic value has been shown.

The Royal Free Hospital, London (RFH) grading system[34] consists of a semiquantitative assessment of the diagnostic features of rejection, defined as immunosuppression responsive inflammation of rejection type, and identified by discriminant analysis. Mixed portal inflammation, eosinophils, endotheliitis, and bile duct damage were found to be independent, statistically significant contributors to the histological diagnosis of acute rejection. Each of the features are scored on a scale of 0 to 3, as in the European grading system, and a total score is derived by adding the individual scores together. Apart from the inclusion of eosinophils, which are of known diagnostic[35,36] and pathophysiological significance[37,38] as a separate variable in the RFH scheme, it is virtually identical to the European grading system. Like the European system, the RFH system offers a quantitative scale for the rejection-related activity, and is reproducible at the home institution.[34] However, neither the European system, nor the RFH system has been shown to have prognostic significance and the numerical cutoff points corresponding to the different degrees of rejection (and consequent therapeutic thresholds) need to be validated. In addition, there are no studies of inter-institutional scoring reproducibility.

The recently published scheme by the National Institute of Diabetes and Digestive Diseases and Kidney Diseases[39] had the advantages of being reproducible with prognostic significance documented at several centers. Unfortunately, the imprecise language used to explain the cutoffs for moderate and severe rejection makes the system difficult to follow, even for those experienced in the field.

INTERNATIONAL GRADING SYSTEM FOR ACUTE LIVER ALLOGRAFT REJECTION (RECOMMENDATIONS OF THE PANEL)

Grading of Rejection. The grading of rejection, as with hepatitis,[33] is a measure of the severity of the necro-inflammatory process. In addition, because rejection is more vasculocentric and vasculodestructive than hepatitis, some estimate of vascular or ischemic damage is needed to assess the full extent of the insult. This can be accomplished either by a global assessment of the biopsy using a "gestalt"[25] approach, or semiquantitatively with the assignment of numerical scores to different histopathological parameters. No data support one approach over the other, and in practice the two methods yield similar results (see below). Moreover, the semiquantitative approach could complement the global assessment by offering a greater degree of precision, by forcing the pathologist to critically evaluate important histopathological features. Conversely, the global approach can temper the semiquantitative analysis in cases with active inflammation and high scores, in which there is little architectural damage.

The panel agreed that existing grading systems for acute liver allograft rejection are conceptually similar, and that like chronic hepatitis, frequent monitoring and reporting of disease activity is an important function of biopsy analysis.[30-33] Therefore, in coming to a consensus, the panel drew upon the strengths, hopefully avoided the pitfalls, and corrected the weaknesses of the currently available grading systems. Portal inflammation, bile duct damage, subendothelial inflammation of portal veins, and terminal hepatic venules, strictly defined inflammatory or necrotizing arteritis and eosinophils (in the proper context) are features that the panel members regard as diagnostic of acute rejection. Portal inflammation, bile duct damage, strictly defined arteritis, and possibly confluent perivenular necrosis associated with perivenular inflammation are features that may also have prognostic significance, based on previous publication,[19,39] or personal experience. However, arteritis, as well as other findings such as bile duct loss, interstitial hemorrhage, and perivenular necrosis without inflammation are not included in the scheme, because they are poorly reproducible findings, considered to be part of chronic rejection, or also encountered frequently in nonrejection-related complications, respectively. If strictly defined arteritis can be shown to be a reproducible observation and present in more than a rare case, the current system can be modified to include it.

TABLE 1. Grading of Acute Liver Allograft Rejection

Global Assessment*	Criteria
Indeterminate	Portal inflammatory infiltrate that fails to meet the criteria for the diagnosis of acute rejection (see text)
Mild	Rejection infiltrate in a minority of the triads, that is generally mild, and confined within the portal spaces
Moderate	Rejection infiltrate, expanding most or all of the triads
Severe	As above for moderate, with spillover into periportal areas and moderate to severe perivenular inflammation that extends into the hepatic parenchyma and is associated with perivenular hepatocyte necrosis

NOTE. Global assessment of rejection grade mode on a review of the biopsy and after the diagnosis of rejection has been established.

* Verbal description of mild, moderate, or severe acute rejection could also be labeled as Grade I, II, and III, respectively.

Being aware of the need for acceptability and thus simplicity, the panel agreed on a verbal grading of acute rejection based on the overall appearance of the biopsy according to the criteria listed in Table 1 (Fig. 2). It should be re-emphasized however, that any grading of acute rejection already presupposes that the diagnosis has been established. For example, use of the "indeterminate" category of acute rejection should be restricted to cases that have minor degrees of cellular infiltration that could possibly represent low grade or early acute rejection, but fail to meet the minimal diagnostic criteria. "Indeterminate" should not be used for cases in which one is unsure whether the inflammation is related to some other condition, such as chronic hepatitis C (see Complicating Conditions). After the global assessment, three specific features, portal inflammation, bile duct inflammation/damage, and venular inflammation, can be more critically evaluated and semiquantitatively scored on a 0 to 3 (mild, moderate, and severe) scale, according to the criteria listed in Table 2. The three are then added together to arrive at a final Rejection Activity Index (RAI) (Table 2), similar to the scoring developed for chronic hepatitis.[30-33] Modifications of the above system [19, 22, 29, 39] were made to arrive at a consensus scheme, so that features given the highest scores on the semi-quantitative analysis were the same as those shown to be of prognostic significance using the overall approach.

Potential problems using this method however, include: 1) the global assessment of rejection may under or overestimate the severity based on a semi-quantitative analysis and 2) the greater degree of "precision" achieved semiquantitatively may occur at the expense of reproducibility. We think that these pitfalls are unlikely to occur because both processes measure the same parameters or endpoints. Moreover, evaluation of a series of 50 posttransplantation liver allograft biopsy specimens using both methods by one of us (AJD) showed no significant differences between the systems. The reproducibility of the semiquantitative analysis will be the subject of future study by this group. The RAI, like other semiquantitative assessments of necro-inflammatory activity, is particularly attractive when evaluating new drugs or other treatment protocols and for comparison with previous biopsy specimens. Thus, it will be most valuable at academic centers involved with new developments in the field. Although strongly recommended for routine patient care, it is not required for day-to-day use if the pathologist chooses otherwise.

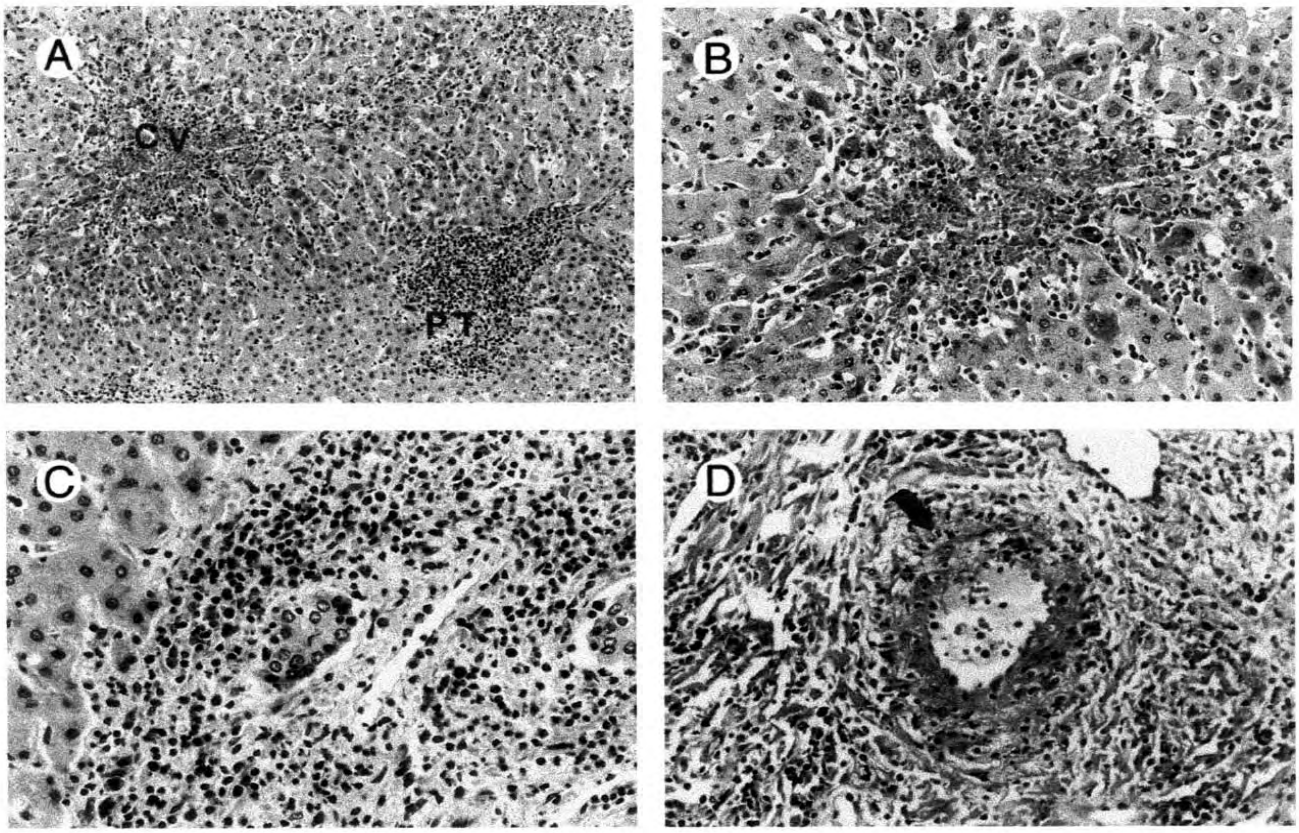

FIG. 2. (A) Low power photomicrograph of a failed liver allograft with severe acute rejection. Note the prominent portal tract (PT) and central vein (CV) inflammation, associated with confluent perivenular necrosis, which is shown at a higher magnification in (B). These findings would elicit a diagnosis of severe acute rejection. (C) In the same liver allograft, the bile duct inflammation and damage was widespread, and there was focal lumenal disruption, eliciting an RAI score of 3 for bile duct damage (Table 2). Both the portal and venous endothelial inflammation were also scored as severe, or "3," resulting in a total RAI score of 9/9. (D) Sections from the hilum of this failed allograft also revealed clear cut necrotizing arteritis (arrow), which is rarely detected with certainty in needle biopsies.

Staging of Rejection. Staging of a biological phenomenon is performed in an attempt to codify a process that is largely unidirectional and evolves in a predictable pattern over a relevant period of time. Acute liver allograft rejection is, for the most part, widely considered to be a completely reversible phenomenon. In the uncommon event of allograft failure from acute rejection, the evolution is relatively rapid. Therefore, acute rejection is not readily amenable to staging. Chronic rejection on the other hand, usually evolves more slowly and is often, but not always,[40,41] unidirectional or irreversible. At this time it is not clear whether acute and chronic rejection represent the ends of a spectrum of alloreactivity, or if they are completely different biological processes. Considerable data suggest the former, because both processes appear to be triggered by alloreactivity, and persistent or severe acute rejection can result in allograft failure from chronic rejection.

Clinicopathological Correlation and Treatment of Acute Rejection. As alluded to in the introductory sections, the histopathological diagnosis of acute rejection does not necessarily imply that the rejection is clinically significant or requires treatment with increased immunosuppression. In fact, Schlitt et al.[42] have shown that up to 40% of patients in whom a biopsy shows acute rejection, according to the criteria of Snover et al.[18,19] did not have clinically apparent graft malfunction or significant elevations of results of liver injury tests, and did not require additional immunosuppressive therapy. Similar conclusions were also reached in a study from Birmingham, in which 70% of histologically mild rejection episodes received no additional immunosuppression, without any adverse outcome.[28,29] A survey of the panel members showed no clear-cut consensus on the therapeutic approach to mild acute rejection (RAI $\leq$ 4) as defined in this report. In contrast, most centers report that patients with histopathological moderate or severe rejection (RAI $\geq$ 6) experience significant elevations of liver injury tests and the vast majority probably should, and usually are treated with additional immunosuppression. At present, no therapeutic recommendations can be inferred from the mild acute rejection grade, although some centers have exercised the option of routinely obtaining a follow-up biopsy after 1 to 2 weeks.

Complicating Conditions. Liver allografts are frequently affected by more than one condition. In the first few weeks after transplantation, preservation-related changes and mechanical problems with the vascular and/or biliary tree are the conditions that most commonly co-exist with acute rejection. Separation of the necro-inflammatory and ischemic damage of rejection from the same type of nonrejection insults is at times problematic, but achievable for the most part. For example, perivenular necrosis can occur in both preservation injury and severe rejection. However, the concomitant presence of mononuclear perivenular inflammation, portal changes of rejection, and absence of perivenular necrosis in a prior biopsy, are features that help to distinguish between the two. In contrast, more than several months after

TABLE 2. Rejection Activity Index

Category	Criteria	Score
Portal Inflammation	Mostly lymphocytic inflammation involving, but not noticeably expanding, a minority of the triads	1
	Expansion of most or all of the triads, by a mixed infiltrate containing lymphocytes with occasional blasts, neutrophils and eosinophils	2
	Marked expansion of most or all of the triads by a mixed infiltrate containing numerous blasts and eosinophils with inflammatory spillover into the periportal parenchyma	3
Bile Duct Inflammation Damage	A minority of the ducts are cuffed and infiltrated by inflammatory cells and show only mild reactive changes such as increased nuclear:cytoplasmic ratio of the epithelial cells	1
	Most or all of the ducts infiltrated by inflammatory cells. More than an occasional duct shows degenerative changes such as nuclear pleomorphism, disordered polarity and cytoplasmic vacuolization of the epithelium	2
	As above for 2, with most or all of the ducts showing degenerative changes or focal lumenal disruption	3
Venous Endothelial Inflammation	Subendothelial lymphocytic infiltration involving some, but not a majority of the portal and/or hepatic venules	1
	Subendothelial infiltration involving most or all of the portal and/or hepatic venules	2
	As above for 2, with moderate or severe perivenular inflammation that extends into the perivenular parenchyma and is associated with perivenular hepatocyte necrosis	3

NOTE. Total Score = Sum of Components. Criteria that can be used to score liver allograft biopsies with acute rejection, as defined by the World Gastroenterology Consensus Document.

transplantation, chronic viral hepatitis and recurrence of autoimmune chronic inflammatory disorders pose considerable difficulties in differential diagnosis and with grading or scoring of rejection related activity.

The problem of differentiating duct damage associated with complicating conditions such as viral hepatitis C from that seen in acute rejection can be minimized by applying strict diagnostic criteria: damage of more than an occasional bile duct, the presence of unequivocal endotheliitis, and absence of significant lobular disarray and necro-inflammatory activity favor a diagnosis of acute rejection. However, problematic cases will still be encountered, and implicit in any grading scheme for acute rejection (including this one), is the notion that grading can be reliably applied to biopsies only when rejection is thought to be the sole or predominant cause of graft damage. Therefore, in cases where other causes of cellular infiltration are suspected, neither the overall grade nor the scores can be reliably applied. In such cases, it is left to the judgment of the pathologist whether apportioning the necro-inflammatory activity to rejection or other concurrent conditions is appropriate.

CONCLUSIONS AND RECOMMENDATION

Although the adequacy of any particular biopsy is ultimately left to the judgment of the pathologist, the panel recommends that at least two hematoxylin and eosin stained sections from at least two different levels, of a core needle biopsy containing at least five triads be examined. The adequacy of the biopsy in the absence of any diagnostic findings when fewer than five portal tracts are identified, is again left to the pathologist's judgment.

The following format for the grading and reporting of acute liver allograft rejection is recommended, although all of this information is not needed in every case. The type of specimen and time after transplantation, if available, should be listed first. This is followed by the histopathological diagnosis(es). Although not necessary, some pathologists may prefer to list first the diagnosis perceived to be of greatest significance, followed by the second most important, and so forth. However, a comment on the presence or absence of acute rejection should be given for every biopsy, either in the diagnosis or comment section. This is followed by reporting of an RAI. The presence of chronic injury, such as bile duct loss or obliterative arteriopathy should also be listed. Lastly, a comparison with the most recent previous biopsy should be made if the pathologist feels that such a comparison is warranted. The following are several examples:

1. Liver allograft, needle biopsy (7 days posttransplantation)
   (a) Moderate preservation injury
   (b) No evidence of rejection (RAI = 0)
   (c) No previous biopsy for comparison
2. Liver allograft, needle biopsy (10 days posttransplantation)
   (a) Acute rejection, moderately active (RAI = 7)
   (b) Significantly worse than previous biopsy (S95-999 of 02/06/95{RAI = 2})
3. Liver allograft, needle biopsy (10 weeks posttransplantation)
   (a) Acute hepatitis, viral type C
   (b) No rejection (RAI = 0)
4. Liver allograft, needle biopsy (18 months posttransplantation)
   (a) Chronic hepatitis, viral type B, moderately active (HAI = 14)
   (b) Acute rejection, mildly active (RAI = 4)
   (c) Duct loss in 5/9 portal triads, suggestive of chronic rejection

We believe that this system will be easy to use and useful for physicians caring for allograft recipients. There already are data available to suggest that it will be both reproducible and have prognostic significance,[39] yet flexible enough to incorporate future development like the inclusion of chronic rejection or staging of rejection. We urge scientific journals to adopt this reporting system, classification, and grading of liver allograft rejection, to overcome the obstacles presented by the multiple schemes that currently exist and facilitate comparisons among different centers.

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SPECIAL ARTICLE

Liver Biopsy Interpretation for Causes of Late Liver Allograft Dysfunction

Banff Working Group[1]

Evaluation of needle biopsies and extensive clinicopathological correlation play an important role in the determination of liver allograft dysfunction occurring more than 1 year after transplantation. Interpretation of these biopsies can be quite difficult because of the high incidence of recurrent diseases that show histopathological, clinical, and serological features that overlap with each other and with rejection. Also, more than one insult can contribute to allograft injury. In an attempt to enable centers to compare and pool results, improve therapy, and better understand pathophysiological disease mechanisms, the Banff Working Group on Liver Allograft Pathology herein proposes a set of consensus criteria for the most common and problematic causes of late liver allograft dysfunction, including late-onset acute and chronic rejection, recurrent and new-onset viral and autoimmune hepatitis, biliary strictures, and recurrent primary biliary cirrhosis and primary sclerosing cholangitis. A discussion of differential diagnosis is also presented. (HEPATOLOGY 2006;44:489-501.)

Distinguishing among potential causes of late liver allograft dysfunction can be difficult because of overlapping clinical, serological, and histopathological features. Most problematic biopsies are obtained more than 1 year after transplantation. Currently, diagnoses are made using center-specific criteria, but a standardized set of criteria has not been generally agreed upon. Availability of standardized criteria[1,2] would enable centers to compare and pool results, improve therapy, and better understand pathophysiological disease mechanisms.

Native disease recurrence is a significant problem and can be categorized as follows: (1) infectious (viral hepatitis A, B, C, D.), (2) dysregulated immunity (autoimmune hepatitis [AIH], primary biliary cirrhosis [PBC], primary sclerosing cholangitis [PSC], and sarcoidosis),[3] (3) malignancies, (4) toxic (*e.g.*, alcohol, adverse drug reactions.), (5) metabolic disorders, including nonalcoholic steatohepatitis, and (6) other diseases, such as idiopathic granulomatous hepatitis,[4] postinfantile giant cell hepatitis,[5] and Budd-Chiari syndrome,[6] that are of uncertain etiology or multifactorial in origin. Recurrent infectious and dysregulated immunity diseases pose the most difficult diagnostic challenges and are addressed herein. Some diseases in the remaining categories can also recur, but because they do not usually present diagnostic challenges they are not discussed further.

Immunological Considerations

Immune recognition of differences in major histocompatibility complex antigens triggers a characteristically robust inflammatory response in the first few months after transplantation referred to as early acute rejection.[2] Like all other immune responses, acute and especially chronic rejection reactions[7,8] evolve over time and diversify via "epitope spreading."[9] Tissue damage during the initial phase releases cryptic antigens that activate endogenous danger signals. Recipient dendritic cell antigen uptake and self-reactive T and/or B lymphocyte priming[10] triggers "autoantibody" production and immunity directed against non–major histocompatibility complex determinants. Some non–major histocompatibility complex cytoplasmic, nuclear, and matrix protein antigens[11-14] (reviewed in Graft[15-18]) are shared by the donor and recipient, whereas others may be donor-specific.

Long-Term Protocol Biopsies

Most programs obtain biopsies when changes in liver tests represent a significant deviation from baseline values. Obtaining protocol allograft biopsies in asymptomatic

Abbreviations: AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis; HCV, hepatitis C virus; LAR, late-onset acute rejection.

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long-term survivors with normal or near-normal liver tests is controversial. Considerations such as potential morbidity and mortality, cost, inconvenience, use of resources, and potential impact of unexplained histopathological findings should be weighed against potential individual and/or societal benefits.[4,19-24] These include (1) early detection of clinically inapparent disease,[19,24] (2) recognition of nonalcoholic steatohepatitis as a significant cause of cryptogenic cirrhosis in the United States[25] but not in England,[26] (3) identification of recipients that might be successfully weaned from immunosuppression,[27] (4) recognition of late-onset rapid hepatitis C virus (HCV) progression,[21] and (5) impact of alcohol use.[20]

Approximately 75% of biopsies from long-surviving recipients with abnormal liver tests or symptoms show significant histopathological abnormalities.[4,19-23] These abnormalities are usually attributable to recurrent disease or biliary tract strictures, some of which occur as a late complication of preservation injury.[4,19-23] The incidence and significance of histopathological abnormalities in long-surviving recipients without abnormal liver tests or symptoms is dependent on the original disease: up to 25% show significant abnormalities when obtained from recipients with original diseases that commonly recur (*e.g.*, HCV, PBC, AIH).[4,19-23]

Even in the absence of recurrent disease, minor histopathological abnormalities appear in approximately two thirds of biopsies obtained from long-surviving asymptomatic recipients with normal liver tests.[4,19-23] These include nodular regenerative hyperplasia changes and thickening/hyalinization of small hepatic artery branches[4,28] (probably side effects of immunosuppression) and "nonspecific" portal and lobular inflammation.[4,22-24] The pathogenesis, significance, and long-term consequences of nonspecific inflammation (*e.g.*, idiopathic posttransplantation hepatitis), portal venopathy, and nodular regenerative hyperplasia are in need of further study.

Recurrent HCV disease progression is significantly more rapid than HCV in native livers. Disease progression rates for recurrent hepatitis B virus, PBC, PSC, nonalcoholic steatohepatitis, and AIH are difficult to study because of the small number of long-term survivors with biopsies and chronic immunosuppression, as well as introduction of new medical therapies. Regardless, nearly all recurrent diseases can potentially cause allograft cirrhosis.

Practical Problems and Approach to Biopsy Interpretation

Most late causes of liver allograft injury are first detected because of abnormalities in routinely monitored liver tests; clinical signs and symptoms are much less common. When signs or symptoms do occur, they are similar to those seen in the general population with the same causes of liver injury. Examples include fever and upper right quadrant pain in ascending cholangitis; fatigue, nausea, vomiting, and jaundice in viral hepatitis; relapsing bacteria in hepatic infarcts, *etc.*

Many late posttransplantation biopsies show portal-based chronic inflammation with variable interface activity. Subtle histopathological differences relied upon to distinguish among several possible specific causes of dysfunction are not always present or reliable. Occasionally, rendering a definitive diagnosis may not be possible in the early stages of a disorder. A caveat of "features suggestive of early" emphasizes a tentative diagnosis.

Laboratory tests used to establish a diagnosis before transplantation may not have the same significance after transplantation. Antimitochondrial antibodies and antinuclear antibodies often persist after transplantation in patients with PBC or AIH, albeit at lower titers, even without histopathological evidence of recurrent disease. Patients without AIH before transplantation can develop autoantibodies either as a complication of otherwise typical rejection[11,12,29] or in association with new-onset AIH.[30-36] "Non–organ-specific" autoantibodies have been detected in up to 71% of patients after liver transplantation,[37] emphasizing the need for clinicopathological correlation.

More than 1 insult can contribute to late posttransplantation dysfunction. Biopsy analysis can help to determine the main component of injury, but careful clinicopathological correlation is needed. Levels of immunosuppression can influence biopsy findings and the severity of recurrent viral hepatitis, AIH, and rejection. For example, late-onset acute rejection (LAR) is often precipitated by inadequate immunosuppression and recipients with AIH and other autoimmune disorders usually require more immunosuppression to prevent rejection and disease recurrence. Too much immunosuppression can trigger cholestatic HCV hepatitis. Lymphoid depletion followed by rapid withdrawal of immunosuppression can precipitate aggressive HCV recurrence.[38]

Biopsy interpretation should include an assessment of adequacy, systematic examination, and thorough clinicopathological correlation. Adequacy is ultimately the subjective opinion of the pathologist, but in general, at least 6 small portal tracts should be sampled. The findings should then be correlated with the original disease, immunosuppression, liver tests, viral serology, and immunology and radiology findings.

Generalized Criteria

Criteria used to distinguish rejection from AIH can be melded into generalized criteria applicable to other causes

Table 1. Incidence, Risk Factors, and Clinical Observations

Diagnosis	Incidence at 5 Years of Recurrent Disease	Risk Factors for Disease Recurrence and/or Severe Recurrent Disease	Clinical/Immunological/Radiological Observations
Recurrent AIH	~30%	Suboptimal immunosuppression; type I > type II disease; severe inflammation in native liver before transplantation; longer duration of follow-up HLA DR3 or DR4 recipient status may reflect more severe disease	Usually need higher baseline immunosuprression (see text) HLA DR3 and/or DR4 genotype often present
De novo AIH	<5%	May be more common in children, but this assumption has been questioned recently	Same as above
Recurrent HBV	100% if HBV DNA is positive; less frequent if HBV DNA is negative	Anti-HBc–positive donor Inadequate anti-HBV treatment HBV mutants	Recurrent HBV disease not usually a significant problem because of treatment with effective antiviral drugs
Recurrent HCV	Nearly universal in those with HCV replication before transplantation	HCV RNA in blood helpful in differential diagnosis (>30,000,000 IU/L); increased risk of cholestatic hepatitis Significant acute or chronic rejection usually occurs only in association with relatively low HCV RNA levels (<5,000,000 IU/L)	Greater viral burden and more rapid progression of fibrosis than in general population Severity of hepatitis often worse with genotype 1 viruses Variable disease progression Subset of recipients with late-onset rapid progression
Recurrent PBC	20%-30%; increases with time	Tacrolimus as baseline immunosuppression; living-related donor; steroid and other immunosuppression withdrawal May recur as AIH	Initial diagnosis often made via biopsy in asymptomatic recipient with or without increased liver tests
Recurrent PSC	20%-30%; increases with time	Male sex; donor–recipient sex mismatch Intact colon at time of transplantation Patients at increased risk of rejection	Cholangiographically confirmed biliary strictures occurring >90 days after liver transplantation Mural irregularity, diverticulum-like outpouchings, and an overall appearance resembling PSC Patient and allograft survival not adversely affected up to 5 years; later outcome uncertain
Acute rejection	Variable; <30% of causes of late dysfunction	Inadequate immunosuppression Treatment with immune-activating drugs (*e.g.*, interferon) History of autoimmune liver disease	Much less common than early after transplantation May be more difficult to treat, perhaps related to delay in diagnosis.
Chronic rejection	~3 %	Inadequate immunosuppression Treatment with immune-activating drugs (*e.g.*, interferon) Refractory acute rejection Chronic rejection in a previous failed allograft	Important cause of late dysfunction Most cases occur within first year Does not appear to increase with time after transplantation, but more follow-up is needed.
Idiopathic posttransplantation hepatitis	5%-60%; wide variation		5%-15% of patients followed for a minimum of 10 years will develop progressive fibrosis resulting in established cirrhosis Incidence varies widely among centers

Abbreviations: AIH, autoimmune hepatitis; HBV, hepatitis C virus; HCV, hepatitis C virus; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

of late liver allograft dysfunction,[39-56] including: (1) histopathological evidence of liver injury showing a pattern compatible with the diagnosis (liver tests are usually elevated in a pattern consistent with the diagnosis); (2) positive serological, molecular biological, immunological, or radiographic evidence of pathogen or possible cause of injury; and (3) other causes of similar histopathological changes and elevated liver tests, if present, have been reasonably excluded.

Table 1 shows approximate incidences, risk factors, and clinical, immunological, and radiological observations for common causes of late dysfunction. Specific diagnoses can be rendered when these observations are combined with histopathological findings (Table 2), tim-

Table 2. Histopathologic Features Most Commonly Detected With Various Causes of Late Liver Allograft Dysfunction

Histopathological Features	Autoimmune Hepatitis*	Acute Rejection	Chronic Rejection	Chronic Viral Hepatitis Types B and C	Primary Biliary Cirrhosis	PSC/BD Strictures
Distribution, severity, and composition of portal inflammation	Usually diffuse; predominantly mononuclear of varying intensity; often prominent plasma cell component	Usually diffuse; variable intensity; mixed "rejection-type" (see text) infiltrate	Patchy; usually minimal or mild lymphoplasmacytic	Patchy; variable intensity; predominantly mononuclear; nodular aggregates	Noticeably patchy and variable intensity; predominantly mononuclear; nodular aggregates and granulomas	Usually patchy to diffuse depending on stage; mild neutrophilic, eosinophilic, or occasionally mononuclear predominant
Presence and type of interface activity	Prominent and defining feature is usually necroinflammatory-type; often plasma cell–rich	Focally present and mild necroinflammatory type	Minimal to absent	Variable; usually not prominent: necroinflammatory- and ductular-type	Important feature later in disease development: ductular and necroinflammatory-type with copper deposition	Prominent and defining feature: ductular-type with portal and periportal edema
Bile duct inflammation and damage	Variable; if present, involves a minority of bile ducts	Present and usually involves a majority of bile ducts	Focal ongoing lymphocytic bile duct damage; inflammation wanes with duct loss	Variable; if present, involves a minority of bile ducts	Granulomatous or focally severe lymphocytic cholangitis is diagnostic in proper setting	Periductal lamellar edema; "fibrous cholangitis"; acute cholangitis; multiple intra-portal ductal profiles
Biliary epithelial senescence changes and small bile loss	Absent or involves only a minority of ducts/portal tracts, but may be focally severe	Absent or involves only a minority of ducts	Senescence/atrophy/atypia involve a majority of remaining ducts (see text)	Absent or involves only a minority of ducts	Small bile duct loss associated with ductular reaction	Small bile duct loss associated with ductular reaction
Perivenular mononuclear inflammation and/or hepatocyte dropout	Variable; can involve a majority of perivenular regions, similar to rejection (see text); may be plasma cell–rich.	Variable, if defining feature should involve a majority of perivenular regions; may also show subendothelial inflammation of vein (see text)	Usually present, but variable	Variable but generally mild; if present, involves a minority of perivenular regions	Variable but generally mild; if present, involves a minority of perivenular regions	Absent
Lobular findings and necroinflammatory activity	Variable severity; rosettes may be present and/or prominent	Variable; if present, concentrated in perivenular regions	Variable; if present, concentrated in perivenular regions	Disarray variable; variable severity; necroinflammatory activity	Mild disarray; parenchymal granulomas; periportal copper deposition and cholatestasis are late features	Disarray unusual; neutrophil clusters; ± cholestasis
Pattern of fibrosis during progression toward cirrhosis	Usually macronodular; posthepatitic pattern	Rare	Uncommon, if present usually a venocentric pattern; may evolve to biliary pattern over time	Usually macronodular, hepatitic pattern; may be micronodular (see text)	Biliary pattern	Biliary pattern

NOTE. The histopathological findings in this table should be combined with clinical, serological, radiographic, and important exclusionary criteria listed in Table 2 to arrive at a final diagnosis.
Abbreviation: PSC/BD, primary sclerosing cholangitis/bile duct.
*The same findings apply to recurrent and *de novo* autoimmune hepatitis.

ing and pattern of liver test elevations, and important exclusionary criteria (Table 3). A discussion of histological findings in late posttransplant biopsies and their differential diagnosis follows.

Late-Onset Acute Rejection. LAR, which occurs more than several months after transplantation, may show slightly different features than typical acute rejection seen early after transplantation (Fig. 1). Fewer blastic lymphocytes, slightly greater interface activity, less venous subendothelial inflammation, and slightly more lobular activity cause biopsies with LAR to resemble chronic hepatitis.[4,57] LAR can also present as isolated perivenular inflammation and hepatocyte dropout (so-called "central perivenulitis")[58-60] and evolve into typical chronic rejection with ductopenia.[61] Subendothelial inflammation of portal or central veins is not a required finding in such cases. LAR, however, is still most commonly characterized by: (1) predominantly mononuclear portal inflammation containing lymphocytes, neutrophils, and eosinophils; (2) venous subendothelial inflammation of portal or central veins or perivenular inflammation; and (3) inflammatory bile duct damage. Previously proposed criteria[2] should be used for grading unless LAR presents as isolated central perivenulitis. For these cases, the following descriptors are recommended:

- minimal/indeterminate: perivenular inflammation involving a minority of central veins with patchy perivenular hepatocyte loss without confluent perivenular necrosis

Table 3. Inclusionary and Exclusionary Criteria for the Diagnosis of Recurrent and New-Onset Chronic Necroinflammatory Diseases After Liver Transplantation and Timing of First Onset and Pattern of Liver Test Elevation

Diagnosis	Original Disease	Serology/Molecular Testing*	Timing and Liver Injury Test Profile†	Important Exclusionary Criteria
Recurrent AIH	AIH	Autoantibodies (ANA, ASMA, ALKM) usually in high titers (>1:160); elevated serum immunoglobulin G	>6 months hepatocellular	Acute and chronic rejection, HBV, HCV infection, as determined via third-generation ELISA and/or serum PCR
De novo AIH	Other than AIH	Same as above	>6 months hepatocellular	Same as above
Recurrent HBV or HCV	HBV- or HCV-induced cirrhosis	HBV or HCV infection using standard, third-generation serological criteria and/or positive molecular testing for HBV or HCV nucleic acids	Usually 6-8 weeks, but as early as 10 days Usually hepatocellular but may be cholestatic	Acute and chronic rejection AIH
Recurrent PBC	PBC	Positive AMA, but little additional benefit because AMA remains elevated in the majority of patients after transplantation	>1 yr Cholestatic	Biliary tract obstruction/strictures
Recurrent PSC	PSC	NA	Usually >1 yr Cholestatic	HA thrombosis/stenosis, chronic (ductopenic) rejection, abnormal surgical anatomy, anastomotic strictures alone, nonanastomotic strictures occurring <90 d after liver transplantation, and ABO incompatibility
Acute rejection	NA (see text for risk factors)	NA	Any time Usually hepatocellular; may be mixed if superimposed on chronic rejection	Inadequate immunosuppression usually, but not always present (see text) Important exclusions: biliary tract obstruction/strictures, HBV, HCV, AIH
Chronic rejection	NA (see text for risk factors)	NA	Any time, but usually <1 yr Cholestatic; rarely hepatocellular in veno-occlusive variant (see text)	Inadequate immunosuppression usually, but not always present (see text) Important exclusions: biliary tract obstruction/strictures, HBV, HCV, AIH
Idiopathic posttransplantation hepatitis	Nonviral and non-autoimmune hepatitis	Negative testing for HBV and HCV infection and autoantibodies	>1 yr Usually hepatocellular	Acute and chronic rejection, all other causes of chronic hepatitis, and biliary tract obstruction/strictures reasonably excluded; all attempts should be made to determine a cause

NOTE. See Table 1 for compatible histopathological findings.

Abbreviations: AIH, autoimmune hepatitis; ANA, antinuclear antibodies; ASMA, anti–smooth muscle antibodies; ALKM, anti-liver–kidney microsomal antibodies; HBV, hepatitis B virus; HCV, hepatitis C virus; PCR, polymerase chain reaction; PBC, primary biliary cirrhosis; PSC, primary sclerosing cholangitis.

*Timing = usual timing of first onset.

†Sustained elevation for more than 1 month. Hepatocellular = alanine aminotransferase and/or aspartate aminotransferase > alkaline phosphatase and/or γ-glutamyltranspeptidase. Cholestatic = alkaline phosphatase and/or γ-glutamyltranspeptidase > aspartate aminotransferase and/or alanine aminotransferase.

- mild: as above, but involving a majority of central veins
- moderate: as above, with at least focal confluent perivenular hepatocyte dropout and mild moderate inflammation, but without bridging necrosis
- severe: as above, with confluent perivenular hepatocyte dropout and inflammation involving a majority of hepatic venules with central-to-central bridging necrosis.

"Minimal" and "mild" cases, as described above, may resolve spontaneously.[60] More severe perivenular changes probably warrant more aggressive treatment, but studies of long-term outcome according to therapy are needed to validate such an approach.

Chronic Rejection. Portal tracts and perivenular regions are primarily affected in chronic rejection, and changes are divided into "early" and "late" stages.[1] In a biopsy specimen, the minimum diagnostic criteria are: (1) biliary epithelial senescence changes affecting a majority of the bile ducts with or without bile duct loss; or (2) foam cell obliterative arteriopathy; or (3) bile duct loss affecting >50% of the portal tracts.[1]

Biliary epithelial senescence changes include cell and nuclear enlargement, multinucleation, uneven nuclear spacing, and cytoplasmic eosinophilia.[62] Some small bile ducts may be only partially lined by biliary epithelial cells. Perivenular hepatocyte dropout and central perivenulitis are typical of early chronic rejection.[63] Variable perivenular fibrosis occasionally progressing to veno-centric cir-

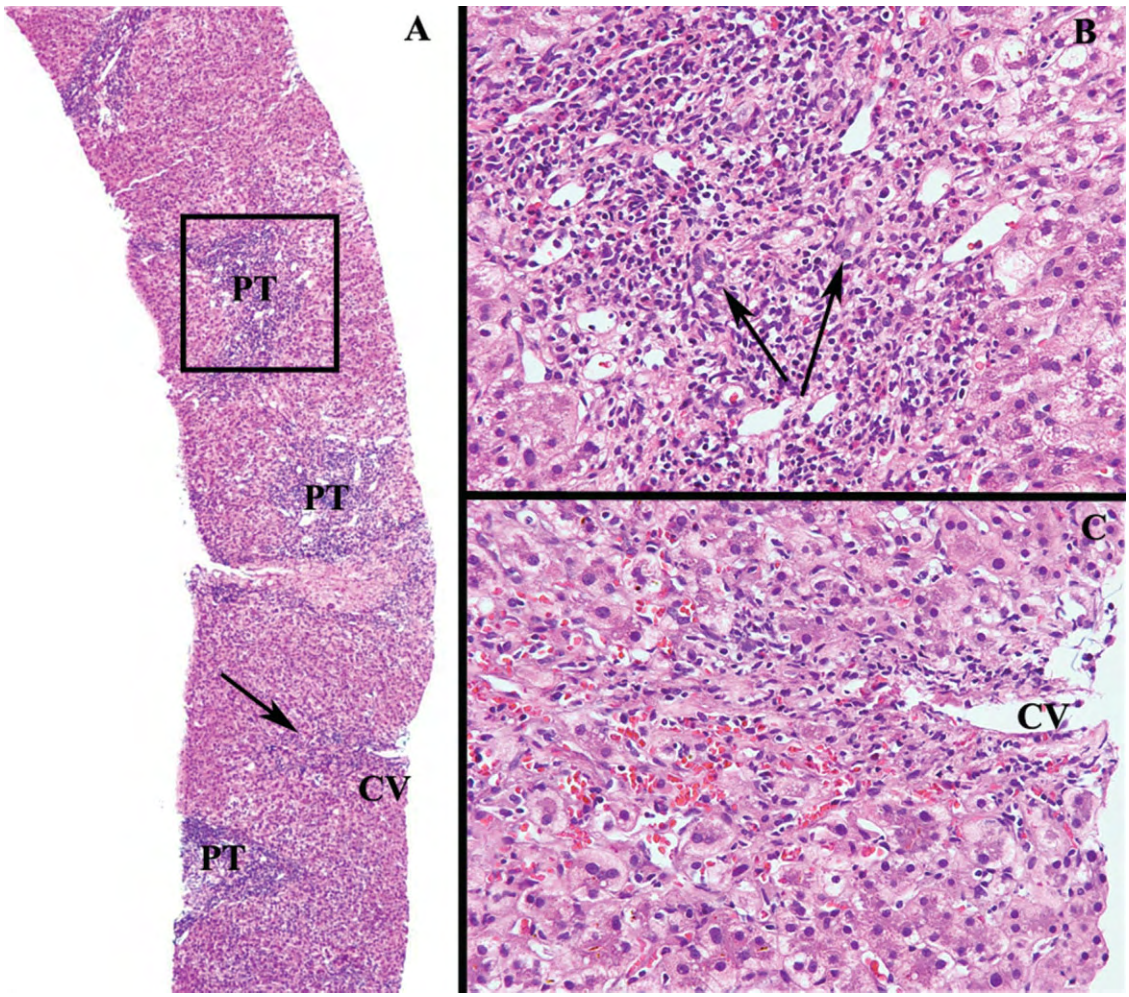

Fig. 1. Composite of late-onset acute rejection occurring more than 1 year after transplantation in a patient with low levels of baseline immunosuppression. (A) Low-magnification view (×20) shows prominent portal inflammation distributed evenly throughout the portal tracts, as well as perivenular mononuclear inflammation. Note also the irregular interface zone around the inflamed portal tracts. (B) Higher magnification (×200) of the portal tract outlined by the rectangle in panel A better illustrates the irregular interface zone and fewer blastic lymphocytes, which causes the biopsy to resemble chronic hepatitis. However, the prevalence and severity of lymphocytic cholangitis (**arrows**) are much worse than would be expected in chronic hepatitis and point toward acute rejection as the correct diagnosis. (C) Higher magnification (×200) of the central vein designated by the arrow in panel A better illustrates the perivenular mononuclear inflammation, or "central perivenulitis." Abbreviations: PT, portal tract; CV, central vein.

rhosis is typical of late chronic rejection.[64] Chronic rejection rarely results in a "posthepatitic" pattern of cirrhosis. If this pattern is present, other insults should be reasonably excluded.

The safest approach to a chronic rejection diagnosis in any setting is to review prior biopsies and correlate the histopathological findings closely with the clinical course. The typical scenario usually includes persistent/unresponsive acute rejection and/or inadequate immunosuppression.

Recurrent Diseases and New-Onset Diseases

Hepatitis C Virus. The predominant features of HCV include mononuclear portal inflammation, often arranged into nodular aggregates, necroinflammatory and ductular-type interface activity, and mild macrovesicular steatosis. Except for an association between steatosis and HCV genotype 3,[65] no histopathological features reliably distinguish among different viral genotypes. Lymphocytic cholangitis, if present, involves a minority of bile ducts without ductopenia. Lobular disarray and necroinflammatory activity are usually mild. Confluent or bridging necrosis with recurrent HCV alone is unusual. Central perivenulitis, if present, involves a minority of central veins.

There are two histopathological patterns of severe chronic HCV: (1) aggressive conventional hepatitis with prominent interface activity and (2) fibrosing cholestatic hepatitis. Features of fibrosing cholestatic hepatitis in-

clude centrilobular hepatocyte swelling and degeneration; cholestasis, hepatocyte apoptosis, and portal expansion because of a ductular reaction; fibrosis; and a mild mixed portal inflammation.[66] Fibrosing cholestatic hepatitis is associated with massive HCV replication (*e.g.*, >40-50 million IU/mL[67,68]).

Recurrent and New-Onset or* De Novo *Autoimmune Hepatitis. AIH is difficult to distinguish, histologically and conceptually, from rejection. Immune responses against self-antigens constitute an autoimmune response, whereas those against foreign antigens constitute rejection. Donor livers undoubtedly contain non–major histocompatibility complex antigens not expressed in the native liver, and theoretically all forms of AIH after transplantation could be classified as rejection.[34,42] Serological and histological findings used to distinguish AIH from rejection may reflect the nature, density, and location of antigenic targets. There are no conventional clinical tests that differentiate an autoimmune response from rejection, and distinctions based on clinical and histopathological findings may not reflect the true pathogenesis. Some new-onset AIH cases might be attributable to polymorphic expression of glutathione S-transferase T1[69]; transplantation of a mismatched graft into a nonexpressing recipient could trigger rejection that closely resembles AIH.

The International Autoimmune Hepatitis Group[70] scoring system and criteria for the diagnosis of AIH in native livers have not been tested in allografts; however, they do provide useful guidelines. AIH is established through a combination of serological, molecular biological, and histopathological findings. Non–organ-specific autoantibodies, a requisite for diagnosis, typically include anti–smooth muscle antibodies and antinuclear antibodies, as well as antibodies to liver kidney microsome type 1.[71] Their occurrence implies activation of immune mechanisms possibly involved primarily in disease pathogenesis or collateral responses to liver cell destruction and nonselective antigen release. Autoantibodies after liver transplantation do not establish the diagnosis of AIH, nor are they accurate parameters of inflammatory activity. Their principal value is to direct attention to the possibility of AIH.

The minimum diagnostic criteria for recurrent or *de novo* AIH in an allograft are: (1) interface hepatitis with portal lymphocytic infiltrates; (2) significant titers (≥1:160) of antinuclear antibodies, smooth muscle antibodies, or antibodies to liver kidney microsome type 1; (3) hyper-gammaglobulinemia; and (4) exclusion of virus-induced or drug-related hepatitis and late acute or chronic rejection. Titers ≥1:160 are unlikely to be nonspecific background reactivities and therefore compel a thorough evaluation for AIH.[70]

Initial manifestations include lobular hepatitis with hepatocyte rosetting[40] that usually evolves into the chronic phase characterized by lymphoplasmacytic portal inflammation with prominent interface activity. Plasmacytic infiltrates characterize AIH, but are not diagnostic requisites. Confluent and bridging necrosis are not uncommon, particularly in patients on suboptimal immunosuppression. Lymphocytic cholangitis, if present, involves a minority of ducts.

Central perivenulitis can occur in acute onset AIH in native livers[72-74] and in otherwise typical LAR. In native livers, perivenular hepatocyte injury associated with AIH usually wanes as interface hepatitis appears,[75] but the evolution of changes has not been studied in allografts. Panacinar hepatitis is also within the spectrum of histological findings in AIH,[70] but a cholestatic form is not recognized.

Idiopathic Posttransplantation Hepatitis. Idiopathic posttransplantation hepatitis is defined as chronic hepatitis that cannot be ascribed to a particular cause. By definition, bile duct damage and venous endothelial inflammation are not conspicuous. In adults, the prevalence is difficult to determine, because most native diseases have the potential to recur with features of chronic hepatitis. In some centers, up to 40% of adult patients subjected to biopsy more than 12 months after transplantation have unexplained chronic hepatitis.[76] A similar prevalence has been observed in the pediatric population, in which recurrent native disease is less of a problem; the frequency of "idiopathic" chronic hepatitis was 20% at 1 year of age, rising to 60% at 10 years of age.[77]

Cases presenting as central perivenulitis probably represent centrilobular-based acute rejection, or AIH if autoantibodies are also present,[57] because allograft dysfunction usually responds to increased immunosuppression.[59-61,78] Some idiopathic posttransplantation hepatitis cases may represent rejection with chronic hepatitic features.[79] However, a diagnosis of idiopathic posttransplantation hepatitis does not usually trigger treatment with increased immunosuppression. In some series, as many as 50% of such cases may develop bridging fibrosis or cirrhosis over a period of 10 years.[77] This observation supports the need for protocol biopsies and clarification of management policies in those with significant activity.[77]

Primary Biliary Cirrhosis. Recurrent PBC findings are nearly identical to those seen in native livers.[80,81] The pathognomonic lesion is noninfectious granulomatous cholangitis in the proper setting, which includes presence of antimitochondrial antibodies and absence of other causes such as infections and biliary strictures. Diagnostic

lesions are not always present. Patchy but easily recognizable and severe lymphocytic cholangitis accompanied by biliary epithelial cell eosinophilia, portal lymphoid nodules containing germinal centers, and development of a "biliary gestalt" can also be diagnostic of recurrent PBC in the proper setting. The biliary gestalt includes a ductular reaction at the interface zone combined with portal and periportal fibrosis, small bile duct loss, periportal edema (halo sign), and lysosomal pigment and copper/protein deposition in periportal hepatocytes. Plasma cell–rich periportal hepatitis may be an early marker predictive of later PBC recurrence.[82] Nonspecific lobular findings include mild spotty hepatocyte apoptosis, slight sinusoidal lymphocytosis, mild nodular regenerative hyperplasia, and Kupffer cell granulomas.

Primary Sclerosing Cholangitis. Findings are identical to those described for native livers with PSC and to other causes of biliary strictures. Subtle histopathological clues that suggest low-grade biliary strictures include mild portal edema; mild nonspecific acute and chronic "pericholangitis" often accompanied by a very mild type I ductular reaction; sinusoidal clusters of neutrophils; and centrilobular hepatocanalicular cholestasis. More significant strictures usually cause lamellar periductal edema, increased portal tract ductal profiles, and/or concentric periductal fibrosis.[83] Later-stage findings include the biliary gestalt. "Fibro-obliterative duct lesions" are not diagnostic of recurrent PSC, because they can also develop in patients with ischemic cholangitis and reflux cholangiopathy.

Differential Diagnosis

Rejection Versus Chronic Hepatitis. This commonly encountered and difficult problem has important therapeutic implications.[67] Unnecessary augmentation of immunosuppression can accelerate fibrogenesis in chronic HCV or trigger cholestatic hepatitis. Untreated acute rejection can progress to chronic rejection, particularly in interferon-treated recipients.

Mononuclear portal inflammation and lymphocytic cholangitis are features of chronic hepatitis and most cases of LAR. In LAR, however, the portal infiltrate tends to be more diffusely distributed throughout the portal tracts and throughout the biopsy rather than aggregated into nodules in occasional portal tracts, as in chronic hepatitis. In LAR and chronic rejection, lymphocytic cholangitis and/or biliary epithelial senescence changes, respectively, should involve a majority of bile ducts.[67] Central perivenulitis involving a majority of central veins also favors rejection. Damage limited to a minority of bile ducts favors acute or chronic hepatitis. Key features of acute and chronic hepatitis are lobular necroinflammatory activity and necroinflammatory and ductular-type interface zone activity, respectively, which are more prevalent and severe than in acute rejection.

Because acute and/or chronic rejection and chronic hepatitis can coexist, the predominant process should be identified. Key features of acute rejection in the context of recurrent HCV are prevalence and severity of mononuclear inflammatory bile duct damage and central perivenulitis. If either feature involves a majority of bile ducts or central veins, then acute rejection is present. However, coexistent acute rejection should be listed as the primary process only when rejection-related changes are obvious. Most such cases are graded as "moderate" according to the Banff schema.[67] Chronic rejection in the context of recurrent HCV is recognized by the same features as in allografts without recurrent HCV: small bile duct loss or biliary epithelial senescence or perivenular inflammation and fibrosis involving a majority of bile ducts or hepatic venules, respectively.

Chronic Rejection. Small bile duct damage and loss and perivenular fibrosis are relied upon for the diagnosis of chronic rejection because arteries with pathognomonic changes are rarely present in needle biopsy specimens.[1] Bile duct injury and ductopenia, however, can also be caused by biliary strictures, hepatic artery pathology, adverse drug reactions, and cytomegalovirus. Isolated ductopenia involving less than 50% of portal tracts can be seen occasionally without significant elevations of liver tests.[80] Whether these uncommon cases are an early phase or subclinical chronic rejection is uncertain. Angiography showing pruning of intrahepatic arteries with poor peripheral filling and segmental narrowing also supports a chronic rejection diagnosis.[84,85]

Perivenular fibrosis can also be caused by suboptimal hepatic venous drainage, adverse drug reactions,[86] and the various causes of veno-occlusive disease and Budd-Chiari syndrome in native livers.[87] In cases of chronic rejection identified by biliary epithelial senescence, bile duct loss, or perivenular fibrosis alone, non–rejection-related causes of ductal injury and loss or perivenular fibrosis should be reasonably excluded, particularly if the clinical scenario is not typical (Table 1).

Biliary Strictures Versus Acute and Chronic Rejection. Significant biliary strictures are usually recognized by the biliary gestalt and are reinforced by preferential elevation of γ-glutamyltranspeptidase and alkaline phosphatase. However, a thorough clinicopathological correlation is needed to distinguish among many underlying causes, such as recurrent PSC, ischemic cholangitis due to injury from prolonged preservation or non–heart-beating donors, imperfect biliary anastomoses, inadequate arterial

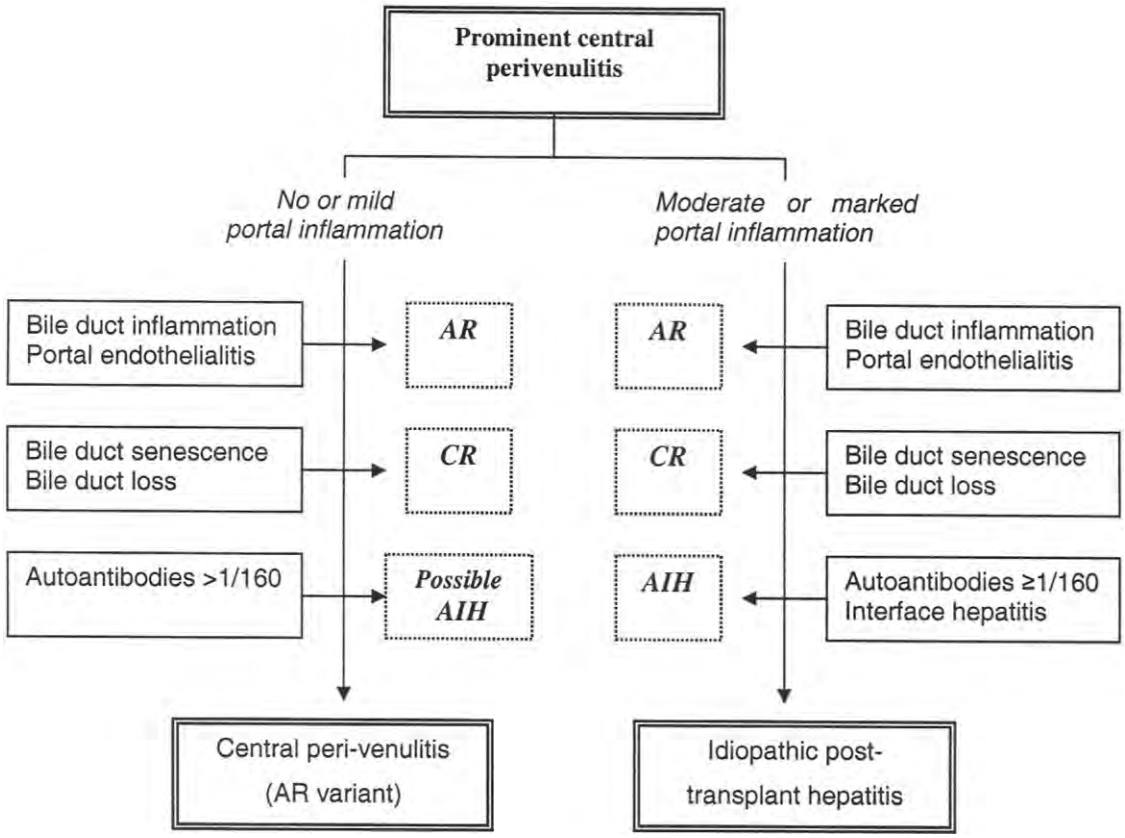

Fig. 2. Approach to biopsies showing posttransplantation central perivenulitis. In cases with no or minor portal inflammation, the differential diagnosis includes acute rejection, chronic rejection, and prediagnostic autoimmune hepatitis. If none of these changes is present, and vascular imaging is normal, the lesion is likely to represent a form of acute rejection. Cases with a more extensive portal inflammatory infiltrate have a similar differential diagnosis. It remains unclear whether idiopathic posttransplantation hepatitis is a form of rejection, and how it is related to pure central perivenulitis. Follow-up biopsies also frequently provide important diagnostic findings. Abbreviations: AR, acute rejection; CR, chronic rejection; AIH, autoimmune hepatitis.

flow, and antibody-mediated rejection.[4,88-92] Periportal hepatocyte copper deposition signals chronic bile flow impediments.

Mononuclear portal inflammation usually favors acute rejection, whereas neutrophilic or eosinophilic portal inflammation, late after transplantation, favors biliary strictures. However, chronic low-grade biliary strictures can occasionally cause predominantly mononuclear portal inflammation. Ductopenia in some portal tracts accompanied by a ductular reaction should raise the suspicion of biliary strictures. Cholangiography and/or angiography may be required to distinguish between chronic rejection and biliary strictures. Acute rejection occurring more than 6 months after transplantation is unusual in adequately immunosuppressed recipients. Therefore, checking baseline immunosuppressive drug levels and the liver test profile often point to the need for cholangiography before increased immunosuppression.

Acute and Chronic Rejection Versus Primary Biliary Cirrhosis. In acute rejection, portal inflammation and lymphocytic cholangitis are usually more diffusely distributed throughout the portal tracts and the biopsy and typically involve small bile ducts (<20 μm). Portal inflammation and lymphocytic cholangitis in recurrent PBC are typically patchy and involve medium-sized bile ducts (>40-50 μm). In the absence of a pathognomonic lesion, recurrent PBC is most commonly recognized by the biliary gestalt occurring in the absence of mechanical biliary strictures. This gestalt is unusual in rejection. Central perivenulitis is not a feature of PBC.

Central Perivenulitis. LAR can manifest primarily as central perivenulitis.[59-61,63,93-96] Because of its association with severe acute rejection[2] and transition to early chronic rejection,[63] central perivenulitis is sometimes portrayed as a poor prognosis lesion, but this is not necessarily correct.[59,60] As in native livers, central perivenulitis in allografts has several causes (Fig. 2), including various forms of rejection (pure perivenular rejection and early chronic rejection), early autoimmune hepatitis,[72,74,97] compromised afferent or efferent blood flow,[73,87,98] and adverse drug reactions. Perivenular rejection can be missed clinically and

present later as ascites because of a Budd-Chiari syndrome or veno-occlusive disease.[63,64,93,96,99]

An acute rejection diagnosis is obvious when central perivenulitis occurs in association with other portal-based changes typical of acute rejection; the severity is graded according to standard criteria.[2] Acute rejection is also the most likely diagnosis when central perivenulitis involves a majority of central veins with minimal or absent portal inflammation, except if the original disease was AIH. In this situation, isolated central perivenulitis may represent early recurrent AIH[34,42,74,75] or new-onset AIH. In native livers presenting with acute AIH central perivenulitis, chronic portal inflammation and interface activity usually develop over time.[72,74,97] Therefore, in allografts, re-examination of the native liver histopathology, serological studies for autoantibodies, and close follow-up for the development of changes more typical of chronic hepatitis[75] are warranted. Because increased immunosuppression effectively treats either rejection or AIH, any differences in assigned diagnoses may be semantic. Hepatic vein outflow obstruction and ischemia can also cause centrilobular necrosis, but any associated lymphocytic inflammation is usually minimal.

Mild focal central perivenulitis can coexist with other causes of late dysfunction. In such cases, central perivenulitis probably represents a focal alloreaction, because similar changes are rarely—if ever—seen with the same disorders in native livers. Therefore, we recommend mentioning its presence or suggesting a diagnosis of "indeterminate for rejection," unless a majority of central veins are involved.

Distinguishing Among the Various Causes of Chronic Hepatitis. Determining a specific cause of chronic hepatitis is not always possible, but subtle differences can suggest a specific etiology. Plasma cell and aggressive interface activity and confluent perivenular or bridging necrosis are suggestive of AIH, macrovesicular steatosis is suggestive of HCV, and viral inclusions are seen only in hepatitis B virus. Because potentially distinguishing features are inconsistently present and not entirely reliable, determining the underlying cause of acute and/or chronic hepatitis should be based on a complete clinicopathological evaluation (Tables 2 and 3). Steatohepatitis can coexist with other causes of injury.

Cholestatic or Biliary Disease Versus Chronic Hepatitis. A single granulomatous duct destructive lesion is diagnostic of PBC in the proper setting. Infectious causes of granulomatous cholangitis should be excluded, but they are uncommon. Portal granulomas without granulomatous cholangitis have been reported in native livers with HCV.[100] In the absence of pathognomonic lesions, recurrent PBC or PSC is most commonly distinguished from chronic hepatitis by a biliary gestalt.

Cholestatic viral hepatitis can be difficult to distinguish from biliary strictures with or without hepatic artery thrombosis. Portal edema and portal—rather than periportal—neutrophilia are common in biliary strictures. Cholangiolar proliferation and acute cholangiolitis without portal edema is more characteristic of cholestatic hepatitis. Lobular disarray and hepatocellular swelling and apoptosis are more usual for cholestatic viral hepatitis.

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Standardized Cardiac Biopsy Grading

Grade	Histopathological Findings
0	No rejection
1	**A** = Focal (perivascular or interstitial) infiltrate without necrosis
	B = Diffuse but sparse infiltrate without necrosis
2	One focus only with aggressive infiltration and/or focal myocyte damage
3	**A** = Multifocal aggressive infiltrates and/or myocyte damage
	B = Diffuse inflammatory process with necrosis
4	Diffuse aggressive polymorphous ± infiltrate ± edema, ± hemorrhage, ± vasculitis, with necrosis

Additional Required Information*

- **Biopsy less than 4 pieces**
- **Humoral rejection (positive IF, vasculitis, or severe edema in absence of cellular infiltrate**

■ **"Quilty" effect**	**A** = No myocyte encroachment
	B = With myocyte encroachment
■ **Ischemia**	**A** = Up to 3 weeks posttransplant
	B = Late ischemia

- **Infection present - biopsy therefore uninterpretable**
- **Lymphoproliferative disorder**
- **Other (specify)**

* Must be added to biopsy report if present

Reference Billingham ME, et al. A working formulation for the standardization of nomenclature in the diagnosis of heart and lung rejection: heart rejection study group. *J Heart Trans* 1990;9(6):587-93.

Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

[FRAMES] [NO FRAMES]

INTERNATIONAL SOCIETY FOR HEART TRANSPLANTATION

With the emergence of many different grading systems for heart and lung biopsy interpretation, the International Society for Heart Transplantation realized the necessity to establish a standardized grading system for the purpose of multicenter trials and for publication so that results from different centers could be compared effectively. Our purpose is not necessarily to change the grading systems in individual centers but to find one to which most grading systems may be extrapolated for the purpose of publications and multicenter drug trials. The charge to the heart and the lung study groups was to provide simple grading systems so that there could be conformity of interpretation of heart and of lung rejection among pathologists. Basic grading systems are presented in the following articles that will now be required for all manuscripts accepted by THE JOURNAL OF HEART AND LUNG TRANSPLANTATION. In taking this step to require standardization of grading systems, the International Society for Heart Transplantation has taken a leadership role and has made a significant contribution to more meaningful scientific results in the field of thoracic organ transplantation.

Margaret E. Billingham, MD
Stanford, California

A Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart and Lung Rejection: Heart Rejection Study Group

The International Society for Heart Transplantation:
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At the Society meeting in Munich in 1989 and again at the Colorado Springs Symposium in June 1989, Bruno Reichart, MD, then president of the Society, cited the many grading systems of rejection in heart and lung transplantation,[1-7] and he made urgent appeals to pathologists to come together for the purpose of establishing a universal grading system for biopsy interpretation. For this purpose, an international meeting of heart and lung pathologists from large transplantation centers from which previous publications on grading

TABLE I Standardized cardiac biopsy grading

Grade	"New" nomenclature	"Old" nomenclature
0	No rejection	No rejection
1	A = Focal (perivascular or interstitial) infiltrate without necrosis B = Diffuse but sparse infiltrate without necrosis	Mild rejection
2	One focus only with aggressive infiltration and/or focal myocyte damage	"Focal" moderate rejection
3	A = Multifocal aggressive infiltrates and/or myocyte damage	"Low" moderate rejection
	B = Diffuse inflammatory process with necrosis	"Borderline/severe"
4	Diffuse aggressive polymorphous ± infiltrate ± edema, ± hemorrhage, ± vasculitis, with necrosis	"Severe acute" rejection

"Resolving" rejection denoted by a lesser grade.
"Resolved" rejection denoted by grade 0.

systems had emerged convened at Stanford University Medical Center in August 1990. The object of the meeting was to find a simple and easily understood grading system that could be used by most pathologists. Furthermore, it was recognized that the grading system should have a high degree of reproducibility among all pathologists. Standardization of grading systems will result in more accurate comparison of results and will allow the establishment of multicenter immunosuppressive trials.

TECHNICAL CONSIDERATIONS

Because acute rejection is often focal, the standardized cardiac biopsy grading requires four to six undivided pieces of myocardium, depending on the size of bioptome used, to reduce sampling error to 2%. The tissue should be fixed in 10% buffered formalin and be paraffin embedded. At least one piece of evaluable myocardium should be frozen, fixed in OCT freezing compound, and saved for all biopsies in the first 6 weeks after transplant. No tissue needs to be routinely fixed for electron microscopic evaluation.

Adequacy of Sample

As to adequacy of sample, four pieces of myocardium are required, 50% of which must be evaluable myocardium and not biopsy site or scar. If a 7F bioptome or smaller is used, then six pieces of myocardium are required, 50% of which should also be evaluable. Biopsy specimens should not be divided.

Histologic Requirement

For histologic examination, a minimum of "three step levels" must be made through the paraffin block with at least three sections of each level. Slides should be stained routinely with hematoxylin and eosin, and one additional slide should be stained with a connective tissue stain such as Masson's trichrome.

GRADING OF CELLULAR REJECTION BY ENDOMYOCARDIAL BIOPSY

A new standardized cardiac biopsy grading is tabulated in Table I.

Grade 0 (No Acute Rejection)

Grade 0 should be used when there is no evidence of acute rejection or myocyte damage on the biopsy specimens. Equivocal findings of rejection should also be graded zero.

Grade 1A (Focal, Mild Acute Rejection)

Grade 1A represents focal, perivascular, or interstitial infiltrates of large lymphocytes that cause no myocyte damage, as seen in Figure 1. One or more pieces of the biopsy tissue may be involved.

Grade 1B (Diffuse, Mild Acute Rejection)

The grade 1B represents a more diffuse, perivascular or interstitial (or both) infiltrate of large lymphocytes with no myocyte damage, as seen in Figure 2. One or more pieces of biopsy tissue may be involved.

Grade 2 (Focal, Moderate Acute Rejection)

Grade 2 should be used when there is only one focus of inflammatory infiltrate (large aggressive lymphocytes with or without eosinophils), which is sharply circumscribed. Architectural distortion with myocyte damage should be present within the solitary focus, as seen in Figure 3.

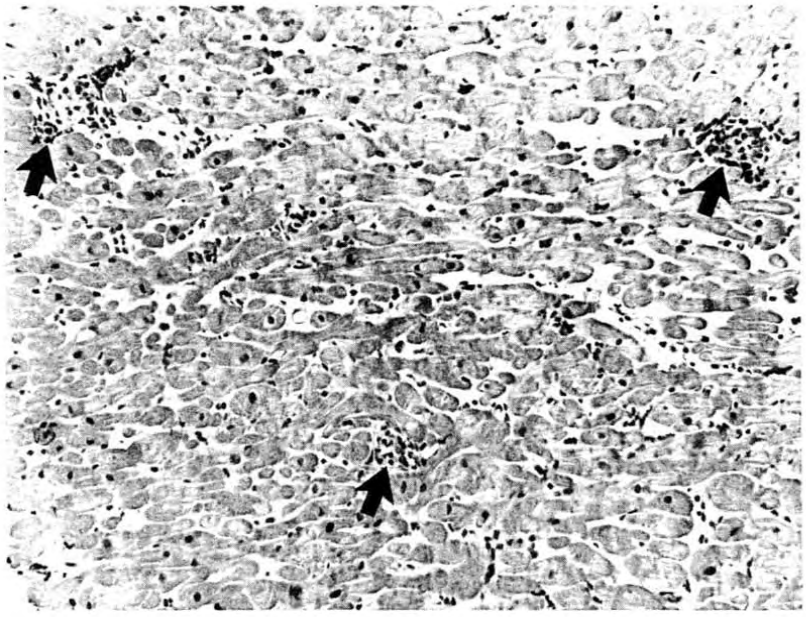

FIGURE 1 Grade 1A (ISHT Standardized Grading System). Section from a cardiac biopsy specimen shows focal, perivascular *(arrows)*, sparse infiltrate of lymphocytes, with no myocyte damage. (Hematoxylin and eosin. Magnification ×250.)

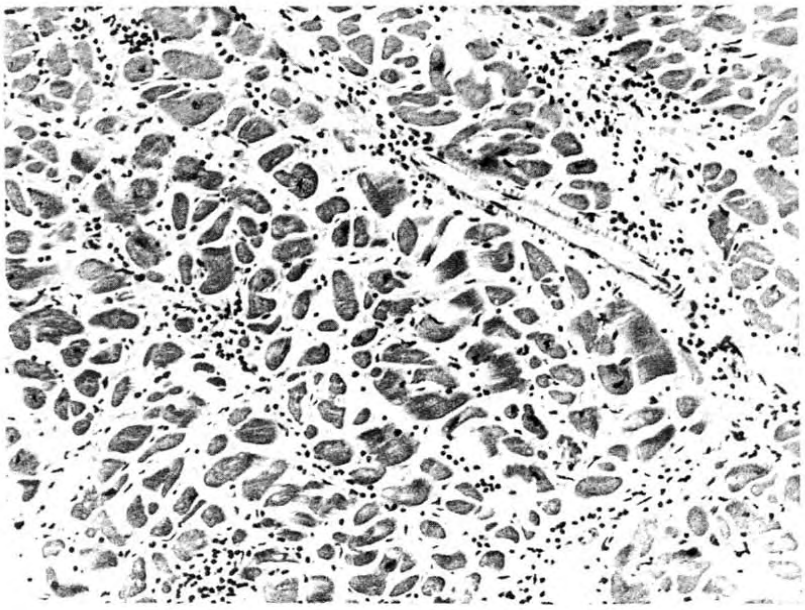

FIGURE 2 Grade 1B (ISHT Standardized Grading System). Section from a cardiac biopsy specimen shows a sparse, diffuse interstitial lymphocytic infiltrate without myocyte damage. (Hematoxylin and eosin. Magnification ×500.)

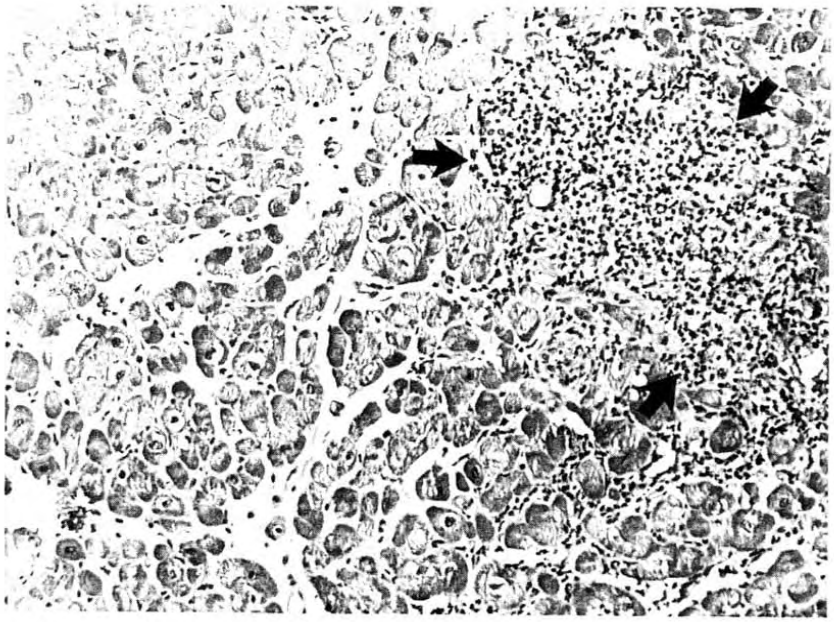

FIGURE 3 Grade 2 (ISHT Standardized Grading System). Section of a cardiac biopsy specimen shows a solitary, circumscribed focus of inflammatory cells *(arrows)*, with myocyte damage. Other pieces from the biopsy were clear of any infiltrate. (Hematoxylin and eosin. Magnification ×500.)

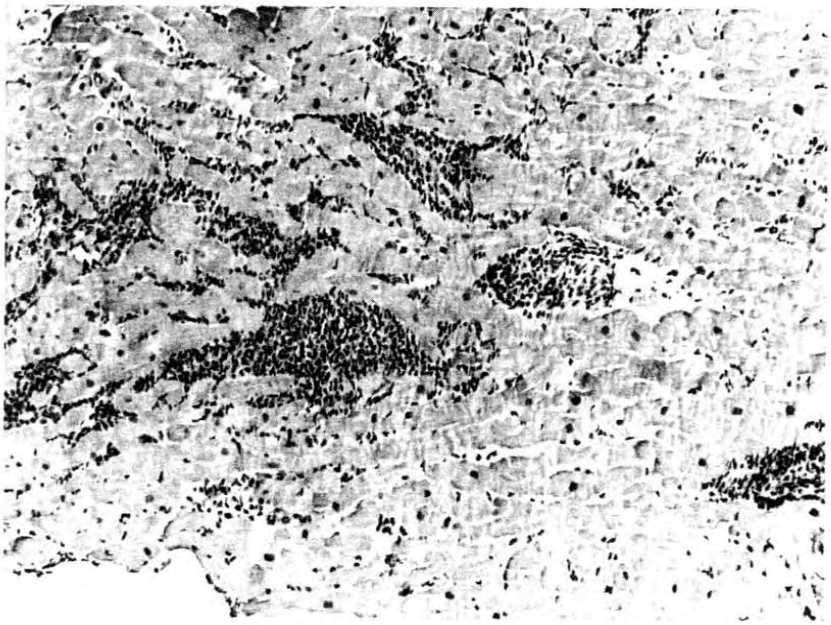

FIGURE 4 Grade 3A (ISHT Standardized Grading System). Section of a cardiac biopsy specimen shows an obvious multifocal pattern of inflammatory infiltrate, some with myocyte encroachment and damage. (Hematoxylin and eosin. Magnification ×250.)

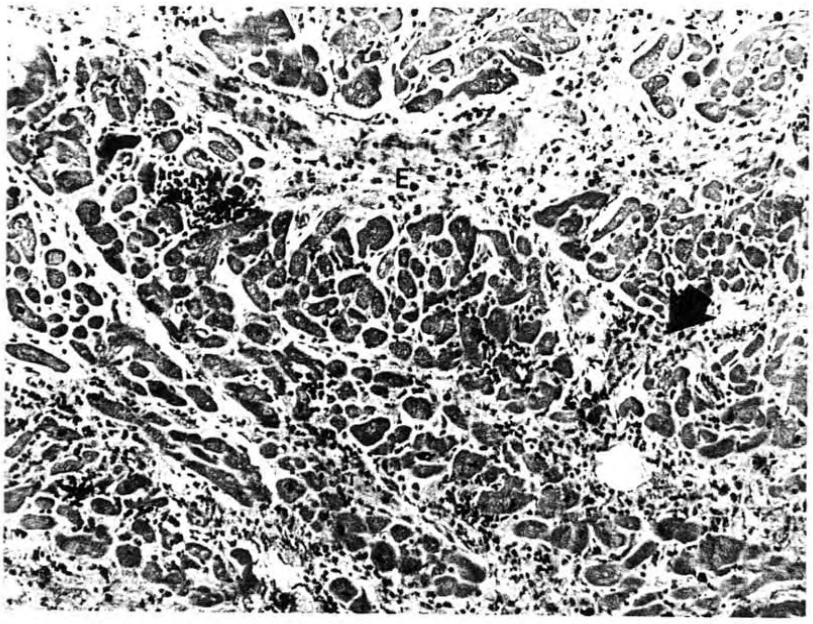

FIGURE 5 Grade 3B (ISHT Standardized Grading System). Section of a cardiac biopsy specimen shows a diffuse inflammatory process with focal myocyte damage *(arrow)* and edema *(E)*. All of the pieces were involved similarly. (Hematoxylin and eosin. Magnification ×250.)

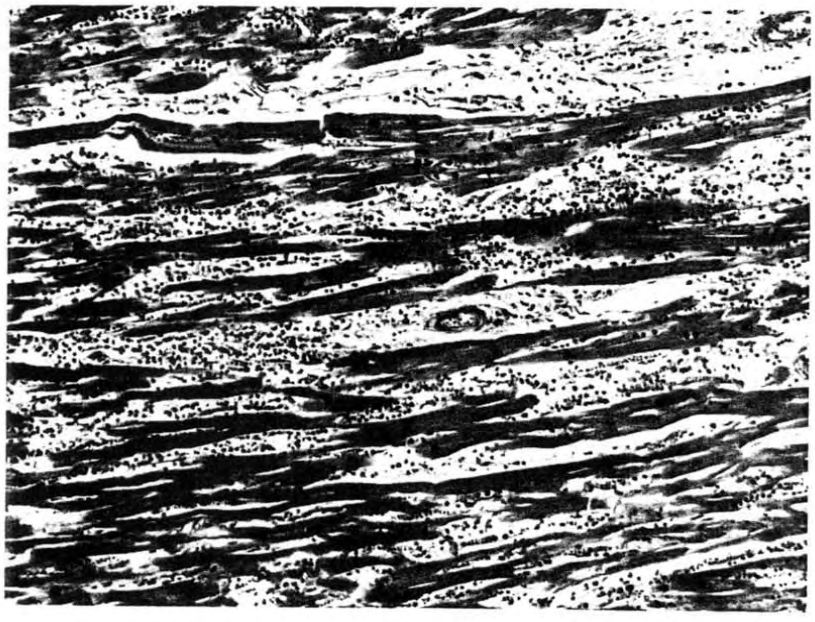

FIGURE 6 Grade 4 (ISHT Standardized Grading System). Section of cardiac biopsy specimen shows edema, hemorrhage, and an aggressive polymorphous inflammatory infiltrate. (Hematoxylin and eosin. Magnification ×250.)

TABLE II Standardized cardiac biopsy grading

Additional required information Check and specify "A" or "B"	
☐	Biopsy less than 4 pieces
☐	Humoral rejection (positive IF*, vasculitis, or severe edema in absence of cellular infiltrate
☐	"Quilty" effect A = No myocyte encroachment B = With myocyte encroachment
☐	Ischemia A = Up to 3 weeks posttransplant B = Late ischemia
☐	Infection present—biopsy therefore uninterpretable
☐	Lymphoproliferative disorder
☐	Other (specify)

*Immunofluorescence.

Grade 3A (Multifocal Moderate Rejection)

Grade 3A represents multifocal inflammatory infiltrates consisting of large aggressive lymphocytes with or without eosinophils. These infiltrates may involve one or more of the pieces of biopsy tissue, as seen in Figure 4.

Grade 3B (Diffuse, Borderline Severe Acute Rejection)

Grade 3B represents a diffuse inflammatory process within several of the pieces of biopsy tissue. Myocyte damage is present, as well as an aggressive inflammatory infiltrate of large lymphocytes and eosinophils with an occasional neutrophil. Hemorrhage is not usually seen in this grade (Figure 5).

Grade 4 (Severe Acute Rejection)

Grade 4 represents a diffuse aggressive, polymorphous inflammatory infiltrate that includes aggressive lymphocytes, eosinophils, and neutrophils (Figure 6). Myocyte necrosis and damage is always seen. Edema, hemorrhage and vasculitis are usually present. In some cases in which the patient has been treated aggressively with immunosuppression for some time, the edema and hemorrhage may be more prominent than the cellular infiltrate.

"Resolving" acute rejection in the old nomenclature will be replaced by denoting a lesser grade than that given in a recent biopsy. In addition, the term "resolving" may be used in parenthesis after the grade number. Likewise, "resolved" rejection should be denoted by grade 0; however, in this case also, "resolved" rejection may be included after the numerical grade in parenthesis.

Table II shows the additional required information that should be added to any biopsy report using the standardized grading system.

—Inadequate biopsy: Check box for biopsies of less than four pieces. The number of pieces should be written in the box.

—Immunofluorescence: If immunofluorescence is performed, and humoral rejection is suspected, the box on humoral rejection should be checked. It is suggested that in those centers where it can be easily done immunoflourescence be performed on all biopsy specimens in the first 6 weeks after heart transplantation, as previously described.[8]

—"Quilty" effect: The Quilty effect has been described many times and is an endocardial infiltrate that may not be associated with acute rejection.[9] Whenever a Quilty effect is present, the appropriate box should be checked. If the Quilty effect encroaches into the myocardium from the endocardium, that should also be designated with the letter "B."

—Ischemia: Evidence of myocyte damage from ischemia at the time of heart transplantation or reperfusion injury is often seen in the first early biopsies after transplantation. If ischemia is present, it should be checked in the appropriate box; the letter "B" should be used for late ischemia occurring 3 or more months after heart transplantation.

—Infection: Whenever infection is found to be present in the endomyocardial biopsy, as evidenced by a mixed inflammatory infiltrate that is not characteristic of acute rejection or organisms (viral,

fungal, or protozoal) are found, this should be indicated in the appropriate box.

—Lymphoproliferative disorder: If the endomyocardial biopsy provides evidence of a lymphoproliferative disorder, as previously described,[10] the appropriate box should be checked.

In conclusion, the above is a proposal for use in interpreting endomyocardial biopsies in cardiac or in combined heart-lung allografts. By using this scheme, different institutions can construct their data to support or argue against certain propositions espoused by other investigators. The International Society for Heart Transplantation will require that this grading system be used in all Society-sponsored publications in the future. It is expected that the study group will meet again in the future to evaluate the adequacies or deficiencies of this proposal.

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A Working Formulation for the Standardization of Nomenclature in the Diagnosis of Heart and Lung Rejection: Lung Rejection Study Group

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The grading of rejection in combined heart-lung, double lung, and unilateral lung allografts has created several nomenclatural problems in the transplant community. Establishing a uniform grading system, which could be used as a working formulation for comparing data from independent institutions, promoting improved patient care, and developing alternative therapies, is essential. Although it is recognized that all grading systems currently in use have individual merits, there is a need for a single comprehensive scheme that

Update on Cardiac Transplantation Pathology

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● ***Context.*—The endomyocardial biopsy is the mainstay for monitoring acute allograft rejection in heart transplantation. Objective and accurate assessment of cellular and humoral types of rejection is important to optimize immunosuppressive therapy, avoid therapeutic complications, and improve patient outcome. The grading system for evaluation of heart transplant biopsies published in 1990 was revised in 2004 after more than a decade of implementation.**

***Objective.*—In this review, we focus on a practical approach to the evaluation of human heart transplant biopsies as diagnostic surgical pathologic specimens. We discuss the revised International Society of Heart and Lung Transplantation working formulation.**

***Data Sources.*—We reviewed pertinent literature, incorporating ideas and vast experience of participants in various work groups that led to the revision of the 1990 grading system.**

***Conclusions.*—The grading system for cellular rejection is presented with detailed light microscopic morphology and comparison of the 1990 and 2004 International Society of Heart and Lung Transplantation working formulations. We show how the pathologic recognition of cellular rejection and antibody-mediated rejection has evolved. We emphasize the interpretation of immunostains for complement components C4d and C3d in the diagnosis of antibody-mediated rejection. Evidence of regulation of complement activation in human heart transplant biopsies is presented in this context. We also discuss the pitfalls, caveats, and artifacts in the interpretation of allograft endomyocardial biopsies. Lastly, we discuss the pathology of human cardiac allograft vasculopathy in practical detail.**

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Heart transplantation remains the most effective therapy for end-stage heart disease of coronary and noncoronary etiology, with continued improvement in survival during the years. The most common indications for cardiac transplantation in the adult have not changed in the last 3 decades; 85% of cases are roughly equally divided between coronary heart disease and nonischemic cardiomyopathies.[1] In the pediatric age group, congenital heart disease is the leading diagnosis for recipients younger than 1 year old. Cardiomyopathy and congenital heart disease are the two most common indications for transplantation in children.[2]

The 10-year survival rate after cardiac transplantation currently approaches 50% and more in high-volume centers.[1,3] The success of heart transplantation, for the most part, has been achieved through better understanding of the immunology of transplant rejection and the application of strategies for the recognition, treatment, and prevention of rejection. In the early years of cardiac transplantation, failure resulted from a high incidence of acute cellular rejection that limited graft survival. The signs and symptoms of acute cellular rejection are often vague and there are no serologic markers of cardiac allograft rejection. The treatment of rejection, in turn, was often complicated by infection, malignancy, and drug toxicities that result from the difficulty in titrating immunosuppression to the desired end point according to the severity of rejection. The introduction of percutaneous transvenous endomyocardial biopsy by Caves et al[4] in 1973 provided an objective means of diagnosing rejection and allowed for careful monitoring and prompt treatment of cardiac allograft rejection.

ENDOMYOCARDIAL BIOPSY

Endomyocardial biopsy (EMB) remains the gold standard for rejection surveillance in the heart transplant patient.[5] It has a high sensitivity and specificity for the diagnosis of acute cellular rejection.[6,7] There are currently no cardiac imaging modalities or serum markers that can replace the performance of surveillance biopsies in the posttransplantation care and management of these patients.[8]

Ideally, an initial biopsy of the donor heart should be obtained in the operating room at the time of transplantation. This biopsy can be valuable because it provides a means to assess the status of the donor myocardium for hypertrophy, ischemia, or the presence of any pathologic process such as myocarditis. The frequency of posttransplant surveillance biopsies varies highly between different institutions. Typically, surveillance biopsies are performed once weekly for the first month, every 2 weeks for the second month, and every 6 to 8 weeks between the third and 12th months. After the first year, the frequency can be decreased to quarterly, biannually, or annually. In some centers, protocol biopsies are not done after 2 or more years unless there is a clinical suspicion of rejection. If rejection is diagnosed, the patient is treated and undergoes repeat biopsy after 1 to 2 weeks.

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The procurement of the tissue is made with a bioptome introduced from either the jugular or femoral vein to sample the right ventricular septal wall. Bioptomes are available in different sizes; therefore, the size of the pieces of tissue retrieved will differ slightly. The common sizes used are 7 F (French) and 9 F in adults and 3 F, 5 F, and 7 F in pediatric-age patients (Figure 1, A through D).[9]

Handling the Biopsy Specimen

To prevent introducing artifacts in EMB, the tissue should not be allowed to sit on filter paper, gauze, or any other surface that is impregnated with saline or other solutions that are not iso-osmotic, for a prolonged period of time. The tissue should be fixed immediately in the desired fixative, the most commonly used being 10% phosphate-buffered formalin that has been allowed to reach room temperature (25°C). Cold fixative enhances contraction band artifact. To avoid crushing artifacts, the tissue should not be handled with forceps or divided with a scalpel. The cardiac catheterization suite personnel should not triage the tissue based on gross appearance. All the pieces obtained should be submitted because they may have valuable information when examined histologically. Pieces that look white, suggesting that they are made up of thick endocardium, or pieces that look like blood clot may harbor a piece of myocardium in their core. Tissue is not routinely fixed in glutaraldehyde for electron microscopy of allograft biopsies.

Adequacy of the Biopsy Specimen

In the 1990 International Society for Heart and Lung Transplantation Working Formulation of Cardiac Allograft Pathology (ISHLT-WF1990), 4 to 6 pieces of tissue, depending on the size of the bioptome used, were required for light microscopic evaluation.[10] Because acute cellular rejection is not uniformly distributed in the heart, it is important to take multiple samples during the biopsy procedure. It has been shown that if 3 biopsy pieces taken show no rejection, there is a 5% and 0% chance of missing a mild and moderate-to-severe rejection, respectively. However, if 4 pieces are examined, the false-negative rate of mild rejection is further reduced to 2%.[11] Other investigators have suggested that the extent of infiltration is also important. Where mild rejection is the most severe grade observed in 3 or 4 fragments, the probability of missing moderate or severe rejection is 25.4% and 28.2%, respectively.[12] The 2004 revised working formulation (ISHLT-WF2004), however, currently recommends an absolute minimum of 3 biopsy pieces for evaluation, each of which must contain at least 50% myocardium and exclude a previous biopsy site or scar.[13] Studies of sensitivity to detect rejection with only 3 biopsy pieces using the current grading system have yet to be performed. Specimens that do not meet these criteria should be diagnosed as ''inadequate biopsy.'' If rejection is noted in a biopsy of fewer than 3 evaluable pieces, the rejection grade may be indicated in a diagnosis comment with the emphasis that a higher grade of rejection cannot be ruled out.

Gross Pathologic Evaluation

In addition to the demographic data of the patient, the gross description should include the number of tissue pieces, an aggregate measurement with the average size, and color. Careful gross examination provides, in most instances, important information regarding the presence of myocardium, thickened endocardium, adipose tissue, blood clot, or chordae tendineae (Figure 1, E and F).[14] It is good practice to state the number of pieces submitted in the requisition form to be verified on gross examination and always correlated with the number of pieces present in the paraffin block and in the hematoxylin-eosin–stained slides.

Histopathologic Evaluation

The current working formulation suggests a minimum of 3 step levels for microscopic examination.[13] No special stains are routinely required for evaluation. Unstained slides can be cut and saved for immunohistochemical staining if needed.

Frozen Section Evaluation

One or more pieces of tissue can be snap-frozen for immunofluorescence or other additional study (such as in situ nucleic acid hybridization, in situ polymerase chain reaction, and gene expression profiling) depending on the needs of a given patient and any research protocol used by the institution.

Following a careful freezing protocol is important in order to achieve the best preservation of morphology possible. The ISHLT-WF1990 suggests freezing 1 biopsy piece in OCT freezing compound (Miles Inc, Diagnostics Division, Elkhart, Ind). There is no specific recommendation in the ISHLT-WF2004 regarding either the manner of freezing or the number of biopsy pieces to be frozen. In our institution, 4 biopsy pieces are routinely obtained and all pieces are frozen. The tissue is quickly and gently blotted to remove any excess moisture before embedding them on a chuck containing partially frozen OCT. After proper orientation, the specimen is fully covered with OCT and submerged in liquid nitrogen until frozen. Three step levels are cut for hematoxylin-eosin staining. This technique yields excellent frozen sections that are comparable to those obtained from paraffin sections. Additional slides can be obtained for the application of immunoperoxidase and immunofluorescence studies. The tissue is then kept frozen and stored at −80°C for future study.

CARDIAC ALLOGRAFT REJECTION: MORPHOLOGIC ASPECTS

As in any other solid organ, cardiac rejection can result from humoral and cellular rejection. These are, in turn, subclassified into hyperacute, acute, and chronic rejection on the basis of mechanism and duration of the process.

HYPERACUTE REJECTION

Hyperacute rejection is graft injury triggered by preformed antibodies and occurs rapidly after implantation of the graft, usually within minutes to hours. In the older literature, hyperacute rejection has also been referred to as *humoral rejection, vascular rejection*, and *antibody-mediated rejection.* This type of rejection is extremely rare in the current practice of allograft cardiac transplantation. The morphologic findings are well described in experimental discordant xenografts[15] with similar findings in autopsy cases of cardiac allograft recipients.[16] Predisposing factors that may play a role are preformed antibodies to epitopes of the ABO and HLA systems and vascular endothelial cells,[17] previous pregnancies, multiple surgeries with the use of blood products and, especially, previous cardiac or

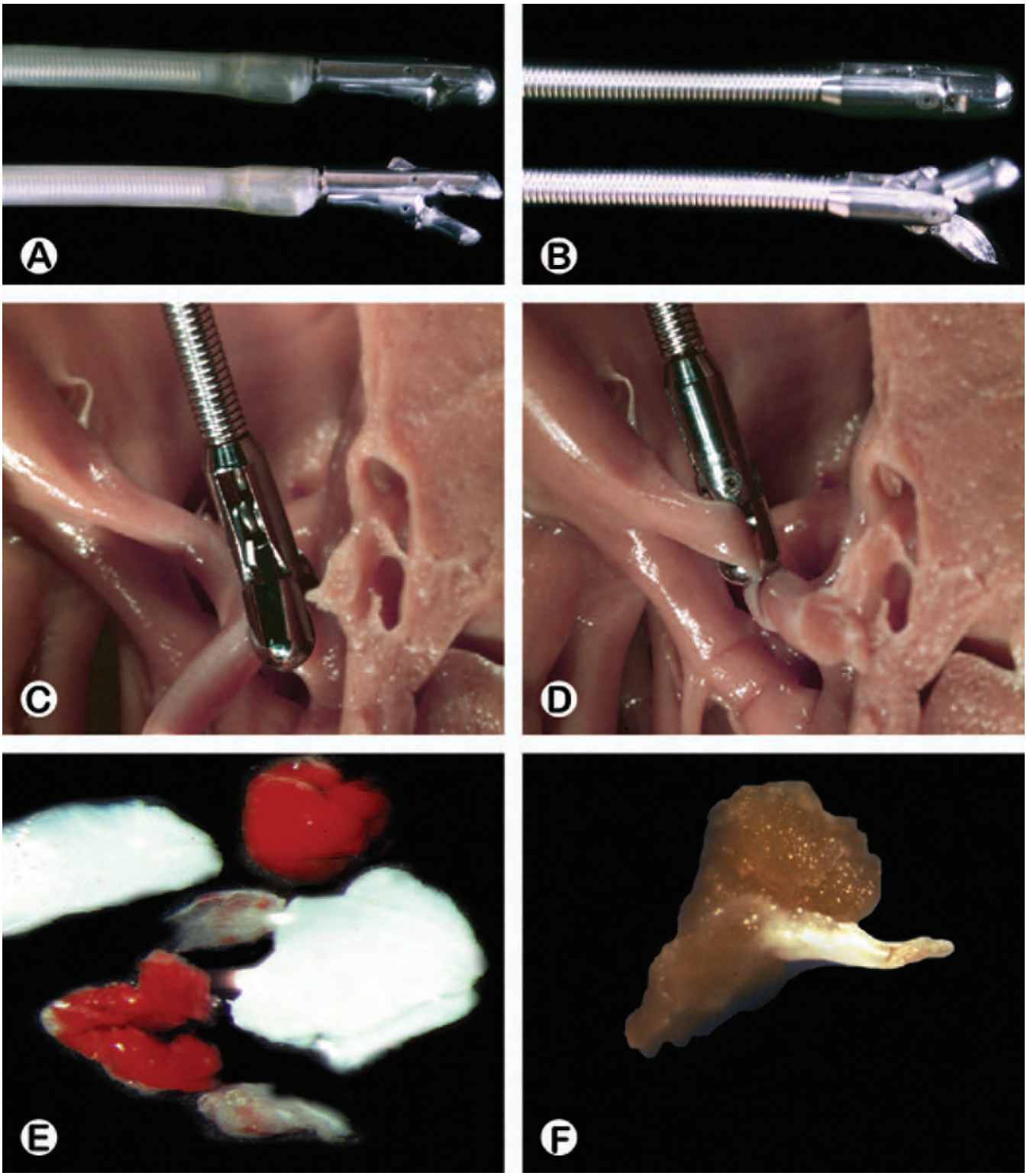


Figure 1. *The endomyocardial biopsy specimen. Bioptomes used for procurement of endomyocardial biopsy specimens are available in different sizes. A, Caves bioptome has a cutting mechanism that is composed of 1 rigid and 1 mobile jaw. B, The Cordis bioptome has 2 flexible jaws. C and D, The bioptome is seen in an open and closed position against the right side of the interventricular septum where trabeculations are usually abundant. E, Pieces of white thickened endocardium and blood clots can be seen in an endomyocardial biopsy specimen. They can be recognized grossly and should also be submitted for histologic examination as they may contain myocardium beneath. F, A fragment of papillary muscle with attached short segment of chorda is shown. Chordae tendineae are sometimes inadvertently sampled during the procedure. Their presence should be mentioned in the report.*

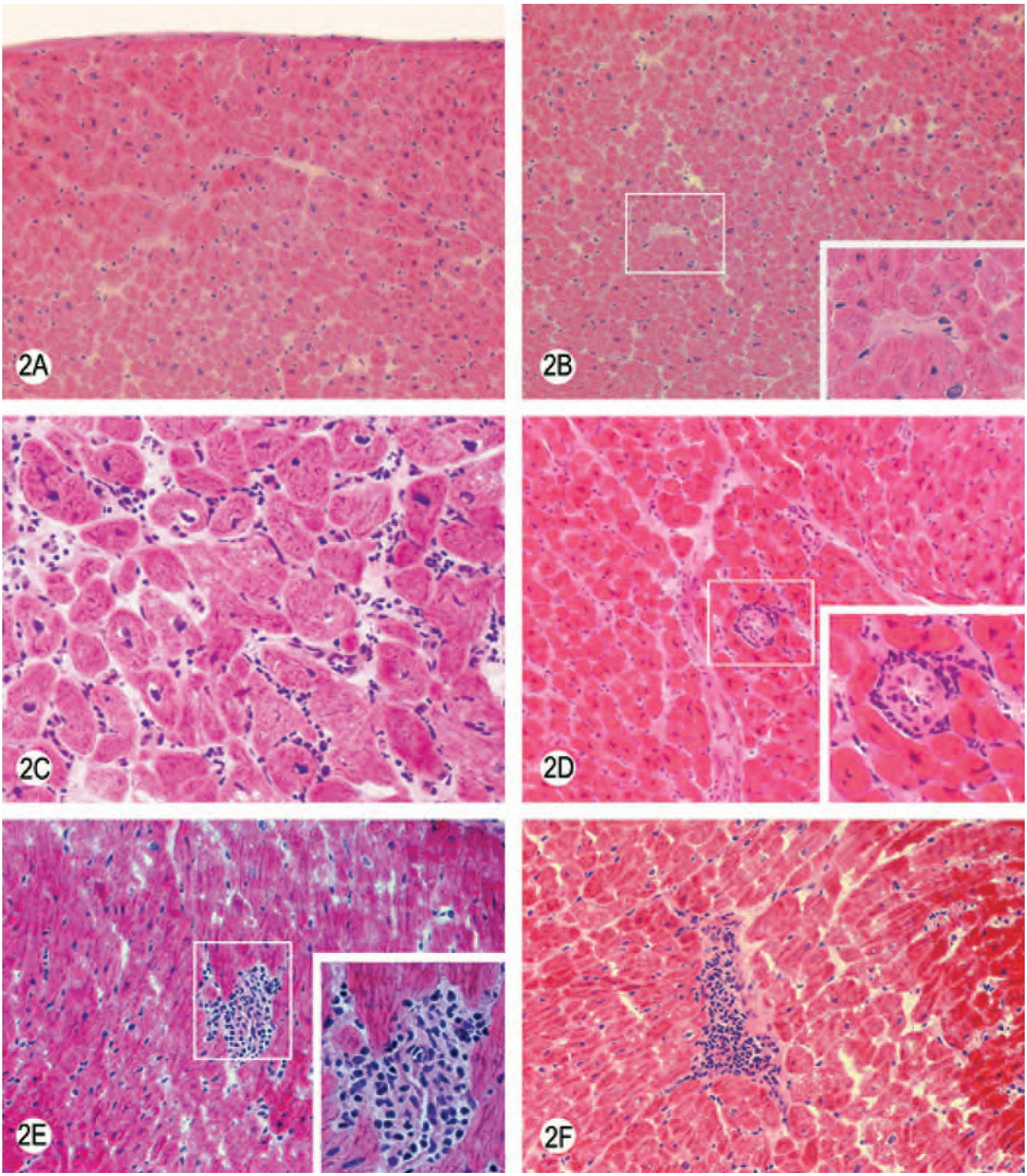


Figure 2 (Part 1). *Grades of cellular rejection (International Society of Heart and Lung Transplantation Revised Working Formulation-2004). A, Grade 0R. A well-oriented fragment of normal myocardium with no evidence of inflammatory infiltrates is illustrated. The endocardium is normal (frozen section, hematoxylin-eosin, original magnification ×100). B, Grade 0R. The inset shows a venule with flattened endothelial lining and no infiltrates in the interstitium or perivascular space (frozen section, hematoxylin-eosin, original magnifications ×100 and ×400 [inset]). C through F, Examples of Grade 1R. Mild rejection is seen as sparse interstitial infiltrates in between myocytes. Note the absence of interstitial expansion by the infiltrates (C, frozen section, hematoxylin-eosin, original magnification ×400). Scant inflammation is demonstrated around an arteriole (D) and a venule (E). Isolated mildly expansile perivascular infiltrate cut in a longitudinal orientation is shown (F). In the absence of significant myocyte encroachment or clear myocyte damage, it is graded as mild cellular rejection (D through F, frozen section, hematoxylin-eosin, original magnifications ×100 and ×400 [insets]).*

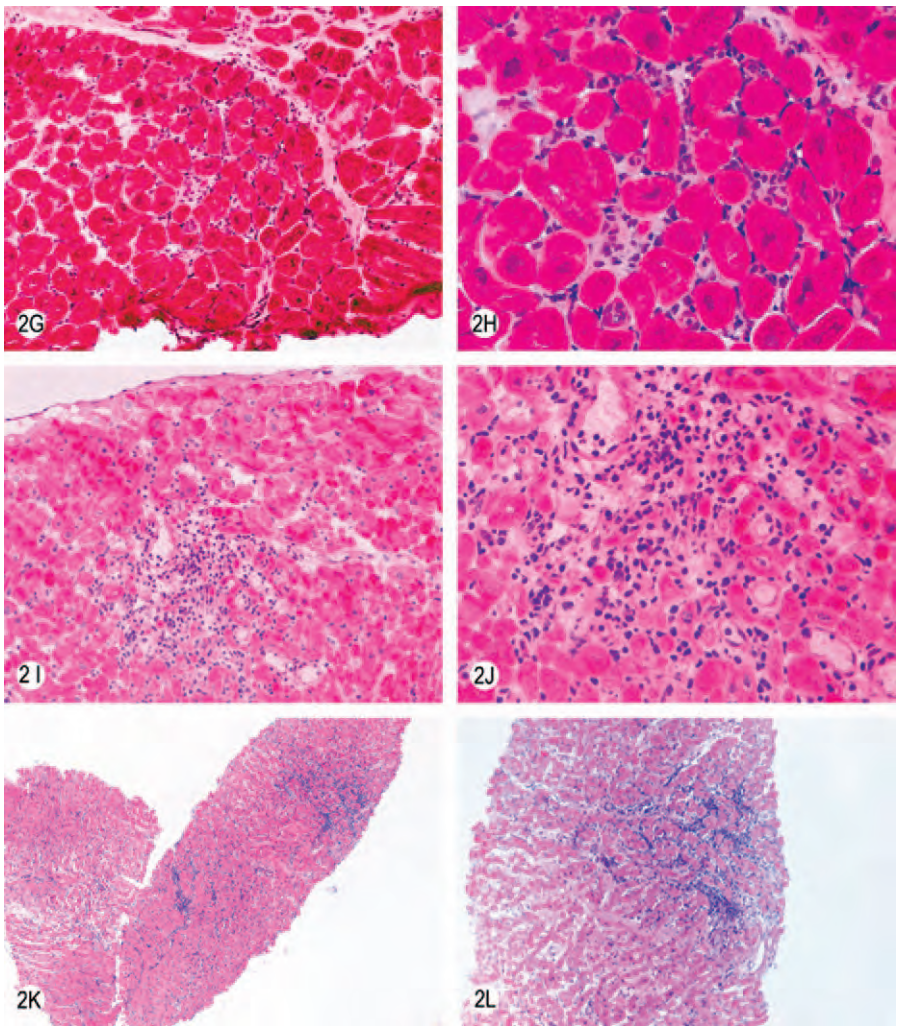


Figure 2 (Part 2). *Grades of cellular rejection (International Society of Heart and Lung Transplantation Revised Working Formulation-2004 [ISHLT-WF2004]). G and H, Grade 1R. A small focus of diffuse, predominantly interstitial mononuclear infiltrate is demonstrated in low and higher magnification (frozen section, hematoxylin-eosin, original magnifications ×200 [G] and ×400 [H]). This interstitial ''chicken wire'' pattern was previously referred to as 1B in the ISHLT-WF1990. I and J, Grade 1R with a focus of myocyte damage, formerly grade 2. A single focus of inflammation is located close to a normal endocardium in this biopsy fragment. At higher magnification (J), this area shows myocyte replacement or dropout, implying myocyte damage. Note the presence of fragmented and attenuated myocyte sarcoplasm (arrowheads) in the midst of the inflammatory cells as well as the loose stroma in the background consistent with an acute process (frozen section, hematoxylin-eosin, original magnifications ×100 [I] and ×200 [J]). K and L, Grade 2R with multifocal myocyte damage. Scanning magnification shows one fragment of tissue containing two distinct foci of more abundant inflammatory infiltrates with an intervening area of myocardium without inflammation (hematoxylin-eosin, original magnifications ×20 [K] and ×40 [L]).*

other organ transplants. The pathogenesis of hyperacute rejection is believed to be an antibody-mediated activation of the complement cascade, producing severe damage to the endothelial cells, as well as platelet activation followed by the clotting cascade and thrombosis. Although the widely accepted concept is injury to the capillary network of the graft, some investigators have suggested that endothelial damage occurs primarily in cardiac venules, resulting in venular thrombosis.[18] On gross examination, the heart is swollen and it is dusky on external inspection. The ventricles are dilated with scattered hemorrhages, mostly in the subendocardium. Histopathologic changes include swelling of the endothelial cells, vascular thrombosis, extravasation of red blood cells, prominent interstitial edema, and subsequent polymorphonuclear inflammatory infiltrates followed by tissue necrosis. These changes initially occur focally but rapidly spread through the organ. Immunohistochemical studies may show deposits of immunoglobulin (Ig) M, IgG, and complement in the vessel walls as well as fibrin deposits.

ACUTE CELLULAR REJECTION

Morphologically, acute cellular rejection consists of a mononuclear inflammatory infiltrate that is predominantly a T-cell–mediated response directed against the cardiac allograft. In severe cases, there is also participation of granulocytes in the rejection process. Characterization of the phenotype of lymphocytes in cardiac biopsy tissue has shown no good correlation between the extent and composition (CD4:CD8 ratio) of T lymphocytes infiltrating the graft and the histologic grading of rejection.[19,20] However, other studies report a good correlation between the mean number of $CD8^+$ T cells and the severity of rejection grade.[21] The discrepancy in these studies may be related to the fact that the immune response to the allograft is a continuous process in flux that is usually dissected in small ''time-lapsed'' views for pathologic study. Some support to this notion is provided by the observation that if subsets of T lymphocytes are further classified on the basis of the presence of naive cells (CD45RA) and memory or activated cells (CD45RO), naive cells of the CD4 phenotype are more abundant in biopsy tissue during mild rejection. A shift toward activated CD8 phenotype is seen in moderate rejection.[22] An increase in the number of antigen presenting cells (ie, macrophages and dendritic cells) is also observed as a function of the severity of rejection.[23–26] B-cell infiltrates are rarely present in mild rejection. However, a substantial increase in activated B lymphocytes and natural killer cells are seen in moderate rejection, suggesting their important role as promoters and effectors of cellular rejection.[26]

Grading of Acute Cellular Rejection

Historically, several methods to assess the histologic grade of rejection have been used by different transplant

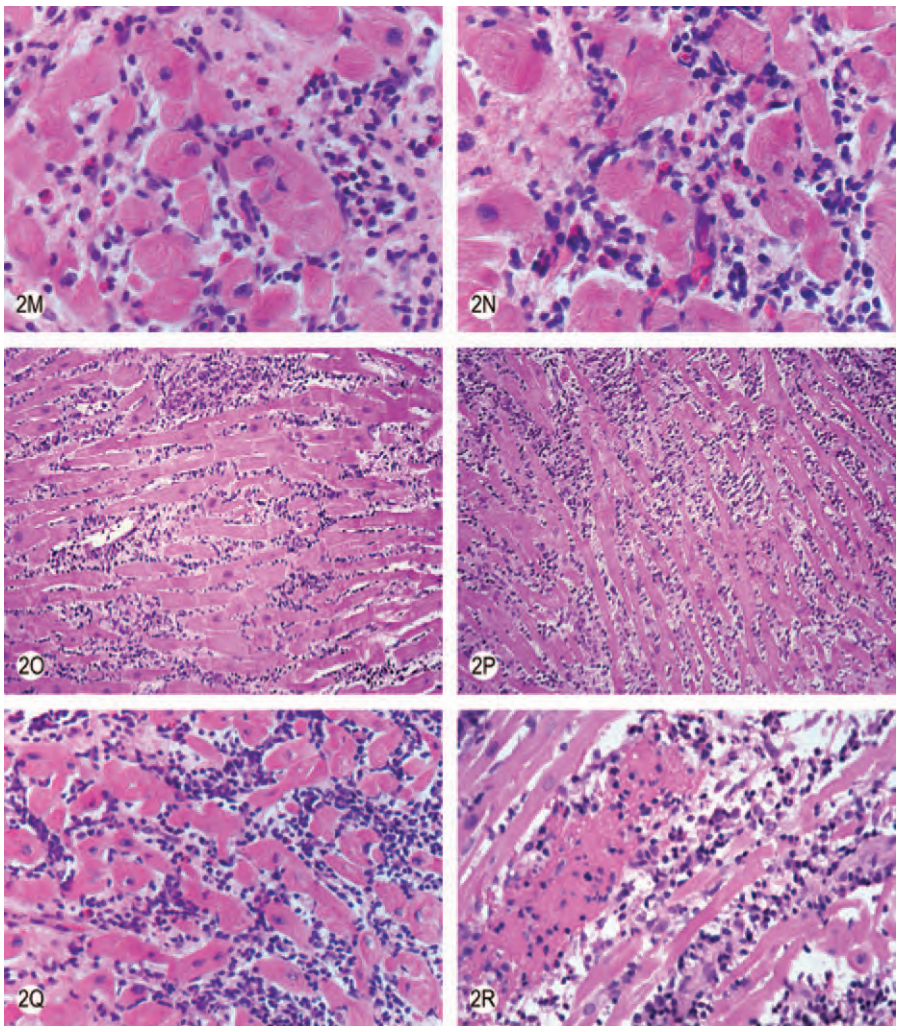


Figure 2 (Part 3). *Grades of cellular rejection (International Society of Heart and Lung Transplantation Revised Working Formulation-2004 [ISHLT-WF2004]). M and N, Grade 2R. Higher magnifications of 2 different areas of moderate rejection in a biopsy demonstrate widening of the interstitium. The lymphocytes appear to be in close contact with the myocyte borders. Numerous eosinophils are present in these images. These lesions would be 3A in the ISHLT-WF1990 (hematoxylin-eosin, original magnification ×400). O through Q, Grade 3R with diffuse inflammation. These myocardial pieces are diffusely infiltrated by dense mononuclear inflammatory infiltrates. These represent a grade 3B in the ISHLT-WF1990 (hematoxylin-eosin, original magnifications ×100 [O and P] and ×200 [Q]). R, Grade 3R with edema and hemorrhage. There is a mixed inflammatory infiltrate including neutrophils in severe rejection. The blood vessel shown here is necrotic. There is interstitial edema that separates damaged myocytes (hematoxylin-eosin, original magnification ×200).*

centers and will not be reviewed here. In 1990, the ISHLT published a standardized international grading system for the purpose of effectively communicating outcomes in multicenter drug trials and among institutions using different treatment regimens. The grades proposed in the ISHLT-WF1990 were mainly based on the amount of inflammatory infiltrate and the presence of myocyte damage.[10] The absence of cellular rejection was called grade 0 (Figure 2, A and B). Because rejection is a patchy process, the severity of inflammation may differ from one fragment to the next. Rejection is generally graded on the worst area of involvement. The pattern of inflammatory infiltration was reflected in the subdivisions A and B in grades 1 and 3. In mild rejection, it played a minor role and does not imply that a diffuse pattern (1B) is worse than a focal infiltrate (1A) (Figure 2, C through H).[27] It must also be noted that the grading of rejection was designed to assess rejection in endomyocardial biopsies and not the whole grafts.

As this grading scheme was widely adopted after its publication, variability in the interpretation of histologic grading among pathologists became evident and resulted in a lack of consensus with regard to the treatment of specific grades of cellular rejection. In 2001, the Banff Allograft Pathology Group invited pathologists, cardiologists, and cardiac surgeons to discuss their experiences after more than 10 years of using the ISHLT-WF1990. These discussions pointed out some of the more difficult

Comparison of the 1990 and 2004 Grading System of the International Society of Heart and Lung Transplantation for Acute Cellular Rejection

1990	2004
Grade 0 (no acute rejection)	Grade 0R (no acute cellular rejection)
Grade 1A (focal, mild acute rejection)	Grade 1R (mild, low-grade, acute cellular rejection): interstitial and/or perivascular infiltrate with up to 1 focus of myocyte damage
Grade 1B (diffuse, mild acute rejection)	
Grade 2 (focal, moderate acute rejection)	Grade 2R (moderate, intermediate-grade, acute cellular rejection): 2 or more foci of infiltrate with associated myocyte damage
Grade 3A (multifocal moderate rejection)	
Grade 3B (diffuse, borderline severe acute rejection)	Grade 3R (severe, high-grade, acute cellular rejection): diffuse infiltrate with multifocal myocyte damage ± edema, ± hemorrhage ± vasculitis
Grade 4 (severe acute rejection)	

issues for clinical practice and for use of the pathology information as end points in clinical trials.[5] In 2004, under the direction of the ISHLT, a working group composed of an international, multidisciplinary team of subspecialists in cardiac transplantation met to review the ISHLT-WF1990 definitions of cellular and antibody-mediated rejection, identify areas of difficulty in interpreting transplant biopsies, and revise the grading system. There was strong consensus that any changes in the formulation should reflect current pathologic practice and should not affect the grading of historic samples. The issue then was not one of changing the 1990 ISHLT grading scales, but one of more clearly defining how pathologists and cardiologists should interpret the grading system.

A major controversy in the ISHLT-WF1990 is the diagnosis and clinical significance of grade 2 rejection (Figure 2, I and J).[28,29] It is a grade that has been used in many transplant centers as a discrete defining point in therapeutic decisions. The misdiagnoses of grade 2 lesions by pathologists and the clinical data indicating that grade 2 rejections resolve without treatment in the majority of cases prompted the working group to now include grade 2 rejection with the revised mild rejection category. The old grade 3A (Figure 2, K through N) has been reclassified as grade 2R in the new working formulation (Table). Disagreement in the diagnosis between grade 3B (diffuse, borderline, severe acute rejection) and grade 4 (severe acute rejection) (Figure 2, O through R) also occurred previously as both of these can show the same severity of diffuse destructive infiltrates. The difference rests mainly on finding additional neutrophilic infiltrates and demonstrating edema and hemorrhage in the biopsy. It seemed more logical then that grades 3B and 4 were placed together in the severe category of the revised grading system because these minor discrepancies do not affect clinical therapeutic decisions.

The different histologic grades in the revised ISHLT-WF2004 classification are indicated by a suffix, ''R'' (Table). Absence of inflammation is reported as no rejection. A perivascular or interstitial infiltrate of mononuclear cells without architectural distortion is considered mild rejection. A focus of inflammation with myocyte damage, previously termed *grade 2* in the ISHLT-WF1990 classification, has been incorporated in the mild rejection category. Moderate, intermediate-grade rejection consists of 2 or more foci of mononuclear cell infiltrates associated with myocyte damage. Eosinophils may be present in moderate rejection. Severe, high-grade rejection is a diffuse process with multiple areas of myocyte damage and often a polymorphous inflammatory infiltrate that may be accompanied by edema and hemorrhages. A comparison of the 1990 working formulation and the revised grading system is presented in the Table.

Pitfalls and Caveats in Evaluating Endomyocardial Biopsies for Cellular Rejection

Although an enormous effort has been put forth to create a standard method for grading rejection that is easily reproducible, there were some controversial points that have been identified by both pathologists and clinicians in using the ISHLT-WF1990 and these warranted further clarification in the revised grading scheme.[5] Some of these controversies are discussed in the following sections.

Definition of Myocyte Damage.—A major source of discordance in histologic grading is the criteria used for the interpretation of ''myocyte damage'' in light microscopy, which is a required feature in higher grades of rejection.[5] The morphologic spectrum of myocyte damage is wide and has subtle changes that can be difficult to ascertain. Various forms of myocyte injury described by experienced cardiac pathologists include vacuolization, perinuclear halo, ruffling of the cytoplasmic membrane, irregular myocyte border, splitting or branching of myocytes, and myocyte encroachment with partial disruption of the myocytes.[6,30] Hypereosinophilia and nuclear pyknosis would indicate myocyte necrosis. Ultrastructural studies have shown that actual myocyte necrosis is rare, and reversible myocyte injury and myocyte regeneration occur even in moderate-to-severe acute cellular rejection.[31–33] In the revised working formulation, myocyte damage is described as ''clearing of the sarcoplasm and nuclei with nuclear enlargement and occasionally prominent nucleoli.''[13] Architectural distortion, myocyte encroachment with irregular myocyte borders, and myocyte dropout also frequently indicate myocyte damage in cellular rejection.

Does Grade 2 Lesion Exist?—One of the criticisms in relying on EMB to monitor rejection is the low interobserver agreement in the diagnosis of grade 2 rejection.[34,35] A corollary to this is the controversy of whether or not grade 2 rejection exists. Recognition of a grade 2 lesion is indeed problematic because of the obvious implications for therapy. Earlier on, most centers treated moderate rejection (grade 2 or higher) with adjustment in the immunosuppressive regimen. It is believed that a major source of confusion in grade 2 rejection is the difficulty in distinguishing the histologic features of this grade from Quilty lesions. Quilty lesions, named after the first patient in whom they were observed at Stanford University, are also known as *endocardial lymphocytic infiltrates*, which we think is a better term (Figure 3, A through D).[36,37] These are collections of predominantly T lymphocytes with admixed B cells, occasional macrophages, and plasma cells seen in the endocardium of transplanted hearts that vary in size from 0.007 to 1.89 mm^2.[38] (The detailed pathology of endocardial lymphocytic infiltrates is discussed under ''Redefinition of the Quilty Effect.'') Small capillaries, sometimes with prominent endothelial cells, and dense endocardial collagen (Figure 3, B) are seen within the infiltrate and are diagnostically useful clues. Quilty infiltrates can extend deep into the subjacent myocardium and the lesion is designated type B in the ISHLT-WF1990 (Figure 3, C and D). Quilty B lesions can be big and may be associated with architectural distortion that does not represent acute rejection. One may imagine how a tangential section through the deeper (myocardial) end of a Quilty B lesion may show inflammatory infiltrates with myocyte encroachment that can easily be mistaken for moderate rejection if only a few levels of section are examined. However, if additional sections are made, one can usually ascertain the continuity of such a lesion from the myocardium to the endocardium. This type of artifact has prompted some observers to question whether or not grade 2 cellular rejection even exists.[39] Our personal experience shows that sectioning through the entire tissue block and examining alternatively stained slides almost always resolves the question (Figure 4).

Another solution offered to this problem is to stain the biopsy section with antibodies to RANTES (regulated on activation, normal T cell expressed and secreted). This is helpful in differentiating a focus of cellular rejection from

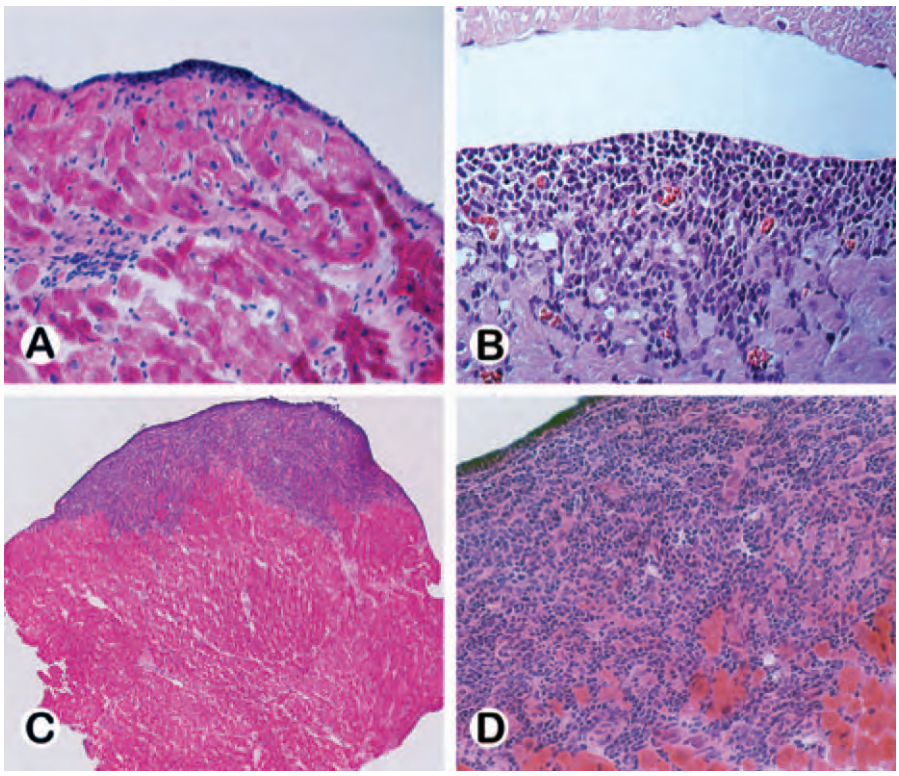


Figure 3. *Endocardial lymphocytic infiltrates (Quilty effect). A, Endocardial lymphocytic infiltrates are confined to the endocardium in this Quilty lesion (frozen section, hematoxylin-eosin, original magnification ×200). B, Another example of a Quilty lesion is shown that extends into the myocardium (invasive Quilty lesion). There are numerous capillaries present within the dense infiltrate. Cytoplasmic vacuoles can be seen in the adjacent myocytes. If this focus is not oriented properly in the biopsy, a tangential cut or a section through the deeper portion of the lesion can easily be misinterpreted as rejection with myocyte damage (hematoxylin-eosin, original magnification ×200). C and D, Large Quilty lesions are frequently seen in biopsies. Proliferation of small blood vessels and fibrous stromal background are typical of these lesions. In contrast, cellular rejection lesions show no fibrosis or small vessel formation during the acute process. In these images, isolated myocytes and small groups of myocytes appear to be entrapped within the lesion. This infiltrative type of Quilty lesion was formerly called Quilty type B in the 1990 International Society of Heart and Lung Transplantation Working Formulation (frozen section, hematoxylin-eosin, original magnifications ×40 [C] and ×200 [D]).*

Quilty B lesions because the RANTES-positive cells are more abundant in acute rejection.[40]

Characterization of the Inflammatory Infiltrate.—In the ISHLT-WF1990, the inflammatory infiltrates are called ''aggressive'' but are not further defined. Pathologists have difficulty in determining what is meant by ''large aggressive lymphocytes.'' This descriptive term is therefore deleted in the current grading system. Immunostains for phenotyping inflammatory cells are not routinely performed for diagnostic or prognostic purposes.

Additional Information to be Included in the Biopsy Report

The following sections show morphologic findings that may be confusing for the novice pathologist in the differential diagnosis of rejection. Some of these features do not represent rejection but need to be clearly recognized. Furthermore, the ISHLT-WF2004 requires that these features be recorded in the report.

Ischemic Injury.—The presence or absence of ischemic damage should always be documented. The ISHLT-WF1990 makes a distinction during allograft monitoring between ischemia commonly seen in the biopsy up to 3 weeks posttransplant representing perioperative injury (ischemia A) and late ischemia that occurs after 3 or more months (ischemia B). In the revised grading system, ischemia is divided into early (up to 6 weeks) and late ischemic injury (Figure 5). Late ischemic injury may explain cardiac allograft dysfunction secondary to severe allograft atherosclerosis.

Perioperative ischemia is seen in a majority of transplanted hearts and is strongly associated with prolonged total ischemic time.[41] Other causes of ischemic injury include events that affect the donor such as catecholamine discharge, pressor therapy given during acute care, severe donor trauma, reimplantation damage, or early postoperative damage. In the early stage, it consists of subendocardial foci of myocytes showing coagulation necrosis (with or without contraction bands) and macrophages with variable amounts of polymorphonuclear leukocytes. These areas are usually sharply demarcated with necrotic myocytes occurring in small groups and highlighted by staining with Masson's trichrome. Some lesions can lack an acute inflammatory reaction (Figure 5, A and B). Ischemic foci may persist for several weeks because of a depressed inflammatory response in these immunosuppressed patients. In the healing phase, these ischemic foci usually show pigment-laden macrophages with a few lymphocytes, a somewhat loose connective tissue stroma, and scant granulation tissue (Figure 5, C). Once they mature, ischemic lesions are indistinguishable from scars produced by previous endomyocardial biopsies (Figure 5, D and E).

Ischemic injury should be differentiated from cellular rejection. The extent of myocyte necrosis is usually out of proportion to the inflammatory infiltrate in ischemic injury, with the infiltrates consisting mostly of neutrophils and macrophages. In cellular rejection, the infiltrates are predominantly lymphocytic. A more difficult distinction to make is between the healing phase of ischemic injury and the resolving phase of moderate rejection in the early posttransplant period. This is usually resolved with clinical correlation and proper communication with the cardiologists.

Most early ischemic injury is clinically silent, but if the injury is extensive, myocyte necrosis can compromise the function of the graft postoperatively. Another possible implication in hearts that had damage during the peritransplant period is the subsequent development of interstitial fibrosis.[42]

Redefinition of the Quilty Effect.—The ISHLT-WF1990

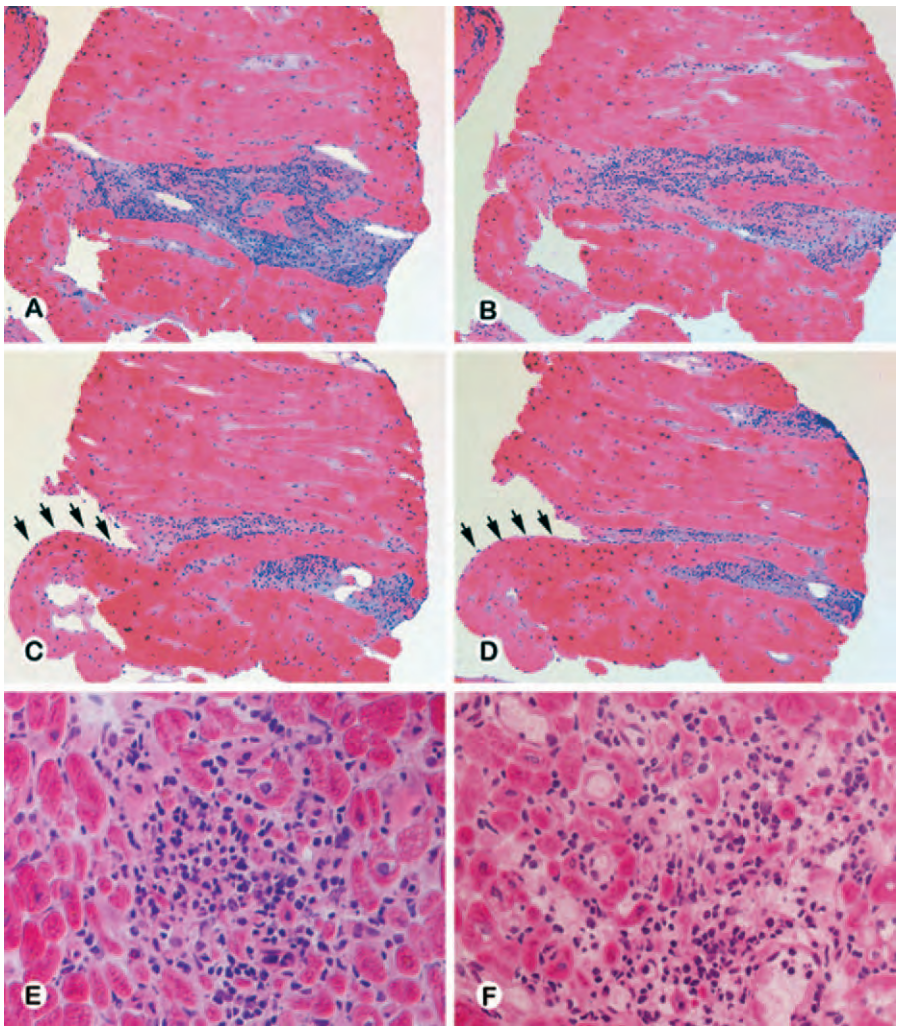


Figure 4. *Endocardial lymphocytic infiltrates versus cellular rejection. A through D, Sequential and deeper sections are helpful in differentiating a focus of rejection with dense inflammatory infiltrates and apparent disruption of the myocytes from a tangential cut of an invasive Quilty lesion. In the first section, a few myocytes are noted on the left side and no endocardial surface is clearly identified around the fragment. Deeper sections show a decrease in the amount of infiltrates. The infiltrate connects to the endocardial surface, which becomes identifiable on the left side in C and D (arrows) (frozen section, hematoxylin-eosin, original magnification ×40). E and F, Differentiation between a Quilty lesion (E) and a focus of rejection with myocyte damage (F) based on a few sections is difficult. Step levels have to be examined. A very useful feature in our observation is the difference in the character of the stroma between the two entities. The stroma in Quilty lesions is fibrotic (frozen section, hematoxylin-eosin, original magnification ×200).*

recommends that the presence or absence of the Quilty effect should be recorded. In the revised grading system, distinction between infiltrates exclusively confined to the endocardium (Quilty A) (Figure 3, A) and those that extend into the underlying myocardium (Quilty B or invasive Quilty) (Figure 3, B through D) is no longer indicated. There appears to be no clinical significance in subtyping Quilty lesions into A and B.[36] Both these lesions are now referred to as the *Quilty effect*. Several hypotheses have been proposed to explain the pathogenesis of these infiltrates and include the use of cyclosporine-based immunosuppression,[43] idiosyncratic responses to cyclosporin A,[37] reduced endocardial levels of cyclosporine A,[44] and concomitant infection with Epstein-Barr virus.[45] None of these have been proven conclusively. One striking observation is that the Quilty effect was not found in the hearts of patients who were also treated with cyclosporin A for other solid-organ transplantation including the liver and kidney.[46] The Quilty lesion seems to be a phenomenon that occurs only in the endocardium of cardiac allografts. Clear and consistent associations of Quilty lesions with grade of cellular rejection, viral infection, subsequent development of vasculopathy, or survival have not been established. As alluded in the section ''Does Grade 2 Lesion Exist?,'' Quilty effect lesions are sometimes misinterpreted by inexperienced pathologists and the diagnosis of rejection is rendered. Serial sections are very useful to differentiate these two lesions, as shown in Figure 4, A through D. Furthermore, the histologic detail of these two lesions is rather distinct. The Quilty lesions usually have extracellular matrix (collagen) between the lymphocytes as these cells are infiltrating the endocardium (Figure 4, E). These lesions frequently show capillaries in the middle of the infiltrate. On the other hand, the rejection lesions that were previously called grade 2 are indeed foci of rejection in which the lymphocytes are attacking the graft and not infiltrating connective tissue. Thus, one does not find collagen bundles surrounding the lymphocytes (Figure 4, F).

Previous Biopsy Site.—A previous biopsy site is a common finding in transplant surveillance biopsies and can be seen in up to 69% of biopsies.[47] This high frequency occurs because, for a given patient, the anatomy of the inflow tract to the right ventricle is constant. During the biopsy procedure using the transjugular approach, the ridges of the atrial or caval anastomotic sites, the right ventricular trabeculations, and the moderator band all contribute to guide the tip of the bioptome toward the same site in the interventricular septum. Figure 6, A through G, illustrates different stages of lesions related to previous biopsy site. Gross examination at autopsy may show a patch of thickened endocardium measuring 1 to 2

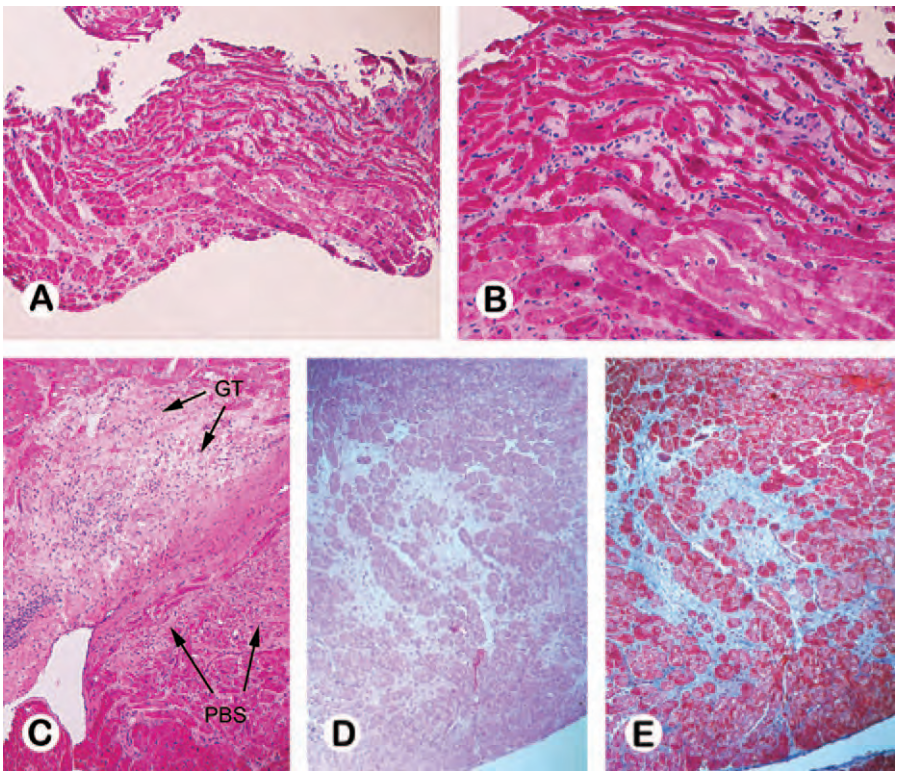


Figure 5. *Acute and healed ischemic injury. A and B, This specimen is the first biopsy taken after transplantation and shows a focus of ischemic myocytes with thin, stretched, and wavy cytoplasm in the upper half of the myocardium. Ischemic myocytes are often found in small groups, subendocardial in location, with absent or pyknotic nuclei and typically hypereosinophilic cytoplasm (frozen section, hematoxylin-eosin, original magnifications ×40 [A] and ×200 [B]). C, Healing ischemic focus with loose granulation tissue (GT) and mild lymphocytic infiltrates in the left upper corner. A previous biopsy site (PBS, also see Figure 7) is also present in the right lower corner (frozen section, hematoxylin-eosin, original magnification ×40). D and E, Interstitial fibrosis and small replacement scars in a transplant biopsy should always raise the suspicion for the presence of allograft vasculopathy (hematoxylin-eosin [D] and Masson trichrome [E], original magnification ×40).*

cm in diameter in the mid third of the right ventricular septum in patients who survived several months to years after the transplant. On light microscopy, the findings of this repetitive sampling of a small area of the septum will include several stages of healing. Recent biopsy sites will show thrombus and granulation tissue (Figure 6, A). Later, there is fibrosis with entrapped myocytes that often exhibit disarray and a variable amount of mononuclear cell infiltrate (Figure 6, E). Old biopsy sites present as endocardial scars (Figure 6, F and G).

Lymphoid Neoplasia.—Posttransplant lymphoproliferative disease has been reported to occur in 1.2% to 9% of cardiac transplant patients, more commonly within the first year of transplantation.[48–50] Recent studies are lacking, and this diagnosis is indeed rare in large-volume centers, perhaps as a result of modern immunosuppression regimens. Identified risk factors for the development of lymphoid neoplasms in these patients are infection with Epstein-Barr virus and type of immunosuppressive regimen received, particularly OKT3.[50–53] Histology of lymphoid neoplasia can range from polymorphic lymphoid hyperplasia to monomorphic malignant lymphomas.[54] Posttransplant lymphoproliferative disease can be diagnosed in the transplant biopsy and should be distinguished from that of acute rejection because early diagnosis and reduction of immunosuppression may lead to regression.[55] Molecular studies can be performed using allograft biopsy material to confirm the diagnosis, including DNA analysis for immunoglobulin gene rearrangement and detection of Epstein-Barr virus genome by in situ hybridization or polymerase chain reaction.[48,56–59] The majority of posttransplant lymphoproliferative diseases seen today are malignant lymphomas of B-cell origin. Their clinical presentation, in decreasing order of frequency, involves lymph nodes, lung, gastrointestinal tract, liver, central nervous system, spleen, and the heart itself.[60] T-cell lymphomas also occur and usually present in extranodal sites.[61–63] Development of multiple myeloma after cardiac transplantation is rare.[64,65]

Opportunistic Infections.—Chronic immunosuppressive therapy to control rejection predisposes transplant patients to a large number of opportunistic infections. Bacterial infection is the most common type of infection, accounting for 47% of the cases. Viral infections are second in frequency (41%), with fungal and protozoal pathogens being responsible for the remaining 12%.[66,67] Identification of infectious pathogens in cardiac biopsy is rare. The two most commonly reported opportunistic infections seen in EMB specimens are *Toxoplasma* and cytomegalovirus (Figure 7, A and B, respectively).[68–70] When examining a biopsy, unusual inflammatory infiltrates such as the presence of granulocytes, plasma cells, and/or macrophages in a focus of inflammation without overt myocyte necrosis or dropout should alert the pathologist to consider a possible infectious process. One should also look for viral inclusions in the nuclei of endothelial cells, smooth muscle cells, or miscellaneous perivascular cells. Cytomegalic inclusions within cardiac myocytes are extremely rare. Both infections can also be associated with a paucity of inflammatory infiltrates and can therefore be easily overlooked. Figure 7, C and D, show examples of fungal infections.

Fibrosis.—Development of interstitial fibrosis in the transplanted heart has been associated with cyclosporine therapy, total ischemic time, rejection episodes, and donor cause of death.[42,71–75] Other investigators, however, did not find a significant association between increase in myocardial collagen and prolonged ischemic time or cyclosporine immunosuppression.[76] The perception of the amount of fibrosis in endomyocardial biopsies may be influenced by the size of the bioptome used; larger pieces of biopsy fragments appear to have lesser quantitated area of fibrosis.[77] Perimyocytic fibrosis is seen most often in areas adjacent

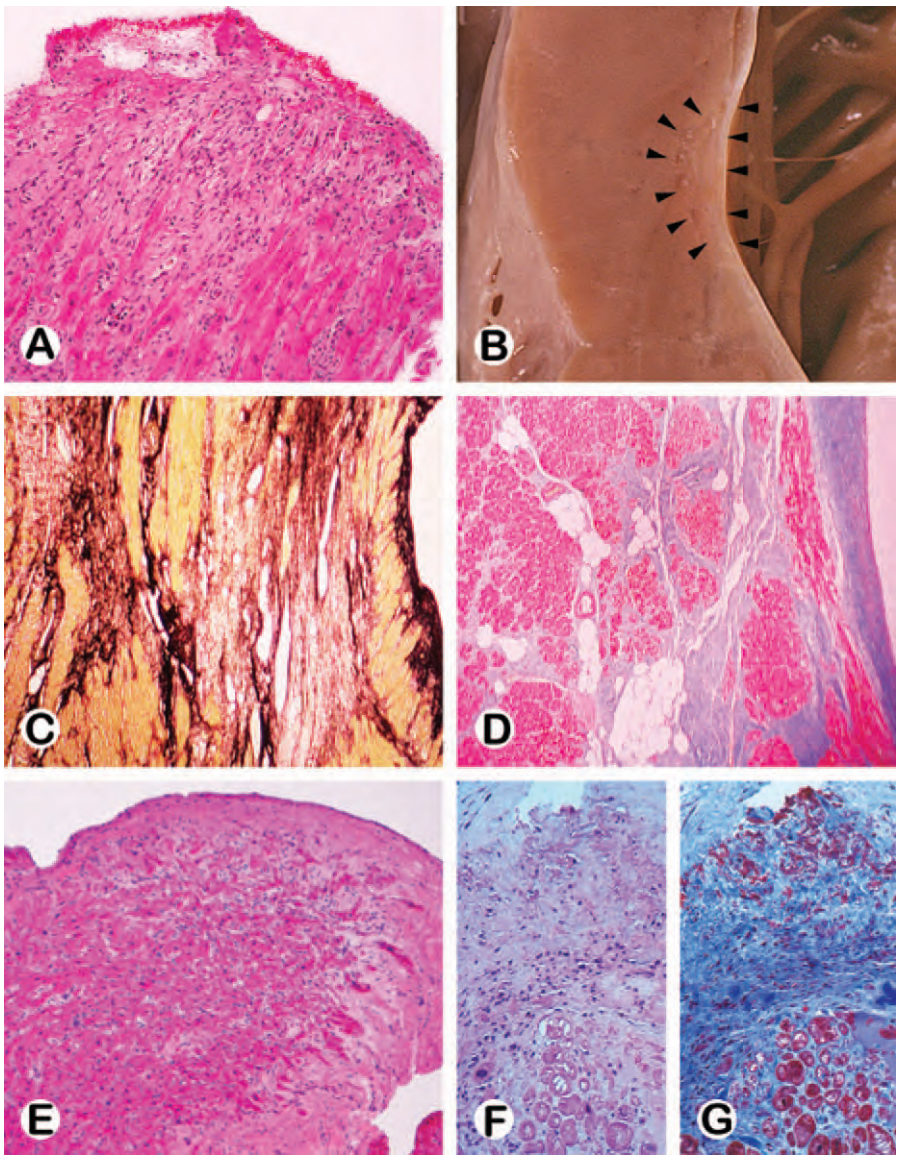


Figure 6. *Previous biopsy sites versus rejection or ischemic injury. A, A recent biopsy site that is about 1 week old and composed of granulation tissue with chronic inflammation is shown. There is a microscopic fibrin clot occurring on the superficial aspect of the biopsy site. Inflammation in biopsy sites or scar is not considered in the evaluation of rejection grade (hematoxylin-eosin, original magnification ×200). B, A slightly depressed, crescent-shaped, scarred myocardium along the septal wall (arrowheads) indicates the site of previous biopsies in the heart of this transplant patient. C and D, Connective tissue stains reveal endocardial fibroelastosis and interstitial fibrosis in the myocardium adjacent to the biopsy site (C, elastic stain, original magnification ×20; D, Masson trichrome, original magnification ×20). E, A healed previous biopsy site shows endocardial thickening with subendocardial fibrosis. Entrapped myocytes show disarray. Variable amount of inflammatory cells can be present (frozen section, hematoxylin-eosin, original magnification ×40). F and G, An old biopsy site with thick fibrotic endocardium is illustrated. Some of the subendocardial myocytes also show colliquative myocytolysis. Previous biopsy sites are common findings in endomyocardial biopsy specimens as the same areas are repeatedly sampled (hematoxylin-eosin [F] and Masson trichrome [G], original magnification ×200).*

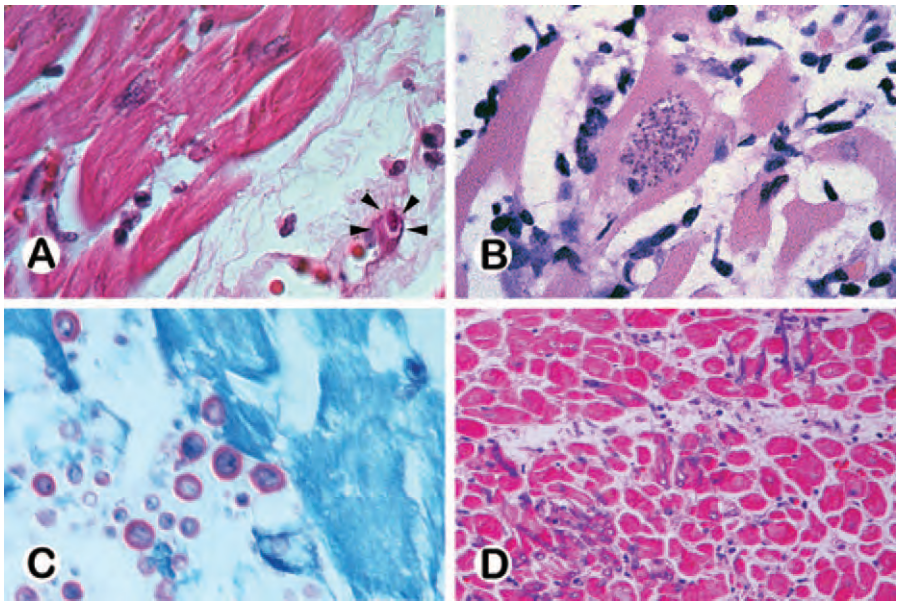


Figure 7. *Opportunistic infections. A, Nuclear inclusion with cytomegaly is noted in an endothelial cell (arrowheads) in a case of cytomegalovirus infection (hematoxylin-eosin, original magnification ×1000). B,* Toxoplasma *bradyzoites are evident as small basophilic structures within the sarcoplasm of a myocyte. Scant lymphocytic infiltrates are present in the interstitium in this image. However, polymorphonuclear leukocytes can also be present (hematoxylin-eosin, original magnification ×1000). C, Variably sized round yeast forms of* Cryptococcus *are present without inflammatory reaction in the myocardium of a posttransplant patient who died of overwhelming infection (mucicarmine, original magnification ×1000). D, Septated fungal hyphal elements invading the myocardium are demonstrated in an autopsy case of invasive* Aspergillosis *(hematoxylin-eosin, original magnification ×200).*

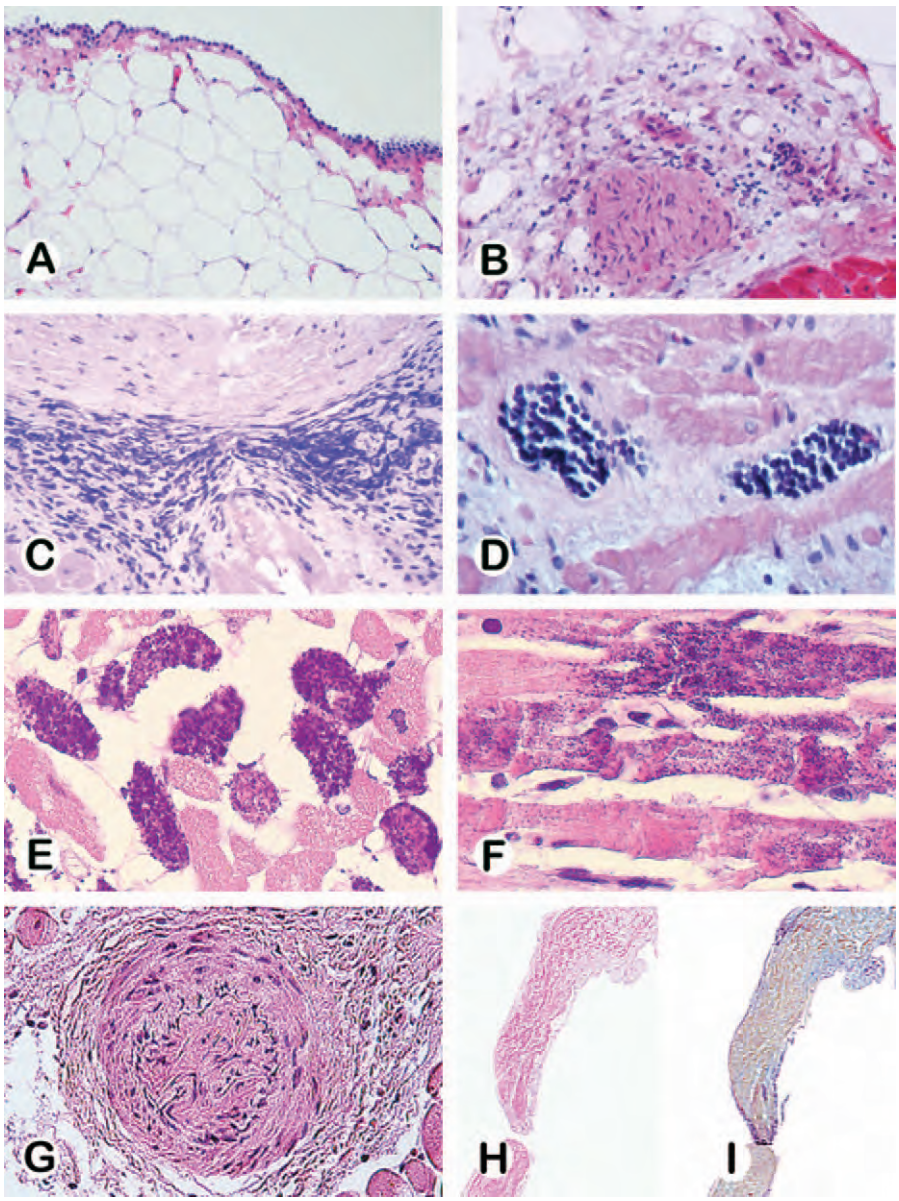


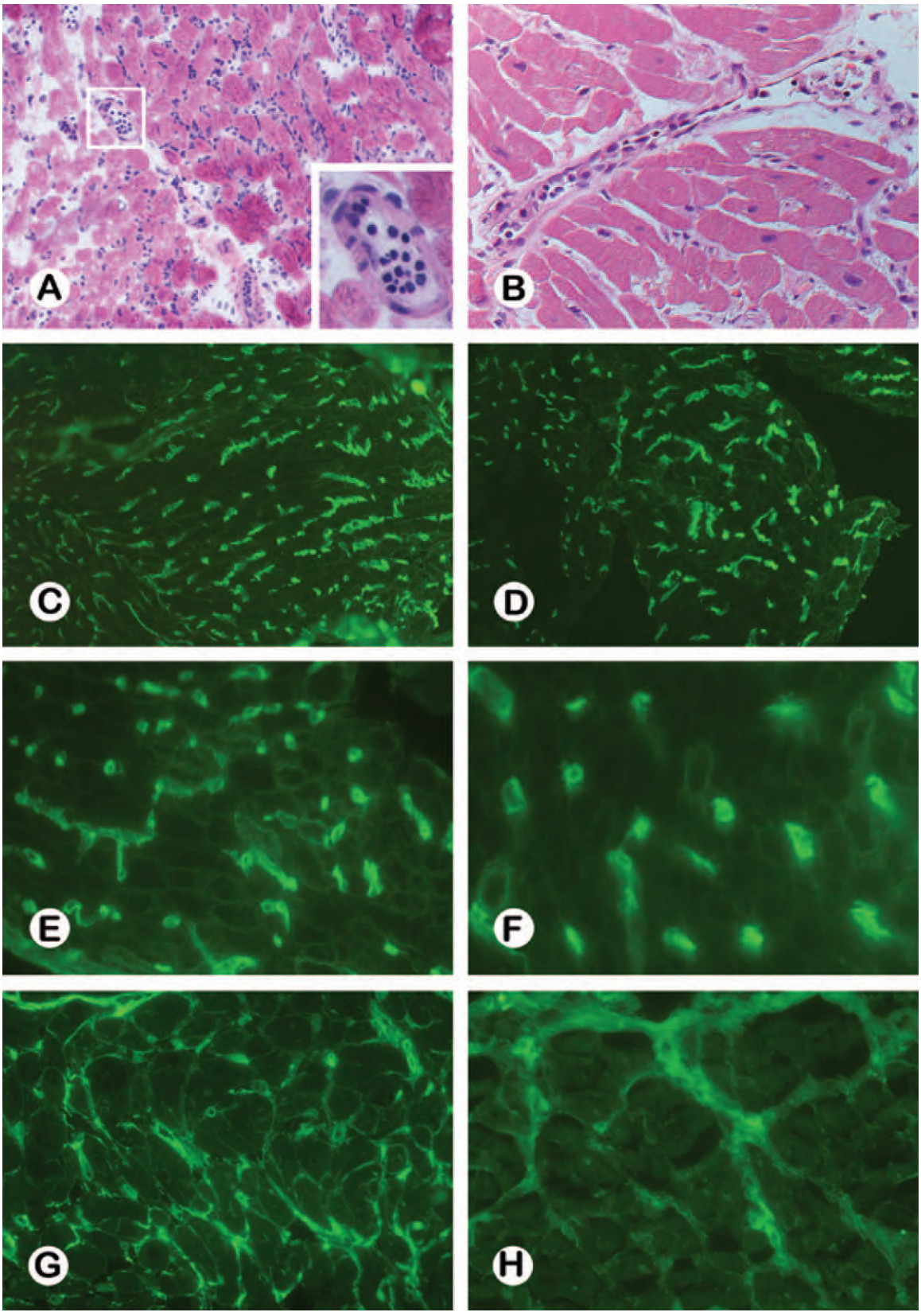
Figure 8. *Interpretation of other findings and artifacts in heart biopsies. A, The presence of mesothelial lining overlying adipose tissue in endomyocardial biopsies is indicative of perforation of the ventricular wall. Mesothelial lining is present in the visceral layer (also called* epicardium*) and parietal layers of the pericardium (hematoxylin-eosin [H&E], original magnification ×100). B, One fragment of epicardial fat with a small nerve bundle and scant inflammation but absent mesothelial lining is noted in a transplant surveillance biopsy. Inflammation in the epicardial fat is commonly seen early in the postoperative period, but in the absence of mesothelial cells, one cannot conclude that this represents a perforation of the ventricular wall (frozen section, H&E, original magnification ×100). C, Crush artifact may be so extensive as to render a piece of myocardium difficult to interpret (H&E, original magnification ×200). D, Occasional lymphatic vessels are seen in biopsies that are distended with lymphocytes. Note that the endothelial cells are not swollen or prominent. This is an infrequent finding. The International Society for Heart and Lung Transplantation Working Formulation does not provide guidelines to interpret this finding (H&E, original magnification ×400). E and F, Mitochondrial calcification appears as basophilic granules in these necrotic myocytes cut in cross and longitudinal sections. Eventually, these myocytes become completely calcified (H&E, original magnification ×400). G, Telescoping (intussusception) within the lumen of this small artery can be confused with luminal occlusion. Note the presence of elastic lamina within the smooth muscle cells that fill up the lumen of the artery (H&E, original magnification ×400). H and I, Chordae tendineae can occasionally be seen in specimens (Figure 1, F) and are characterized by parallel arrays of dense collagen fibers covered by thin endocardium in all their surfaces (H&E [H] and Movat pentachrome [I], original magnification ×100).*

to previous biopsy sites. A causal relationship between interstitial fibrosis and diastolic dysfunction in the cardiac allograft is still uncertain.[78] Furthermore, fibrosis may in fact be a feature already present in a donor heart. Low availability of hearts has led cardiologists and surgeons to the practice of accepting hearts from older donors, which, in some instances, show interstitial and/or replacement fibrosis despite having ''no history'' of coronary artery disease.

Adipose Tissue, Perforation Versus Infiltration.—Adipocytes are normal cellular components of the heart, mostly present in the epicardium. In addition, microscopic foci of adipose tissue are usually present in the subendocardium and, less frequently, within the myocardium.

→

Figure 9. *Antibody-mediated rejection (AMR). A and B, On visible light microscopy, this sample shows a striking low magnification feature, which is the presence of conspicuous endothelial cell nuclei in the interstitial compartment. The small arterioles also appear to be filled with cells. The inset shows mononuclear inflammatory cells within the lumen of an arteriole (A, frozen section, hematoxylin-eosin, original magnifications ×200 and ×400 [inset]; B, hematoxylin-eosin, original magnification ×400). C and E, C4d staining shows intense linear deposits in capillaries that are mostly oriented longitudinally in this frozen section (C, fluorescein isothiocyanate [FITC] anti-C4d, original magnification ×100). Higher magnification demonstrates cross sections of the capillaries (E, FITC anti-C4d, original magnification ×400). D and F, The same biopsy also shows an identical pattern of intense deposits in capillary endothelium with anti-C3d (FITC anti-C3d, original magnifications ×100 [D] and ×400 [F]). G, Repeat biopsy of the same patient after 1 week shows persistent but weaker staining in capillaries. In addition, the biopsy now shows linear staining around myocytes, indicating that the complement split products (C4d and/or C3d) are redistributed to the interstitium. This type of pattern is commonly seen in resolving AMR and after therapy with plasmapheresis (FITC anti-C4d, original magnification ×200). H, After resolution of AMR, a biopsy of the same patient shows artifactual staining in the perimysial collagen. There is no staining in vascular endothelium. The biopsy is now negative for AMR. This staining pattern can persist for several weeks (FITC anti-C4d, original magnification ×200).*


A
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These foci can be seen in all chambers but are more commonly found in the right ventricular wall. In obese patients, older patients, and patients taking steroid hormones, fat infiltration is more common and can be grossly visible. Thus, the presence of adipose tissue per se is not pathologic. The goal of the right ventricular biopsy procedure is to obtain samples from the right side of the interventricular septum; however, on rare occasions, the bioptome may actually sample the right ventricular free wall. Therefore, when a focus of adipose tissue is found in an EMB, the pathologist should make an effort to determine if this is subendocardial or subepicardial adipose tissue. This can sometimes be easily determined by looking for the presence of mesothelial cell lining, indicating the epicardial surface (Figure 8, A). Because of the fibrinous and eventually fibrous pericarditis that usually develops after the transplant, it may be difficult to find mesothelial cells; in the latter case, the presence of nerves and ganglion cells or inflammation in the fat is suggestive of epicardial location (Figure 8, B). In time, the organized pericarditis usually forms a dense, fibrous, protective layer around the myocardium that prevents the development of tamponade if there is perforation. In one study, the presence of adipose tissue was reported to occur in 4.62% of transplant biopsies.[79] There is also some tendency to see fat deposits in areas of previous biopsy site or foci of healing ischemic damage. Whether the use of steroids for the treatment of rejection increases the amount of adipose tissue in the subendocardium is not known.

Nonrejection Lymphocytic Infiltrates.—Lymphocytes from Quilty lesions can be trapped in previous biopsy sites and then are crushed during subsequent biopsies (Figure 8, C). In other instances, lymphocyte clusters can be seen in postcapillary venules that become engorged with lymphocytes as these prepare to migrate into the interstitial space of the graft (Figure 8, D).

Dystrophic Calcification.—There have been reports of various forms of calcification in the heart after transplantation. In some patients, evidence of calcification has been shown histologically in biopsy tissue and radiographically in the native atria.[80,81] In our experience, it is also uncommon to see dystrophic calcification of the ventricular myocardium in biopsies. Calcium deposition within mitochondria is known to occur during ischemia and catecholamine-induced myocardial injury. In the posttransplant patients, a relationship between calcification and cyclosporine therapy has been suggested.[82] In some cases, several episodes of rejection requiring therapy, temporary uremia, and septicemia appear to be associated with the development of dystrophic calcification.[80] On light microscopy, the dystrophic calcification of the mitochondria is easily recognized as dark blue granular material in the cytoplasm of myocytes, ranging from 1 to 2.5 μm in diameter (Figure 8, E and F). The granules may be seen in perinuclear location and in between the myofibers. When they are abundant, they follow the contour of the whole myocyte. Dystrophic calcification is usually found in the subendocardium, affecting single myocytes or small groups of myocytes.

"Telescoping" or Intussusception of Small Arteries.—When a small muscular artery is sampled by the bioptome, telescoping or intussusception occurs. Just before the jaws of the bioptome completely cut through the tissue, the small artery is stretched and then recoils into its own lumen as soon as it is severed. This can give the appearance of an occluded vessel or a small artery with vasculopathy. The birefringent internal elastic lamina within the lumen can be recognized easily on closer examination of small arteries (Figure 8, G).

Chordae tendineae and valvular tissue.—Fragments of chordae tendineae are occasionally seen in the biopsy specimen and should be described in the report when present (Figure 8, H and I). Chordae to the tricuspid valve can arise from the septum and thus can be entrapped, torn, or biopsied during the procurement of tissue. Chordal rupture may or may not result in clinically significant tricuspid regurgitation.[14,83–85]

Procedural Artifacts

Procedural artifacts are common and should be recognized in the interpretation of the endomyocardial biopsy.[86] *Contraction bands* are a very common artifact seen in transplant and nontransplant heart biopsies. Several factors may influence the presence of contraction bands in the biopsy. It may be the result of trauma to the myocardium induced when the bioptome cuts the tissue. It may also be induced by poor osmolarity of the medium in which the biopsy is placed before and during fixation, as well as the cool temperature of the medium. We rarely observe contraction bands in frozen sections. Because of the high likelihood of finding contraction bands, they should never be the only criterion used to make a diagnosis of myocyte necrosis or ischemic damage in heart transplant biopsies. *Pinching or forceps artifact* represents mechanical distortion of the tissue induced by the bioptome itself during extraction. It can also be induced during processing of the tissue in the pathology laboratory. An effort should be made to handle biopsy tissue with care because this artifactual deformation may render the specimen uninterpretable. *Foreign bodies* introduced at the time of the transplant, such as gelatin foam, occasionally can be seen. At other times, actual sampling of fragments of indwelling catheters or the soft plastic cover of pacemaker leads may occur. *Pseudohemorrhage* occurs when red blood cells are embedded into the tissue by the pressure of the bioptome on the myocardium being sampled. This produces artifactual pools that mimic hemorrhage. They are usually not accompanied by inflammatory cells or pathologic changes in the myocytes, thus making the distinction between artifact and rejection fairly easy.

ANTIBODY-MEDIATED REJECTION

Transplants are capable of eliciting strong cellular and humoral immune responses. Antibody-mediated rejection (AMR) is an immunopathologic process in which injury to the graft is, in part, the result of activation of the complement system. This was first recognized in kidney transplantation as a distinct clinicopathologic entity characterized by acute allograft rejection associated with the production of antidonor reactive antibodies and poor prognosis.[87] It is poorly responsive to conventional immunosuppression, which targets the cellular arm of the immune response. Old terminology such as *vascular rejection, microvascular rejection,* and *humoral rejection* should be avoided as it has only led to confusion in the literature. The preferred terminology in the ISHLT-WF2004 is *AMR.*

Risk factors for developing AMR include pregnancy, previous transplantation, blood transfusions, sensitization by OKT3 induction therapy, use of ventricular assist devices, presence of positive B-cell flow cytometry cross-

match, and elevated panel-reactive antibodies.[13,88,89] The long-term outcome of AMR is not yet fully established in heart transplantation but it has been associated with the development of cardiac allograft vasculopathy (CAV) and with decreased survival.[90,91]

A detailed pathologic classification of ''humoral rejection'' in biopsies was not well defined in the ISHLT-WF1990. Consequently, the true incidence of AMR is unknown and recognition of AMR as a real entity was not widely accepted for several reasons. There was no uniform set of diagnostic criteria provided to guide different transplant programs in the detection of this entity. The antibodies used in evaluation of immunofluorescence changed over time. Positive immunofluorescence with the markers suggested then (IgG, IgM, C3, C1q, and fibrinogen) did not always correlate with hemodynamic compromise or incidence of CAV, which resulted in decreased usefulness of this test.[92] Lastly, it was believed that most AMR occurs early and the ISHLT-WF1990 recommends AMR monitoring by immunofluorescence on all biopsies up to 6 weeks posttransplant only. This is clearly incorrect, as it is now known that AMR can and most commonly does occur months and even years after transplantation.

Diagnostic Criteria

The histologic features that allow for the identification of this type of rejection on endomyocardial biopsies as defined in the ISHLT-WF2004 and its companion article on AMR include: ''capillary endothelial changes (swelling or denudation with congestion), macrophages in capillaries [Figure 9, A and B], neutrophils in capillaries, interstitial edema and/or hemorrhage and fibrin in vessels.'' [93] If these features are observed in the biopsy and there is unexplained cardiac dysfunction, the revised working formulation proposed that immunofluorescence or immunohistochemistry, in the absence of frozen tissue, be performed. Immunopathologic evidence of AMR include[13]

''—Immunoglobulin (IgG, IgM and/or IgA) plus complement deposition (C3d, C4d and/or C1q) in capillaries by immunofluorescence on frozen sections; and/or
—CD68 staining of macrophages within capillaries (CD31- or CD34-positive) by immunohistochemistry; and
—C4d staining of capillaries by paraffin immunohistochemistry.''

Examples of the capillary pattern of complement deposition are shown in Figure 9, C through F.

It is also recommended that these patients undergo assessment for circulating antibodies to HLA class I or II as well as non-HLA donor antigens. An EMB with no histologic or immunopathologic evidence of AMR is graded 0 (AMR 0). If the immunofluorescence or immunohistochemical staining supports the histologic features of AMR, the biopsy is considered positive (AMR 1).

Mixed Acute Cellular and AMR

Although most AMRs are associated with absent, or at most mild, acute cellular rejection, mixed rejections have also been reported that carry a significant risk of mortality.[94,95] Mixed rejections usually occur early in the course of transplantation and are also associated with allograft dysfunction.

Practical Issues in the Diagnosis of AMR

Histologic Features of AMR.—The ISHLT-WF2004 recommends that if histologic features suggestive of AMR are not seen, no further testing (immunofluorescence or immunohistochemical) needs to be pursued. However, a recent report[96] describes that the sensitivity of histologic criteria (ie, light microscopic features such as endothelial cell swelling, intravascular macrophages, edema, and hemorrhage) is too low to serve as screening parameters for AMR. The authors thus recommend the addition of immunostaining to screen for the presence of AMR.

Diagnostic Considerations of Complement Split Products.—Immunofluorescence methods for detection of AMR in tissues have evolved in the last decade. Some complement components, specifically C3d and C4d, are found to be more readily detected than antibodies and serve as very sensitive markers of rejection in endomyocardial biopsies for several reasons.[97] Antibodies bind to antigens with different avidity and either dissociate at varying rates or are eliminated by shedding or internalization. In contrast, the process of complement activation yields split products of C4 and C3 that bind to the tissue where complement was activated. This increases the sensitivity of complement detection by prolonging their half-lives. Among the components of the complement system, C3 is present in the highest concentration, followed by C4; therefore, their split products are also deposited in tissues in the largest quantities (Figure 10).[98] Furthermore, the amplification steps in the complement cascade results in the generation of more C3 split products.[99]

Although complement is activated through antibody in the classic pathway, one must remember that complement can also be activated during procedures such as extracorporeal circulation during surgery,[100,101] by ischemia/reperfusion injury,[102] and by induction therapy before transplant with antithymocyte globulin.[103] Thus, the mere presence of C4d and/or C3d in capillaries should not be equated with AMR.

In our experience, the use of C4d immunostaining alone is not a reliable tool. Instead, evaluation of endomyocardial biopsies for AMR should include staining for both C4d and C3d (Figure 9). A recent prospective study of heart transplant patients evaluated the usefulness of IgG, IgM, IgA, C1q, C4d, and C3d as markers for the diagnosis of AMR.[104] In this study, the authors performed routine staining of all biopsies for these five markers. These authors' institution reported 3% incidence of AMR in 165 nonsensitized patients. Immunoglobulin G, IgM, IgA, and C1q did not prove to be useful in discriminating patients with AMR. Conversely, the usefulness of C4d and C3d was confirmed. Immunostaining for C4d alone can be misleading because about 10% of the patients showed either C4d or C3d deposits alone in capillaries without clinical evidence of dysfunction of the allograft. Within the study period of 3 years, some patients demonstrated persistent activation of complement with C4d deposition over time without the development of allograft dysfunction. Another important observation made was that AMR occurred many months to years after transplantation in most patients. This study showed that the diagnosis of AMR must be a correlative diagnosis in which pathologic and clinical criteria play a role.

Discrepancy Between Pathology and Clinical Presentation.—Activation of the complement cascade detected

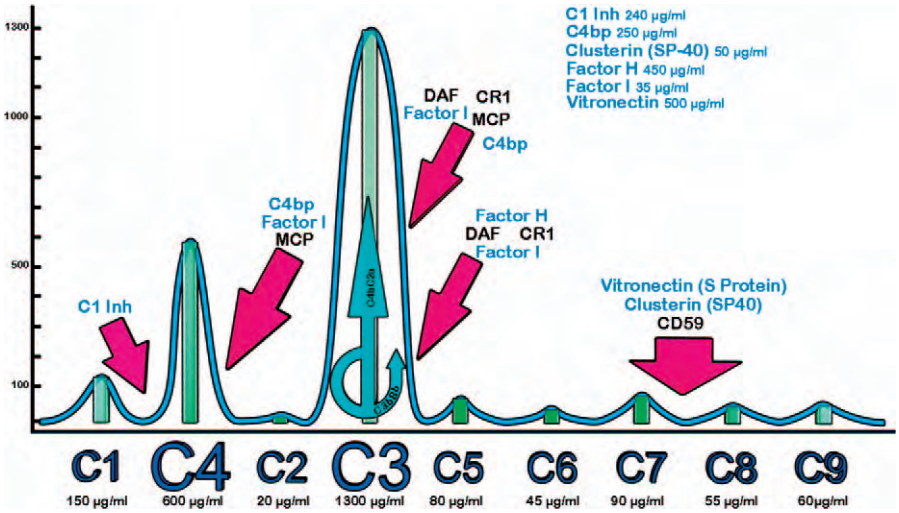

Figure 10. *Serum concentration of complement components and regulators of complement activation. This figure shows the serum concentration of the different complement factors in micrometers per milliliter. The activation of C3 is critical as it augments both cellular and humoral immune response. C3 is enzymatically cleaved and activated by C4b2a of the classic pathway and C3bBb through an amplification loop of the alternative pathway. Its activation is an important amplification step because C3 is present in a larger molar amount and, once activated, it can further increase the activation of the rest of the cascade. Regulators of complement activation (RCA) are composed of both plasma (blue letters) and membrane (black letters) proteins that inhibit the proteolytic subunits of classical and alternative pathways, thereby preventing the progression of the complement pathway to the membrane attack complex (MAC) formation. MCP indicates membrane cofactor protein; DAF, decay accelerating factor; CR1, complement receptor 1; C1 Inh, C1 inhibitor; and C4bp, C4 binding protein.*

by immunostains for C4d and/or C3d is not always accompanied by dysfunction of the graft. Some authors have referred to this apparent lack of graft injury despite evidence of complement activation as ''accommodation'' in animal models[105] and in ABO-incompatible renal transplants.[106] One possible explanation is that complement activation is interrupted by a protective mechanism in the host. This suggests that unless the complement cascade proceeds to the formation of the membrane attack complex, there is no expected injury to the allograft. This complex is needed to form a ''pore'' that leads to loss of integrity of the cell membrane. In humans, it is well known that there are regulators of complement activation that can prevent the completion of the complement cascade at different stages of activation.

Regulators of complement activation exert their effects at different points in the complement activation cascade, whether the activation occurs through the classic, alternate, or mannose binding lectin pathways. All these pathways converge at the point of generation of the enzymatic complexes known as the *C3 convertases*, which, in turn, proceed to activate the remaining complement components required for the formation of the membrane attack complex. There are two main types of proteins that can regulate the activation of complement. These can be divided into the membrane-bound and soluble types. In humans, the membrane-bound regulators are CD35 or complement receptor 1, CD46 or membrane cofactor protein, CD55 or decay-accelerating factor, CD59 or protectin, and C8-binding protein or homologous restriction factor.[107,108] The soluble factors include the C1 inhibitor, C4 binding protein (C4bp), factor I, factor H, clusterin, and S protein (vitronectin). Their points of action are shown in Figure 10.

There is little information about the expression of these regulators of complement activation molecules in human heart transplantation. A recent abstracted study shows that decay-accelerating factor or CD55 is expressed locally in the myocardium in heart transplant patients. In this study, a group of patients with complement deposition in endomyocardial biopsies was examined. The biopsies were stained by immunofluorescence for C4d, C3d, and decay-accelerating factor (Figure 11, A through F). There were 2 subgroups identified on the basis of present or absent allograft dysfunction. All patients had biopsy-proven C4d (Figure 11, A and C) and C3d (Figure 11, B and D) deposits. Patients with good response to therapy and resolution of the AMR episode showed intense tissue expression of CD55 in the endothelium of the allograft (Figure 11, E and F). Patients with poor outcome had low or absent tissue expression of CD55. Thus, the local expression of decay-accelerating factor correlates with absence of allograft dysfunction in spite of C4d and C3d deposition in capillaries.[109] In the same study, there was no evidence of detectable CD35, CD46, or CD59 in the biopsy tissue of this cohort of patients. At this juncture, there are no studies published that address the role of the soluble regulators of complement activation in human heart transplantation.

Complement Staining Artifacts.—Common artifactual staining seen in immunofluorescence microscopy of transplant biopsy includes autofluorescent lipofuscin deposits (Figure 12, A), nonspecific binding to collagen in the interstitium (Figure 12, B), and to the internal elastic lamina of arteries (Figure 12, C). Necrotic myocytes likewise bind complement (Figure 12, D).

CARDIAC ALLOGRAFT VASCULOPATHY

Currently, the most challenging problem in attaining a long-term successful outcome in cardiac transplantation is the development of CAV (Figure 13, A through E), also known as graft coronary artery disease, graft coronary vascular disease, transplant coronary artery disease, accelerated graft arteriosclerosis, and chronic rejection. This problem is not unique to the heart; it occurs in other solid organ grafts in a somewhat similar manner.[110,111] Cardiac allograft vasculopathy develops in a majority of transplanted hearts at a variable rate, sometimes as early as 3 months after transplantation.[112] According to the most recent ISHLT registries, only 47% of adults are free of CAV as detected by angiography at 9.5 years; in children, the incidence is much lower compared with adults, with 75%

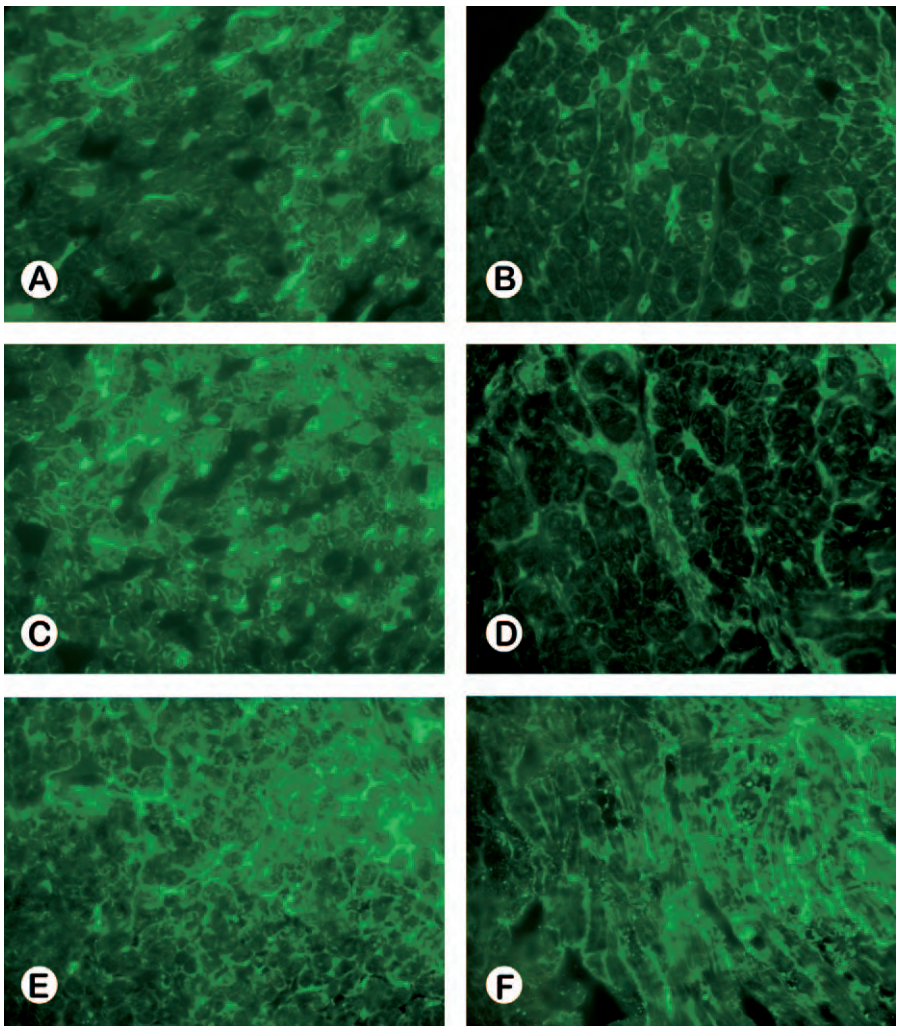

Figure 11. *Immunofluorescence detection of regulators of complement activation (RCA). Injury to the graft can be limited by regulating complement activation in the tissue. This panel illustrates a female patient who presented with hemodynamic compromise at 154 weeks posttransplant with evidence of capillary staining with C4d (A) and C3d (C). Two weeks after therapy (B and D), there was rapid recovery with normal ejection fraction on echocardiography. C4d remained positive with linear perimyocytic staining (B) while C3d shows rare positive capillaries and nonspecific interstitial staining (D). Staining for the membrane-bound RCA CD55, also known as decay accelerating factor (DAF), shows focal granular staining with DAF along capillaries at 154 weeks (E). Clinical improvement is accompanied by increased number of capillaries expressing DAF at 156 weeks (F) (fluorescein isothiocyanate, original magnification ×400).*

of patients free of CAV at 7 years posttransplant.[113,114] Most patients cannot experience typical angina associated with myocardial infarction or ischemia because of denervation of the donor heart. Therefore, CAV commonly presents clinically as congestive heart failure, ventricular arrhythmias, and sudden death. Risk factors for developing early CAV (occurring within 3 years posttransplant) include donor hypertension, infection within 2 weeks posttransplant requiring intravenous antibiotics, and rejection during the first year.[1,2,115] Risk factors associated with late CAV (occurring within 7 years posttransplant) include donor history of diabetes and intracranial hemorrhage as donor cause of death. Independent continuous risk factors for both early and late CAV are donor age, recipient age (inverse relationship), center volume, and recipient pretransplant body mass index.[1,116] Female donors are associated with a lower risk.[1]

The events leading to this type of vasculopathy are complex and interdependent. The mechanisms can be divided into immunologic and nonimmunologic. Endothelial cells express major histocompatibility complex class I and class II antigens, and thus appear to be primary targets of cell-mediated and humoral immune response.[117–119] Activated T lymphocytes secrete cytokines (interleukins, interferons, and tumor necrosis factors), which promote proliferation of alloreactive T cells, activate monocytes and macrophages, and stimulate expression of adhesion molecules by endothelial cells.[120] Macrophages are then recruited to the intima where they elaborate cytokines and growth factors, leading to smooth muscle cell proliferation and synthesis of extracellular matrix.[121] The role of humoral immune response in CAV relates to the antibody production against HLA and endothelial cell antigens.[122–125] The relationship between acute cellular rejection, histocompatibility mismatch, and development of CAV remains controversial.[126–132] Endothelial cell dysfunction resulting from sustained inflammatory injury also predisposes to thrombosis, vasoconstriction, and vascular smooth muscle proliferation.[133–135]

Some of the nonimmune factors that have been associated with the development and progression of CAV include myocardial ischemia,[136,137] donor-transmitted coronary atherosclerosis,[138,139] cytomegalovirus status,[140–143] lipid profile,[144,145] arteritis,[146] deficient fibrinolysis,[147,148] hormonal milieu,[149] and immunosuppressive therapy.[150–152] Excellent reviews of the pathobiology of vasculopathy have been written.[110,153–155]

Allograft vasculopathy involves both epicardial and intramural coronary arteries. The whole length of the coronary vessels is usually affected. Formation of collateral vessels is lacking. In some patients, the pathology predominantly involves only the small intramyocardial branches.[156] In these cases, early diagnosis is limited by

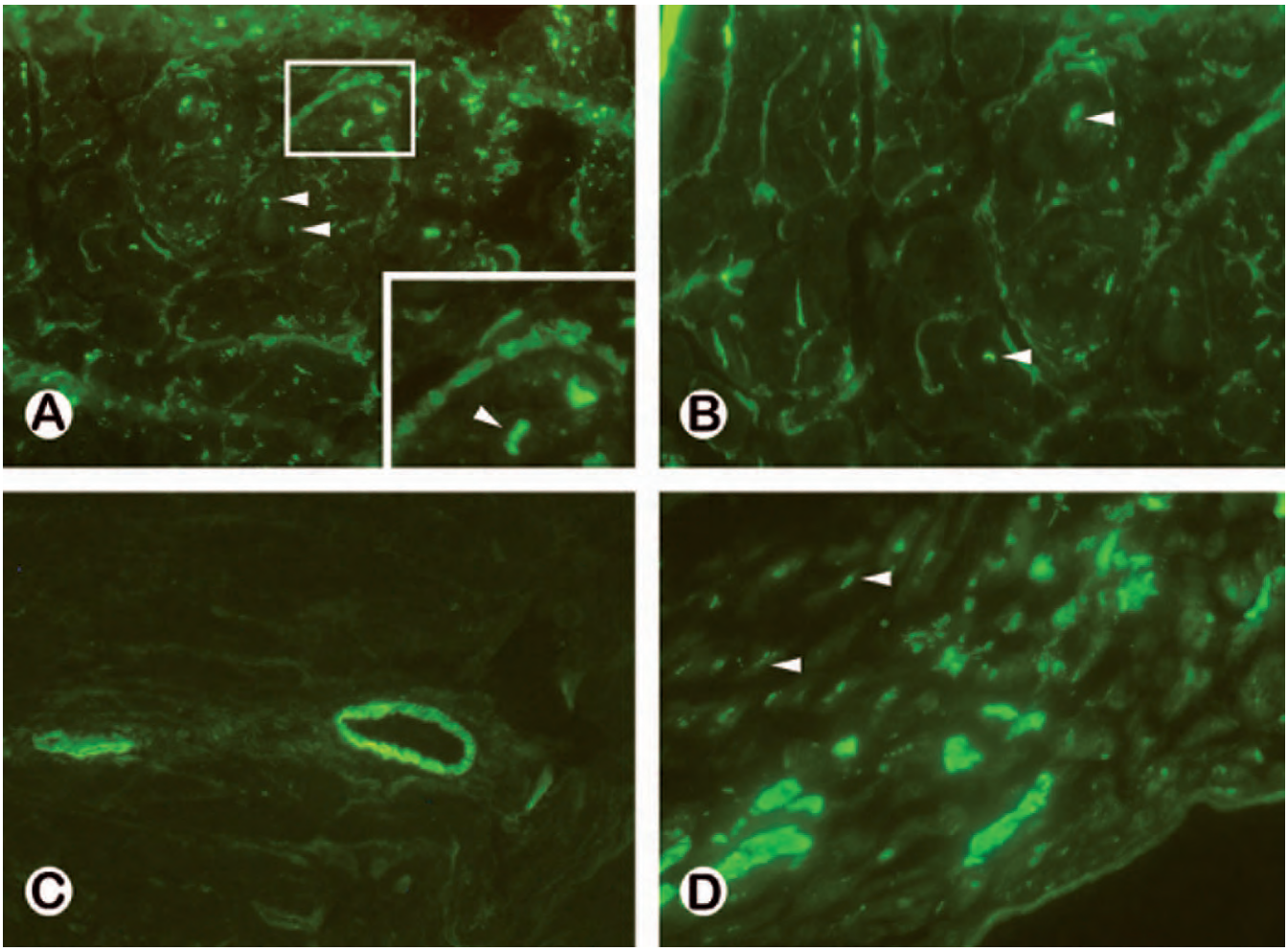

Figure 12. *Artifacts in immunofluorescence microscopy. A, Lipofuscin pigments (arrowheads) appear as irregularly shaped autofluorescent coarse granules that are distributed within the myocytes (fluorescein isothiocyanate [FITC] anti-C4d, original magnifications ×200 and ×400 [inset]). Lipofuscin is also evident in two other parts in this figure (B and D). B, Interstitial collagen can be artifactually stained with complement in some patients and appear as interrupted linear to squiggly strands around myocytes with uneven staining intensity (FITC anti-C4d, original magnification ×400). C, The internal elastic lamina of small arteries stains positive with complement (FITC anti-C4d, original magnification ×200). D, Necrotic myocytes are easily identified in immunofluorescence microscopy. They are artifactually stained intensely with complement and to a lesser extent with immunoglobulin G because of nonspecific absorption (FITC anti-C4d, original magnification ×200).*

inaccessibility of distal lesions to evaluation by coronary angiography or intravascular ultrasonography.

The endomyocardial biopsy has limited sensitivity in the recognition of vasculopathy because it samples only the smallest of intramyocardial arteries and arterioles, which often do not show histologic features of CAV.[157] Proliferative intimal lesions are usually not prominent in the coronary microvasculature (vessels less than 100 μm in diameter) within the first few years posttransplantation when most of the surveillance biopsies are being performed on a regular basis.[158] Moreover, investigators have suggested discordant progression of CAV because of differences in the structural and functional abnormalities between small intramyocardial and large epicardial arteries.[158–160] Reported histologic changes seen in the small arteries in endomyocardial biopsies include concentric intimal thickening with or without foamy macrophages, subendothelial accumulation of lymphocytes (called by some, *endothelialitis*), and perivascular fibrosis.[156,157] Evidence of myocardial ischemia, such as colliquative myocytolysis, frank coagulation necrosis, and healing ischemic lesions as well as interstitial, perivascular, and replacement fibrosis, can be seen in endomyocardial biopsies.[161] Identification of myocardial injury should raise the suspicion of CAV as the cause of graft dysfunction. Absence of these findings, however, does not necessarily rule out the presence of CAV. One study of the predictive value of endomyocardial biopsies in a series of patients with CAV confirmed on autopsy reveals a sensitivity of only 21% for the detection of myocardial ischemic changes.[162]

The classic feature of CAV is that of diffuse concentric narrowing with luminal stenosis (Figure 13, A and B). If atherosclerotic plaques were present in the donor heart prior to the transplantation, the morphology of the lesion is one of eccentric atheromatous plaques with superimposed intimal proliferation of transplant-related vasculopathy. Sometimes, long-term lesions of epicardial coronary arteries may eventually look like conventional atherosclerosis and be indistinguishable from CAV. Careful examination of the cut surfaces of ventricles often reveals intramural arteries (with a range in diameter from 0.2 to 0.5 mm) that are thickened with abundant perivascular

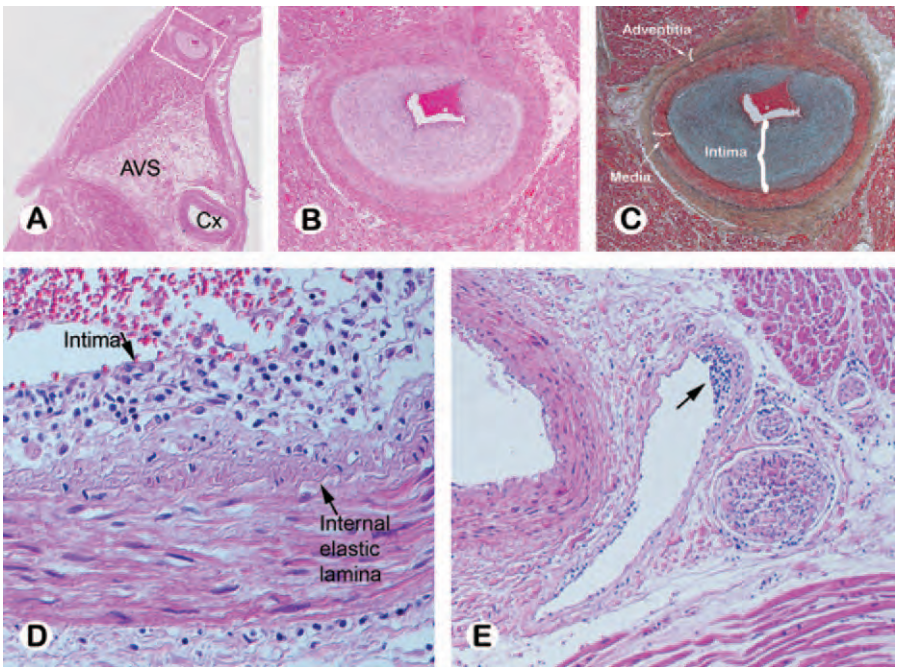

Figure 13. *Cardiac allograft vasculopathy (CAV). A, Scanning photomicrograph of left atrium and ventricle with the left circumflex (Cx) artery noted at the atrioventricular sulcus (AVS) from a patient who died of CAV 3 years posttransplant. The epicardial coronary arteries did not show occlusive lesions (hematoxylin-eosin, original magnification ×10). B and C, Intramyocardial branch of circumflex artery (from inset in A) supplying the atrium shows marked intimal proliferation with preservation of the elastic lamina and normal medial layer (hematoxylin-eosin [B] and Movat pentachrome [C], original magnification ×20). D and E, Subendothelial lymphocytic infiltration is seen in a small epicardial artery (D) and in an intramyocardial vein (arrow, E). This lesion has been called* endothelialitis *and is part of a spectrum of pathologic changes seen in CAV. Its clinical significance, however, is uncertain (hematoxylin-eosin, original magnifications ×200 [D] and ×40 [E]).*

fibrosis. In addition, focal areas of myocardial scarring may be evident.

The histology of allograft vasculopathy is slightly different in epicardial arteries compared with medium-sized or small arteries.[146,163,164] Microscopically, allograft vasculopathy in large epicardial vessels shows concentric intimal proliferation composed of smooth muscle cells and less-differentiated spindled cells (myofibroblasts or ''myointimal'' cells) (Figure 13, B and C). There is accompanying abundant deposition of proteoglycans with different immunohistochemical staining pattern and distribution, compared with conventional atherosclerosis, and more similar to angioplasty-related restenotic lesions (Figure 13, C).[165] Calcification and large pools of extracellular lipid are rare unless associated with atheromatous plaques that may develop in long-term survivors. Early lesions tend to be more cellular than those in the late stages, where the smooth muscle cells decrease in number and the intima becomes fibrotic. Mononuclear inflammatory cells are usually present in variable number, consisting mostly of T lymphocytes admixed with macrophages and foam cells. The internal elastic lamina is intact or only focally disrupted. The media is of normal thickness and shows little to no lipoprotein deposition. Medial fibrosis in the outer half is associated with lymphocyte-mediated injury of the vasa vasorum. An adventitial cuff of fibrous tissue with or without mononuclear inflammatory infiltrates is commonly observed (Figure 13, B and C). Atheromatous plaques, if present, are found in the proximal to middle segments of large epicardial arteries, produce an eccentric type of luminal stenosis, and histologically are indistinguishable from those of conventional atherosclerosis.

In the small epicardial and intramyocardial branches, allograft vasculopathy is also concentric but foam cells are not prominent. Endothelialitis is frequently observed in autopsy material (Figure 13, D and E). Occasionally, vasculitis with transmural inflammation by lymphocytes and plasma cells and disruption of internal elastic membrane is present in distal coronary arteries, usually associated with acute cellular rejection in the myocardium.[164,166] A second pattern of vasculitis that is characterized by severe inflammation in the adventitia that extends to the medial layer with destruction of external elastic membrane and is correlated with myocardial rejection has also been recognized by some investigators.[146] Fibrinoid necrosis of the media and thrombosis in small epicardial and intramural arteries can sometimes be seen.[146,163,164] Recanalized vessels may represent healed vasculitis with thrombosis. It is not clear whether this necrotizing vasculitis is due to cellular or humoral rejection or a combination of both.

The myocardium oftentimes show bilateral, patchy microscopic acute and healing ischemic injury[161] because it is believed that intramyocardial vessels are totally occluded first before the large epicardial arteries become critically stenosed. Chronic ischemic changes including myocytolysis and interstitial fibrosis are also frequent. Large myocardial infarcts are uncommon in the absence of thrombosis in the major epicardial vessels.[164] The pathology of CAV in children is practically identical.[166]

POSTTRANSPLANT MORBIDITY AND MORTALITY

Complications of chronic immunosuppression include drug toxicities, development of malignancy, and increased risk of infection. In time, most patients also develop hypertension, hyperlipidemia, diabetes mellitus, and renal insufficiency. Other notable adverse effects of therapy include bone marrow suppression and gastrointestinal symptoms.

Despite the use of newer and less toxic immunosuppressive drugs that decrease the incidence of acute cellular rejection, immunosuppression is still a significant cause of morbidity and mortality in the first year posttransplant. The majority of patients will have at least one episode of rejection. The rates for freedom from rejection at 1 year ranged from 10% to 23% covering the era before and after the introduction of cyclosporine in the Stanford experience.[167] Other centers have shown a significant decrease in the incidence of moderate and severe cellular rejection at-

tributed to improved immunosuppressive therapy in the 1990s.[168] Other major causes of death within the first year are early graft failure, multiorgan failure, and infection other than cytomegalovirus.[1,169] Early graft failure in the absence of cellular rejection can be the result of right ventricular failure from pulmonary hypertension, acute humoral rejection, and ischemia related to donor atherosclerosis, prolonged ischemic time, or poor donor preservation.[116]

Infections after cardiac transplantation often occur in the first few months after transplantation, with the highest risk of death at approximately 2 months after transplantation. Risk factors identified for fatal infections are very old and very young recipients, ventilator support at time of transplant, older (more than 50 years) donor heart, and prolonged donor ischemic time.[67] The most common site of infection reported was the lung.[66] Late infection is usually associated with recurrent high-grade rejection that requires augmentation of the immunosuppressive regimen.

Between 1 and 4 years, rejection, malignancy, and CAV account for most number of deaths. After 5 years, malignancy and CAV remain as the leading causes of death. A study involving 7290 patients who received transplants in multiple institutions between 1990 and 1999 reported malignancy (29%) as the leading cause of death, followed by CAV (23%) after the fourth year following transplantation.[169] The ISHLT registry with an 8-year cumulative data shows a 26% incidence of malignancy.[114] Transplant recipients do not appear to have increased risk of developing common cancers, including carcinomas of the lung, breast, prostate, and colon. However, an increased incidence of lymphoproliferative disorders, squamous cell carcinomas of the skin, sarcoma including Kaposi sarcoma, renal cell carcinoma, carcinomas of the vulva and perineum, and hepatobiliary tumors are observed.[170] The etiopathogenesis of posttransplant malignancies can be multifactorial. Association between the development of lymphoproliferative diseases and cytolytic induction therapy has been reported in renal and cardiac transplant recipients.[53,171] In another study, no increase in the incidence of malignant neoplasms was found between patients who received Thymoglobulin induction therapy and those who did not. However, it has been reported that patients who are treated with rabbit anti-thymocyte immunoglobulin develop malignant neoplasms earlier than those without induction therapy and have worse prognosis of their malignancies.[172] Long-term use of azathioprine has also been implicated in the development of myelodysplastic syndrome and acute myelogenous leukemia.[173]

Oncogenic viral infection may also play an important role in the development of malignancy in the immunocompromised hosts, including Epstein-Barr viruses, human papillomavirus, and hepatitis B and C virus. Recurrence of prior malignancy in a transplant patient may be because of defective immune surveillance. Transmission of cancer from donor to recipient is also possible. Transmitted malignancies in recipients of cardiac allografts include high-grade primary brain tumors, choriocarcinoma, lung adenocarcinoma, and melanoma.[174]

Autopsy studies of sudden unexpected cardiac deaths in transplant recipients reveal arrhythmias, CAV with diffuse involvement of distal coronary arteries, and cellular or humoral rejection as the most frequent causes of death.[175,176]

THE FUTURE

Endomyocardial biopsy is an invasive procedure that carries a low but finite risk of complications. There is associated patient discomfort with frequent biopsies. It is also a costly procedure and is resource-intensive. In addition, criticisms for EMB are the lack of concordance among pathologists in grading acute cardiac allograft rejection and failure to recognize AMR because of poorly defined histologic diagnostic criteria. However, no viable alternative exists to date.

Noninvasive monitoring of allograft rejection is an area of active research.[177] The need for proteomic and genomic markers to predict cardiac transplant rejection, correlation with outcomes, and risk of graft failure has been well recognized.[5] A recent study correlated gene expression profiling in peripheral blood mononuclear cells to the presence of acute cellular rejection in endomyocardial biopsies.[178] Their results indicating a high negative predictive value for the test show a promising diagnostic role for molecular testing. Reproducibility of these results in large-scale clinical settings has to be further demonstrated.[179]

In summary, the ultimate goal of any heart transplant team is a successful long-term outcome for the patients. This can be achieved with the pathologist working closely with the cardiac transplant team before and after the transplant. The recent revision of the ISHLT grading scheme should improve the interobserver reproducibility for cellular rejection and allow objective recognition of AMR. This, in turn, will result in more accurate diagnosis and better assessment of the effectiveness of therapy. Targeting proper therapy for host cellular or humoral response to the allograft may reduce the development and progression of CAV and other causes of graft dysfunction.

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Revised Working Formulation for Classification and Grading of Lung Allograft Rejection - 1995

Acute Rejection*

Grade	Histopathological Findings
A0 (None)	No mononuclear inflammation, hemorrhage or necrosis
A1 (Minimal)	Scattered infrequent perivascular mononuclear infiltrates not obvious at low magnification (40X). Blood vessels, particularly venules, are cuffed by small round, plasmacytoid, and transformed lymphocytes forming a ring of 2 to 3 cells thick in the perivascular adventitia.
A2 (Mild)	Frequent perivascular mononuclear infiltrates surrounding venules and arterioles readily recognizable at low magnification and usually consist of activated lymphocytes, small round lymphocytes, plasmacytoid lymphocytes, macrophages, and eosinophils. Frequent subendothelial infiltration by the mononuclear cells with hyperplastic or regenerative changes in the endothelium (endotheliitis); although there is expansion of the perivascular interstitium by inflammatory cells, there is no obvious infiltration by mononuclear cells into the adjacent alveolar septae or air spaces. Concurrent lymphocytic bronchiolitis is not uncommon. A solitary perivascular mononuclear infiltrate of significant intensity to be noted at low magnification still warrants a diagnosis of grade A2 (or greater) rejection
A3 (Moderate)	Readily recognizable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates, which are usually associated with endothelialitis; eosinophils and occasional neutrophils are common. By definition, there is extension of the inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septae and air spaces. Collections of alveolar macrophages are common in the airspaces in the zones of septal infiltration.
A4 (Severe)	Diffuse perivascular, interstitial, and air space infiltrates of mononuclear cells and prominent alveolar pneumocyte damage usually associated with intra-alveolar necrotic cells, macrophages, hyaline membranes, hemorrhage, and neutrophils; there may be associated parenchymal necrosis, infarction, or necrotizing vasculitis. The obvious presence of numerous perivascular and interstitial mononuclear cells seen with grade A4 rejection permits distinction from peri-operative (reperfusion/ischemic) lung injury.

* Pathologists should mention airway inflammation and may choose to grade B lesions (see below).

Chronic Airway Rejection (Bronchiolitis Obliterans)

Classification	Histopathological Findings
Active	In addition to the fibrosis, there are intra and/or peribronchiolar submucosal and peribronchiolar mononuclear cell infiltrates usually associated with ongoing epithelial damage
Inactive	Dense fibrous scarring without cellular infiltrates; this represents old cicatricial change in the small airways with a lack of significant submucosal and peribronchiolar inflammatory infiltrates

Chronic Vascular Rejection

Refers to the vaso-obliterative process affecting arteries and veins, that affects most solid organ transplants, and reflects accelerated atherosclerosis with fibrointimal thickening of the subendothelial area by loose myxomatous connective tissue. A mononuclear cell and foamy cell infiltrate is common

Airway Inflammation§

Grade	Histopathological Findings
B0 (None)	No airway inflammation
B1 (Minimal)	Rare scattered mononuclear cells within the submucosa of the bronchi and/or bronchioles
B2 (Mild)	Circumferential band of mononuclear cells and occasional eosinophils within the submucosa of bronchi and/or bronchioles unassociated with epithelial cell necrosis (apoptosis) or significant transepidermal migration by lymphocytes
B3 (Moderate)	Dense band-like infiltrate of activated mononuclear cells in the lamina propria of bronchi and/or bronchioles including activated lymphocytes and eosinophils, accompanied by evidence of satellitosis of lymphocytes, epithelial cell necrosis (apoptosis) and marked lymphocyte transmigration through epithelium
B4 (Severe)	Dense band-like infiltrate of activated mononuclear cells in the lamina propria of bronchi and/or bronchioles, associated with dissociation of epithelium from the basement membrane, epithelial ulceration, fibrinopurulent exudates containing neutrophils, and epithelial cell necrosis
BX	Ungradeable because of sampling problems, infection, tangential cutting, etc

§ All cases of acute rejection should have a designation indicating whether coexistent airway inflammation is present and may choose to grade the intensity.

Reference Yousem SA, et al. A revision of the 1990 Working Formulation for the classification of pulmonary allograft rejection: Lung Rejection Study Groug (LRSG) *J Heart Lung Transplantation* 1996;15:1-15.

Please mail comments, corrections or suggestions to the *TPIS administration* at the UPMC.

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LEAD ARTICLE

Revision of the 1990 Working Formulation for the Classification of Pulmonary Allograft Rejection: Lung Rejection Study Group

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In 1990, an international grading scheme for the grading of pulmonary allograft rejection was instituted. The use of this classification has resulted in a uniformity of grading which has allowed inter-institutional collaborations and communication unique in allograft monitoring. In 1995 an expanded group of international pathologists convened and revised the original proposal. This article summarizes the updated classification for pulmonary allograft rejection. In brief, acute rejection is based on perivascular and interstitial mononuclear infiltrates. Each grade of acute rejection should mention the presence of coexistent airway inflammation, the intensity of which may also be graded. Chronic rejection is divided into bronchiolitis obliterans—active or inactive—and vascular atherosclerosis—accelerated arterial or venous sclerosis. J HEART LUNG TRANSPLANT 1996;15:1-15.

In 1990, the International Society for Heart and Lung Transplantation sponsored a workshop to develop guidelines for the standardization of nomenclature in the histologic diagnosis of lung rejection.[1] At this meeting, a core group of pathologists developed a grading scheme for pulmonary allograft rejection which could be used to compare data from independent institutions. The intention was to develop a simple, easily taught, and readily reproducible grading system that incorporated the advantages and benefits of the multiple grading systems used at that time. The working formulation was readily adopted at most institutions performing lung transplantation in 1990, and, over the ensuing 5 years, it was accepted de novo by most new lung transplant programs. For the most part, it has been perceived as an excellent classification and grading formula. To respond to new developments in the field and an increasing wealth of experience by a greater number of pathologists, the Lung Rejection Study Group (LRSG) held a second meeting at the University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, in March 1995. The purpose of this meeting was to critically assess the merits of the initial working formulation and, on the basis of published data and, to a lesser extent, experience collected at multiple transplantation centers, improve the initial working formulation. The goal was

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TABLE I Working Formulation for classification and grading of pulmonary allograft rejection

A. Acute rejection		B.* Airway inflammation – lymphocytic bronchitis/bronchiolitis
Grade 0 – None	With/without	
Grade 1 – Minimal		
Grade 2 – Mild		
Grade 3 – Moderate		
Grade 4 – Severe		

C. Chronic airway rejection – bronchiolitis obliterans
a. Active
b. Inactive

D. Chronic vascular rejection – accelerated graft vascular sclerosis

*Pathologists may choose to grade B lesions (see text).

to maintain scientific and biologic accuracy while striving to streamline the classification into one that would be pathologically, clinically, and therapeutically relevant. The revised classification was designed so that it was based on histologic findings of acute and chronic lung rejection primarily using transbronchial biopsy as the means of allograft monitoring in both adults and children.[2-8] Although the importance of a morphology-based system for the interpretation of pathologic abnormalities was emphasized, it was noted that all biopsy data needed to be interpreted in an integrated clinical context to allow optimum patient management and clinical decisions.[9] It was also noted that infection and rejection often occur together and can be confused histologically.[10,11] For this reason, exclusion of infection was believed to be essential for accurate and reproducible interpretation of allograft biopsies.

HISTOLOGIC GRADING OF PULMONARY ALLOGRAFT REJECTION

The LRSG recognized that alloreactive injury to the donor lung can affect the vasculature and the airways in both acute and chronic rejection. Acute rejection is characterized by perivascular and subendothelial mononuclear infiltrates and by lymphocytic bronchitis and bronchiolitis. Chronic rejection, by definition, manifests as bronchiolitis obliterans – dense eosinophilic fibrous scarring of the bronchioles – and accelerated vascular sclerosis affecting pulmonary arteries and veins.[2,3,12] Although it is probable that a continuum of vascular and airway histopathologic changes exists in the pulmonary allograft, these changes have been divided into histologic grades based on the intensity of the cellular infiltrate and the presence of dense eosinophilic hyaline fibrosis (Table I). It should be emphasized that the presence of such irreversible hyaline eosinophilic scarring of the airways and vessels represents the key histologic discriminator between acute and chronic rejection.

A. Acute Rejection

The diagnosis of acute rejection is based exclusively on the presence of perivascular and interstitial mononuclear cell infiltrates. In grading acute rejection, attention should be directed at the intensity of the perivascular mononuclear cell cuffs which surround the blood vessels and at whether the mononuclear cells extend beyond the vascular adventitia and percolate into the adjacent alveolar septa. This latter feature denotes a higher grade of rejection. Although rejection processes usually affect more than one vessel, solitary perivascular infiltrates should be evaluated with criteria that are identical to those which are applied to multiple infiltrates, as outlined later. Infiltrates surrounding small vessels in the submucosa of airways are interpreted as part of the spectrum of airway inflammation and are not diagnostic of acute rejection.

Grade 0 (no acute rejection)

In grade A0, normal pulmonary parenchyma is seen without evidence of mononuclear infiltration, hemorrhage, or necrosis.

Grade A1 (minimal acute rejection)

In grade A1, there are scattered infrequent perivascular mononuclear infiltrates in alveolated lung parenchyma that are not obvious at low magnification (40× magnification); blood vessels, particularly venules, are cuffed by small round, plasmacytoid, and transformed lymphocytes forming a ring of two to three cells in thickness in the perivascular adventitia (Figure 1).

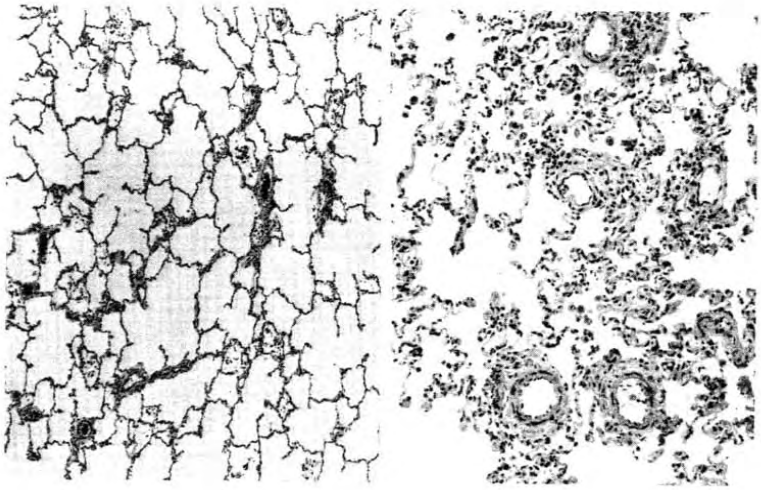


FIGURE 1 Minimal acute cellular rejection (A1): At *left,* no obvious perivascular infiltrates are seen at low magnification. At higher power *(right),* rare isolated mononuclear cells cuffed small pulmonary vessels, primarily venules.

Grade A2 (mild acute rejection)

In grade A2, frequent perivascular mononuclear infiltrates surrounding venules and arterioles are readily recognizable at low magnification (Figure 2); they usually consist of activated lymphocytes, small round lymphocytes, plasmacytoid lymphocytes, macrophages, and eosinophils. There is frequently subendothelial infiltration by the mononuclear cells with hyperplastic or regenerative changes in the endothelium ("endothelialitis"); although there is expansion of the perivascular interstitium by inflammatory cells, there is no obvious infiltration by mononuclear cells into the adjacent alveolar septae or air spaces. Concurrent lymphocytic bronchiolitis is not uncommon.

Mild acute rejection is distinguished from minimal acute rejection by the presence of unequivocal mononuclear infiltrates which are identified at scanning magnification. Additional helpful features which suggest mild rejection are the presence of subendothelial mononuclear infiltrates, eosinophils, and coexistent airway inflammation. It is also important to note that a solitary perivascular mononuclear infiltrate of significant intensity to be noted at low magnification still warrants a diagnosis of grade A2 (or greater) rejection.

Grade A3 (moderate acute rejection)

Grade A3 shows readily recognizable cuffing of venules and arterioles by dense perivascular mononuclear cell infiltrates, which are usually associated with endothelialitis (Figures 3 and 4); eosinophils and occasional neutrophils are common; by definition, there is extension of the inflammatory cell infiltrate into perivascular and peribronchiolar alveolar septae and air spaces (Figure 5). Collections of alveolar macrophages are common in the airspaces in the zones of septal infiltration.

Grade A4 (severe acute rejection)

In grade A4, there are diffuse perivascular, interstitial, and air space infiltrates of mononuclear cells and prominent alveolar pneumocyte damage usually associated with intraalveolar necrotic cells, macrophages, hyaline membranes, hemorrhage, and neutrophils (Figures 6 and 7); there may be associated parenchymal necrosis, infarction, or necrotizing vasculitis.

Grade A4 acute rejection may be separated from posttransplantation acute lung injury (diffuse alveolar damage) by the obvious presence of numerous perivascular and interstitial mononuclear cells, which are not present in perioperative (reperfusion/ischemic) lung injury.

B. Airway Inflammation – Lymphocytic Bronchitis/Bronchiolitis

In the 1990 classification, acute rejection was divided into four grades based on the presence and intensity of perivascular and interstitial mononuclear infiltrates. Under each grade, four suffixes were offered to reflect coexistent airway inflamma-

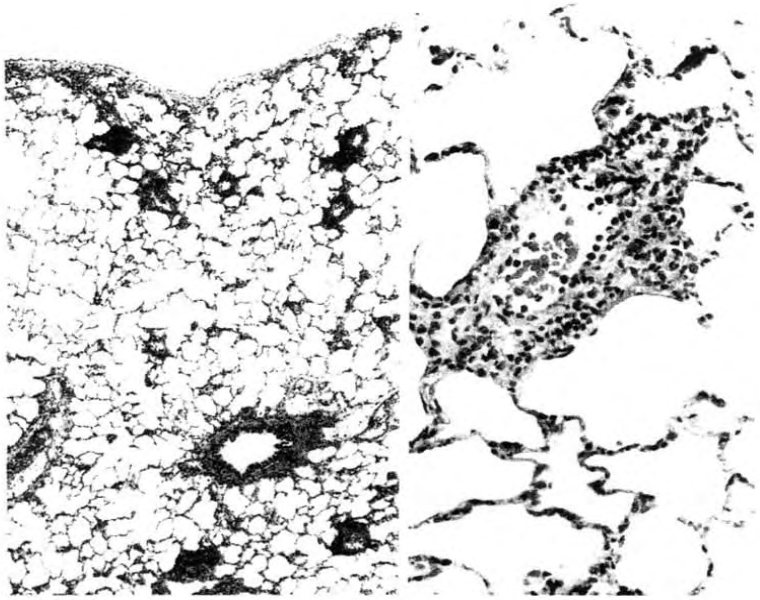


FIGURE 2 Mild acute cellular rejection (A2): At *left,* obvious perivascular infiltrates are present as one scans the lung parenchyma, and they are restricted to the perivascular adventitia. At *right,* the perivascular mononuclear infiltrate is five to eight cells in thickness. No alveolar septal infiltration is seen.

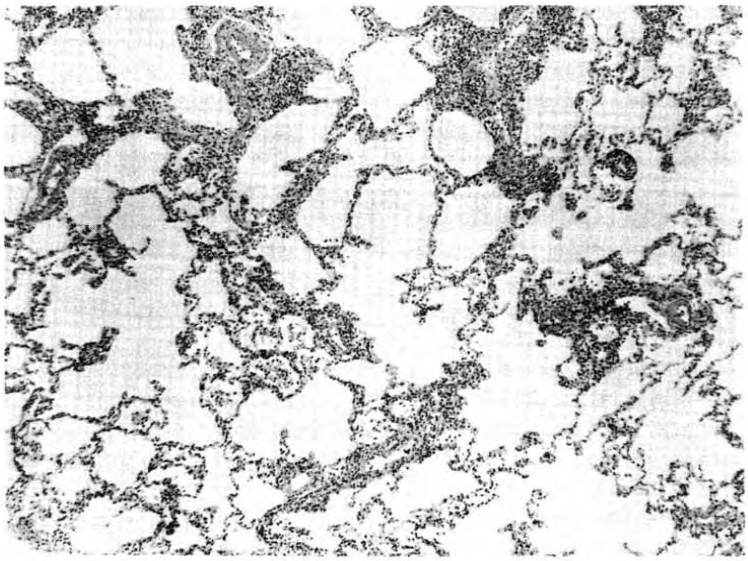


FIGURE 3 Moderate acute rejection (A3): In moderate acute rejection, the mononuclear infiltrates extend beyond the perivascular zones and trickle into adjacent alveolar septa.

tion. These designations did not reflect the intensity of the inflammatory infiltrate and were believed to be cumbersome by the 1995 LRSG. In the 1995 modification of the working formulation of pulmonary allograft rejection, it is recommended that perivascular infiltrates remain the determining factor on which to grade acute rejection. It is also recommended that the presence and intensity

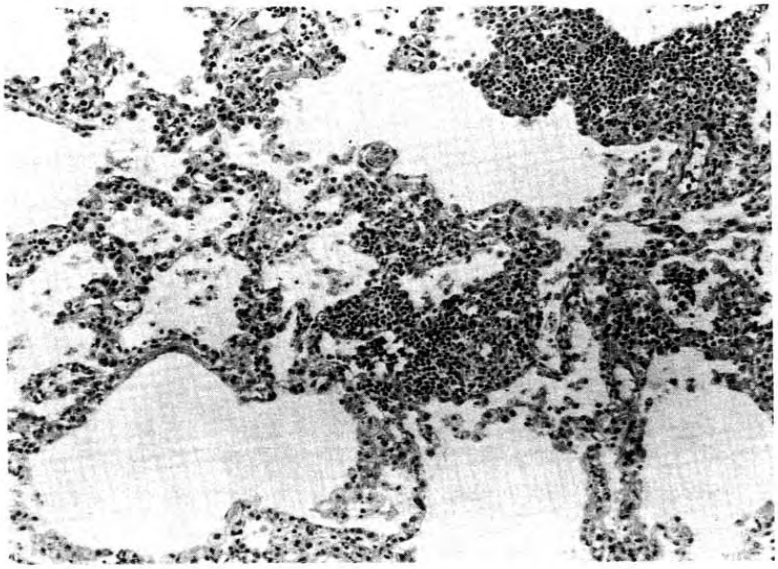


FIGURE 4 Moderate acute rejection (A3): The perivascular and septal mononuclear infiltrates are frequently accompanied by a lymphocytic bronchitis and bronchiolitis. The thickened septa define alveolar sacs which contain numerous macrophages.

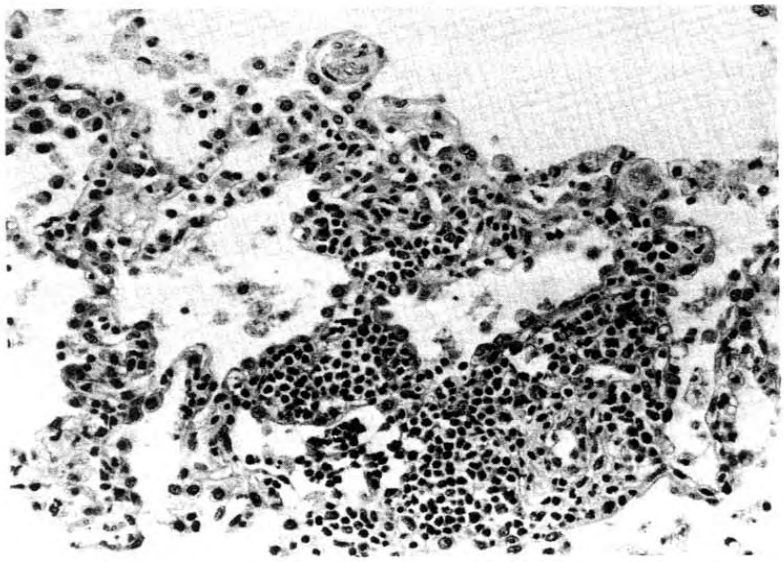


FIGURE 5 Moderate acute rejection (A3): In A3 rejection, endothelialitis and eosinophilic infiltrates are usual. Alveolar pneumocytes may have a hyperplastic or hobnail configuration.

of combined large and small airway inflammation should be noted histologically and recognized as a possible harbinger of bronchiolitis obliterans.[13] The airway inflammation should be listed as a "B" category and can be divided into five grades or simply designated as present or absent, at the discretion of each institution.

B0: No airway inflammation
B1: Minimal airway inflammation—rare scattered mononuclear cells within the submucosa of the bronchi or bronchioles (Figure 8)
B2: Mild airway inflammation—a circumferential band of mononuclear cells and occasional eosinophils within the submucosa of bronchi

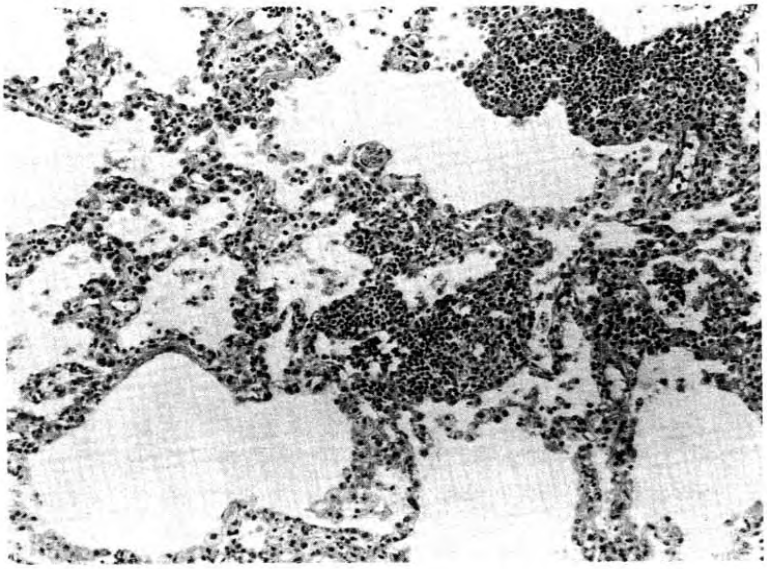
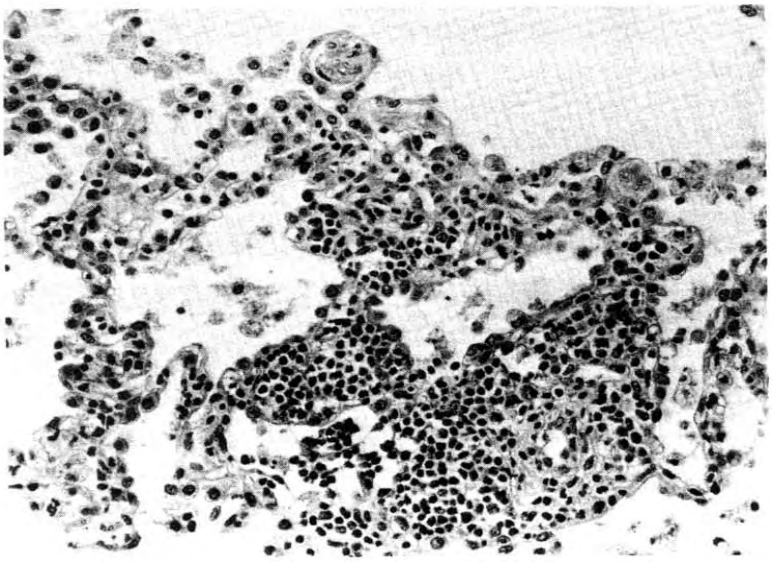


FIGURE 4 Moderate acute rejection (A3): The perivascular and septal mononuclear infiltrates are frequently accompanied by a lymphocytic bronchitis and bronchiolitis. The thickened septa define alveolar sacs which contain numerous macrophages.



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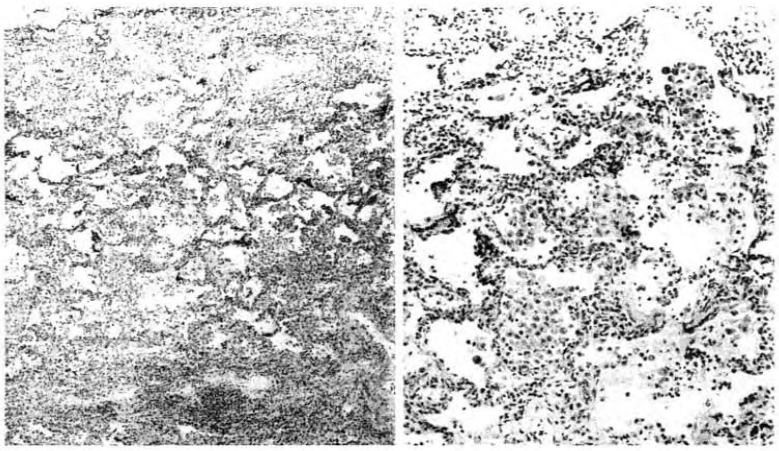

FIGURE 6 Severe acute cellular rejection (A4): Severe acute cellular rejection shows obvious perivascular, peribronchiolar, and alveolar septal mononuclear infiltrates at low magnification *(left)*. At more intermediate powers *(right)*, prominent alveolar macrophages with airspace fibrin, hemorrhage, and hyaline membranes are seen. Neutrophils are prominent and alveolar septa appear thickened and edematous.

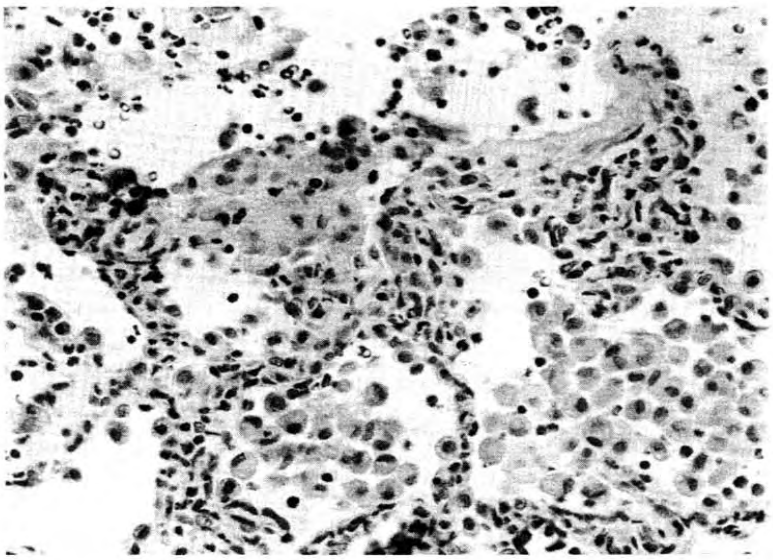

FIGURE 7 Severe acute cellular rejection (A4): Alveolar septa contain a mixed mononuclear and neutrophilic infiltrate that spills into air sacs. These alveoli contain macrophages, blood, fibrin, and hyaline membranes in severe rejection. The mononuclear infiltrate helps discriminate severe rejection from diffuse alveolar damage of other causes.

and/or bronchioles unassociated with epithelial cell necrosis (apoptosis) or significant transepidermal migration by lymphocytes (Figure 9)

B3: Moderate airway inflammation—a dense band-like infiltrate of mononuclear cells in the submucosa of bronchi and/or bronchioles including activated lymphocytes and eosinophils, accompanied by evidence of satellitosis of lymphocytes, epithelial cell necrosis (apoptosis), and marked lymphocyte transmigration through epithelium (Figures 10 and 11).

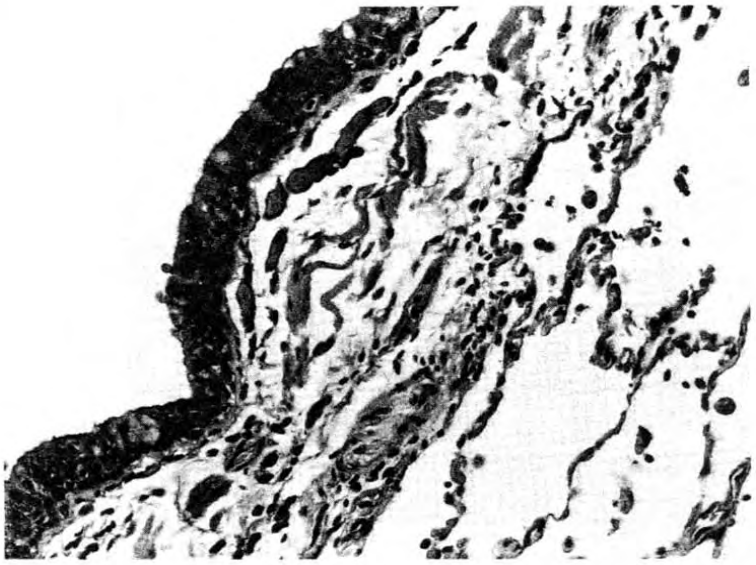

FIGURE 8 Airway inflammation—lymphocytic bronchitis/bronchiolitis, mininal (B1): A scant infiltrate of mononuclear cells is seen in the lamina propria of the airways.

B4: Severe airway inflammation—a dense band-like infiltrate of activated mononuclear cells in bronchi and/or bronchioles, associated with dissociation of epithelium from the basement membrane, epithelial ulceration, fibrinopurulent exudates containing neutrophils, and epithelial cell necrosis (Figures 12 and 13).

BX: Ungradable because of sampling problems, infection, tangential cutting, etc.

Although some members of the LRSG believed that grading the presence and intensity of airway inflammation in acute rejection was important because of the increased risk of developing bronchiolitis obliterans, many other members did not.[13-15] These latter individuals believed that clinicopathologic evidence did not convincingly prove that airway inflammation solely could be used to grade rejection because of its frequent coexistence with airway infection and problems with biopsy adequacy. For these reasons, some institutions may choose only to note the presence of airway inflammation and decline to grade its intensity. Still other centers may opt to focus on the separation of large and small airway inflammation. It should also be highlighted that dense scarring of the bronchioles is not accepted under the "B" designation.

The LRSG proposes to designate the diagnosis of acute rejection with coexistent airway inflammation as follows: Acute rejection grade—, with airway

FIGURE 9 Airway inflammation—lymphocytic bronchitis/bronchiolitis, mild (B2): At low magnification, an obvious mononuclear infiltrate is seen in the lamina propria. Rare intraepithelial cells are seen but are unassociated with epithelial cell necrosis (apoptosis).

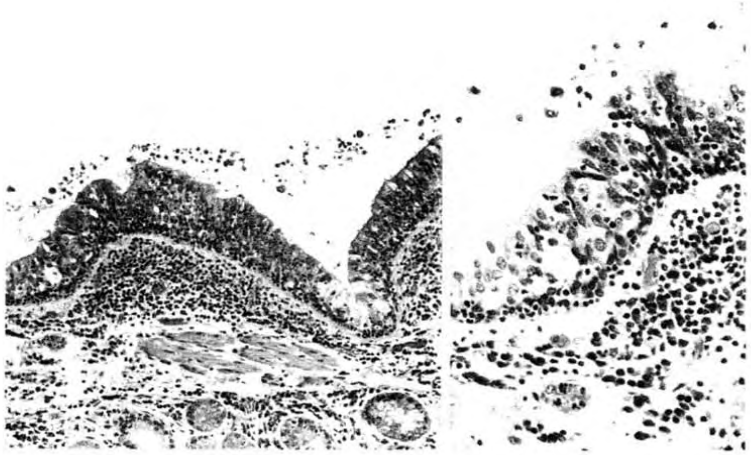

FIGURE 10 Airway inflammation—lymphocytic bronchitis/bronchiolitis, moderate (B3): At scanning power, an obvious band of mononuclear cells fills the lamina propria (left) and infiltrates the overlying epithelium (right). Within the epithelium individual cell necrosis and dropout is seen and a mild luminal exudate is present.

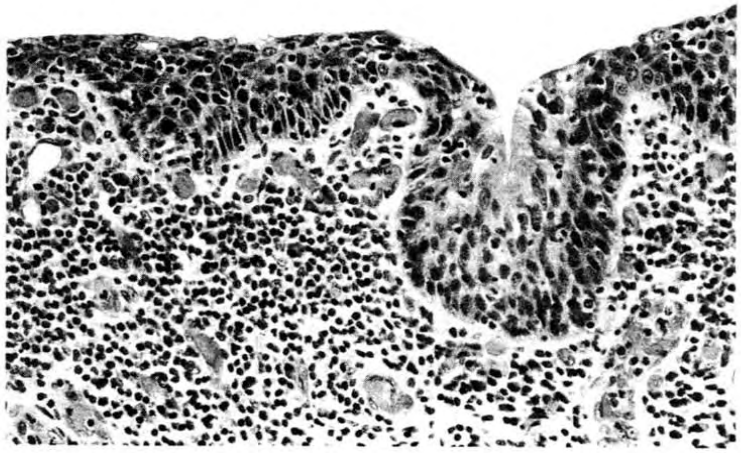

FIGURE 11 Airway inflammation—lymphocytic bronchitis/bronchiolitis, moderate (B3): In some cases, squamous metaplasia with lymphocyte emperipoiesis and epithelial injury may be seen, especially in the large cartilaginous airways.

inflammation, grade—. For example, mild acute rejection in which there is an intense airway infiltrate with epithelial cell necrosis would be diagnosed as "Mild acute rejection, grade A2, with airway inflammation, grade B3". Similarly, the 1990 category of "B lymphocytic bronchitis/bronchiolitis" would now be graded as a "A0 B—" in the 1995 classification. This format emphasizes the need to retain perivascular infiltrates as the primary focus in the histologic classification of acute lung rejection.

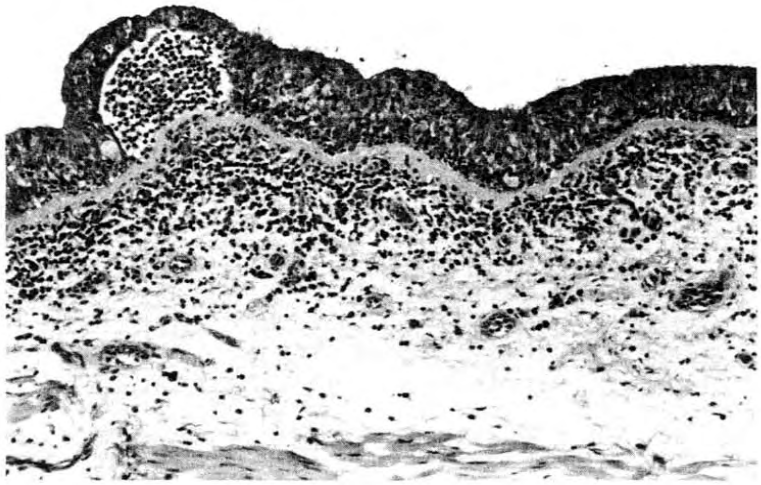

FIGURE 12 Airway inflammation—lymphocytic bronchitis/bronchiolitis, severe (B4): In the early phase of severe airway injury, the epithelium becomes dissociated from the basement membrane. At the site of fracture, a mixed infiltrate of mononuclear and polymorphonuclear cells is present in the subepithelial "blister".

C. Chronic Airway Rejection—Bronchiolitis Obliterans

Bronchiolitis obliterans is a term restricted to membranous and respiratory bronchioles, and, in the context of a pulmonary allograft, refers to dense eosinophilic hyaline fibrous plaques in the submucosa of the small airways which results in partial or complete luminal compromise (Figure 14).[2,3] This scar tissue may be concentric or eccentric, may be associated with fragmentation and destruction of the smooth muscle wall, and may extend into the peribronchiolar interstitium. Mucostasis or foamy histiocytes in the distal airspaces (endogenous lipidosis) are common. Bronchiolitis obliterans rarely develops within the first 3 months after transplantation, usually developing at the end of or after the first postoperative year.

In contrast to the 1990 scheme, the current LRSG believed that a distinction between "subtotal" and "total" forms of bronchiolitis obliterans was not worthwhile in the evaluation of the pulmonary allograft by transbronchial biopsy; however, it was believed that estimation of the relative activity of the inflammatory infiltrate was worthwhile. In those instances of coexistent acute and chronic rejection, the pathology report should reflect these processes in the following manner: "acute rejection, grade −, with active/inactive bronchiolitis obliterans, grade C a/b."

- **a. Active:** In addition to the fibrosis, there are intrabronchiolar and/or peribronchiolar submucosal and peribronchiolar mononuclear cell infiltrates usually associated with ongoing epithelial damage (Figure 15)
- **b. Inactive:** Dense fibrous scarring without cellular infiltrates; this represents old cicatricial change in the small airways with a lack of significant submucosal and peribronchiolar inflammatory infiltrates (Figure 16)

At present the significance of large airway fibrosis is uncertain.[16] In the opinion of the LRSG, this finding is nonspecific and does not warrant a diagnosis of chronic rejection. Inflammation and scarring of the bronchioles is believed to be a more significant reflection of chronic allograft injury.

D. Chronic Vascular Rejection—Accelerated Graft Vascular Sclerosis

In chronic vascular rejection, there is fibrointimal thickening of arteries and veins (Figures 17 and 18). The significance of this change is uncertain but seems to correlate with the presence of coronary artery disease in allograft hearts in combined heart-lung procedures and bronchiolitis obliterans

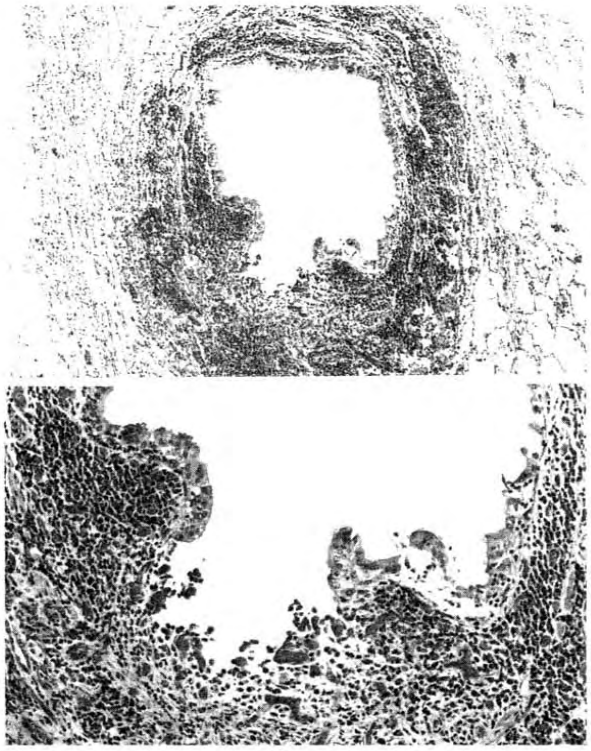

FIGURE 13 Airway inflammation—lymphocytic bronchitis/bronchiolitis, severe (B4): Bronchioles show an intense mural mononuclear infiltrate *(top)* which progresses to ulceration and is accompanied by a neutrophilic infiltrate and luminal exudate *(bottom)*.

in isolated pulmonary allografts.[12] There may also be an "active" component consisting of subendothelial, intimal, and/or medial mononuclear cell infiltrates.

The 1995 LRSG believed that the 1990 category of "vasculitis" was probably not worth retaining, as most cases of vascular injury reflect either severe acute rejection reactions or active graft vascular sclerosis.

RECOMMENDATIONS

Adequacy of Specimens

Transbronchial biopsy remains the mainstay of allograft evaluation.[17,18] It was the uniform opinion of the LRSG that at least five pieces of alveolated lung parenchyma each containing bronchioles and greater than 100 air sacs are necessary to confidently grade acute and chronic rejection. In our experience, it may be necessary for the bronchoscopist to obtain more than five biopsies to provide this minimum number of adequate alveolated pieces. Furthermore, if bronchiolitis obliterans is a consideration, more than five pieces are frequently needed to sample the small bronchioles adequately. Specimens should be gently agitated in formalin to inflate the biopsy fragments and tenderly handled by histotechnicians to avoid crush artifacts when embedding tissue in paraffin.

When appropriate sampling has not occurred, it is essential to note in the pathology report that the biopsy findings may not be fully representative of the changes in the pulmonary allograft.

Histology

Histologic evaluation should include a minimum of (1) sections from three levels of the paraffin block containing the specimens for hematoxylin and eosin stains, (2) connective tissue stains to evaluate the submucosal fibrosis essential for the diagnosis of arteriosclerosis/bronchiolitis obliterans, and (3) silver stains for fungi/pneumocystis. Beyond this minimum workup, individual investigators may augment their evaluation with a wide spectrum of histochemical, immunohistochemical, and in situ hybridization studies.

Review of Clinical History and Previous Biopsies

All biopsies should be studied by the pathologist with full knowledge of the native recipient disease and the results of the last biopsy and current bronchoalveolar lavage. In many instances this requires simultaneous review of prior slides with the current specimen. The biopsy should be reported in this context. Individual institutions may choose to use the term *ongoing rejection* for biopsies which remain unchanged from previous ones; *resolving rejection* for those biopsies where the mononuclear infiltrate is reduced in intensity and the number of activated cells has subsided as compared with a previous biopsy; or *resolved rejection* if the infiltrates have completely disappeared. In these contexts, it is recommended that a rejection score still be included.

Differential Diagnosis of Perivascular and Interstitial Infiltrates

Perivascular and interstitial mononuclear infiltrates are not specific for acute rejection, and other conditions may simulate or mimic alloreactive injury.[11,19] Differential considerations include the following:

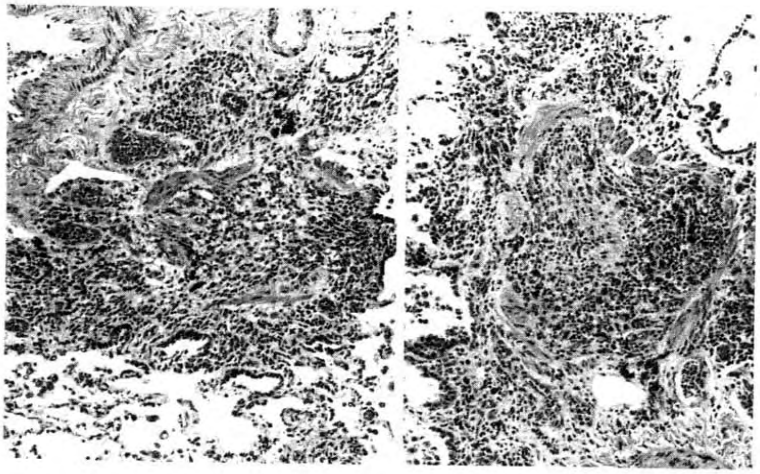

FIGURE 14 Bronchiolitis obliterans, active (Ca): At *left* the bronchovascular bundle is engulfed in an inflammatory process. The bronchiole is completely obliterated, and, at *right*, the lumen contains dense eosinophilic collagen and abundant mononuclear cells. The presence of an airway is confirmed by its position adjacent to a pulmonary artery and its curvilinear interrupted bands of smooth muscle which define the outer circumference of the airway lumen. The mononuclear infiltrate should precipitate an "active" designation to this case of bronchiolitis obliterans.

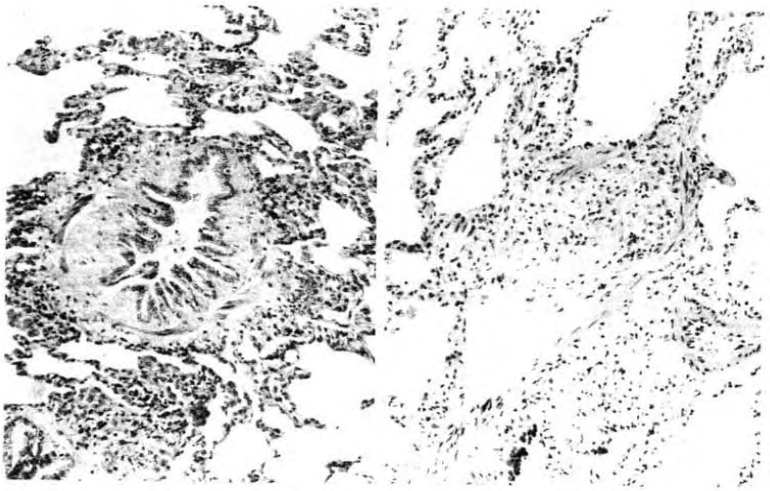

FIGURE 15 Bronchiolitis obliterans, active (Ca): At *left*, the airway contains a subtle diffuse increase in the amount of hyaline collagen throughout the lamina propria, and the airway lumen is partially compromised. At *right*, a bronchiole contains an eccentric deposit of dense scar tissue and its epithelium is flattened and attenuated. Residual smooth muscle defines the airway circumference.

Cytomegalovirus pneumonitis
Pneumocystis carinii pneumonia
Posttransplantation lymphoproliferative disease ranging from pneumonitis to active lymphoproliferative disorders[20]

Bronchial-associated lymphoid tissue, seen particularly in the submucosa and at the bifurcations of large airways, where it is well circumscribed, unassociated with epithelial injury or eosinophils, and frequently accompanied

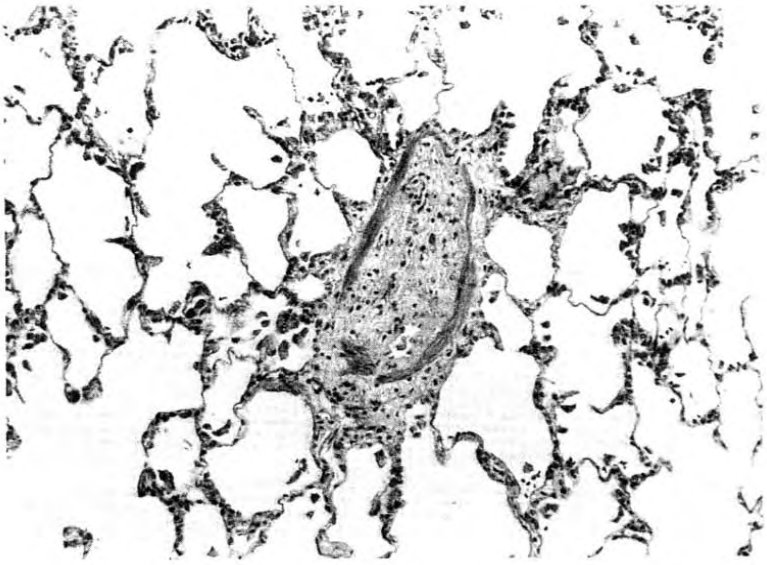

FIGURE 16 Bronchiolitis obliterans, inactive (Cb): In this instance, the lumen of the bronchiole is totally occluded by dense eosinophilic collagen. The luminal scar is ringed by residual smooth muscle.

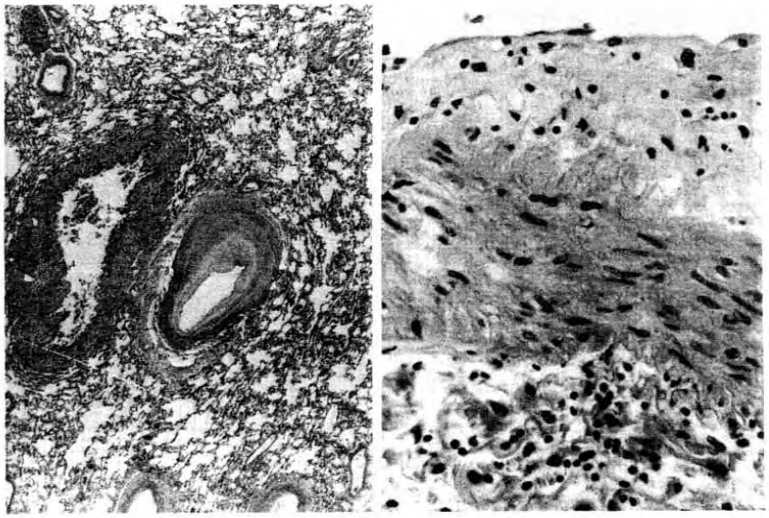

FIGURE 17 Accelerated graft vascular sclerosis (D): At *left*, a small bronchus is dilated while the adjacent pulmonary artery shows a subendothelial fibroelastotic plaque. At *right* the intima is expanded by a mixture of lymphocytes, plasma cells, histiocytes and foamy macrophages in the myointimal connective tissue.

by histiocytes containing particulate matter
Previous biopsy sites
Recurrent primary disease (e.g., sarcoidosis)
Ischemia (preservation injury)

Differential Diagnosis of Airway Inflammation

There are many causes of airway inflammation and scarring of the airways, which may result in complete obliteration in some instances. The following con-

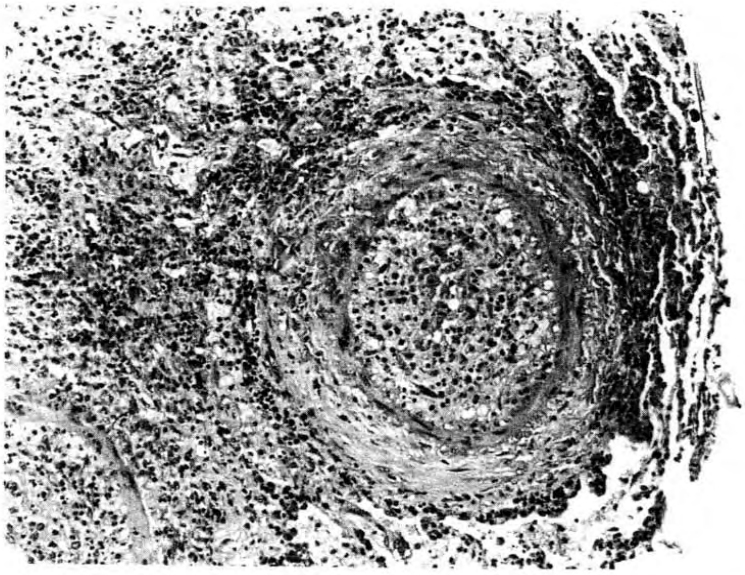

FIGURE 18 Accelerated graft vascular sclerosis (D): At times, the arteriosclerotic process may be highly cellular and be associated with an active mural and adventitial infiltrate. Complete occlusion of pulmonary arteries and veins is uncommon.

ditions represent some nonrejection causes of lymphocytic bronchiolitis/bronchitis and bronchiolitis obliterans.[2,3,21-24]

Infection: Perhaps the most frequent cause of acute and chronic inflammation of the airways is low-grade infection of the respiratory tract, particularly viral, bacterial, mycoplasmal, fungal, and chlamydial in nature.

Aspiration: Because of the loss of cough reflex and tracheal sensitivity after the transplant procedure, patients with pulmonary allografts are predisposed to recurrent aspiration; helpful features in diagnosing aspiration include the identification of exogenous material with an associated foreign body giant cell reaction within the airways, distal organizing pneumonia, and supportive radiologic studies.

Granulation tissue reactions are common in the pulmonary allograft and are manifested as intraairway and intraalveolar plugs of fibromyxoid connective tissue.[20,25] These loose edematous polyps should be distinguished from the dense eosinophilic scarring of bronchiolitis obliterans. Particular settings in which these reactions occur are resolving infection (organizing pneumonia), aspiration, the proliferative phase of diffuse alveolar damage, active or resolving acute rejection reactions, and miscellaneous other conditions. Rare reports of idiopathic organizing pneumonia in pulmonary allografts are another potential cause.[21] A variety of nonspecific reactions may occur in the pulmonary allograft. Although they usually do not cause diagnostic confusion with rejection, awareness of these injury patterns may be helpful in the evaluation of potential causes of graft dysfunction. Such reactions are listed in Table II.

Hyperacute Rejection

The LRSG believed that a morphologic definition of hyperacute lung rejection was not possible at this point in time and that it would require an integrated evaluation of clinical findings, histology, serologic studies, and immunofluorescence evaluation of allograft tissue.

Concomitant Acute Rejection and Infection

The LRSG recognized that the histologic characteristics of acute rejection and infection overlap, especially in cytomegalovirus and *Pneumocystis carinii* pneumonitis.[11,18] In some instances there may also be coexistent infection and rejection. In these cases, we continue to recommend that the pathologist indicate that he or she favors infection or rejection as the predominant process and that a follow-up biopsy be obtained after appropriate antimicrobial therapy to adequately

TABLE II Pathologic alterations in the pulmonary allograft—Nonrejection related

1. Diffuse alveolar damage/patchy acute alveolar injury—usually a manifestation of ischemic/reperfusion injury or other perioperative insults
2. Acute alveolar hemorrhage
3. Alveolar hemosiderosis—usually seen in a previous biopsy site, in resolved high grade acute rejection reactions where endothelial damage was significant, sites of prior infection, or in individuals with coagulation abnormalities
4. Post transplantation lymphoproliferative disorder
5. Recurrent native disease (e.g., sarcoidosis, lymphangioleiomyomatosis, giant cell interstitial pneumonia)
6. Smoker's macrophages in air spaces (if donor was a smoker or recipient continues/begins smoking).

assess the presence of simultaneous rejection.

Some histologic features are so distinctive that they strongly suggest infection in the context of perivascular or interstitial mononuclear infiltrates.[19] Granulomatous inflammation and punctate zones of necrosis are unusual in rejection and should raise the possibility of mycobacterial, fungal, or pneumocystis infection in the former and herpes simplex or cytomegalovirus in the latter context. Pneumocystis and fungal disease can readily be diagnosed with silver stains, as can mycobacterial infection by acid fast stains and culture. Viral infection may be confirmed by immunohistological studies or culture. Clues to cytomegalovirus infection include perivascular edema that outstrips the degree of mononuclear infiltration; disproportionate alveolar septal cellular infiltrates in comparison with perivascular cuffing; the presence of abundant neutrophils with the formation of microabscesses; marked atypia of alveolar pneumocytes; and acute airway inflammation. Finally, airspace consolidation or heavy interstitial infiltrates of eosinophils may suggest fungal infection.[26]

This revised working formulation for the classification of pulmonary allograft rejection is a proposal that is intended to simplify the original classification scheme based on clinicopathologic study and empiric observations. What has been achieved is that the formulation has been stripped of some of its complexity, in terms of the numerous suffixes under acute rejection, the subtotal/total designations of bronchiolitis obliterans, and the presence of "vasculitis." The significance of airway inflammation without fibrosis, in the context of acute rejection, is unclear in the literature. In the 1995 proposal we encourage continued investigation of its significance. As part of this study, it may also be worthwhile to grade the intensity of the latter process, if desired by the institutional pathologist and the clinical care team. This flexibility was emphasized in the original proposal and should be noted once more: the working formulation scheme should not dissuade individuals from constructing their own local classifications but should be used as a means of facilitating communication among clinicians, pathologists, disparate departments, and institutions. Furthermore, the system should always be used in the context of the individual patient, with the histopathology representing one piece of a therapeutic puzzle that includes symptomatology, physical and radiographic findings, pulmonary function studies, and microbiological culture results.

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Bronchiolitis Obliterans Syndrome 2001: An Update of the Diagnostic Criteria

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Bronchiolitis obliterans (BO) is a major cause of allograft dysfunction in lung and heart lung transplant recipients.[1,2] Clinically, progressive airflow limitation develops because of small airway obstruction. The disease has a variable course. Some patients experience rapid loss of lung function and respiratory failure. Others experience either slow progression or intermittent loss of function with long plateaus during which pulmonary function is stable. Histologic confirmation is difficult because transbronchial biopsy specimens often are not sufficiently sensitive for diagnosis. Because BO is difficult to document histologically, in 1993 a committee sponsored by the International Society for Heart and Lung Transplantation (ISHLT) proposed a clinical description of BO, termed *bronchiolitis obliterans syndrome* (BOS) and defined by pulmonary function changes rather than histology. Although this system does not require histologic diagnosis, it does recognize it.[3]

Transplant centers worldwide have adopted the BOS system as a descriptor of lung allograft dysfunction. This allows centers to use a common language to compare program results. In the years since publication of the BOS system, transplant scientists have studied basic and clinical aspects of lung transplant BO. In this document, we update and summarize new information obtained from this research and incorporate, where appropriate, the results into the BOS criteria.

The document will include the following topics: (1) criteria for BOS, (2) BOS considerations in pediatric patients, (3) risk factors for BOS, (4) pathology of BO, (5) surrogate markers for BOS, (6) confounding factors in making a BOS diagnosis, and (7) assessment of response to treatment of BOS.

CRITERIA FOR BOS

Background

When the original definition of BOS was formulated in 1993, the working group had several goals. The group aimed to provide a classification system for airway disease after lung transplantation that did not rely on histopathologic findings, was sensitive and specific, relied on diagnostic techniques available to all lung transplant physicians, and was relatively simple to understand and apply. The resulting classification system defined post-transplant

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pulmonary function using the forced expiratory volume in 1 second (FEV_1) as the primary parameter. For each lung transplant recipient, a stable post-transplant baseline FEV_1 is defined as BOS Stage 0. In patients who experience a decrease in FEV_1, progressive stages of BOS, from 1 to 3, are defined according to the magnitude of the decrease. An additional notation can reflect histologic findings: "a" designates that no BO has been identified, or that no biopsy has been done; and "b" designates that BO has been identified.[3]

Although the ISHLT classification system for BOS has gained universal acceptance, several limitations have been identified. First, the current grading system—which defines BOS 1 as a >20% decrease in FEV_1 from baseline—was not sensitive enough to pick up early, small, but potentially important changes in pulmonary function.[4–6] In addition, the mid-expiratory flow rate ($FEF_{25–75}$) was not used for defining airflow obstruction because the wider intrasubject variability of this index, in particular in recipients of unilateral transplants,[7] and the very high values observed in some patients early after surgery were considered as potential limitations. Yet several reports in recipients of bilateral and heart–lung grafts have shown that $FEF_{25–75}$ is more sensitive than FEV_1 for early detection of airflow obstruction in BOS[4–6] (one study also included recipients of single lung transplants but results in these patients were not reported specifically[8]). These observations have led to a critical re-examination of the BOS criteria, and formulation of the revised classification system as detailed in this document.

Recommendations

1. Definition of BOS: We use the term *bronchiolitis obliterans syndrome* to connote graft deterioration secondary to persistent airflow obstruction (however, note that not all patients in whom airflow obstruction develops have BOS—see confounding conditions discussed below). It is widely presumed, but unproved, that chronic rejection often contributes to functional deterioration. BOS does not necessarily require histologic confirmation; in contrast, the term *bronchiolitis obliterans* is used for a histologically proven diagnosis.
2. Definition of equipment: Spirometric measurements must be made with equipment that conforms to the American Thoracic Society standards for spirometric testing.[9]
3. Definition of baseline: The *baseline value*, to which subsequent measures are referred, is defined as the average of the 2 highest (not necessarily consecutive) measurements obtained at least 3 weeks apart, such measurements being made without the use of an inhaled bronchodilator preceding the study. The *baseline date* is defined as the date of the first measurement used to compute the baseline. The values used to compute the baselines for FEV_1 and for $FEF_{25–75}$ may be obtained on different days. Because spirometric values may increase with post-operative time, the baseline should be recalculated using the highest values achieved. The definition of baseline, and hence of BOS stages, is expected to be more accurate as more functional tests are performed.
4. Definition of confounding conditions: Patients are evaluated under this system only after evaluation of other conditions that may alter graft function and after treatment of these conditions if found. Interpretation of changes in lung function should take into account confounding conditions, which are discussed below.
5. Definition of variables: In the original staging system, a ≥20% decrease in FEV_1 from previous baseline was used to diagnosis BOS. Studies of intrasubject variability of spirometry in lung transplant recipients indicate that using a 10% to 15% decrease in FEV_1 may be more appropriate for early detection of BOS.[5–7] In addition, evidence suggests that $FEF_{25–75}$ deteriorates before FEV_1 in most bilateral and heart–lung transplant recipients with BOS.[4–6] Therefore, a *potential-BOS stage* (BOS 0-p), defined by a 10% to 19% decrease in FEV_1 and/or by a ≥25% decrease in $FEF_{25–75}$ from baseline is added to the original staging system. This potential-BOS stage alerts the physician to the need for close functional monitoring and in-depth assessment, which might include surrogate markers for BOS (see below).
6. Definition of BOS stages: For the purpose of staging, a significant decrease in FEV_1 or $FEF_{25–75}$ will be determined by the average of 2 measurements made at least 3 weeks apart, without patient use of an inhaled bronchodilator. Patients having a single measurement of decreased FEV_1 or $FEF_{25–75}$ are not evaluated until a second measurement is obtained at least 3 weeks after the initial data point. Because BOS is meant to represent a persistent alteration in lung function, additional values of FEV_1 or $FEF_{25–75}$, which may be obtained during this 3-week period,

TABLE I Original and proposed classifications of BOS

Original classification		Current proposition	
BOS 0	FEV_1 80% or more of baseline	BOS 0	$FEV_1 > 90\%$ of baseline and $FEF_{25-75} > 75\%$ of baseline
		BOS 0-p	FEV_1 81% to 90% of baseline and/or $FEF_{25-75} \leq 75\%$ of baseline
BOS 1	FEV_1 66% to 80% of baseline	BOS 1	FEV_1 66% to 80% of baseline
BOS 2	FEV_1 51% to 65% of baseline	BOS 2	FEV_1 51% to 65% of baseline
BOS 3	FEV_1 50% or less of baseline	BOS 3	FEV_1 50% or less of baseline

BOS, bronchiolitis obliterans syndrome; FEF_{25-75}, mid-expiratory flow rate; FEV_1, forced expiratory volume in 1 second.

should also show a significant decrease from baseline value. The date at which a patient enters the new BOS stage is the date of the first of the 2 measurements used to confirm the stage. In case of a concomitant decrease in vital capacity (VC) and FEV_1, a restrictive ventilatory defect should be excluded before categorizing the patient in a new BOS stage (see confounding conditions discussed below).

7. Definition of functional decline: Because a universal table for converting the absolute value of FEV_1 and FEF_{25-75} to "percent predicted" does not exist, a fractional decrease in FEV_1 and FEF_{25-75} should be determined from absolute values. The fractional decrease in FEV_1 and FEF_{25-75} shall be expressed as the percent of decrease from the previously established baseline, i.e., the highest previous baseline value is used for all subsequent calculations.
8. Definition of staging system: A proposed staging system is outlined in Table I. Within each of the staging categories is an "a" and a "b" subcategory. These relate to histologic findings of biopsy specimens. This staging system is intended to describe the recipient's current status. Although BOS is considered irreversible, a minority of patients may show improvement in lung function over time. When a patient experiences such improvement in BOS stage, the worst stage that the patient has ever achieved may be noted in parentheses, if desired for study purposes. Therefore, BOS 1(2) will indicate a patient currently in BOS 1 who has been in BOS 2 at some point in the past.

BOS CONSIDERATIONS IN PEDIATRIC PATIENTS

Background

Approximately 2.5% of lung transplant candidates are ≤17 years of age. In terms of the number of transplants, number of patients on the waiting list, and number of active centers, pediatric lung transplantation lags behind adult lung transplantation and other pediatric solid-organ transplantation. Published reports indicate an incidence of BO similar to that of adults,[10–12] except in children <3 years old, in whom it may be lower.[10]

Airway inspection is particularly important in children to assess for stenosis and/or malacia at the anastomotic site. In general, the BOS criteria can be used in children who can perform pulmonary function tests reproducibly (usually at least 5 years of age). However, in defining functional decline, a decrease in percent predicted rather than a change in absolute value (see 7 above) should be used. The use of percent predicted values for FEV_1 and FEF_{25-75} should be a more accurate indicator in children because absolute values of lung function should increase with the child's growth. In older children who can perform reproducible respiratory maneuvers, the adult criteria with the use of predicted values should be easily applied. Because of the difficulty in performing pulmonary function studies in some pediatric patients, surrogate markers for BOS may assume more importance. Infants and young children require lung function testing by other techniques, most commonly through the rapid compression technique. The combined use of forced expiratory flow at functional residual capacity, normalized by the measured functional residual capacity, is a useful technique to separate anastomotic complications from peripheral airflow obstruction. Techniques for lung function testing in infants and young toddlers provide tools for performing serial lung function testing in lung transplant recipients of this age.[13,14] Experience with such techniques is limited to 1 pediatric lung transplant center,[15] and further clinical research with newer techniques is clearly indicated.

Recommendations

1. Pediatric patients suspected of having BO should undergo bronchoscopic examination of the airways and transbronchial biopsy when possible. On occasion in young patients or in those with obscuring clinical or large airway pathology, an open lung biopsy to assess for histopathology may facilitate early therapeutic intervention.
2. In general, the criteria for BOS can be applied in children who can complete pulmonary function tests satisfactorily. However, declines in function should be expressed in terms of percent predicted instead of absolute values because of lung and airway growth. Newer techniques facilitate measurements in infants and have been used to assess for BOS.

RISK FACTORS FOR BOS

Background

Many factors have been reported as risk factors for BOS. However, quality of data is often a problem because almost all existing information derives from retrospective studies with no control groups and reflects the experience of single centers. Numbers are small and often difficult to interpret. In some cases, risk factors seem to have been more important in the earlier years of lung transplantation, e.g., cytomegalovirus (CMV) infection. This may reflect a change in the risk environment because of the use of prophylactic antimicrobial regimens, changing immunosuppressive approaches, or the increasing experience of transplant management teams.

Alloimmunologic injury directed against endothelial and epithelial structures have been thought to mediate BOS, but non-alloimmunologic inflammatory conditions including viral infections or ischemic injury may also play a role. Risk factors reported in the literature will be designated as (1) probable risk factors, (2) potential risk factors in need of further analysis, and (3) hypothetic risk factors.

Probable Risk Factors

Acute rejection and lymphocytic bronchitis/bronchiolitis belong to this category. Six separate publications document the increased incidence of BOS in patients with acute rejection episodes, especially when multiple and/or long-lasting and/or high-grade episodes occur.[16–21] Two additional publications document the role of late acute rejection in the development of BOS.[22,23] Five publications report that lymphocytic bronchitis/bronchiolitis is a risk factor for BOS, when infection has been excluded as a cause of an inflammatory airway process.[18,20,24–26]

Medication non-compliance is a known risk factor for rejection and graft loss after kidney, heart, and liver transplantation.[27–30] Medication non-compliance also is perceived as a risk factor after lung transplantation, although results supporting this have not been published.

Cytomegalovirus is difficult to interpret as a risk factor for 2 main reasons: the pattern of CMV has changed with the widespread use of prophylactic strategies directed against the virus and with varying definitions of infection, disease, and pneumonitis among institutions. Eight reports consider CMV a risk factor for BOS,[16,19,22,25,31–34] whereas 4 other studies reported no impact of the virus.[18,20,21,35] Four other studies document a decreased risk of CMV in the development BOS—either decreased incidence or delay in onset—after the use of CMV prophylaxis.[17,36–38] However, data from the pre-prophylaxis era in which CMV pneumonitis was more prevalent strongly correlates pneumonitis as a BOS risk factor.

Potential Risk Factors

Potential risk factors are so designated because of conflicting data, suggestive but not definitive data, or differences in definitions of the specific risk factor between centers so that available data cannot be interpreted. These factors include (1) organizing pneumonia; (2) bacterial, fungal, and non-CMV viral infection; (3) older donor age; (4) longer graft ischemic time; and (5) donor antigen-specific reactivity.

Two centers report that organizing pneumonia is a risk factor for BOS. One of these centers reported that it was a univariate risk factor for BOS. The data are from small numbers and not complete enough to designate it a probable risk.[18,19]

A surprisingly small body of data has been published that report the impact of bacterial, fungal, and non-CMV viral infections. One center reported bacterial and P carinii pneumonia as risks during the period before broad-spectrum prophylaxis in lung transplantation.[17] In a more recent report, bacterial or fungal pneumonia was not associated as an univariate risk with an increased rate of BOS, but did increase the acute rejection score in a multivariate model.[18] A peak incidence of BOS onset in the respiratory virus season suggested to one set of authors that common respiratory viral infections may trigger the complication.[39] Treatment of respi-

ratory syncytial and parainfluenza viruses decreased the incidence of BOS in one center.[40]

Donor age did not correlate with BOS in a large population in the United Kingdom; however, the ISHLT 2000 Registry identified donor age as a risk factor.[19,41] The Registry identified graft ischemic time as a second donor risk factor, a finding also differing from the findings of the UK study.

Persistent donor antigen-specific reactivity has reportedly led to increased rates of BOS, and conversely, donor-specific hyporeactivity was reported as protective.[42,43] Preliminary experience from the Pittsburgh Transplant Group has shown that the infusion of donor bone marrow in combination with lung transplantation increases donor cell chimerism and donor antigen-specific hyporeactivity, and is associated with a lower incidence of BOS.[44]

Hypothetic Risk Factors

Hypothetic risk factors include factors supported by theoretical considerations but having scanty clinical evidence to date. These factors include (1) underlying disease, (2) genotype of the recipient for certain cytokine gene polymorphisms, (3) HLA-mismatching, and (4) gastroesophageal reflux with aspiration.

Two studies suggested that underlying diagnosis is a risk factor and that patients with pulmonary hypertension may be more at risk of BOS; in a third study, this was not the case.[17,25,33] The ISHLT 2000 Registry identifies emphysema patients as having the best survivals but does not identify freedom from BOS as the reason.[41]

Data are emerging on the potential role for genotypic susceptibility to development of BOS. Cytokine gene polymorphisms of tumor necrosis factor (TNF)–α, interferon γ, IL-10, IL-6, or TGF-β genes may play a role.[45] Available data are scant and conflicting.[46]

Data also conflict on HLA mismatching, with most series showing no association.[17,18,20] One institution has documented an increased risk of BOS with the development of anti-HLA Class I antibodies.[47] Confusion in this area arises in part from the small number of transplantations performed in individual centers and because no attempt at HLA matching is made. Therefore, it is uncommon for any center to have more than a few HLA-matched recipients. In the largest study yet reported that involves HLA matching, 3,549 lung transplantations were reviewed using the United Network for Organ Sharing (UNOS)/ISHLT Registry database. Only 164 patients had 2 or fewer mismatches. No significant association could be found between HLA mismatching and BOS development.[48]

TABLE II Risk factors for BOS

Probable risk factors:
Acute rejection
Lymphocytic bronchitis/bronchiolitis
CMV pneumonitis
Medication non-compliance
Potential risk factors:
CMV infection (without pneumonitis)
Organizing pneumonia
Bacterial/fungal/non-CMV viral infection
Older donor age
Longer graft ischemic time
Donor antigen-specific reactivity
Hypothetic risk factors
Underlying disease
HLA-mismatching
Genotype of recipient
Gastroesophageal reflux with aspiration

BOS, bronchiolitis obliterans syndrome; CMV, cytomegalovirus.

Case reports and small series have suggested an incremental risk from gastroesophageal reflux disease with aspiration and from impaired mucociliary clearance.[49–52]

Several additional factors, including history of smoking or asthma in the donor, head injury as cause of death, airway ischemia, and diffuse alveolar damage (reperfusion injury), have been proposed as risk factors for late organ dysfunction. However, convincing data to support the role of these factors are lacking.[20,53–56]

A differential in the prevalence of BOS among unilateral, bilateral, and heart–lung grafts has not been documented.

Recommendations

1. Many factors have been reported as potential risk factors for BOS, but proven causal relationships are difficult to establish.
2. Based on available information, Table II summarizes the probable, potential, and hypothetic risk factors.

PATHOLOGY OF BO

Background

Bronchiolitis obliterans is a cicatricial process that affects the small airways of the allograft lung. Conceptually, BO is thought to result from chronic lung rejection, although not exclusively. It progresses through a sequence of lymphohistiocytic-mediated

cytotoxicity directed at the respiratory epithelium. The initial process is a lymphocytic infiltrate of the sub-mucosa of the airways with migration of lymphocytes through the basement membrane into the epithelium.[57] At this site, epithelial cell necrosis occurs with denudation of mucosa. A secondary cascade of non-specific inflammatory mediators and cytokines attracts other cells, including neutrophils. The reaction stimulates migration of fibroblasts and myofibroblasts into the luminal exudate. Formation of an intraluminal fibromyxoid granulation tissue polyp results. In some instances, macrophage collagenases may dissolve the polyp. The diagnostic fibrous scarring can be eccentric with formation of a fibrous plaque in the wall of the airway; concentric with the interposition of a "donut" of collagen tissue; or the granulation tissue may completely obliterate the lumen of the airway, reducing the air passages to stenotic cords of scar tissue ("vanishing airways disease").[58] At the time of histologic diagnosis, the airway injury may be temporally heterogeneous with some airways showing only cellular infiltrates, some displaying active fibroplasia, and others demonstrating inactive fibrosis.

Bronchoscopy may exclude other causes of deteriorating lung function, but diagnosing BO with transbronchial biopsy specimens may be extremely difficult. It requires multiple, large fragments, and even then, diagnostic lesions may be missed. Trichrome and elastic tissue stains may assist in recognizing the damaged or obliterated airway. When the clinical diagnosis is unclear and transbronchial biopsy specimens have not offered an unequivocal answer, open lung biopsy may be necessary.

The initial document describing BOS used an "a" sub-category to designate no pathologic evidence of BO (or no pathologic material for evaluation) and a "b" sub-category to mean that pathologic evidence of BO was obtained. The usefulness of these designations has not yet been validated.

Recommendations

1. Histologic activity may not reflect the clinical activity monitored by pulmonary function tests.
2. The term *bronchiolitis obliterans* should be used only when histology demonstrates dense fibrous scar tissue affecting the small airways.
3. The presence of only lymphocytic sub-mucosal infiltrate or intraluminal granulation tissue is not sufficient for a diagnosis of BO.
4. If the obliterative lesion is associated with a mononuclear infiltrate, it is defined as active; fibrosis without inflammatory cells is defined as inactive.
5. An "a" sub-category designates no pathologic evidence of BO (or no pathologic material for evaluation). A "b" sub-category means that pathologic evidence of BO has been obtained.

SURROGATE MARKERS FOR BOS

Background

The diagnostic criteria for BOS are based on a decrease in lung function. Various indirect measures or analyses have been undertaken to identify alternative early markers of a decrease in graft performance. Perhaps these markers can provide a surrogate means of predicting disease or of monitoring disease activity, with the aim of enabling early therapy to block a relentless decrease in lung function.

Bronchoalveolar lavage (BAL) analysis

A number of cross-sectional studies[59–64] and 3 prospective studies[7,60,64] indicate an association between BOS and BAL neutrophilia, and they indicate that this alteration may actually precede the 20% decrease in FEV_1 required for the spirometric diagnosis of BOS.[7,60,64] In addition, a persistent increase in BAL neutrophilia is an independent predictor of mortality after lung transplantation.[65] Other preliminary studies implicate various BAL markers or mediators in the pathogenesis of BOS (e.g., IL-8, markers of oxidative stress, neutrophil elastase, TGF-β, platelet derived growth factor (PDGF), collagen I/III, insulinlike growth factor-1). Although these markers may provide useful concepts for exploring the mechanisms behind development of chronic allograft rejection, they are not yet sufficiently robust tests to contribute to the clinical diagnosis of BOS.

Exhaled nitric oxide

Exhaled nitric oxide (eNO) provides a potentially useful tool in diagnosing acute and chronic allograft rejection in lung transplant recipients. Several lung transplant centers have evaluated eNO and found it to be reproducible, repeatable, and reflective of NO levels in the lower airways.[66,67] The source of eNO in allograft pathology remains to be identified, but potential sources include epithelial cells and infiltrating leukocytes;[67–69] eNO has a close link with BAL neutrophilia.[67] A cross-sectional study of 104 lung transplant recipients noted elevated eNO in

lymphocytic bronchitis and BOS Stage 1 but not in BOS Stages 2 and 3.[70] Other studies have reported a variable association between increased eNO and BOS.[71,72]

Air trapping shown on expiratory computerized tomography scans

Imaging is a potentially simple and repeatable means of assessing BOS. High-resolution computerized tomography (CT) scanning is the most accurate imaging tool for diagnosing BOS. On inspiratory scans, several abnormalities have been associated with BOS, including bronchial dilatation, bronchial wall thickening, and mosaic perfusion pattern, although these findings lack sensitivity.[73–76] In contrast, the presence of air trapping on expiratory CT scans is an accurate indicator of the bronchiolar obliteration underlying BOS.[77–80] In patients with BOS, the pulmonary lobules that have normal airways increase in density during the expiratory phase, whereas areas with diseased airways cannot empty and remain radiolucent secondary to the obstructive bronchiolar inflammatory and fibrotic changes. In a recent prospective study that included 111 expiratory CT scans in 38 heart–lung transplant recipients, the presence of air trapping >32% had a 87.5% sensitivity and specificity for the diagnosis of BOS, and in some patients this preceded the spirometric criteria for BOS.[79] Conversely, having <32% of air trapping had a high negative predictive value until the fifth post-operative year. In another, smaller study, an air-trapping score provided a sensitivity of 74% and a specificity of 67% for histopathologically proven OB.[80]

Bronchial hyper-responsiveness.

Bronchial hyper-responsiveness has been reported in patients who have undergone lung transplantation, although some studies have been negative for this finding.[81–89] In a recent longitudinal study that included 111 patients undergoing bilateral lung transplantation, Stanbrook and Kesten[89] reported that 30% of patients had a positive methacholine challenge at 3 months after transplant and were significantly more likely to have BOS; the mean time to development of BOS was 16.9 months. A retrospective study of 94 lung transplant recipients showed that the presence of a bronchodilator response at low lung volume had a sensitivity of 51%, a specificity of 87%, and a positive predictive value of 81% for the diagnosis of BOS.[90] This study also noted that the bronchodilator response may precede BOS by months.

Distribution of ventilation.

Two recent prospective studies have shown that indices of ventilation distribution (e.g., the alveolar plateau slope obtained for nitrogen or helium during single-breath washout) may detect BOS earlier than do conventional pulmonary function tests.[6,7] Reynaud-Gaubert et al[6] considered a nitrogen slope >3% as abnormal, whereas Estenne et al[6] considered significant a 100% increase above baseline.

Problems with and quality of data.

In addition to the limitations that clinical trials in lung transplant recipients frequently encounter (small sample size, retrospective study, lack of adequate control group), 3 specific limitations should be mentioned in the context of the surrogate markers for BOS:

1. Many of the markers discussed above have been used and validated primarily in recipients of heart–lung and double-lung grafts, e.g., air trapping on expiratory CT and indices of ventilation distribution. No clear effect on eNO caused by the type of surgical procedure or the type of disease in the native lung has been demonstrated in transplant recipients who are stable or who have BOS. This point deserves further study.
2. Specificity of the markers discussed here for the diagnosis of BOS is low, e.g., BAL neutrophilia may be caused by infection, and eNO or indices of ventilation distribution may increase in acute rejection or infection.
3. Thresholds indicating a significant alteration from the stable state, particularly for BAL neutrophilia and eNO, have not been clearly established. These thresholds must be determined on the basis of standardized baseline values[91] using intrasubject coefficients of variation.

Recommendations

1. BAL neutrophilia and elevated cytokine levels, eNO, air trapping on expiratory CT scans, bronchial hyper-responsiveness, and measures of an altered distribution of ventilation have all been identified as early markers of BOS. However, none is specific or sensitive enough to be used reliably for diagnosing BOS.
2. The presence of an abnormal level of a surrogate marker should alert the clinician to the potential for BOS onset.

CONFOUNDING FACTORS IN DIAGNOSING BOS

Background

Lung function is exquisitely sensitive to complications that affect the allograft, such as rejection, infection, and anastomotic complications. These complications often produce some degree of airflow obstruction and may lead to a pattern of functional deterioration, which is qualitatively similar to that seen in BOS. In addition, several complications that affect the native lung and disease progression in the native lung may contribute to changing pulmonary function. This section addresses (1) confounding factors in the graft that apply to all types of transplants, (2) confounding factors that affect the native lung in single lung transplants, and (3) confounding factors that cause a restrictive ventilator defect.

Factors that affect the graft.

- Infection and rejection: Symptoms characteristic of infection frequently herald the onset of BOS, and a community-acquired respiratory bacterial or viral infection may be documented. Similarly, some patients with recurrent or refractory acute rejection (including acute cellular rejection and lymphocytic bronchitis/bronchiolitis) progress to BOS. Therefore, the presence of infection or acute rejection, which may produce airflow obstruction,[92] does not exclude the diagnosis of BOS and may confound its early diagnosis. If the lung function change persists after appropriate treatment, the diagnosis of BOS can be made.
- Anastomotic complications: Complications at the site of the tracheal or bronchial anastomosis (e.g., stenosis, dehiscence, and malacia) may alter forced expiratory flows and volumes. Because these complications occur early after surgery, they are generally recognized before the diagnosis of BOS is suspected. Yet interpretation of functional changes in the presence of anastomotic complications may be difficult because it is not always easy to determine whether stenosis/malacia or the development of BOS is responsible for a decrease in lung function. The final diagnosis is left to the discretion of the individual physician.
- Disease recurrence: Some primary diagnoses have recurred in the lung graft. These include sarcoidosis, lymphangioleiomyomatosis, Langerhans cell histiocytosis X, alveolar cell carcinoma, desquamative interstitial pneumonitis, panbronchiolitis, and giant cell interstitial pneumonitis.[93–99] Disease recurrence may cause graft dysfunction, may confuse the diagnosis of BOS, or may coexist with BOS. In other cases, e.g., sarcoid, recurrent disease may have little functional effect. In the context of recurrent disease, the diagnosis of BOS must be made with caution unless histologic confirmation is available.
- Aging: In long-term survivors, the physiologic aging process of the lung is expected to significantly decrease both FEV_1 and $FEF_{25–75}$. However, making firm recommendations as to how to account for this factor is not possible because the rate of functional decline with age in an otherwise normal graft remains unknown.

Factors affecting the native lung.

- Native lung hyperinflation: Acute native lung hyperinflation is a complication reported in patients with emphysema who receive single lung transplants.[100–104] If acute native lung hyperinflation occurs early after surgery, it does not interfere with the diagnosis of BOS. However, intermediate- and long-term, progressive hyperinflation of the emphysematous lung may be associated with graft dysfunction.[105] Studies in stable recipients of single lung transplants for emphysema have shown that the total lung capacity of the graft is decreased to 66% to 79% of the predicted normal values.[106,107] In a small sub-set of patients, hyperinflation of the native lung may worsen over time and lead to clinical and functional changes similar to those produced by BOS (e.g., dyspnea, worsening airways obstruction, hypoxemia, accentuated radiologic shift of the mediastinum toward the graft, and V/Q mismatch). In this context, lung volume reduction or lobectomy of the native lung may improve lung function in selected individuals.[108–112] The mechanisms underlying delayed native lung hyperinflation have not been precisely identified, and more importantly, no easy means exist to distinguish between this complication and BOS. Moy et al[113] suggested that measuring lung resistance during inspiration may be helpful in this context, but further studies must validate the use of this variable. From a practical standpoint, if a patient with emphysema who has undergone single lung transplantation has worsening airflow obstruction without another specific cause, the patient should be considered to have BOS.
- Disease progression in patients without emphysema: Disease progression in the native lung may contribute partially to a change in overall lung function. However, because the native lung usually makes only a minor contribution to maximal expiratory flows and volumes, disease progression

is not expected to be a frequent confounding factor for the diagnosis of BOS.

- Other complications: Several complications may occur in the native lung and affect approximately 25% to 40% of the recipients.[114–117] Infectious complications are more frequent, and recipients who have emphysema seem to be at increased risk. However, complications affecting the native lung are easy to identify and generally do not interfere with the diagnosis of BOS.

Factors causing a restrictive ventilatory defect

Several diseases may decrease static and dynamic lung volumes in recipients of lung transplants. These conditions include increased body mass index,[118] respiratory muscle weakness unrelated[119] or related to generalized neuromuscular disorders, pleural effusion, rib fractures, chronic post-operative pain, and pulmonary edema. The functional impact is expected to be a decrease in both VC and FEV_1. Therefore, in the presence of a decreased FEV_1, an unchanged FEV_1/VC ratio should alert the clinician to exclude the above-mentioned conditions before considering the diagnosis of BOS. In the presence of a concomitant decline in VC and FEV_1 with an unchanged FEV_1/VC ratio, the baseline for FEV_1 and for $FEF_{25–75}$ may be reset to a lower value.

Recommendations

1. Infection, acute rejection, disease recurrence, and anastomotic complications can confound the diagnosis of BOS. These diagnoses should be excluded or treated before assigning a designation of BOS.
2. Following single lung transplant for emphysema, native lung hyperinflation occasionally results in a functional and physiologic picture similar to BOS. In this setting, a precise diagnosis may be impossible and each case should be judged on its individual characteristics.
3. A number of conditions can occur that cause decreases in both the VC and the FEV_1 (e.g., an increase in body mass index, muscular weakness, pleural effusion, etc.) without a decrease in the FEV_1/VC ratio. Such comorbidities must be excluded before assigning a diagnosis of BOS.

ASSESSING BOS RESPONSE TO THERAPY

Background

Although the fibrous obliteration of the bronchioles seen in BO probably is irreversible, the histologic lesions are often heterogeneous, with some airways showing inflammatory infiltrates potentially amenable to treatment. This probably explains why some patients show functional stabilization or improvement with treatment. Assessing response to therapy is difficult in individual patients because of the high variability of the disease response of an individual to an intervention.[9,120–125] This document proposes methods of assessing populations and study purposes. Retrospective and non-randomized designs, small sample size, absence of a control group, and relatively short follow-up have weakened published studies of treatment for BOS. Given the variable natural course of BOS, an appropriate number of patients in randomized studies with both a treated and a control arm is mandatory, and the method used to assess the response to therapy must be standardized. Designing multicenter studies with a large number of patients may allow stratification according to several factors that may affect response to therapy, e.g., BOS stage, association with acute rejection or lymphocytic bronchiolitis, rate of functional decrease, association with infection, time from transplantation to development of BOS, etc.

Recommendations

1. Assessing response to therapy should be based on the diagnostic criteria for BOS, i.e., FEV_1. Absolute values of FEV_1 measured before and after the therapeutic intervention should be plotted over time, and the slopes should be obtained by linear regression analysis. At least 3 measurements with a negative slope, obtained over 1 to 3 months, should be used to compute the slope before treatment. This slope should be calculated using all the data points obtained in the 1 to 3 months before initiation of treatment; the first point used should be the first measurement below the BOS threshold. The slopes after treatment should include all data points obtained after initiation of treatment and for at least a period of 6 months (see Appendix). A decrease in the rate of functional decline after initiation of treatment may be coincidental (i.e., reflect the natural history of the disease) and may not reflect a therapeutic benefit. This underscores the difficulty in interpreting the response in individual patients and emphasizes the need for control groups in prospective studies.
2. Stability may occur spontaneously after onset of BOS. This results in a flat FEV_1 slope (instead of a negative slope), and assessment of therapeutic intervention is problematic. Because this course of the disease occurs relatively frequently, pro-

spective studies assessing intervention probably will require large numbers of patients and prolonged study periods.
3. Comparisons of frequency of occurrence and progression through BOS grades are appropriate end-points for assessing therapy. In individuals, improvement in BOS grade is not expected or consistent with the current understanding of this syndrome.

FUTURE STUDIES

The committee recognizes that although BOS is the most common complication leading to chronic graft dysfunction and death of lung transplant recipients, it remains poorly understood. However, the course of disease progression may be quite variable for individual patients, suggesting a heterogeneous pathogenesis. Although lung function may decrease rapidly, leading to respiratory failure and death in some patients, other patients may survive for years with either stable or slowly progressive loss of lung function. Therefore, we recommend use of this document to stimulate collection of data and to underlie prospective studies that will lead to better understanding of and eventually prevention of this devastating complication. We suggest the following research priorities.

Risk Factors

1. Collation of existing large data bases to better define risk factors
2. Collaborative prospective collection of data in a centralized database to subsequently correlate with development of BOS

Criteria for BOS

1. Prospective collaborative studies to validate the usefulness of the new BOS 0-p stage, in particular in recipients of single lung transplants.
2. Prospective collaborative studies to evaluate survival and quality of life after BOS onset at each stage.
3. Prospective collaborative studies to define different courses of disease progression, risk factors for disease progression, and time of onset.
4. Prospective collaborative studies to evaluate the relative impact on survival, quality of life, and exercise capacity in double vs single lung transplant recipients.

Surrogate Markers

1. Prospective collaborative studies comparing surrogate markers with lung function and ability to predict future decreases in lung function.
2. Prospective collaborative studies to establish normative data and thresholds for significant change in markers such as BAL neutrophilia and eNO; prospective collaborative studies correlating changes in different surrogate markers.

APPENDIX

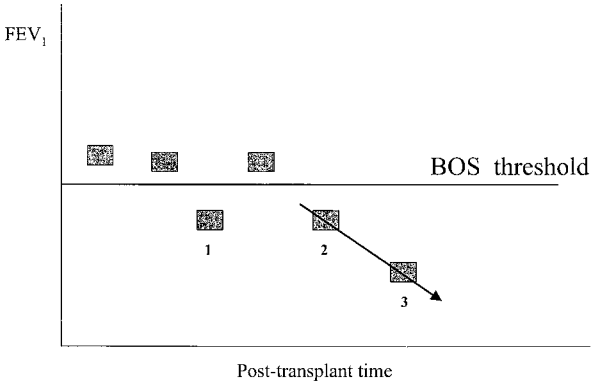

FIGURE 1 Event 1: drop below BOS threshold, not validated by second measurement. Event 2: first BOS measurement and time of onset of BOS defined by validating event #3. FEV_1 decline = slope of values 2 and 3 and any additional measurement over a 1–3 month period.

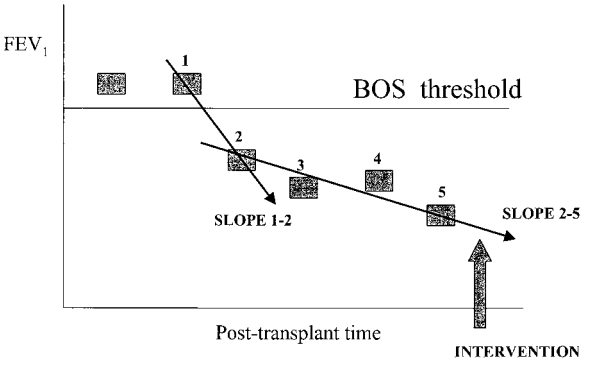

FIGURE 2 Though initial decline below BOS threshold shows a steep decline (slope 1–2), preintervention value 2 which defines BOS onset (and is validated by subsequent values) and subsequent values 3–5 define the slope prior to intervention. Benefit of therapeutic intervention will be defined by comparison with the slope 2–5.

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Lung Transplantation: Current Status and Challenges

Richard N. Pierson III

The lung is an anatomically complex vital organ whose normal physiology depends on actively regulated ventilation and perfusion, and maintenance of a delicate blood–air barrier over a huge surface area in direct contact with a potentially hostile environment. Despite significant progress over the past 25 years, both short- and long-term outcomes remain significantly inferior for lung recipients relative to other "solid" organs. This review summarizes the current status of lung transplantation so as to frame the principle challenges currently facing end-stage lung-failure patients and the practitioners who care for them.

Keywords: Lung transplant, Lung allocation, Reperfusion injury, Acute Lung injury, Chronic rejection, Bronchiolitis obliterans syndrome.

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Evolution of Current Practice

Successful clinical lung transplantation was first achieved in the 1980s (*1*, *2*) after nearly 40 instructive failures over almost 20 years (*3–5*). Reduced steroid doses, a dominant risk factor for both infection and airway anastomotic dehiscence (*3*, *6*, *7*), became possible with cyclosporine-based immunosuppression (*8*). Encouraged by preclinical results (*8–11*), Reitz and colleagues at Stanford achieved long-term survival after combined (en bloc) heart-lung transplantation, and demonstrated that rejection and various infections of the lung could be accurately distinguished and thus more safely and successfully treated (*1*, *12*). Subsequently, single and then double lung transplantation were reintroduced clinically at Toronto by Cooper and colleagues (*2*). Improved donor management, lung preservation, immunosuppression, and antimicrobial therapy contributed to 1-year survival approaching 70% by 1990, justifying broad acceptance in the pulmonary medicine community, and explosive growth of the waiting list through the 1990s. Worldwide activity increased from a few patients per year at one center in 1984 to over 1,500 at about 100 centers in the mid-1990s. Annual lung transplant activity in the United States (about 1,000) and the rest of the world (about 500) has remained constant over the past decade (*13*, *14*), while the U.S. waiting list has grown from about 1,500 to nearly 4,000. Consequently, only about 25% of wait-listed patients will receive a transplant in the next year, down from 60% in the mid-1990s; not counting candidates removed from the waiting list (whose reason for delisting and subsequent fate are not tracked), at least 15% will die before a suitable lung becomes available. These statistics document the practical consequences associated with the donor lung shortage but not its human toll.

Surgical practice in lung replacement has evolved considerably over two decades. Excellent clinical results have been reported with several different bronchial anastomotic techniques (*15–19*). Thus, at least in the context of improving graft preservation and immunosuppression and as long as a technically accurate anastomosis is performed without tension between well-vascularized tissues, intussusception, absorbable sutures, and interrupted technique are not critical. Neither omental nor intercostal pedicle flaps nor primary bronchial artery revascularization were found to decrease the incidence of bronchial anastomotic complications or the incidence or kinetics of chronic rejection (*20*, *21*). More recently, lung transplantation through a limited access anterior thoracotomy has been introduced to minimize surgical trauma (*22*).

Concomitant uncorrectable cardiac pathology in the setting of end-stage lung disease still warrants en bloc replacement of the heart and lungs. However, bilateral sequential ("double") lung transplantation has largely replaced heart-lung transplant as the preferred approach for those patients in whom chronic suppuration mandates replacement of both lungs, as in cystic fibrosis or "wet" bronchiectasis. This shift occurred based on evolving priorities for allocation of increasingly scarce hearts and lungs; simpler logistics and superior outcomes (although in expert hands results are similar (*23*, *24*)); and ethical issues associated with assessing the quality of "domino" heart donors and the added risk for a lung recipient of cardiac allograft rejection and vasculopathy. In the United States, more patients now die on the heart-lung waiting list each year than receive a transplant (*13*).

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The optimal procedure for the majority of end-stage lung failure patients remains controversial. For most candidates, either a single or bilateral sequential procedure provides symptomatic relief, independence from supplemental oxygen, and minimal activity restriction, and 1-year mortality and quality of life are similar (*13*, *14*). On the other hand, 5-year survival (38% vs. 47%), time to onset of chronic rejection, exercise tolerance, objective measures of lung function, and quality of life tend to favor double-lung recipients (*25*). However, this observation is based on retrospective nonrandomized data, and ignores prevalent programmatic practices whereby two lungs go preferentially to younger, healthier candidates who have a better prognosis independent of their transplant outcome (*26*). Even if results are superior, as seems biologically plausible, especially for younger candidates, the net impact on access and outcomes must be considered for the entire population of lung transplant candidates (*27*).

Living donor lobar lung transplantation, pioneered by Starnes and colleagues (*28*), has established a niche primarily for small candidates, because an adult lower lobe is sufficient in size to occupy the pleural space only for children or small adults. It is usually performed bilaterally for patients with cystic fibrosis or ventilator dependence of various etiologies who likely will not survive the wait for a suitable cadaveric donor and have two willing, healthy, biologically compatible donors. Short- and long-term survival are similar to cadaveric transplantation despite application in extraordinarily high-risk recipient populations (*28*, *29*). However, the procedure is ethically and logistically complicated (*30*), putting three lives at risk to save one, and thus is performed in significant numbers at only a few centers.

Short- and intermediate-term retransplantation outcomes similar to primary transplant have been reported for carefully selected patients (<2% of lung transplants performed) at a few programs, particularly when performed for chronic rejection (obliterative bronchiolitis [OB]) rather than primary graft failure (*14*, *31*, *32*). Because anecdotally the second allograft usually develops OB within a shorter interval than did the first, retransplantation seems sensible mainly for young patients with normal renal function and late-onset OB in the first graft.

Lung Donor Demographics

In the United States, about 15% of 6,500 cadaveric organ donors each year yield lungs that are transplanted (*13*, *14*). As a consequence of international initiatives to reduce ethanol-related road traffic accidents, the composition of the donor pool has changed adversely: the average age (now well over 30 years) and incidence of significant comorbidities (hypertension, diabetes) and other putative risk factors for initial lung dysfunction (smoking history, radiologic abnormalities, high A-a O_2 gradient, prolonged intubation) have increased among donors whose lungs are used. The proportion of organ donors whose lungs are transplanted varies dramatically by geographic region, from 5–10% in many US organ procurement areas to about 40% in Ontario and Australia. These striking disparities reflect differences in regional demographics of the donor and recipient pools, heterogeneous donor management strategies, and variably aggressive transplant program practices. Expansion of the lung donor pool will require the lung transplant community to identify and disseminate "best practices" to optimize lung function in organ donors (*33*); to systematically match high-risk donors with physiologically appropriate consenting recipients; and to optimally preserve, assess, and even "resuscitate" marginal or deceased donor lungs (*34*, *35*). Lung donation after cardiac death is feasible (*35–39*), but not yet widely disseminated.

Lung Donor Allocation

The U.S. rules for lung allocation have recently changed dramatically (*40*). Until 2005, lung allocation was driven almost exclusively by accumulated time since listing (*27*, *41*). Based on extensive data analysis, modeling, and iterative interactions among the lung transplant community, in April 2005 the United Network for Organ Sharing (UNOS) grouped wait-listed patients into five relatively homogeneous categories. Disease-specific relative risk criteria collected over the previous 6 months were used to estimate for each waitlist candidate the risk of dying before transplant, which was mathematically combined with the probability of survival for that patient after transplant to derive an integrated allocation priority score. Importantly, each patient-specific component of the score is an objective test result that can be obtained serially and updated as often as the transplant center chooses to do so, allowing rate of patient decline to be reflected in prioritization. The allocation model is intended to be revised to address inadvertent inequities or incorporate evolving priorities; it is expected to more fairly balance access for the sickest recipients with efficacy (net increase in years of life) over the entire population of waiting candidates.

Other countries utilize a wide variety of allocation algorithms, few of which incorporate objective recipient disease severity measures or probability of survival after transplant. Most allow transplant programs local to the donor to make allocation decisions before offering organs on a regional or national basis (*42*).

Primary Graft Dysfunction

Clinically evident ischemia/reperfusion (I/R) injury remains quite common after lung transplantation, with interstitial infiltrates and increased A-a O_2 gradient observed in a substantial minority of lung recipients (*43–46*). Although I/R injury delays withdrawal of ventilator support in less than 30% of recipients, when severe (in about 10% of recipients), "primary graft failure" significantly prolongs intensive care unit and hospital stay and is associated with 20–30% additional 90-day mortality and 30% of deaths within 30 days (*14*). How the mode of brain injury influences lung function in the donor and early and late outcomes is poorly understood (*48*); imperfect preservation of organ function during explant and storage, and reperfusion injury following the obligatory ischemic interval also contribute to the pathogenesis of this clinically important problem. It has persisted despite introduction of an extracellular preservation solution specifically tailored to the lung (Perfadex), and wide adoption of improved procurement techniques such as retrograde perfusion through the pulmonary veins (*49*, *50*). Controlled reperfusion with leukocyte-depleted autologous blood is a clinically available, technically straightforward approach that appears to be associated with significantly lower incidence of initial graft dysfunction, and deserves study on a broader scale (*51*).

Although never evaluated in a prospective, randomized study, over the past decade extracorporeal membrane oxygenation (ECMO) has increasingly been used to rescue patients with severe primary allograft failure and is usually associated with lung recovery (*52–55*), perhaps because of reduced ventilator-associated barotrauma.

Acute lung injury (ALI)—whether associated with I/R, systemic infection, transfusion, or hemorrhagic shock—is mediated by a variety of cytokines, chemokines, and adhesive ligand/receptor interactions (*56–62*). Complement activation, oxygen free radical and eicosanoid generation, and coagulation pathway interactions also contribute to inflammation, cell injury, and loss of endothelial barrier function. In the context of lung transplantation, various parenchymal cell populations as well as passenger donor macrophages or neutrophils sequestered in the lung may be primed by brain death and associated stressors. Targeting each of these cell types and pathways is logistically impractical. Rather, pivotal common mechanisms governing pathogenic pulmonary responses to inflammation represent attractive therapeutic targets. In experimental systems, S-1-P (*63*, *64*), PAR-1 (*64*, *65*), and adenosine-2 receptor agonists (*66*, *67*) can reverse established ALI, perhaps by modulating the balance between Rac- and Ras-mediated signaling pathways, and might thus reverse even established primary graft dysfunction.

Once established, ALI may resolve with minimal sequelae, or the lung may undergo fibroproliferative remodeling with loss of compliance and diffusing capacity via mechanisms that are poorly understood. Recently developed scoring systems for stratifying donor risk and recipient lung injury severity (*45–47*) and multicenter cooperative study groups should facilitate expeditious evaluation of preventive approaches, like soluble complement receptor type 1 (*68*), or candidate therapeutic agents. Expression of various protective proteins during the ischemic interval after lung harvest (*69*, *70*) awaits advances in efficient industrial-scale vector development (G.A. Patterson, personal communication, 2006).

Recipient Selection

The proportion of patients receiving lung transplant with emphysema and A1AT deficiency (50%), cystic fibrosis (15%), and idiopathic pulmonary fibrosis (15%) have remained fairly constant since 1990, with primary pulmonary hypertension, sarcoidosis, retransplant, and an assortment of other diagnoses accounting for the remainder (*13*). Recipient selection criteria (*71*) have been substantially relaxed at many programs (*72*). As one consequence, the average age of lung candidates and recipients is steadily increasing (*13*, *14*, *40*). Of note, some U.S. registry studies suggest that patients with emphysema derive no survival benefit from transplantation (*73–75*), a phenomenon perhaps related to U.S. organ allocation strategies because it is not suggested in a European analysis (*76*). Improving medical therapies for pulmonary hypertension have dramatically reduced the need for transplant for this diagnosis (*40*).

Recipient Management and Associated Outcomes

One-year survival has improved slightly over the past 10 years, from about 75% to 82% (*40*). Since use of extended criteria and older donors has expanded during this interval and in older recipients (increasing recipient age is an independent risk factor (*14*)), expected adverse consequences of these donor trends have apparently been mitigated by improving program practices in other areas (organ preparation, perioperative support, immunosuppression). However, 5-year survival remains stubbornly below 50% (*13*, *14*, *40*).

In the absence of one clearly superior approach, a wide variety of maintenance immunosuppression strategies are currently being used in lung recipients. Mycophenolate mofetil and FK506 may be associated with reduced rates of acute infection and/or improved survival relative to azathioprine and cyclosporin, respectively (*77–79*), but these observations are not universally replicated (*80–82*). Few immunosuppressive agents have been formally studied in this population, but from the ever-broadening array of agents approved by the U.S. Food and Drug Administration or European Standards Agencies for use in kidney recipients, a regimen can now be tailored to each lung recipient's risk factors, evolving clinical circumstances, and financial situation. Newer immunosuppressive agents are now being evaluated for lung transplant indications, in part because the room for improvement in 1-year survival remains substantial and favorable effects are thus easier to measure (*83*). Calcineurin inhibitor-associated renal insufficiency is very common among lung recipients within 5 years (creatinine >2.5 mg/dl in >30%; dialysis or renal transplant in 5–10%). Because their antiproliferative effects may also prevent or retard progression of OB, many centers are exploring conversion from calcineurin inhibition to a "renal-sparing" target-of-rapamycin inhibitor, but a high incidence of bronchial anastamotic dehiscences halted substitution of sirolimus at transplant (*84*, *85*). Another approach to minimize cumulative pharmacologic toxicities utilizes "induction." Although popular, interleukin (IL)-2R blockers have not yet consistently decreased acute rejection or infectious complications (*86*). High-dose ATG or Campath 1H (*87*) with low-dose conventional immunosuppression do not prevent chronic rejection despite profound long-term lymphocyte depletion, and at intermediate-term follow-up mortality and infectious complications appear similar to other regimens. Rapid steroid withdrawal or avoidance (*84*) is rarely attempted in lung allograft recipients because acute rejection is common and a strong risk factor for chronic rejection, whereas chronic rejection is prevalent despite relatively intense current immunosuppressive regimens.

Looking forward, aerosols deliver high concentrations of various drugs directly to the lung, thereby increasing their therapeutic index (*88*), an important opportunity unique to the lung. Because its phosphorylated form is an Edg-1 receptor agonist and should promote enhanced endothelial barrier function, preoperative loading with FTY720 may be particularly useful to prevent primary graft dysfunction in lung recipients, in addition to any effects it may have on cell trafficking and adaptive immunity (*89*). Induction therapy could provide a foundation for peripheral or central tolerance based on immunomodulatory costimulation (*90*, *91*) or chemokine pathway blockade (*92*, *93*).

Control of infection, particularly of herpes-family viruses, remains a particularly important issue for lung allograft recipients, perhaps because their immune suppression is relatively intense, and the lung is relatively vulnerable to local

and systemic insults that accompany acute or recrudescent infection with these organisms. Prolonged prophylaxis for cytomegalovirus with ganciclovir or an orally bioavailable variant usually prevents viremia and disease during therapy, but after prophylaxis is stopped viral activation is prevalent and, anecdotally, the organism is more often resistant to conventional pharmacotherapy. Based on the notion that recipient immunity is integral to long-term control, and taking advantage of increasingly reliable tests to diagnose presymptomatic infection, an expectant "bait-and-switch" approach was effective at preventing symptomatic cytomegalovirus disease and facilitating short- and long-term viral control, at significantly reduced fiscal and physiologic costs (*94*). Likewise, invasive aspergillus, either at the bronchial anastomosis or occasionally in native or graft parenchyma, is a highly morbid complication; prophylactic inhaled or systemic antifungal therapy is probably unnecessary unless airway ischemia at bronchocopy, sputum culture results, or environmental circumstances (e.g., construction or preoperative colonization) alter a particular patient's a priori risk. When less common viruses such as adenovirus and respiratory syncytial virus invade the lung, survival is unusual because, at present, these organisms are essentially untreatable except by supportive measures.

OB describes fibrotic occlusion of small airways, the pathologic hallmark of chronic lung allograft rejection (*95*). OB can usually only be proven by histologic demonstration of OB on large tissue samples obtained at open lung biopsy, retransplant, or autopsy. The typical physiologic correlate of OB, bronchiolitis obliterans syndrome (BOS)—defined as a decline in FEV1 of more than 20% not attributable to acute infection or rejection—has been generally accepted as a valid proxy for OB (*96*). However, about 50% of patients with BOS do *not* have OB when pathologic material is comprehensively audited (*25*, *97*). In these patients recent evidence shows that BOS may be caused by silent gastroesophageal reflux and chronic aspiration (*98*), and performing early antireflux surgery reduces the incidence of BOS (*99*). Adaptive immunity mediated by antibody and T-cell mechanisms clearly plays a central role in OB pathogenesis (*100–103*), as do immunity to lung autoantigens (*104*) and innate immune activation (*105–109*). Pharmacologic approaches to inhibit innate immune system activation, such as azithromycin (*110*), are moving into the clinic.

Malignancy accounts for about 10–15% of deaths during late follow-up, similar to other organ recipient populations. Given the terrible results with chemotherapy for posttransplant lymphoproliferative disease in lung recipients (*111*), the advent of relatively safe, effective treatment with anti-CD20 offers a welcome alternative when reduction in immunosuppression is not curative (*112*).

On balance, the evidence to date supports the logical notion that antibody reactive with donor antigens contributes to both acute and chronic lung injury. In a large series of Harefield's heart-lung recipients, a positive NIH lymphocytotoxic crossmatch was associated with 50% survival among 32 patients, compared to 61% survival with a negative result (*113*). Strikingly, in a subset of patients with a positive T cell–directed crossmatch, zero of four patients survived beyond 70 days, compared to 68% of 100 patients with a negative crossmatch, suggesting that antibody detected by this assay is highly injurious to the graft. Among 656 first-time lung recipients from the combined Toronto and Duke experience, 20 (3%) who had a panel-reactive antibody (PRA) titer >25% exhibited significantly decreased median (1.5 vs. 5.2 years) and 1-month survival (70% vs. 90%) (*114*). Thus, a high PRA (anti-HLA antibodies) reflects a propensity to humoral alloreactivity and is a risk factor for acute and chronic allograft injury (*102*). Recent reports suggest that antibody directed against non-HLA antigens can also trigger acute lung injury (*115*, *116*). Although two ABO-incompatible lung reported in the popular press were associated with early death (*117*, *118*), two published cases demonstrate that anti-ABO antibody can be effectively managed by available therapy (*119*, *120*).

In addition to crude survival statistics and costs, patients and health care payors are increasingly focused on quality of life and return to work as important measures of successful transplant outcome (*121*, *122*). Although over 80% of surviving lung recipients report no activity restrictions at 1 and 5 years, less than 40% return to work (*14*): in the United States, return to gainful employment is often impeded by astronomical ongoing medication costs and unaffordable insurance premiums, effectively trapping patients on disability. Although piecemeal remedies specific to transplantation are conceivable, resolution of this catch-22 will probably require fundamental restructuring of U.S. health care financing, a daunting challenge.

Future Advances

Xenotransplantation from genetically modified pigs offers the most likely near-term prospect for alleviating the lung donor shortage, but lung xenografting poses formidable problems (*123–125*). Whether triggered primarily by cellular adhesive interactions or coagulation pathway incompatibilities, to fully prevent acute lung injury in this context, substantial additional work appears necessary. Investment in primate heart and lung allograft tolerance models should yield new knowledge applicable to xenografts, and to address chronic allograft rejection issues that are specific to thoracic organs (*104*, *126*, *127*). In the longer term, durable fully implantable artificial lung technology will probably require evolution of ECMO to a self-renewing biological interface propagated on new biocompatible materials (*128*, *129*).

SUMMARY

Stable overall lung transplant activity for the past decade reflects the net effect of competing forces. Some restrict activity and patient access (low lung donation rates, adverse donor demographics, preferential double lung use), while countervailing influences include liberalization of recipient age and comorbidity criteria, relaxing donor acceptance standards, and initiatives to disseminate optimal donor management practices. Incremental improvement in 1-year lung transplant outcomes have been achieved despite use of older donors in older, sicker recipients, likely due to improved donor management and increasing availability of newer antibiotic and immunosuppressive regimens. However, 5-year survival remains disappointing at below 50%, with OB, infection, renal insufficiency, and malignancy all contributing to late attrition. These persistent problems un-

derscore the imperative to develop tolerance induction strategies for clinical lung transplantation, and to better understand the contribution of innate immune and nonimmune mechanisms to BOS and OB. Improved policies based on wait-list and posttransplant risk factors are being implemented to fairly allocate organs, and may aid patients and their physicians in deciding whether to accept marginal organs. In addition, socioeconomic barriers will need to be addressed for the full therapeutic potential of lung transplantation to be realized.

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Grading of Acute Pancreas Allograft Rejection

Grade	Histopathology
Grade 0 (NORMAL)	Unremarkable pancreatic parenchyma without inflammatory infiltrates
Grade I (INFLAMMATION OF UNDETERMINED SIGNIFICANCE)	Sparse, purely septal mononuclear inflammatory infiltrates. No venous endotheliitis or acinar involvement identified
Grade II (MINIMAL)	Purely septal inflammation with venous endotheliitis (attachment of lymphocytes to the endothelium with associated endothelial damage and lifting of the endothelium from the basement membrane). In the absence of venous endotheliitis a constellation of at least 3 of the following 4 histologic features: a. Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes b. Eosinophils c. Acinar inflammation in rare (up to 2) foci d. Ductal inflammation (permeation of inflammatory cells through the ductal basement membrane)
Grade III (MILD)	Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes with associated acinar inflammation in multiple (3 or more) foci. Eosinophils, venous endotheliitis, ductal inflammation and evidence of acinar single cell injury may be seen depending on sampling. The latter is manifested as cellular drop-out (apoptosis-pyknotic cell death), or necrosis (oncotic cell death)
Grade IV (MODERATE)	Arterial endotheliitis and/or necrotizing arteritis (vasculitis). Features described in Grade III are usually present
Grade V (SEVERE)	Extensive acinar lymphoid or mixed inflammatory infiltrates with multicellular focal or confluent acinar cell necrosis. Depending on sampling vascular and ductal lesions may be demonstrated

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EVALUATION OF PANCREAS TRANSPLANT NEEDLE BIOPSY: Reproducibility and Revision of Histologic Grading System

[Clinical Transplantation]

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Outline

Graphics

Abstract

Background. **Tissue samples for the diagnosis of pancreatic allograft rejection are now obtained routinely through the application of the percutaneous needle biopsy technique. The availability of biopsy material (89% adequate for diagnosis in our setting) presents a challenge for pathologists who are asked to provide a fast and accurate diagnosis of rejection and its severity, while at the same time being able to differentiate rejection from other causes of graft dysfunction**.

Methods. **To differentiate rejection from other pathologic processes, 26 histologic features were assessed in 92 biopsies performed for confirmation of clinical diagnosis of rejection and the results were compared with 31 protocol biopsies, 12 allograft pancreatectomies with non-rejection pathology, and 30 native pancreas resections with various disease processes**.

Results. **Based on these comparisons, a constellation of findings relating to the vascular, septal, and acinar inflammation was identified for the diagnosis of rejection. Application of these features led us to revise our scheme for grading rejection (ranging from 0-normal to V-severe rejection) to include the categories of "inflammation of undetermined significance" and "minimal rejection." The scheme was used by five pathologist to grade 20 biopsies independently of any clinical data and the interobserver level of agreement was highly significant ([kappa]=0.83, *P*<0.0001). This grading scheme was applied blindly to all (183) biopsies from 77 patients with 6-52 months of follow-up. The correlation of the highest degree of rejection on each patient and ultimate graft loss (0% for grades 0-I, 11.5% for grade II, 17.3% for grade III, 37.5% for grade IV, and 100% for grade V) was highly statistically significant (*P*<0.002). The fraction of grafts lost due to pure immunologic causes increased proportionally to the grade of rejection (0, 50, 66, and 100% for grades II, III, IV, and V, respectively)**.

Conclusions. **This study provides strong support for the proposed pancreas rejection grading scheme and confirms its potential for practical use**.

The major cause of graft loss with pancreas transplants has been irreversible rejection (1). This finding is particularly true for pancreas after kidney (PAK *) and pancreas transplant alone (PTA) cases because the clinical diagnosis of rejection remains relatively nonspecific. When a simultaneous kidney transplant (SPK) is performed in a uremic diabetic, the cotransplanted kidney is thought to provide a reliable indicator for rejection through serial determinations of the recipient's serum creatinine. Isolated pancreas rejection in combined kidney pancreas transplantation is not unusual, however (2). Parameters used for the diagnosis of pancreas rejection include decrease in urinary insulin and C peptide (3, 4); increase in serum amylase, lipase, and anodal trypsinogen (5-7) and pancreas specific protein (8, 9); and cytologic evaluation of pancreatic juice (10, 11) and urine (12, 13). In bladder drained grafts, urinary amylase has been used as a measure of pancreas exocrine function (14-19). Other methods used for the diagnosis of pancreas rejection are ^{99m}Tc DTPA scintigraphy (20) and uptake of indiumlabeled platelets (21). None of these modalities is sufficiently specific to be used without the risk of occasional under- or overtreatment of rejection.

The 1-year pancreas graft survival for SPK transplants performed between 1992 and 1993 is

81% (22). In contrast, the results of pancreas transplants performed in patients who have had a prior successful kidney transplant (pancreas after kidney, PAK) or in patients who have never had uremia (PTA) lagged behind the results obtained in SPK cases, with a 1-year success rate of 61% for both PAK and PTA cases performed between 1992 and 1993 (22). The results of PAK and PTA collectively have continued to improve particularly since the general availability of the newer immunosuppressive agents, tacrolimus, and mycophenolate mofetil. The steady improvement in the success of PTA cases is in part due to the restriction of cases to superior HLA match between the donor and recipient. This strategy markedly decreases the possibility that recipients will experience a rejection episode; however, most patients will undergo a prolonged waiting period before this degree of match can be achieved, even with the existing pancreas sharing scheme that emphasizes HLA match (22). In most cases a compromise is necessary if the transplant is ever to be accomplished. Despite better immunosuppression and HLA matching some patients will experience allograft dysfunction that must be diagnosed.

The success of renal, hepatic, and cardiac transplantation, has been dependent on the ability to differentiate nonimmunologic causes for graft dysfunction from rejection by reliance on a confirmatory biopsy (23). Biopsy material from transplanted pancreas grafts was obtained in the past during laparotomy (23, 24) or through cystoscopically guided transduodenal pancreatic biopsy (25-27). Our center has applied a method for routine ultrasound guided percutaneous pancreatic biopsy under local anesthesia (28, 29). This approach has yielded tissue for histologic analysis in greater than 88% of attempts, with complications in fewer than 2% of cases (2, 30). We have previously proposed a system for grading pancreas allograft rejection that differs from other schemes (31, 32) in that it includes the diagnosis of milder forms of rejection in the absence of arterial vascular rejection. Our original report (32) was based on our experience with the application of the percutaneous needle biopsy technique on patients with graft dysfunction; subsequently protocol biopsies from patients with normal function were also available for evaluation, enabling us to test the specificity of the various morphological parameters. In this study we attempted to identify specific histologic features of milder as well as more severe forms of rejection and devised a detailed system for their practical application in the diagnostic process. The differential diagnosis of rejection is also discussed.

MATERIALS AND METHODS

Between July 2, 1992, and May 31, 1995, 129 bladder drained pancreatic transplants were performed at the University of Maryland Hospital. Of these 129 cases, 64 patients (44 SPK, 15 PAK, and 5 PTA) had 138 pancreatic biopsies, 123 (89%) of which were adequate for diagnosis (see criteria below). Biopsies were performed from 2 days to 48 months after transplantation (mean, 17.1 months) and originated from 43 males and 21 females. The ages ranged from 23 to 56 years (mean, 35 years). The number of biopsies per patient ranged from 1 to 7 (mean, 1.3). The biopsies were obtained using an 18-gauge automated biopsy needle with a 17-mm specimen notch.

The 123 biopsies were performed in two types of circumstances. Ninety-two biopsies were performed on 38 patients for confirmation of clinical diagnosis of rejection. This constitutes the main study group and was designated the Rejection biopsy (bx) group. The clinical indications prompting biopsy required fulfillment of one of the following criteria: (1) a twofold or greater increase in serum amylase (mean increase, 3.5-fold) or lipase (mean increase, 8.5-fold); (2) a sustained 40% or greater decrease in urinary amylase (mean, 45%); 3) loss of glycemic control. Baseline laboratory values were calculated as the means of all values obtained in the 4 weeks preceding the episode of rejection. Twenty-eight simultaneous renal and pancreatic transplant

biopsies were performed in combined kidney-pancreas allograft recipients. In 24 of these instances, concurrent acute increase in serum creatinine and abnormal pancreatic function were observed.

The first episode of minimal rejection (grade II) was treated with pulse steroids. Subsequent rejection episodes or mild, moderate, and severe rejection were treated with OKT3 i.p. The Protocol bx group consisted of 31 consecutive protocol pancreatic transplant biopsies. These biopsies were performed in the absence of any clinical sign of rejection as part of a randomized trial comparing tacrolimus and cyclosporine based immunosuppression in simultaneous pancreas-kidney transplants (33).

The Protocol bx group and two pancreatectomy control groups were used for comparison with the Rejection bx group. The Nonrejection tx pancreatectomy group consisted of 12 partial or complete transplant pancreatectomies for nonrejection related problems and included 2 cases of posttransplant lymphoproliferative disorders (PTLD), 4 cases of chronic pancreatitis (3 obstructive, 1 related to ethanol abuse), 2 of cytomegalovirus duodenopancreatitis, 2 cases of peripancreatic abscesses, and 2 cases of early graft thrombosis.

The Native pancreatectomy group consisted of 30 control native pancreases with various diseases. This group included tissue from partial pancreatectomies for recent traumatic injury from 5 young males, 17 partial pancreatectomies in cases of chronic pancreatitis (14 obstructive type, 3 calcifying type), 4 partial pancreatectomies for acute pancreatitis, 2 autopsy pancreases in cases of cystic fibrosis, and 2 partial pancreatectomies for acute infectious pancreatitis.

For the needle biopsies, three serial 4-µm-thick hematoxylin-and-eosin-stained sections from each case were examined. Biopsies with 2-3 mm^2 or more of pancreatic acinar parenchyma present/biopsy surface area were considered adequate. From the pancreatectomies 4-µm-thick hematoxylin-and-eosin-sections from two blocks per case were examined.

The presence of 26 histologic features was evaluated in the main study group and in the three control groups (Table 1). The results in the various groups were compared with those of the Rejection bx group, using the Fisher's exact test. The results were corrected for the absence of specific structures (ducts, vessels, islets, and nerves) in the needle biopsies.

	Rejection bx	Protocol bx	Native pancreas	Nonrejection Tx
Number of specimens	92	31	30	12
Septal inflammation[b]	85	7 (0.0001)	21 (0.006)	9 NS[c]
Acinar inflammation[b]	65	0 (0.0001)	3 (0.0001)	5 NS
"Activated lymphocytes"[b]	60	0 (0.0001)	3 (0.0001)	6 NS
Plasma cells	33	3 (0.005)	17 NS	6 NS
Eosinophils[b]	61	0 (0.0001)	6 (0.0001)	3 NS
Neutrophils	36	0 (0.0001)	6 NS	2 NS
Acinar single cell injury[b]	34	0 (0.0001)	0 (0.0001)	2 NS
Venous endotheliitis[b]	32	0 (0.0001)[d]	0 (0.0001)[d]	1 NS
Arterial endotheliitis[b]	11	0 NS[d]	0 NS[d]	0 NS
Arteritis[b]	8	0 NS[d]	0 NS[d]	0 NS
Confluent acinar necrosis[b]	7	0 NS	1 NS	2 NS
Ductal inflammation[b]	52	0 (0.0001)[d]	15 NS[d]	2 NS
Dilated ducts[e]	2	0 NS	18 (0.0001)	3 (0.006)
Angulated-compressed ducts[e]	0	0 NS	18 (0.0001)	2 (0.008)
Ductal cell necrosis	16	0 NS	4 NS	0 NS
Ductal epithelial prolif.[e]	0	0 NS	8 (0.0001)	1 NS
Ductal cell atypia	25	0 (0.0001)	3 NS	1 NS
Proliferation of small ducts[e]	0	0 NS	17 (0.0001)	1 NS
Ductal squamous metaplasia[e]	0	0 NS	6 (0.0001)	1 NS
Interstitial edema	27	5 NS	9 NS	3 NS
Nerve inflammation	10	0 NS[d]	13 NS[d]	0 NS
Lamellar fibrosis[e]	1	0 NS	23 (0.0001)	6 (0.0001)
Acinar atrophy[e]	1	0 NS	23 (0.0001)	4 (0.0002)
Acinar enzymatic necrosis[e]	0	0 NS	4 (0.004)	3 (0.001)
Calcification[e]	0	0 NS	3 (0.0002)	0 NS
Islet inflammation	10	0 NS	4 NS	0 NS

[a] Numbers in parentheses refer to the *P* value of comparison between the respective group with the Rejection bx group.
[b] Features used in diagnosis of rejection.
[c] NS, not significant.
[d] Corrected for the presence of the specific structure in the biopsies.
[e] Features used in diagnosis of pancreatitis.

Table 1. Histologic features[a]

For the evaluation of the histologic features, all cases were examined independently by two pathologists (C.B.D. and J.C.P.), who were blinded as to the clinical status of the patients. To test the reproducibility of the grading scheme, one hematoxylin and eosin section from 20 percutaneous pancreas biopsies were evaluated independently by five pathologists blinded to the clinical data. Three of them were transplant pathologists (L.C.R., C.B.D., and J.C.P.) whereas two had no previous experience with transplantation pathology (R.J.C. and O.B.I.). The results were evaluated statistically by the [kappa] analysis for the measurement of interrater agreement.

At the closing of this study (May 1996), the total number of patients with pancreas transplant biopsies for which at least 6 months of follow-up was available amounted to 77. The grading scheme was applied blindly to the 183 biopsies from these patients, and the highest grade of rejection on each patient was correlated with the fate of the pancreatic graft. The linear trend of decreasing graft survival was analyzed with the Pearson's chi square test.

RESULTS

Differentiation between rejection and other pathologic processes. As seen in Table 1, the occurrence of septal inflammation, acinar inflammation, activated lymphocytes, eosinophils, acinar single cell injury, and venous endotheliitis differs significantly between biopsies from patients with clinically suspected rejection (Rejection bx group), the Protocol bx group, and the Native pancreatectomies group. Arterial endotheliitis and arteritis are features that are associated almost exclusively with rejection, but occur far less frequently than the features above and for that reason they do not show statistical significance in the respective comparisons.

The presence of ductal inflammation is not significantly different between the cases with presumed rejection and the native pancreas diseases or nonrejection pancreatectomies. Significant differences are noted, however, between the Protocol bx group and the Rejection bx group.

The presence of plasma cells, neutrophils, ductal cell necrosis, ductal cell atypia, interstitial edema, and nerve and islet inflammation is not significantly different between biopsy samples from patients with clinical suspicion of rejection and samples from other pancreatic pathologic processes.

Spotty or diffuse coagulation necrosis of the acinar parenchyma with associated enzymatic fat necrosis was seen in the cases of early graft thrombosis; no significant inflammatory infiltrates were observed in these cases. In contrast, enzymatic necrosis associated with prominent predominantly neutrophilic infiltrates was seen in acute pancreatitis.

Dilated, angulated, and compressed ducts, ductal epithelial cell proliferation, small duct proliferation, ductal squamous metaplasia, lamellar interstitial fibrosis, acinar atrophy, acinar enzymatic necrosis, and calcification were notable for their rarity or virtual absence in the biopsies from the Rejection bx group. In contrast, these findings were commonly seen in the Native pancreatectomies group, yielding highly significant statistical differences. From the above statistical analysis, a constellation of histological features was identified, which was not seen in the diseases of the native pancreas or in the biopsies from patients with normal graft function (Table 1). The concurrent finding of a minimum of these features in biopsies from patients with clinically presumed rejection was considered as suggestive or diagnostic of rejection (see grading scheme, Table 2). Applying the same principle, a group of histological features remarkable for their absence in rejection but commonly seen in acute and chronic pancreatitis was identified and therefore considered useful for the differential diagnosis (Table 1).

Grade 0—Normal
Unremarkable pancreatic parenchyma without inflammatory infiltrates.

Grade I—Inflammation of Undetermined Significance
Sparse, purely septal mononuclear inflammatory infiltrates.
No venous endotheliitis or acinar involvement identified.

Grade II—Minimal Rejection
Purely septal inflammation with venous endotheliitis (attachment of lymphocytes to the endothelium with associated endothelial damage and lifting of the endothelium from the basement membrane).
In the absence of venous endotheliitis a constellation of at least three of the following four histologic features:
(a) Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes.
(b) Eosinophils.
(c) Acinar inflammation in rare (up to two) foci.*
(d) Ductal inflammation (permeation of inflammatory cells through the ductal basement membrane).

Grade III—Mild Rejection
Septal inflammatory infiltrates composed of a mixed population of small and large ("activated") lymphocytes with associated acinar inflammation in multiple (3 or more) foci.*
Eosinophils, venous endotheliitis, ductal inflammation and evidence of acinar single cell injury may be seen depending on sampling. The latter is manifested as cellular drop-out (apoptosis-pyknotic cell death), or necrosis (oncotic cell death).

Grade IV—Moderate Rejection
Arterial endotheliitis and/or necrotizing arteritis (vasculitis). Features described in grade III are usually present.

Grade V—Severe Rejection
Extensive acinar lymphoid or mixed inflammatory infiltrates with multicellular focal or confluent acinar cell necrosis.
Depending on sampling vascular and ductal lesions may be demonstrated.

* Inflammatory focus is defined as a collection of at least 10 mononuclear cells.

Table 2. Grading scheme

Histologic features used for grading. In the Rejection bx group, septal inflammation (Fig. 1 and 2) was the most commonly observed finding (85/92, 92%) (Table 3).

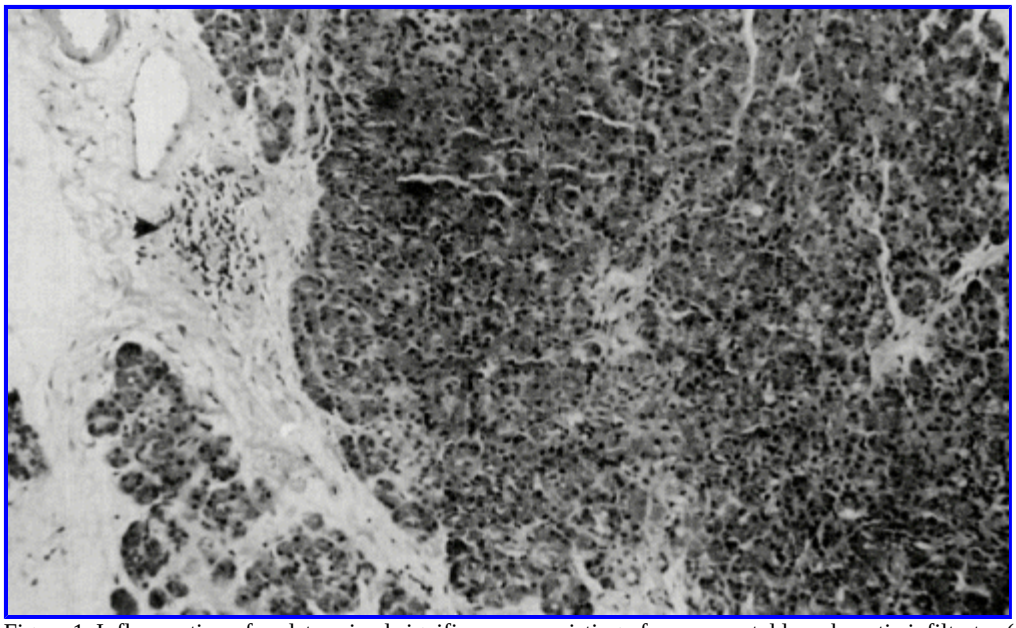

Figure 1. Inflammation of undetermined significance, consisting of sparse septal lymphocytic infiltrates (arrowhead). The acinar parenchyma and neighboring vessels are free of inflammation.

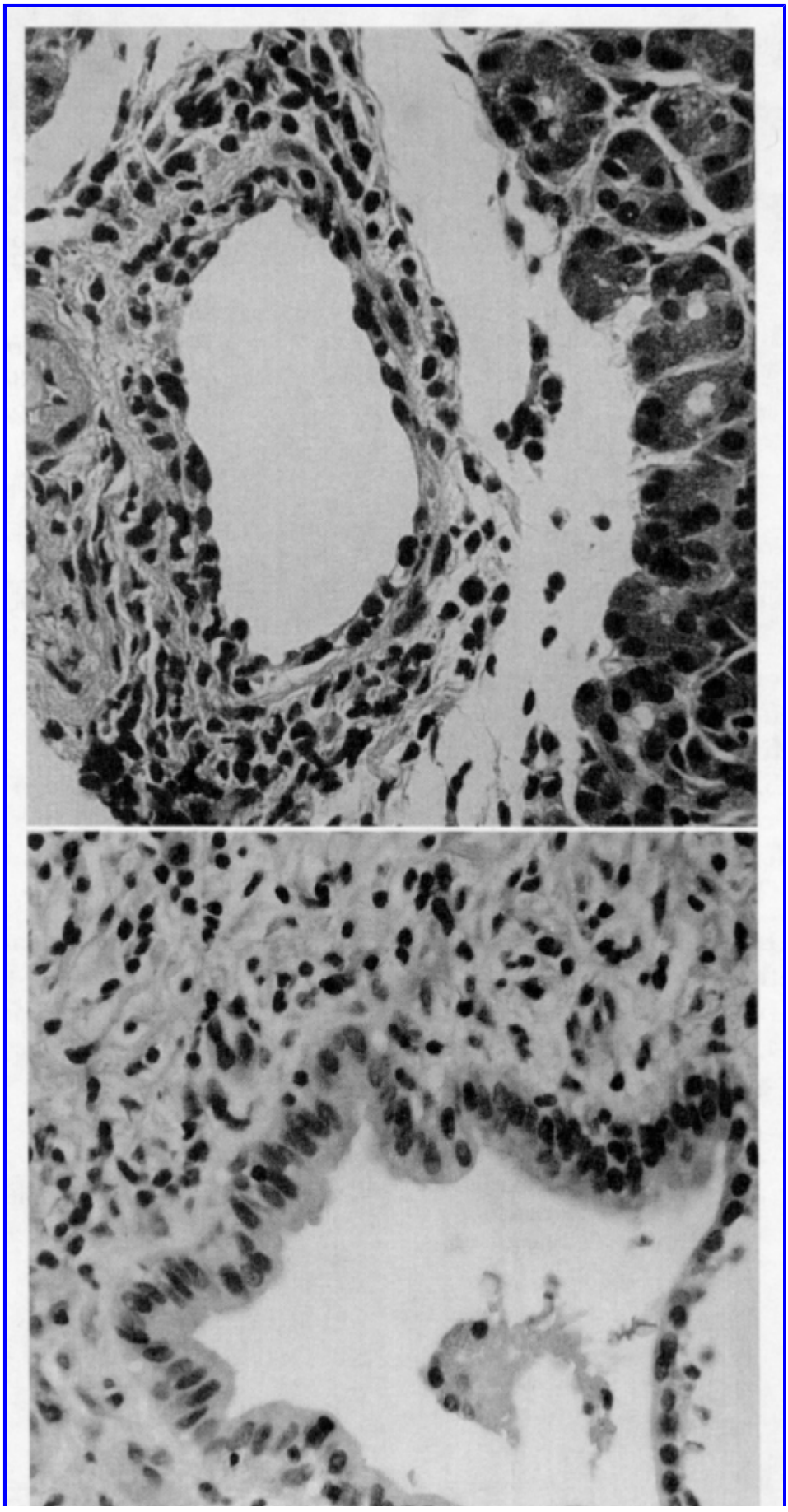

Figure 2. Septal infiltrates associated with venous endotheliitis (A) and ductal inflammation (B).

	Total	0	I	II	III	IV	V
Septal inflammation	85	—	14/14	29/31	27/27	8/8	7/7
Acinar inflammation	65	—	—	23/31	27/27	8/8	7/7
Ductal inflammation	53	—	—	19/31	23/27	7/8	4/7
Eosinophils (septal, acinar)	61	—	—	25/31	23/27	8/8	5/7
Activated lymphocytes	60	—	—	18/31	27/27	8/8	7/7
Venous endotheliitis	32	—	—	6/31	15/27	5/8	6/7
Acinar single cell injury	34	—	—	—	19/27	8/8	7/7
Arterial endotheliitis	11	—	—	—	—	8/8	3/7
Arteritis (vasculitis)	8	—	—	—	—	6/8	2/7
Confluent acinar necrosis	7	—	—	—	—	—	7/7

Table 3. Histologic features useful for grading of rejection (92 nonprotocol needle biopsies)

In 73 of 92 (79%) of the biopsy samples, the inflammatory infiltrates involved additional structures present in the tissue. Ductal inflammation and acinar inflammation were seen in 53 of 92 (64%) and 65 of 92 (71%) of the biopsy samples, respectively (Figs. 2B and 3). Concurrent ductal inflammation and acinar inflammation occurred in 49 of 92 (53%) biopsies. Acinar inflammation with unremarkable ducts was observed in 10 of 92 (11%) cases. Ductal inflammation in the context of septal inflammation and associated venous endotheliitis was seen in 5% of cases with no associated acinar involvement.

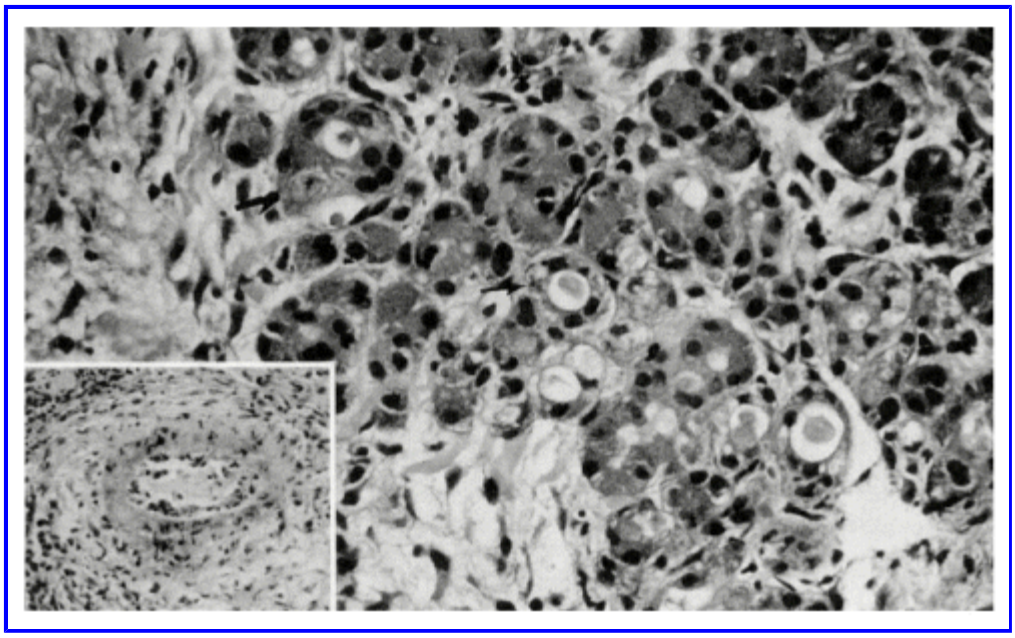

Figure 3. Acinar inflammation with associated acinar cell damage (arrows). Inset shows arteritis.

Eosinophils were identified in fibrous septa and acini in 82% of the biopsies with features of rejection. “Activated” appearing lymphocytes (showing enlarged and convoluted nuclei, increased amounts of cytoplasm) were observed in 60 of 92 cases (65%). Venous endotheliitis consisting of dense perivenular infiltrates with lifting and swelling of endothelial cells was seen in 32 of 92 (35%) biopsies (Fig. 2A). Acinar single cell injury in the form of necrosis (oncotic cell death) or drop-out (apoptosis) was observed in cases with significant inflammation (34/92, 37%, Fig. 3). Arterial endotheliitis, consisting of lifting-up and damage of endothelial cells by lymphocytic or mixed inflammatory infiltrates was seen in 11 of 92 (12%) biopsies. Permeation

of the arterial wall by inflammatory cells and associated fibrinoid necrosis (arteritis) was seen in 8 of 92 (9%) biopsies (Fig. 3, insert). Acinar multicellular necrosis and extensive (nonenzymatic) confluent necrosis were seen in severe rejection (7/92, 8% biopsies, Fig. 4). Necrosis in association with mixed inflammatory infiltrates was most typical of severe acute rejection.

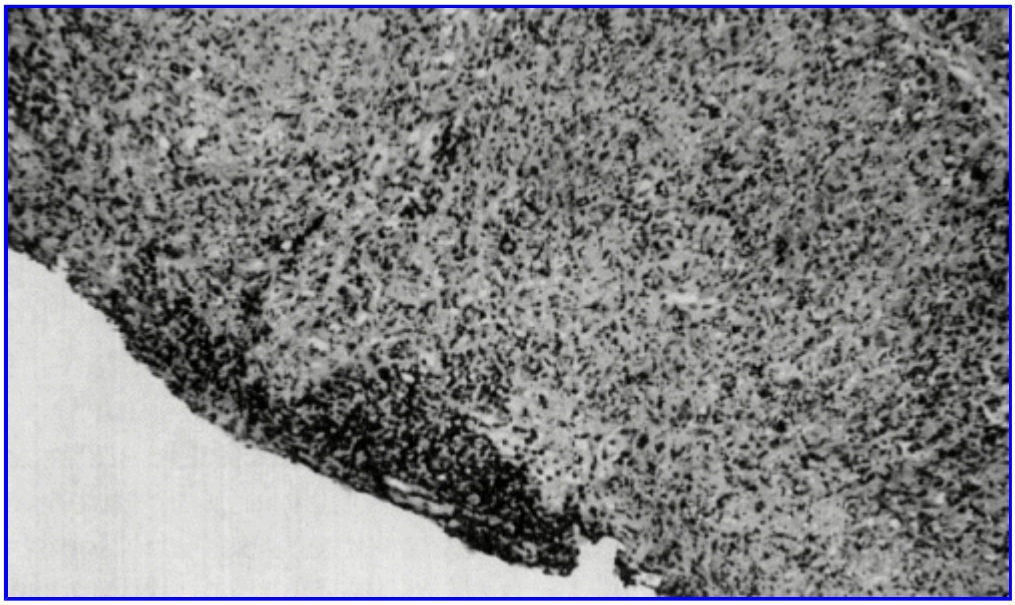

Figure 4. Extensive inflammatory infiltrates associated with confluent necrosis.

The rest of the histologic features evaluated were not useful in the diagnosis and grading of rejection. Interstitial edema, when present, was usually associated with acinar inflammation (22/27 cases). Lack of edema, however, was seen in cases with significant acinar inflammation.

Inflammation involving nerve branches was seen in 10 of 92 cases, and the degree of inflammation was proportional to the septal inflammation. In 10 biopsies, the acinar inflammation extended to involve the islets.

Additional features observed inconsistently in the biopsies from patients with clinical suspicion of rejection are listed in Table 1 and include presence of plasma cells, neutrophils, ductal epithelial cell necrosis, and atypia.

Correlation between grade of rejection and ultimate graft loss. After a mean follow-up (±SEM) of 19.3±1.95 months (range, 6-52 months) the percentage of ultimate graft loss in 77 patients was 0% for patients with biopsies of grades 0-I, 11.5% for grade II, 17.3% for grade III, 37.5% for grade IV, and 100% for grade V (Table 4). The linear trend for decreasing graft survival correlating with the histologic grade, was statistically significant ($P<0.002$) by the Pearson's chisquare method. No difference in graft loss was found between grade II cases with or without venous endotheliitis. The percentage of graft loss due to pure immunological causes increased proportionally to the histological grade (0, 50, 66, and 100% for grades II, III, IV, and V, respectively). Table 5 lists the correlation of immediate clinical outcome with corresponding biopsy grade.

Highest degree of rejection	Graft loss	Functioning	Total no. of patients
Severe (V)	7 (100%), 7[a] 0[b]	0 (0%)	7
Moderate (IV)	3 (37.5%), 2[a] 1[b]	5 (62.5%)	8
Mild (III)	4 (17.3%), 2[a] 2[b]	21 (82.7%)	23
Minimal (II)	3 (11.5%), 0[a] 3[b]	23 (88.5%)	26
Undetermined (I)	0 (0%)	3 (100%)	3
Normal (0)	0 (0%)	10 (100%)	10
Total			77

[a] Graft failure due to immunological causes (acute and/or chronic rejection).

[b] Graft failure due to nonimmunological causes (cardiac death, n = 4, drug overdose, n = 1, late graft thrombosis, n = 1).

Table 4. Ultimate graft loss/highest degree of rejection

Grade, no. of cases	No. of patients treated for rejection	Clinical response[a]	Persistent transplant dysfunction[b]
0, 7	0	—	2
I, 12	3	2	1
II, 31	31	22	9
III, 27	27	20	7
IV, 8	8	4	4
V, 7	7	0	7

[a] Graft function returned to baseline after antirejection treatment.

[b] Persistent graft dysfunction despite antirejection treatment.

Table 5. Correlation of immediate outcome with grade

Correlation of findings in simultaneous pancreatic and renal biopsies. The findings in 22 of the 28 simultaneously biopsied pancreas and kidney transplants correlated with each other (both organs showed minimal rejection in 18 cases and mild rejection in 4 cases). The noncorrelative biopsies showed isolated pancreas rejection in four cases and isolated renal rejection in two cases.

Correlation of number of rejection episodes with severity of rejection and ultimate graft survival. Patients with biopsies showing grades 0-III as highest degree had an average of 1.2 episodes of graft dysfunction (range, 1-2). Patients with one or more grade IV biopsies had an average of 2.1 rejection episodes (range, 1-7). Patients with one or more grade V biopsies had 3.4 rejection episodes (range, 3-5). Patients with failed grafts had 2.2 rejection episodes (range, 1-6), whereas patients with functioning grafts had 1.9 rejection episodes (range, 1-5).

Degree of interobserver agreement between pathologists. Overall high level of agreement was obtained by all five pathologists ([kappa]=0.83, $P<0.0001$). Similar [kappa] values were obtained

for grades I ([kappa]=0.85, *P*<0.0001) and IV ([kappa]=0.79, *P*<0.0001). The reproducibility of grades 0 and V was close to perfect ([kappa]=0.90, *P*<0.0001, and [kappa]=0.94, *P*<0.0001, respectively), whereas substantial levels of agreement were obtained for grades II and III ([kappa]=0.66, *P*<0.0001, and [kappa]=0.72, *P*<0.0001). Excellent discrimination between the diagnosis of rejection versus nonrejection was achieved ([kappa]=0.96, *P*<0.0001), with only one discrepancy by one pathologist who diagnosed one case as grade II (minimal rejection) and the other four pathologists diagnosed it as grade I (inflammation of undetermined significance).

DISCUSSION

By retrospectively examining failed and functioning pancreatic grafts, Nakhleh and Sutherland (30) identified histologic features associated with higher probability of graft failure and proposed a classification scheme for the grading of rejection. In this significant study, the authors indicated that to diagnose rejection vascular changes must be seen. In their classification the latter changes were indicated as arterial endotheliitis in mild rejection and vasculitis in severe rejection.

From our experience with pancreas transplant needle biopsies, however, we developed the concept that a significant number of milder forms of rejection will be underdiagnosed if the presence of arterial changes is a condition for the diagnosis of rejection. In our study we attempted to characterize the features of both mild as well as advanced degrees of pancreas rejection by analyzing needle biopsies from patients with and without clinical evidence of rejection. We compared these features with the morphologic features seen in nontransplant related pancreas diseases and prospectively evaluated their relevance in the graft outcome.

In designing this scheme for histological grading of rejection, we took into account a sequence of events that has been described in unmodified transplant rejection in canine and rodent animal experimental models. The first significant changes in this context occur on days 2 to 3 and consist of septal inflammation and perivascular infiltrates around veins and capillaries (34-36). Capillary-venous endotheliitis also starts to appear at that stage, although it can be somewhat more delayed in the presence of immunosuppression (35). Progressive involvement of the acinar parenchyma and arterial endotheliitis ensue in the following days (34-37). Our findings on needle biopsies support the concept that the most subtle evidence of rejection in human pancreas transplants consists of perivenular infiltrates (often associated with venous endotheliitis) and associated septal inflammatory infiltrates. The small vascular structures (venules) are the point of entry of lymphocytes into the affected tissues (38) and their endothelial cells can be the target of immune attack. Subsequently, the infiltrates involve other interlobular structures (ducts, nerves), as well as the acinar parenchyma and arterial branches, as is shown in the animal models (34, 36). In accordance with this concept, we based the grading of allograft rejection on the progressive involvement of septal, acinar, and arterial vascular structures. This grading concept does not, however, imply a necessary or exclusive pathophysiologic sequence in individual patients.

Our study shows that acinar inflammation is a very sensitive feature of rejection. Thus, it occurs far more often in the context of suspected rejection to a highly significant degree (*P*<0.0001). In particular, the identification of acinar single cell injury secondary to immune attack is a specific feature of rejection (*P*<0.0001), that can be compared with venous endotheliitis, arterial endotheliitis, and vasculitis. In this study, the diagnostic value of eosinophils was again confirmed, as we had previously shown for kidney allograft rejection (39).

Although ductal inflammation shows a significant clinicopathological correlation and appears to be an integral component of pancreas rejection (35, 40) (as is the case with hepatic ducts and

renal tubules in liver and kidney allograft rejection, respectively), it is also often seen in chronic pancreatitis. Ductal inflammation by itself is therefore considered nonspecific if it is not associated with acinar inflammation, venous endotheliitis, eosinophils, or “activated” appearing lymphocytes.

Taking into account the sampling variations and limitations inherent to small needle biopsies, as pancreatic biopsies typically are, we propose a grading scheme that uses a combination of the various useful histological parameters, to maximize the sensitivity and specificity of the diagnosis of minimal rejection (grade II, Table 2). Previous studies have emphasized the specificity of endotheliitis (30, 31)-in particular arterial endotheliitis-for the diagnosis of rejection. Endotheliitis, however, was shown to be related to the degree of inflammation present (30) and therefore would be seen in general in more advanced stages of rejection. The absolute requirement for its identification for the diagnosis of rejection would lead to histological diagnosis of mostly the higher grades whereas the lower grades would be probably treated empirically according to the clinical findings.

From our results we conclude that biopsies with grades II and III identify populations of patients with milder forms of cellular rejection that in the appropriate clinical context should be treated. All 31 biopsies diagnosed as minimal evidence of rejection (grade II) corresponded to patients with acute allograft dysfunction and clinical suspicion for acute rejection (18 cases from this group had concurrent renal biopsies showing mild rejection by the Banff criteria (41). The laboratory abnormalities reversed to baseline after antirejection treatment in 71% of the cases diagnosed as minimal rejection (Table 5). In 19% of the cases there was persistent graft dysfunction, and despite antirejection treatment a higher degree of rejection was seen in a subsequent biopsy. All of our protocol biopsies from patients with normal graft function failed to show features of rejection, being either totally normal (grade 0) or showing inflammation of undetermined significance (grade I).

In the lower grades (0-II), due to the focality of the early rejection process, sampling may cause potential diagnostic problems. Two patients with normal biopsies (grade 0) had persistent transplant dysfunction and showed rejection in subsequent biopsies.

In our initial grading scheme (32), we considered the presence of any degree of interstitial infiltrates as borderline changes (in an analogous manner to the borderline category in the Banff scheme for grading of kidney rejection) (41). With the possibility of evaluating protocol biopsies it became evident that this finding is nonspecific. In rare cases, however, sparse septal infiltrates preceded the development of rejection or occasionally were noted in resolving treated rejection. Thus, we renamed the grade I as inflammation of undetermined significance. Recent papers have addressed the potentially ambiguous meaning of inflammation in grafts, particularly in patients without clinical evidence of organ dysfunction (42).

The evaluation of multiple controls and the statistical analysis described above led us to divide the grades in a slightly different fashion from the original classification in search of increased specificity and sensitivity in the diagnosis of rejection. We introduced the “minimal” category, which is diagnosed by the observation of well defined venous endotheliitis in the context of purely septal infiltrates. To increase the sensitivity of the system we also diagnose minimal rejection in the absence of venous endotheliitis but with the concurrent presence of the constellation of other features described in Table 2. Biopsies that fall short of fulfilling these criteria are classified as inflammation of undetermined significance.

The higher grades (III-V) in this scheme correspond to unequivocal allograft rejection and

therefore pose no problem from a differential diagnostic point of view. Less pronounced acinar or septal inflammation not associated with definite vascular changes should, however, be differentiated from other conditions affecting the graft. The statistical analysis indicates that it should be possible in most cases to differentiate mild rejection from drainage related problems associated with chronic pancreatitis. The latter cases display fibrosis, acinar atrophy and ductal dilation and proliferation, as is the case with chronic pancreatitis in general. On the other hand, it may be impossible to differentiate only on a morphological basis cellular rejection from other conditions acutely affecting the graft. A high degree of caution should therefore be applied to all biopsy samples to rule out the viral cytopathic changes typical of cytomegalovirus pancreatitis, the presence of the atypical infiltrates (particularly atypical immunoblasts) that may suggest PTLD or any other feature indicative of a disease process different from a pure rejection reaction (43). The presence of peripancreatic abscesses or other similar conditions that affect the surgical bed may cause significant changes in the superficial pancreatic tissue, which is the portion usually sampled with the percutaneous biopsy technique. These changes consist of significant mixed septal inflammatory infiltrates associated with early septal fibrosis and occasional acinar involvement. In a different clinical setting many of these features would be indicative of cellular rejection (43). The algorithm proposed here (Fig. 5) emphasizes the importance of first correctly diagnosing rejection before assigning the appropriate grade. Strict adherence to the proposed guidelines leads to highly reproducible results that have clinical significance.

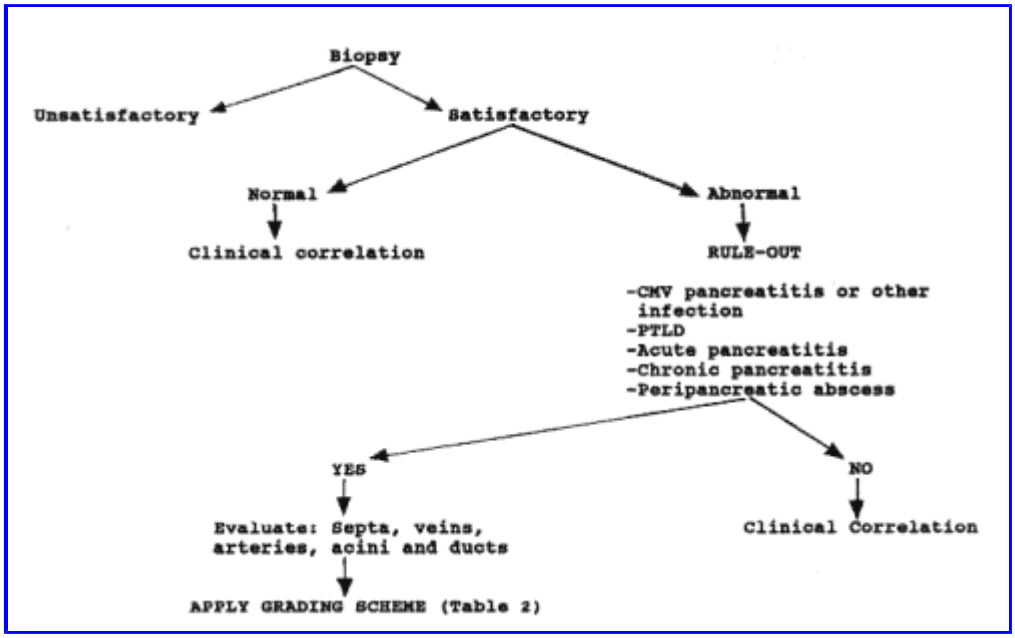

Figure 5. Algorithm for the evaluation of pancreas allograft biopsies.

Although our study deals mainly with diagnosis of acute rejection, it should be stressed that findings indicating chronic rejection (increased septal fibrosis, acinar parenchymal loss, and chronic transplant vasculopathy) have additional prognostic implications as previously described (44), and should be stated in the pathology report. We were not able to demonstrate evidence of primary isletitis or of recurrent diabetes mellitus (45).

In summary, in addition to the well established concept that acute arterial vascular changes are diagnostic of advanced pancreas allograft rejection, we propose that early rejection starts in the connective tissue septa often with associated venular inflammation. With the subsequent

infiltration by a mixed population of inflammatory cells, ducts and acinar parenchyma are affected. These early changes should be recognized and treated as clinically indicated. The progressive morphologic findings leading to our grading system are supported conceptually by experimental studies in animals and further confirmed by the clinical outcome in this group of patients.

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Footnotes [Context Link]

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PANCREAS TRANSPLANTATION: THE HISTOLOGIC MORPHOLOGY OF GRAFT LOSS AND CLINICAL CORRELATIONS

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Background. **Graft losses due to leaks, bleeding, thrombosis, infections, and early pancreatitis are grouped together under the category of technical failure. Among these complications, massive vascular thrombosis continues to be the most important cause of early graft loss due to technical failure. Pathological evaluation of most allografts lost early in the posttransplantation period shows vascular thrombosis with associated proportional parenchymal necrosis. The morphological findings in allografts that are considered to be lost due to technical failure has not been systematically addressed. In particular, the role of acute rejection in early graft loss has not been well studied.**

Methods. **Seventy-four consecutive pancreas graft pancreatectomies were studied histologically to evaluate for thrombosis (recent versus organized), type of vessel involved by thrombosis (arteries, veins, or both), acute rejection grade, chronic rejection grade, endotheliitis, transplant arteritis, coagulation necrosis, acute pancreatitis, presence of infectious organisms, transplant (obliterative) arteriopathy, neoplasia, relative proportions of alpha and beta islet cells, and immunoglobulin and complement deposition. The histological findings were correlated with donor and recipient data as well as clinical presentation.**

Results. **In 23 out of 39 grafts lost in the first 4 weeks posttransplantation, the only pathological changes found were vascular thrombosis and bland ischemic parenchymal necrosis. In these cases, no underlying vascular pathology or any other specific histological change was identified. Most of these grafts (78%) were lost in less than 48 hr and all in the first 2 weeks posttransplantation. Massive vascular thrombosis occurring in an otherwise histologically normal pancreas was the most common cause of graft loss in the first 4 weeks posttransplantation (59%). In most of the remaining cases (33%), although the clinical presentation suggested technical failure, there was clear histological evidence that the massive thrombosis resulted from vascular injury due to immune damage (acute and hyperacute rejection). Increased incidence of early graft thrombosis was seen in grafts from older donors and longer cold ischemia times. After the first month posttransplantation, graft pancreatectomies revealed a wider variety of pathological processes that included severe acute rejection, combined acute and chronic rejection, chronic rejection, and infections. Acute and chronic vascular thrombosis in large and small vessels was commonly seen at all times posttransplantation; chronic, organized thrombosis was strongly associated with chronic rejection.**

Conclusions. **(a) Early acute thrombosis occurring in a histologically normal pancreas defines a true technical failure. This study showed that acute rejection leading to massive thrombosis, which clinically simulates technical failure, results in a significant proportion of early graft losses. (b) Systematic histological evaluation of failed grafts is absolutely necessary for the accurate classification of the cause of graft loss. (c) There is morphological evidence that chronically ongoing thrombosis is an important, common, contributing factor for late graft loss.**

INTRODUCTION

Refinement of surgical techniques, potent immunosuppressive drugs, accurate diagnosis of rejection, better treatment of infections, and careful selection of donors and recipients have all resulted in the widespread use of pancreas transplantation with improving long-term results (*1–24*).

Whereas excellent rates of 1-year graft survival have been achieved in recent years for all types of technically successful pancreas transplants (simultaneous pancreas kidney (SPK), pancreas transplant alone (PTA), pancreas after kidney (PAK)) (*1*, *21*), a significant obstacle in pancreas transplantation continues to be the high incidence of graft loss in the early postimplantation period, due to a variety of surgical complications (*13*, *15*, *25–36*). As reported by the Pancreas Transplant Registry/UNOS, the marked decrease in the rejection rate has caused the relative risk of graft loss to be higher for technical failures than for rejection (*5*). Among the peritransplantation complications, thrombosis continues to be the leading cause of nonimmunological graft loss (5.8–16.4%) with higher rates seen in PAK and PTA cases with enteric drainage (*5*).

The pancreas has intrinsically a low blood flow compared with other solid organs. Perioperative inflammation and edema (*29*), as well as microvascular and endothelial damage relating to donor factors and organ preservation, all contribute to further compromise blood flow in the early posttransplant period (*34*), which leads to thrombosis. Correspond-

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ingly longer cold ischemia times have been associated with increased incidence of graft thrombosis (*30*, *36*).

Abnormalities in coagulation factors have been found in association with increased risk of thrombosis (*37*, *38*), and anticoagulation has been proposed to prevent clotting (*29*, *39*). This treatment, however, seems to be less important than better surgical technique and decreased pretransplantation graft damage in diminishing the risk of graft thrombosis (*13*, *15*).

Acute rejection is suspected to play a role in some patients with early graft thrombosis (*5*, *32*). HLA mismatches seem not to impact on the incidence of graft loss due to technical failures, however, HLA mismatch does have an overall negative impact on graft survival (*6*, *40*, *41*).

In this study, we performed a detailed, histological evaluation of all the pancreatectomies performed during the first 8 years of the pancreas transplant program at the University of Maryland. The objective of this study was to correlate the morphological findings with the clinical course. Specifically, we attempted to (a) determine the morphological changes associated with early graft thrombosis (up to 4 weeks); (b) evaluate the spectrum of morphological changes in pancreatectomies performed at later times, with emphasis on the relationship between graft loss and acute rejection, chronic rejection, and acute and chronic thrombosis; and (c) determine the overall pattern of graft loss in relationship to posttransplant time and specific pathological processes (e.g., rejection, infection, PTLD).

MATERIALS AND METHODS

Between April 1, 1991 and April 1, 1999, 301 pancreas transplants were performed at the University of Maryland Hospital (154 SPK, 114 PAK, 32 PTA). During the same time, 74 pancreas graft pancreatectomies were performed in 69 patients (5 retransplants). In all but two patients, the transplants were performed for insulin-dependent diabetes mellitus type I. Two patients had diabetes secondary to chronic pancreatitis. Five to fourteen routinely prepared hematoxylin and eosin stained sections from each case were reviewed by two pathologists, who were blinded to any clinical data. Available frozen tissue from 10 organs that failed within 7 days posttransplantation were evaluated by immunofluorescence studies for deposition of immunoglobulins and complement (IgG, IgM, IgA, C3). Immunoperoxidase stains for insulin and glucagon (Dako, Carpinteria, CA) were performed on 1 paraffin section from each of the 74 cases to determine the proportion and distribution of alpha and beta cells.

The histological parameters that were evaluated are thrombosis (recent and organized), type of vessel involved by thrombosis (arteries, veins, or both), acute rejection grade, chronic rejection grade, endotheliitis, transplant arteritis, coagulation necrosis, acute pancreatitis, presence of infectious organisms, transplant (obliterative) arteriopathy, neoplasia, and proportion of alpha and beta islet cells on peroxidase stains.

Data made available after the histological evaluation included pertinent clinical history, recipients' age and gender, dates of transplant and pancreatectomy, first serum amylase, and peak lipase in the first 24 hr posttransplantation. Donors' age, gender, weight, serum amylase, serum lipase, cause of death, and HLA mismatch were also recorded.

The diagnosis of infectious pancreatitis was based on the morphological pattern and the microbiology culture results. The diagnosis of acute rejection, chronic rejection, and posttransplant lymphoproliferative disorder was based on the previously described criteria (*42–44*).

The immunosuppression schemes were as follows: all patients received 10–14 days of induction therapy with either ATGAM (Upjohn, Peapack, NJ) or OKT3 (Orthoclone, OrthoBiotech, Raritan, NJ). Maintenance therapy was initiated when the nasogastric tube was discontinued (PAK, PTA) or when a dropping serum creatinine clearly indicated renal transplant function (SPK). Triple maintenance therapy consisted of either cyclosporine (Sandimune or Neoral) or tacrolimus (Prograf), prednisone and azathioprine (Immuran), or mycophenolate mofetil (Cellsept). Target blood concentration of cyclosporine or tacrolimus in the immediate postoperative period were 300–400 ng/ml and 12–20 ng/ml, respectively; by 1 year, the target levels were tapered to 200 ng/ml and 8–10 ng/ml, respectively. Rejection episodes were treated with 500 mg of intravenous methylprednisolone followed by a taper over 2 weeks. ATGAM (Upjohn) or OKT3 (Orthoclone, OrthoBiotech) were administered at standard doses for 10–14 days according to clinical parameters. The first episode of minimal (grade II) or mild (grade III) rejection was treated with corticosteroids. Recurrent episodes of rejection and moderate or severe rejection (grades IV and V) were treated with a combination of corticosteroids and ATGAM or OKT3.

Starting in 1998, all pancreas transplant patients received low doses of heparin for 5 days posttransplantation and then were placed on either ASA indefinitely (SPK) or Coumadin (PAK, PTA) for 3 months.

The morphological parameters were correlated with the patient and donor data and the time at graft loss and analyzed with the 2-tailed Pearson's correlation test and *t* test. Relative risks were calculated with the Cox regression analysis.

RESULTS

Demographic Data

The 74 graft pancreatectomies (including 5 retransplants) were performed on 69 patients; 29 females and 40 males; 7 African-Americans and 62 Caucasians; ages 26–59 with a mean of 28.5 years (SD 12.2 years). Time of graft pancreatectomy ranged from 1 hour posttransplantation to 81 months (mean of 6.4 months, SD 13.8 months). The pancreatectomies corresponded to 39 SPK, 21 PAK, and 14 PTA; 34 grafts were bladder drained and 40 were enteric drained. The donors had a mean age of 27.9 (range 10–54, SD 1.49); these were 14 African-Americans and 55 Caucasians; 25 females and 44 males. The donors' weights ranged from 110 to 229 lb (mean 149, SD 3.4); they had a mean serum amylase and mean serum lipase of 146.6 U/L and 164.4 U/L at the time of procurement (range 15–754, SD 164.4 and range 9–910, SD 72.04, respectively). The mean cold ischemia time was 1137 min (range 540–1950, SD 372 min). The degree of mismatch ranged from 0–6 (mean 3.38, SD 1.74). The cause of death was traumatic/accidental in 67 cases and cerebrovascular in 7 cases.

Histological Findings

Based on the histological findings the cases were classified in the following groups.

Pure vascular thrombosis in an otherwise normal pancreas. In 23 grafts, the only pathological changes found were vascular thrombosis and bland ischemic parenchymal necrosis. No underlying vascular pathology or any other specific histological change was identified in these cases (Fig. 1). The majority of these grafts (78%) were lost in less than 48 hr after transplantation (n=18) and all 23 were lost in the first 2 weeks posttransplantation.

Three of the 5 patients that required retransplantation lost both first and second grafts to this type of thrombosis. The other patients lost their first grafts to infection and

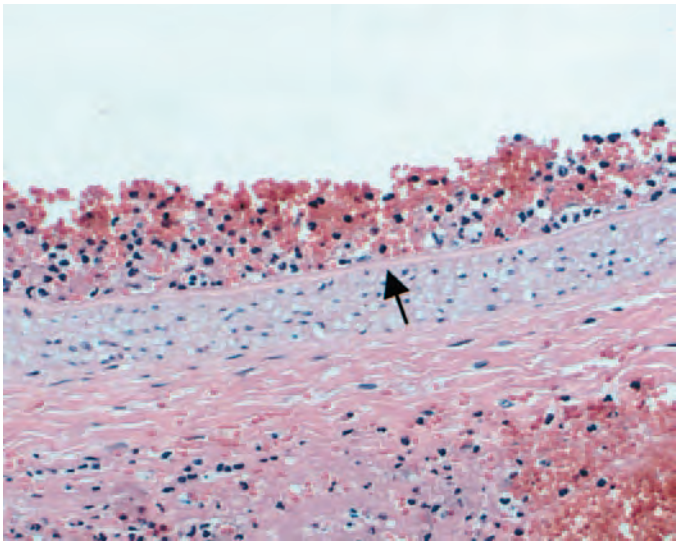


FIGURE 1. Acute thrombosis occurring in otherwise normal pancreas (early thrombosis). The vascular wall is intact. Arrow marks the internal elastic lamina, overlying the normal muscular layer.

rejection, respectively, and their second grafts to pure vascular thrombosis. The time intervals between the first and second grafts were 11, 12, 24, 8, and 7 months, respectively.

Hyperacute allograft rejection. Two pancreatectomy specimens, resected at 1 and 12 hr posttransplantation, respectively, showed fibrinoid necrosis of arteries and veins, indicating hyperacute allograft rejection (Fig. 2A); there was associated massive vascular thrombosis and parenchymal necrosis. Immunohistochemical studies indicated deposition of IgG and C3 in the wall of blood vessels (Fig. 2B).

The first patient with hyperacute rejection was a 50-year-old woman that underwent a PAK transplant. Within minutes of anastomosis, the graft became cyanotic, hemorrhagic, and had no blood flow. Repeat crossmatch and panel reactive antibodies were negative. Additional workup demonstrated (in the recipient's serum) antikeratinocyte antibodies reacting against 20% of a panel of samples.

The second patient with hyperacute rejection was a 40-year-old woman with history of high panel-reactive antibodies; she underwent plasmapheresis before a PAK. Despite an initially negative pretransplant crossmatch, within hours after transplantation the graft was tender, swollen, hemorrhagic, and lacked blood flow. The posttransplantation serum was positive for anti-HLA antibodies.

The cold ischemia time in these 2 patients was 1150 and 1740 min, with a mismatch 5 and 3 antigens, respectively. The donors' amylase and lipase were within normal limits.

Acute allograft rejection. The histological changes in 15 pancreatectomies resected between 1 week to 4 months posttransplantation (mean 5.1 weeks) consisted of endotheliitis and various degrees of necrotizing arteritis (acute rejection grade IV and V). Immunohistochemical studies in eight of these cases failed to show any significant immunoglobulin or complement deposition in the grafts.

Seven patients in this group (46.6%) had a posttransplantation course complicated by systemic and/or peripancreatic infections that required reduction of immunosuppression.

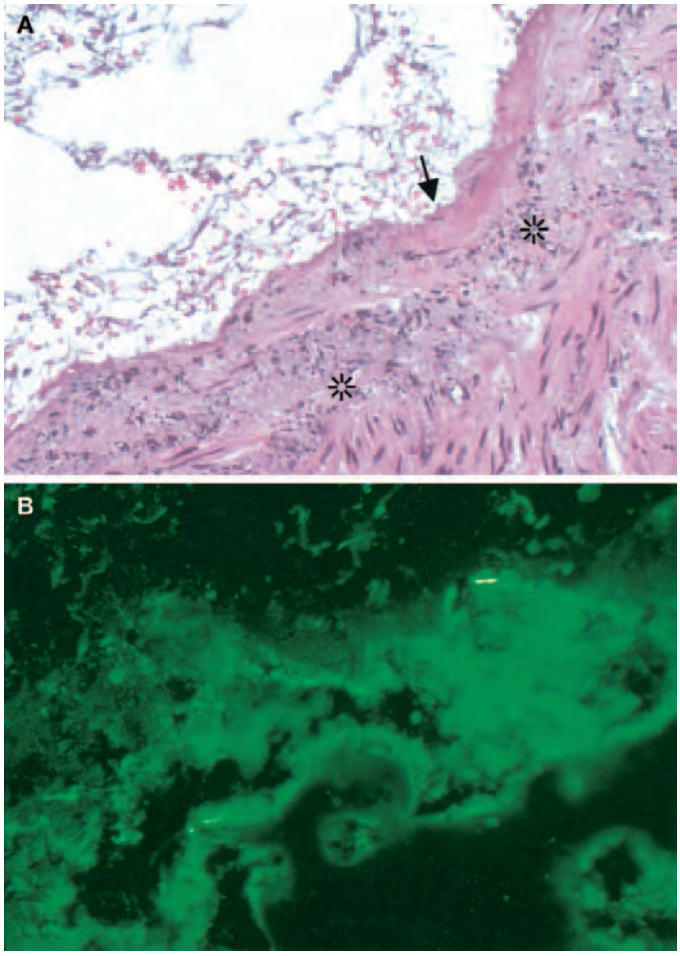


FIGURE 2. (A) Hyperacute allograft rejection with transmural necrosis of the arterial wall. Asterisks mark neutrophilic infiltrates. Early thrombus formation and fibrin strands are attached to the wall (arrow). (B) Hyperacute allograft rejection. Deposition of IgG is in the vascular walls.

Duodenal leaks were seen in five of these patients. Graft losses due to a combination of infection and rejection occurred between 3–6 weeks posttransplantation. In addition to the features of rejection, for patients with peripancreatic infection, the grafts showed increased fibrosis.

Acute and chronic rejection. Cases with persistent (biopsy proven) acute allograft rejection showed early interstitial fibrosis and acinar loss consistent with chronic rejection (*44*), starting in the second month posttransplantation. Six grafts showing these combined features of acute and chronic rejection were lost at times ranging from 6 weeks to 20 months (mean 6.6 months). Three patients that lost their grafts within 4 months posttransplantation due to acute rejection superimposed on accelerated chronic rejection received less than optimal immunosuppression due to persistent infectious complications.

Pancreatitis and peripancreatitis. Five grafts had necrotizing, infectious duodeno-pancreatitis and were resected at 1, 1, 2, 3, and 11 months, respectively (mean 3.6 months); these corresponded to 3 bacterial (*Enterobacter cloacae*, *Proteus mirabilis*, Methicillin resistant *Staphylococcus aureus* (MRSA)), 1 fungal (Candida glabrata), and 1 mixed infection

(Candida albicans/MRSA). Figure 3 demonstrates the wall of a pancreatic abscess.

Chronic rejection. Fifteen graft pancreatectomies showed extensive interstitial fibrosis and acinar atrophy in a pattern consistent with chronic rejection (Fig. 4) and were lost at a mean time of 28.6 months (range 4–81 months). These grafts did not show any significant concurrent acute rejection.

Posttransplant lymphoproliferative disorder. Epstein-Barr related posttransplant lymphoproliferative disorder (PTLD) resulted in five graft losses. Allograft pancreatectomies for PTLD were performed in four patients in the second month posttransplantation and in one patient in month 12 (mean 5 months).

Incidence of Vascular Thrombosis and Relationship with Rejection

Sixty-four grafts, out of the total of 74 (86.4%), displayed some degree of recent thrombosis; in 39 of these grafts, the recent thrombosis was extensive and was associated with focal or diffuse ischemic (coagulative) or hemorrhagic necrosis. Arteries and veins were affected in 44 grafts with recent thrombosis; venous thrombosis only was seen in 4 cases and arterial thrombosis only in 14 cases. There was no correlation between the time of graft loss and the type of vessel affected by the thrombosis.

Recent thrombosis was seen to some degree in all cases of early graft loss due to acute allograft rejection with vascular involvement (endotheliitis or arteritis) (Fig. 5A).

Eight functioning pancreas allografts had to be resected between 10 and 36 months posttransplantation due to acute (recent) thrombosis occurring in larger arteries. In these cases, the thrombosis always occurred in abnormal blood vessels, either showing transplant arteriopathy or lesions consistent with healing vasculitis/endotheliitis (Fig. 5B).

All cases with chronic rejection showed scattered vessels with thrombosis (acute and chronic in 13 cases and only chronic (old, organized) in 2 cases); this typically involved medium size to small arteries and veins (Fig. 5C).

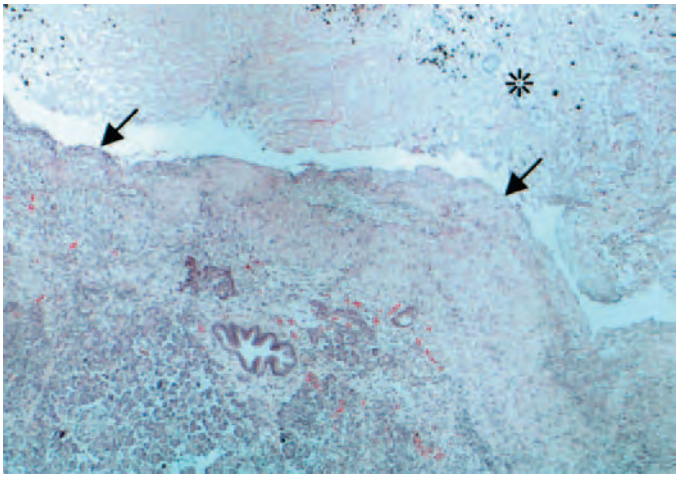


FIGURE 3. Necrotizing bacterial pancreatitis with abscess formation. The cavity of the abscess (asterisk) is lined by a wall composed of granulation tissue and acute and chronic inflammatory exudates (arrows).

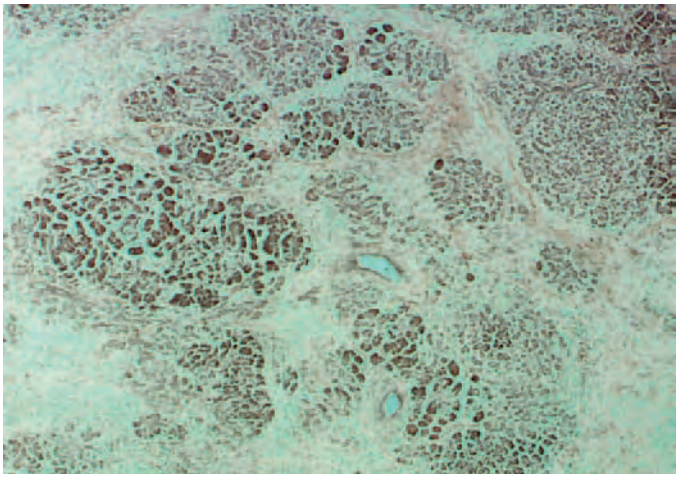


FIGURE 4. Chronic rejection is characterized by an increase in parenchymal fibrosis and concurrent atrophy of the acinar component.

Thrombosis was insignificant in the pancreatectomies performed for infectious processes.

Immunohistochemical Stains for Insulin and Glucagon

The viable components of pancreatectomies that showed no fibrosis or atrophy showed similar strength of staining and pattern of distribution for insulin and glucagon to the controls. Pancreatectomies with chronic rejection displayed fragmented or hyperplastic islets; alpha and beta cells were present in all cases as previously described (*45*). We did not find selective loss of beta cells or evidence of insulitis in any of the pancreatectomies.

Statistical Correlations Between Histological Findings and Other Data

Older donor age and longer cold ischemia time were associated with increased occurrence of early graft thrombosis ($r=0.240$, $p=0.01$, and $r=0.275$, $p=0.02$, respectively). Thrombosis overall occurred with increased frequency in grafts from older donors ($r=0.253$, $p=0.03$).

Higher donor amylase levels were associated with an increased overall incidence of acute rejection ($r=0.323$, $P=0.009$); high donor amylase levels were also associated with higher histological grades of acute rejection (grades IV and V, $r=0.260$, $P=0.03$). The mean donor amylase corresponding to grafts that did not show acute rejection was 98 U/L (SE 17.4), whereas the mean donor amylase corresponding to grafts that showed acute rejection was 216 U/L (SE 38.3), $P=0.003$.

Higher peak lipase values in the first day posttransplantation were associated with increased incidence of acute allograft rejection occurring within the first month posttransplantation ($r=0.377$, $P=0.006$).

The presence of transplant arteriopathy (one of the histological features of chronic rejection) was strongly associated with recent and organized thrombosis ($r=0.278$, $P=0.01$, and $r=0.469$, $P<0.000$, respectively). Correspondingly old (organized) thrombosis was seen almost invariably in pancreatectomies with chronic rejection ($r=0.378$, $P=0.001$). As expected, progressive graft fibrosis (correlating with increasing

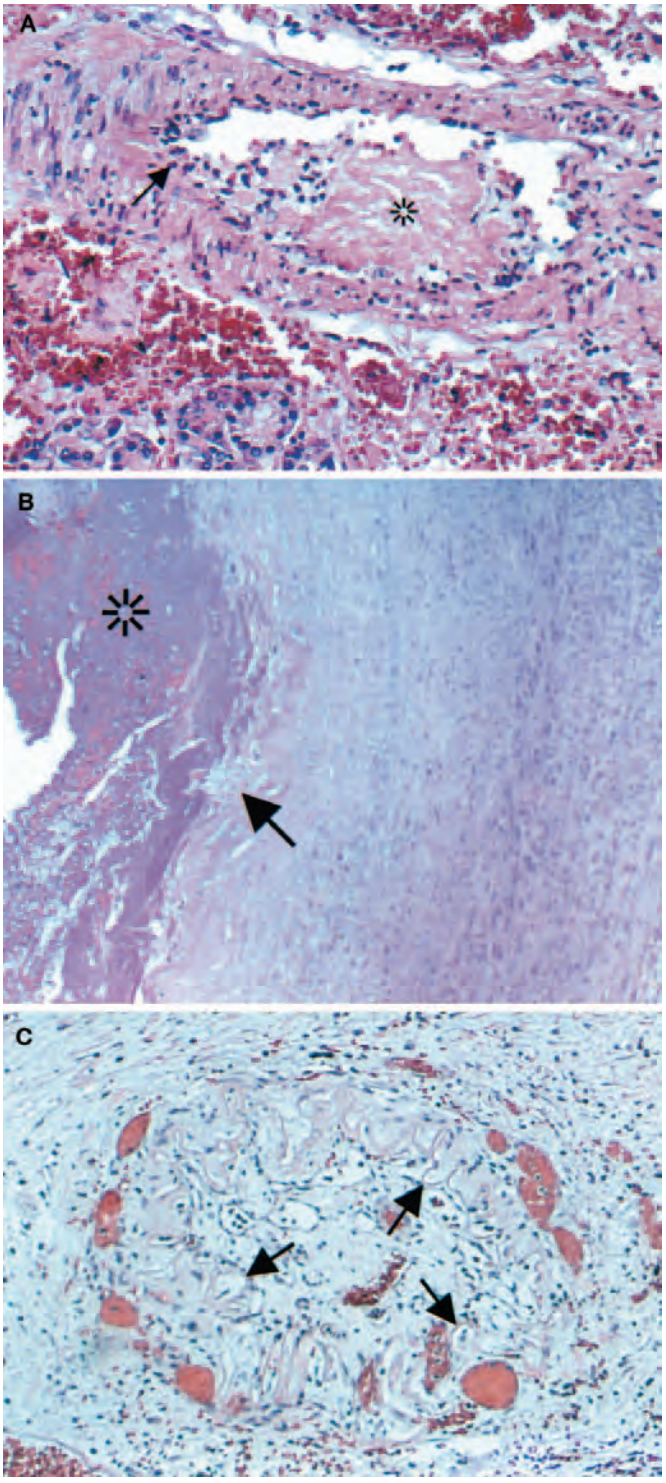


FIGURE 5. (A) Early endotheliitis (arrows) associated with recent fibrin and platelet thrombi (asterisk). (B) Recent (non-organized), massive thrombosis (asterisk) occurring 28 months posttransplantation. The thrombosis occurred in association with abnormal endothelium overlying transplant arteriopathy. The arterial wall is thickened, fibrotic, and there are clusters of subendothelial foam cells (arrow). (C) Organized thrombosis with recanalization in medium-size artery. Arrows mark the internal elastic lamina. Note multiple vascular lumina embedded in fibrous tissue occupying what used to be the original lumen. These changes are commonly seen in pancreatectomies with chronic rejection.

grades of chronic rejection) and the presence of transplant arteriopathy were directly related to the time elapsed after transplantation ($r=0.518$, $P=0.000$ and $r=0.699$, $P<0.0001$, respectively).

There were no significant differences in the donor and recipient data or type of transplant between the group of patients with simple (idiopathic) graft thrombosis occurring early posttransplantation, the group of patients with acute allograft rejection that lost their grafts within 6 months posttransplantation, and the group of patients with successful grafts at 12 months posttransplantation. With respect to drainage type, there was a trend suggesting an association between enteric drainage and idiopathic, early thrombosis, but this did not reach statistical significance ($P=0.057$). No correlation could be found between the type of death in the donor (traumatic versus cerebrovascular).

The type of graft loss in relationship to time of pancreatectomy is summarized in Table 1. Early thrombosis occurring in histologically normal organs and thrombosis due to acute/hyperacute rejection caused 92% of graft losses in the first month posttransplantation. Chronic rejection was the most important cause of graft loss after the first 6 months posttransplantation. Table 2 summarizes donor and recipient data for each histological group.

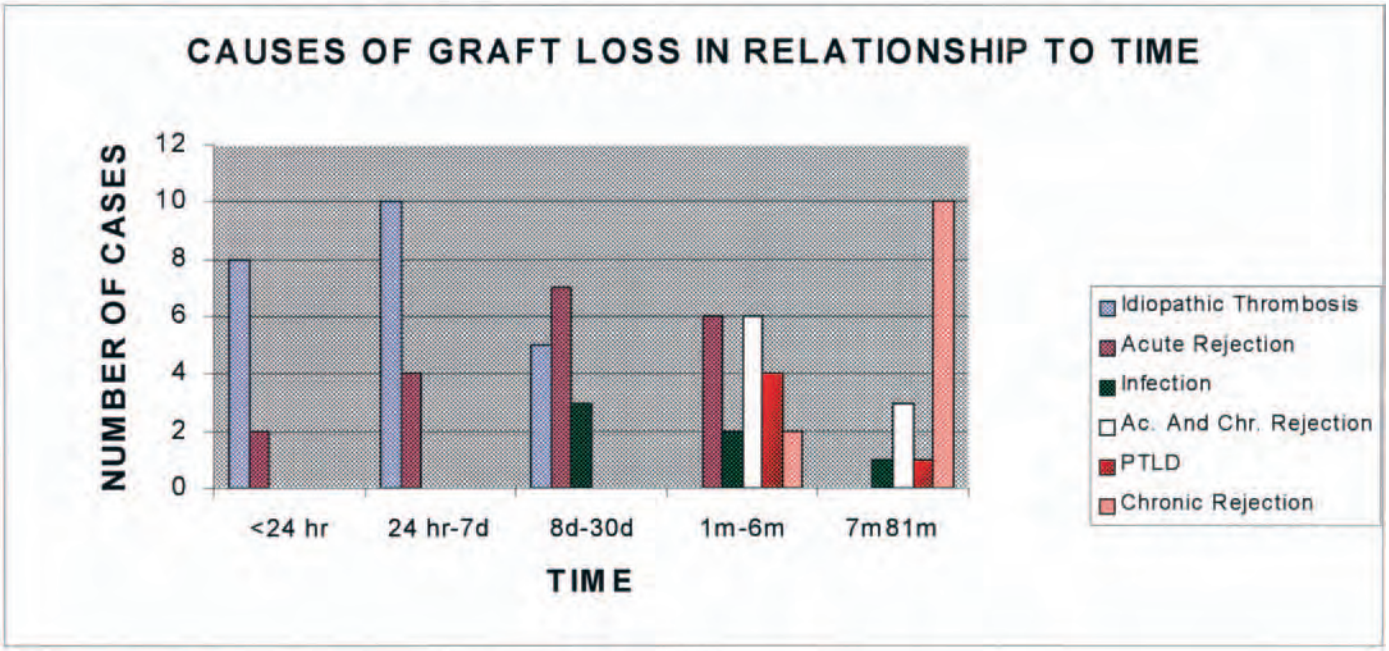
DISCUSSION

Graft losses due to leaks, bleeding, thrombosis, infections, and pancreatitis are grouped together under the category of technical failure. Among these complications, massive vascular thrombosis continues to be the most important cause of early graft loss due to technical failure (*5*). Many factors have been implicated in the occurrence of early graft thrombosis, including old donor age, long cold ischemia time, and poor surgical technique (*15*, *23*, *29*, *33*, *34*, *36*, *45*). The possibility of immunological factors involved in technical failures was raised in the 1994–1998 summary report from the International Pancreas Transplant Registry (*5*). The possibility of thrombosis occurring relatively late due to relatively early rejection arose from the fact that for PAK the risk for technical failure was "inexplicably high as the number of HLA mismatches increased," whereas apparent protection against technical failure was seen with the use of tacrolimus and mycophenolate versus other less potent immunosuppressants (*5*).

The morphological findings in allografts considered to be lost due to technical failure had not been systematically addressed. On the other hand, pathological evaluation of most allograft pancreatectomies performed in the first weeks posttransplantation period do show a common feature, which is the presence of some degree of vascular thrombosis with associated proportional parenchymal necrosis. In this study, we sought to define additional histological features that could help to better understand the mechanisms of graft failure requiring pancreatectomy.

The main histological feature present in the majority of the grafts lost in the first month posttransplantation was extensive vascular thrombosis. In 59% of these grafts, massive thrombosis occurred in otherwise structurally normal pancreas. Thrombosed pancreas with no underlying histopathological abnormalities represent the group of true technical failures.

TABLE 1



In contrast to the group showing early thrombosis in otherwise normal pancreas, in 33% of cases lost within 1 month posttransplantation, extensive vascular thrombosis occurred superimposed on immunological endothelial damage (acute rejection) in the form of endotheliitis or arteritis. Although the need for pancreatectomy in these cases was determined by the occurrence of massive vascular thrombosis, due to the presence of definite acute allograft rejection, these cases cannot be considered idiopathic in nature or true technical failures. The exact determination of the cause of graft loss is further complicated by the fact that in more than half of these patients, the immunological graft loss resulted not from rejection refractory to treatment but from lowered antirejection prophylaxis because of serious infections affecting these patients.

With the exception of rare cases of hyperacute allograft rejection, immune-type losses occurred more often after the second week posttransplantation. This contrasted with the timing of graft pancreatectomy in idiopathic graft thrombosis (true technical failure) that occurred very early in the posttransplantation period, usually within 48 hr and always within the first 2 weeks.

Hyperacute allograft rejection in pancreas transplantation is considered a rare occurrence (*46*). The morphological features in the two cases in this series correspond to the findings described in experimental hyperacute allograft rejection in the pancreas. Hyperacute rejection in the pancreas is indistinguishable from hyperacute rejection affecting other organs (*47*), and it is characterized by necrosis of arteries and veins with secondary massive and immediate thrombosis and parenchymal necrosis.

Thrombosis in pancreas allografts complicates not only the early posttransplantation period but may also occur at later times (*33*). In this study, we found a clear relationship between thrombosis and the presence of acute or chronic damage to vascular walls at all posttransplantation times; the vascular damage occurs in the form of endotheliitis/arteritis in acute rejection and as transplant (obliterative) arteriopathy in chronic rejection. Other more subtle forms of endothelial damage, seen with high levels of cyclosporine, have been implicated in the formation of thrombi in pancreas allografts in one study (*33*).

In the case of early thrombosis, the lack of obvious histological changes associated with the thrombosis does not rule out ultrastructural or subtle functional damage in these organs, because older donor age and longer cold ischemia times were associated with increased risk for early thrombosis. Our findings confirm other studies that showed an association between increasing donor age with long cold ischemia time and technical failures (*33*, *36*, *48–50*). Increased risk for graft thrombosis with older donor age is probably related to preexisting vasculopathy (i.e., atherosclerotic disease). This idea is supported by the fact that donors' cardiovascular disease is associated with worst graft outcome (*33*).

Previous studies have shown that increase in donor amylase levels have no significance for graft survival or immediate function (*47*, *51*, *52*). In this study, we found that there was a statistical association between high donor amylase levels and acute rejection. Although at this time specific data are lacking, it may be speculated that increased exposure of cellular antigens secondary to cellular damage during procurement and preservation could increase the risk of acute rejection.

In this study, there was a strong statistical association between organized (old) thrombosis and the presence of interstitial fibrosis and acinar loss (histological parameters that define chronic rejection). We believe that minor but repetitive episodes of vascular thrombosis contribute to graft sclerosis due to chronic ischemia. Judging by the histological appearance of these vessels, the process seems to be cyclic with initial thrombosis occurring in blood vessels damaged by endotheliitis or transplant arteriopathy followed by organization/recanalization and further formation of clots in the now markedly narrowed blood vessels. This process, which is inherently chronic, tends to

TABLE 2. Donor and recipient data for each histological group

	All cases	Early th[a]	AR early[b]	AR late	AR+CR[c]	PTLD[d]	Infection	CR
Cases	74	23	13	6	9	5	6	12
Gender	29F[e]	9F	7F	3F	2F	2F	2F	5F
Mean age	28.5	39.5	42.1	40.3	39.4	35.4	39.7	39.7
Race	7A,[f] 62C[g]	2A, 21C	1A, 6C	6C	1A, 8C	2A, 4C	1A, 5C	1A, 11C
Mean time of loss	6.4m	3.3d	2.8w	3.8m	6.6m	5m	4.2m	28m
Tx type	21pak,[h] 14pa,[i] 39spk[j]	7pak, 9pa, 7spk	1pak, 7pa, 4spk	1pak, 5spk	1pa, 8spk	1pak, 1pa, 3spk	1pa, 5spk	4pak, 2pa, 6spk
Drainage	34B,[k] 40E[l]	3B, 10E 3B, 10E	3B, 3E	7B, 2E	4B, 1E	3B, 3E	8B, 4E	
Mean 1st amylase	292.2	270.4	426.2	434	237.7	156.2	203.2	237
Mean 1st lipase	1918	2009.4	1920.2	4638	1742.5	843	1793.4	1068.9
Mean Donor age	27.9	32	25	28	29	22	25	26
Mean Donor amylase	146.6	84	100	283	183	313	140	142
Mean Donor lipase	61	59	56	56	99	101	57	40
Mean CIT[m]	1137	1204	970	884	1039	766	1083	1064
Mean MM[n]	3.3	3.8	3	4	4	3.8	3.3	3.5

[a] Early th, thrombosis occurring in normal pancreas.
[b] AR, acute rejection.
[c] CR, chronic rejection.
[d] PTLD, posttransplant lymphoproliferative disorder.
Early, <4 weeks.
Late, >4 weeks.
[e] F, female.
[f] A, African American.
[g] C, Caucasian.
[h] pak, pancreas after kidney.
[i] pa, pancreas alone.
[j] spk, simultaneous pancreas kidney.
[k] B, bladder drained.
[l] E, enteric drained.
[m] CIT, cold ischemia time.
[n] MM, HLA mismatch.

disproportionately affect medium and small sized arteries and veins. In contrast, abrupt graft dysfunction and thrombosis requiring pancreatectomy that occurs in grafts with vessels damaged by transplant arteriopathy or ongoing endotheliitis affects the main arteries. Thus, thrombosis is a common histological finding associated with most forms of graft injury during the whole posttransplantation period.

After the first month posttransplantation, graft pancreatectomies were performed as the result of a wider variety of pathological processes that included severe acute rejection, combined acute and chronic rejection, chronic rejection, and infections; the latter included bacterial, fungal, and EBV-related lymphoproliferative disorders. From the clinical perspective, a similar pattern of graft loss has been reported previously by Stratta (*17*, *18*).

Summary

(a) Massive vascular thrombosis is the most common cause of pancreas allograft loss. It can be present in completely normal pancreas or can result from immunological damage to blood vessels. In the early posttransplantation period, both of these processes can clinically present as technical failures. Acute early thrombosis occurring in normal pancreas represents the morphological definition of a true technical failure; this type of idiopathic thrombosis was never seen after 2 weeks posttransplantation. (b) Systematic histological evaluation of failed grafts is necessary for accurate classification of the cause of graft loss. Minimum histological sampling should include cross-sections of all large vessels and several sections from the parenchyma to include an adequate number of medium size and small vessels. (c) The consistent presence of recent and organized thrombosis in pancreas allografts with chronic rejection underscores the importance of acute and chronic thrombosis as a contributing factor for late graft loss. Further studies are necessary to establish the practical significance of these findings and to determine if some form of long-term anticoagulation therapy can be potentially useful to prolong pancreas allograft survival.

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The Long-term Management of Pancreas Transplantation

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Diabetes mellitus (DM) is a major health problem worldwide, which affects 18.2 million individuals (6.3% of the population) in the United States. Currently, the prevalence of Type 1 DM in the United States is estimated to be 1,000,000 individuals, and 30,000 new cases are diagnosed each year. In addition to end-stage renal disease (ESRD), DM is associated with blindness, accelerated atherosclerosis, dyslipidemia, cardio- and cerebrovascular disease, amputation, poor quality of life, and overall lifespan reduction. It accounts for more than 160,000 deaths per year in the United States alone. In 2002, the annual national direct and indirect costs of Types 1 and 2 DM exceeded $130 billion, which included hospital and physician care, laboratory tests, pharmaceutical products, and patient workdays lost because of disability or premature death. Hyperglycemia alone or in concert with hypertension is the primary factor influencing the development of major diabetic complications. From 1990 to 2001, the number of existing ESRD cases to DM increased by more than 300%, while the rate per million populations increased from 167% to 491%. The number is expected to grow 10-fold by 2030 to 1.3 million accounting for 60% of ESRD population. To date, DM is the leading indication for transplantation and is the cause of ESRD in more than 40% of all transplant recipients each year.

Keywords: Pancreas transplant, Survival, Management, Complications.

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Prior to 1922, a patient diagnosed with Type 1 diabetes mellitus (DM) had an average life expectancy of only two years. The success of insulin, however, changed DM from a rapidly fatal condition to a chronic, incurable illness, revealing the long term complications associated with Type 1 DM (e.g., nephropathy, vasculopathy, retinopathy and neuropathy) in survivors 10 to 20 years after the disease onset. Presently, there is no practical insulin delivery method that could replace the function of the impaired β-cells to produce a near constant euglycemic state. Therefore, persons with Type 1 DM typically exhibit constant wide deviations of plasma glucose levels and patients tend to live with relative chronic hyperglycemia as evidenced by elevated HbA_{1C} levels.

The treatments that have been demonstrated to influence the progression of secondary complications of DM (by normalizing or near normalizing HbA_{1C} levels) are β-cell replacement therapy with pancreas or islet transplantation and intensive insulin therapy. Pancreas transplantation is superior to intensive insulin therapy with regard to normalization of HbA_{1C} and has the added physiological properties of proinsulin and C-peptide release. It has been shown to reverse the diabetic changes in the native kidneys of patients with very early diabetic nephropathy, prevent recurrent diabetic nephropathy in patients undergoing a simultaneous pancreas-kidney (SPK) transplant, reverse peripheral sensory neuropathy, stabilize advanced diabetic retinopathy, and significantly improve the quality of life. Similar glycemic control can also be achieved through islet cell transplantation, which has recently gaining popularity.

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Pancreas Transplantation

After decades of controversy surrounding the therapeutic validity of pancreas transplantation, the procedure has become accepted as the preferred treatment for select patients with Type 1 DM. It is currently the only available form of total endocrine replacement therapy that reliably achieves an insulin-independent euglycemic state and normal glucose homeostasis. Tradeoffs include for normal glucose homeostasis is the operative risks of the pancreas transplantation procedure and the need for chronic immunosuppression. Free islet grafts have the same potential and have been gaining popularity in the recent years.

In 1967, Lillehei et al. (*1*) pioneered the first vascularized pancreas transplantation. The major surgical problem to be overcome was appropriate exocrine drainage of the transplanted pancreas. Fortunately, with introduction of the bladder drainage technique, further improvement in surgical techniques, and better monitoring for rejection has resulted in a significant increase in patient and graft survival.

There are three circumstances where consideration for pancreas transplantation is reasonable: i) for select medically suitable patients with Type 1 DM who are also excellent candidates for kidney transplantation (SPK), ii) for patients with Type 1 DM who enjoy good function of a kidney transplant and are receiving immunosuppression (pancreas after kidney

transplant [PAK]), and iii) for select patients with Type 1 DM who have well preserved native renal function but suffer from the severity of hypoglycemic unawareness and (pancreas transplant alone [PTA]). The most common type of pancreas transplantation is a SPK, which is followed by isolated pancreas transplantation (PAK and PTA). Living donor pancreas transplantation has also been performed. Living donor pancreas transplantation has a decreased incidence of rejection, but the technical failure rate is similar to that of deceased donor transplantations.

The International Pancreas Transplant Registry (IPTR), organized in 1980, provides historical and current data on clinical pancreas transplantation. From December 16, 1966 to December 2004, more than 17,000 pancreas transplants performed in the United States and 6,000 abroad. The number of SPK transplants has remained static since 1995, but the waiting list has doubled in size. In 2004, a total of 87,284 patients were waitlisted to receive an organ transplant; of these, 1,644 were for isolated pancreas transplant (PTA and PAK transplant) and 2,441 for SPK. From January 1988 to October 2004, of the 16,090 pancreas transplants performed in the United States, the majority of the cases, 75% (n=12, 053), have been SPKs while 25% (n=4037) have been isolated pancreas (PAK and PTA) transplants.

Long-term Management of the Pancreas Recipient

Long-term outpatient surveillance of the SPK recipient, the PTA recipient or the PAK recipient should generally follow the guidelines already outlined for outpatient renal transplant surveillance by the American Society of Transplantation (*1a*) and, where appropriate, the clinical practice guidelines for the diabetic patient published by the American Diabetic Association (*2*). However, there are issues unique to the pancreas recipient that also must be addressed.

Long-Term Outcome and Cause of Death in Pancreas Transplant

Five- and 10-year outcomes can now be calculated for pancreas transplant recipients. In the past, the larger series as well as the registry reports have concentrated on SPK (*3–12*). Furthermore, enteric and bladder drainage has been compared for long-term survival benefits (*3–10*). The United Network for Organ Sharing (UNOS)/IPTR reports annually. As of December 31, 2004, more than 23,000 pancreas transplants had been reported to the IPTR; of these, more than 17,000 cases were in the United States and almost 6,000 were from outside the United States (*4*). An analysis of U.S. pancreas transplants performed between 1988 and 2003 showed a progressive improvement in pancreas graft survival rates. In this report, the five-year graft survival worldwide for SPK performed in 1998/1999 was 69%. For PAK and for PTA, the five-year graft survival was 58%. These rates may be increasing. The 10-year graft survival rate for grafts performed in 1992/1993 was 46% for SPK, 17% for PAK and 17% for PTA (*4*). This corresponds to the current immunosuppressive era. Long-term immunosuppression reported during the latest time period consisted of triple therapy with the calcineurin inhibitors (CNI) cyclosporine or tacrolimus, mycophenolate mofetil (MMF), and steroids. Individual large center reports must be examined for details regarding cause of death and co-morbidities. Sutherland et al. (*5*) reported on experience at the University of Minnesota in 1,194 pancreas transplant performed between December 1966 and March 2000. There were 498 SPK, 404 PAK, 291 PTA, and one combined pancreas-liver transplants. The analyses were divided into five eras: era 0, 1966 to 1973 (n=14), historical; era 1, 1978 to 1986 (n=148), transition to cyclosporine, duct managements and solitary transplants; era 2, 1986 to 1994 (n=461), all categories (SPK, PAK, PTA), bladder drainage, and cyclosporine (CyA) based triple therapy; era 3, 1994 to 1998 (n=286), tacrolimus and MMF therapy; and era 4, 1998 to 2000 (n=275), daclizumab induction therapy. Pancreas graft survival rates one year have significantly improved by category and era were as follows: SPK era 2 (n=214) versus eras 3 and 4 combined (n=212), 64% versus 79%; PAK era 2 (n=610 versus era 3 (n=84) versus era 4 (n=92), 76% versus 98% verus 81; PTA era 2 (n=72) versus era 3 (n=30) versus era 4 (n=40), 67% versus 100% versus 88%. Sollinger et al. reported their experience at the University of Wisconsin in 500 SPK performed since 1986 (*6*). One-, five-, and 10-year patient survival were 96.4%, 88.6%, and 76.3%, respectively. The one-, five-, and 10-year kidney graft survival was 88.6%, 80.3%, and 66.6% respectively. The one-, five-, and 10-year pancreas graft survival was 87.5, 78.1, and 67.2%, respectively. Since 1995, patients have been treated with MMF and this group reports a one-year patient survival of 98.1%, one-year kidney graft survival of 94.2%, and one-year pancreas graft survival of 93.1%. Death with functioning graft occurred in 25 patients. Of the 53 deaths in this series, 20/53 (38%) were cardiac, 3/53 (5.5%) were cerebrovascular, 9/53 (17%) were infectious, and 5/53 (9%) were due to malignancy. Comparable success rates are reported at the other centers with similar causes of death (*10*, *11*). In contrast, 120 recipients of kidney-pancreas transplant followed for 10 years by Nankivell et al. noted a patient survival at one, five, and 10 years of 96.7, 94.0, and 84.4%, respectively. Cardiovascular events accounted for 64.3% of the deaths. Death-censored pancreatic graft functioning at one, four, and 10 years was 87.4, 86.5, and 86.5%, respectively (*12*). The same center reported death-censored kidney graft survival at 1, 5, and 10 years of 99.2, 98.2, and 95.2%, respectively. In these patients, ^{99m}TcDPTA measured GFR at one, five, and 10 years was 60.8±17.7, 50.5±27.1, and 49.4±22.8 ml/min with serum creatinine of 129±28, 148±69, and 156±48 mmol/L, respectively (*13*).

Two analyses have been done utilizing large national databases. Ojo et al. investigated the United States Renal Data System (USRDS) database (*14*). Between October 10, 1988 and June 30, 1997 a total of 4,718 patients underwent SPK. The 10-year patient survival was 67%. Cause of death was 33.4% cardiovascular, 7.1% cerebrovascular, 21.5% infectious, and 3.3% malignancy. Reddy et al. (*15*) examined the UNOS for the time period of 1987 through 1996. A total of 4,602 patients are listed as recipients of SPK. Eight-year patient survival was 72%. Cause of death was 24.1% cardiac, 6.7% cerebrovascular, 15.2% infectious, and 3.9% malignancy.

In the United States, there have been 2,427 PAK and 1,008 PTA performed between October 1987 and June 2004 (*4*). Long-term data is available on these patients. The UNOS and IPTR registry reports 10-year patient survival of 40% for PAK and 74% for PTA recipients transplanted in 1992/1993. Similarly the 10-year graft survival for the 1992/1993 eras are

17% for PAK and 17% for PTA (*4*). The major cause of death after 12 months is cardio- and cerebrovascular in both populations (*4*). Two recently published reviews have echoed these data (*16*, *17*). Gruessner et al. (*16*) reviewed the data on PAK done at the University of Minnesota. In the most recent era corresponding to use of tacrolimus plus induction therapy, the one-year graft survival in PAK has increased to over 80%. Hariharan et al. (*17*) reviewed the world experience in PAK and PTA and found that in patients transplanted in 1998–2000, the one year graft survival was 72% for PAK and 71% for PTA with patient survival at one year of 94% for PAK and 98% for PTA. A decision analysis of treatment options utilizing the UNOS database recently demonstrated that PAK added a 17.21 year increase in life expectancy and a 10 year increase in quality adjusted life years (QALY) as compared to a 11.44 year increase in life expectancy and 6.53 year QALY for deceased donor kidney only with a 6.53 year increase in life expectancy and a 4.52 QALY for dialysis (*18*).

Finally, Humar et al. (*19*) retrospectively analyzed 321 SPK, 389 PAK, and 204 PTA (mean follow-up of 39 months) and report that the second most common cause of pancreas graft loss was chronic rejection, with technical failure being the most common cause. Interestingly, chronic rejection, defined as "a gradual loss of exocrine and then endocrine function" accompanied by biopsy changes of arteriopathy and atrophic lobules separated by expanded fibrous septa, occurred in 11.3% of PTA, 11.6% of PAK, and only 3.7% of SPK. Risk factors for chronic rejection included acute rejection, isolated pancreas transplant (PAK or PTA), CMV, retransplantation and one- or two-antigen mismatch at the B loci.

Conclusions and Recommendations

Recipients of SPK can expect 10-year patient survival in the range of 67–84%. Predominant cause of death in SPK, PAK, and PTA continues to be cardio- and cerebrovascular disease. In addition, when compared to type I diabetic recipients of kidney alone (*11*, *14*), there appears to be an increase in infectious deaths. Patients surviving beyond the first year after any pancreas transplant require continuous monitoring for cardiac and cerebrovascular disease as well as infectious complications. Despite normalization of metabolic parameters (see below), excess cardiovascular disease exists in this population. Interventions directed at preventing or reversing these abnormalities have not been specifically studied. No specific studies have been designed to intervene in this patient group. In the absence of these studies patients should be treated according to published guidelines for detection and treatment of hypertension (*20*, *21*), and for detection, evaluation, and treatment of high blood cholesterol in adults (*22*). Specific monitoring for infectious complications including routine physical exams, urinalysis, routine urine cultures, and routine laboratory work such as complete blood count (CBC) is recommended. After one year, these should be performed at a two- to four-month interval. From two years on, they should be performed on a three- to six-month basis. A consensus definition of chronic rejection or chronic allograft dysfunction of the pancreas graft is needed along with additional long-term data.

Survival in Diabetics Receiving Pancreas Transplant Compared to Living Donor Transplant (LDT), Deceased Donor Transplant (DDT) or Dialysis (D)

Several studies have addressed long-term survival in patients receiving SPK versus other forms of therapy for ESRD (LDT, DDT, or D) (*7*, *14*, *15*, *18*, *23–26*). Tydén et al. (*7*) compared 14 SPK patients to 15 diabetic kidney only patients over a 10-year period. At eight years, the patient survival was 20% on the kidney alone group versus 80% in the SPK group. This group further refined their analysis to include nondiabetic kidney recipients and SPK patients who lost the pancreas within two years of transplant. The 10-year patient survival of nondiabetic kidney transplant recipients was 72% versus 60% for SPK (*24*). However, the SPK patients in whom the pancreas transplant had failed within two years had a 10-year survival of only 33%. Ojo et al. examined the USRDS database and compared diabetics who received SPK vs. LDT vs. DDT. Adjusted 10-year survival was 67% for SPK, 65% for LDT, and only 46% for DDT (*12*). A subanalysis of patients over the age of 50, however, negated the advantage of SPK. Reddy et al. (*15*) examined the UNOS database and found similar results. The eight-year survival was 72% for SPK, 72% for LDT, and 55% for DDT. The group from Wisconsin has the largest individual series reported to date (*23*, *25*). Their series clearly show that SPK when compared to DDT increased the lifespan and decreased the annual mortality rate from 6.27% in DDT to 1.5% in SPK. LDT recipients were similar to SPK. The major cause of death in their patients was cardiovascular. Knoll et al. (*18*) used the UNOS database to determine the optimal treatment strategy based on a decision analytic Markov model. This approach takes into account all complications resulting from transplant as well as the outcome of patients who lose their grafts. The outcome measures were life expectancy (LY) and quality adjusted life expectancy (QALY). LDT was associated with 18.30 LY and 10.29 QALY; Pancreas after kidney transplant was associated with 17.21 LY and 10.00 QALY; SPK was associated with 15.74 LY and 9.09 QALY; DDT was associated with 11.44 LY and 6.53 QALY; and dialysis was associated with 7.82 LY and 4.52 QALY. This approach demonstrates that SPK is better than DDT but the difference is not as dramatic as one would expect from the other analytic approach. Bunnapradist looked at the UNOS database in a different manner (*26*). They compared SPK to DDT in a multivariate analysis. They found that the recipients of SPK received higher quality kidney grafts than diabetic recipients of DDT. For the overall group, SPK enjoyed a survival advantage to DDT (5-year patient survival of 85% in SPK versus 76% in DDT). However, when the groups were compared as "low-risk group" (recipients under the age of 41 and donors under the age of 36), there were no differences in patient survival at five years.

Conclusions and Recommendations

SPK offers a survival advantage to diabetic recipients when compared to DDT. However, it has no survival advantage in diabetics compared to LDT. Furthermore, if diabetic recipients under the age of 41 years receive DDT from low-risk donors (<36 years), the advantage of SPK versus DDT may be negated. It must be, however, be pointed out that compared to DDT or LDT, SPK may be associated with better

quality of life as manifested by an increase in QALY, removal of diabetic monitoring, removal of need for exogenous insulin administration, more dietary freedom, etc.

Long-term Consequences of Bladder versus Enteric Drainage in SPK

Several series have compared bladder drainage (BD) versus enteric drainage (ED) of pancreas secretions for differences in long-term survival. The most recent UNOS/IPTR registry reports no difference in patient, kidney graft or pancreas graft survival between the techniques (*3*). Recent individual center reports demonstrate no difference in long-term survival between the techniques (*6*, *9*, *10*, *27–30*). This is in contrast to reports from the early 1990's that reported increased infections (mainly intra-abdominal), increased technical failure, and worse graft outcome for ED compared to BD (*31–36*). Sollinger et al. (*6*) demonstrated no difference if graft survival in BD versus ED. However, of the 388 BD grafts, 62.5% had urinary tract infection (UTI), 17.7% had hematuria, 15.4% had duodenal segment/bladder leak, 2.8% had urethral stricture and 2.5% had urethral disruption. By comparison, only 8% of the ED grafts suffered from leak and only 11.7% had urinary tract infections. The rate of both fungal and cytomegalovirus (CMV) infections was also higher in the BD grafts. The rate of conversion from BD to ED was 23.8%. The main indications for conversion were leak (44%), urethral complication (23%), hematuria (19%), and recurrent UTI (11%). Kuo et al. (*9*) from the University of Maryland obtained similar results. The rate of UTI was 52% in BD versus 25% in ED. Bloom et al. compared the course of 37 BD versus 34 ED (*10*). They found a higher incidence of leak, pancreatic ascites, acute rejection, graft pancreatitis, UTI and other infections in BD. The ED grafts had a higher incidence of abscess and hypertension. Furthermore, they documented more volume contraction, hematuria, and acidosis in the BD grafts. This is more consistent with the known volume and bicarbonate wasting of the BD grafts. They reported a conversion of BD to ED of 18.7%. BD grafts have significant urologic complications. In the series reported from Minnesota, Hakim et al. (*30*) reported that 20% of patients with BD had complications related to the duodenal anastomosis including leaks, hematuria, recurrent UTI, and bladder stones. Rejection of the duodenal stump can lead to duodenal rupture with severe hematuria or urine extravasations (*37*, *38*). Two centers have reviewed their experience in urologic complications in BD grafts (*37*, *38*). Del Pizzo et al. (*39*) reported on 140 consecutive BD grafts. They reported that 50% of the patients had urologic complications necessitating intervention including bladder tumors, duodenitis, bladder calculi, reflux pancreatitis, and urethral problems. In their series, 21% required conversion from BD to ED. Gettman et al. (*40*) reported on the Mayo Clinic experience in 65 consecutive cases and found similar complications in 79% of the patients. The group from the University of Washington reported that only 33/236 (14%) of BD patients required conversion, most commonly for recurrent UTI (*41*). Interestingly, they also performed reduction cystoplasty in 21 patients for bladder dysfunction. Finally, there have been two case reports of bladder carcinoma in BD grafts, one of which may have been instigated by BK virus (*42*, *43*). One center has documented late duodenal complications with ED. Nymann et al. (*44*) followed 53 patients who were more than one-year posttransplant with ED. Four of the patients had duodenal complications (8%) consisting of leaks.

Conclusions and Recommendations

With current techniques and immunosuppression, patient and graft survival appears equal with either BD grafts or ED grafts. However, BD grafts appear to have a higher incidence of metabolic abnormalities, urologic complications, and infectious complications. The latter may play a role in the increased death from infectious causes in pancreas recipients. ED grafts appear to have a higher rate of intra-abdominal infections and hypertension. BD pancreas patients need to be monitored long-term for infections, metabolic abnormalities, and urologic complications including tumors. Patients who develop these manifestations may safely undergo conversion to ED without compromising graft or patient survival.

Long-term Consequences of Systemic versus Portal Venous Drainage in SPK

Historically, venous drainage of the pancreas graft was performed systemically using an anastomosis to an iliac vein (*45*). Due to the concern of hyperinsulinemia and its metabolic consequences associated with systemic venous drainage (*46–49*), Gaber et al. (*50*) began the clinical use of portal venous drainage in 1992. According to the International Pancreas Transplant Registry (IPTR) 2002 Annual Report, of 4,309 deceased donor pancreas grafts performed from 1996–2002, about 25% (1,091) were drained portally (*51*). There were no differences in one-year pancreas graft survival rates between systemic and portal venous drainage. Patients randomized to systemic-enteric (SE) or portal-enteric (PE) surgery were reported by Stratta et al. (*52*). No significant differences in patient or graft survival were found with a mean follow-up of 17 months. A similar randomized study of SE versus PE drainage by Petruzzo et al. (*53*) demonstrated no differences in pancreas graft survival at one-year, and no significant differences in two-year creatinine, fasting glucose, HbA_{1C}, fasting insulin, or fasting C-peptide levels. Perez et al. (*54*) reviewed UNOS data from 15 transplant centers with portal drainage experience. The 539 patients with systemic drainage had equivalent graft survival to the portally drained group, but six-month rejection frequency was 48% with systemic versus 36% with portal drainage ($P<0.001$). Two-year rejection data is available from Philosophe et al. (*55*), who found more rejection in the 62 systemic drained SPK patients (40%) than the 67 portal drained SPK (10%, $P<0.05$). The same group (*56*) looked at three-year data and report pancreas rejection of 21% for portal and 52% for systemic venous drainage ($P<0.0001$). Pancreas graft survival at three years was 79% for portal versus 65% for systemic venous drainage ($P=0.008$). Other short-term studies have not shown this immunologic advantage (*52*, *53*, *57*).

Hyperinsulinemia complicates pancreas transplant with systemic drainage and most of these studies have average follow-up of one year or less (*46*, *48*, *49*, *57*, *58*). Nankivell et al. (*49*) show an association of insulin resistance to corticosteroid therapy, body weight, and time posttransplant. In contrast, Petruzzo et al. (*59*) found little difference in insulin metabolism between portal and systemic drainage recipients

with both groups falling in the normal range. In this study, average time since transplant was 48 months for the systemic drained group and 27 months for portal venous drainage. HbA_{1C}, hepatic glucose production, C-peptide, and glucose oxidation were not significantly different between the groups. Basal glucose concentrations were slightly lower in systemic drained recipients (4.63 mM) than portal drained (5.07 mM, $P=0.03$) and insulin levels were slightly higher (6.58 versus 4.56 mU/L respectively, $P=0.005$). Low-density lipoprotein and triglyceride levels were significantly lower in the systemic drained (2.32 and 758 mM) versus portal drained patients (3.12 and 1043 mM, $P=0.049$ and 0.026, respectively). There were no significant differences in high-density lipoprotein and cholesterol. The discrepancies between this study and others may lie in the immunosuppression (prednisone 5 mg per day and cyclosporine levels of 100–130 ng/dl), length of follow-up, and size of patients (mean body mass index [BMI] of 21.3 and 22.8 in systemic and portal drained patients).

Conclusions and Recommendations

Portal venous drainage of pancreas allografts reconstitutes physiologic insulin circulation. Most studies show no pancreatic graft survival advantage when compared to systemic venous drainage. Additional long-term studies are required to settle conflicting data when comparing portal and systemic venous drainage in regard to rejection rates and insulin/lipid metabolism.

Long-term Monitoring and the Role of Pancreas Biopsy

Early after pancreas transplant, the status of the pancreas can be monitored with urinary amylase and bicarbonate levels in the case of BD grafts (*60*, *61*). In the case of SPK with ED, one may rely on the kidney to act as a sentinel organ for monitoring rejection. Long-term monitoring of PTA or PAK grafts as well as long-term survival of SPK grafts can also be monitored with urinary amylase in case of BD grafts. However, as demonstrated in the most recent international registry, only 18% of SPK, 28% of PAK, and 43% of PTA had BD (*4*). Pancreas transplant biopsy was introduced as a tool for evaluating pancreas graft rejection (*62*). Furthermore, cystoscopically directed biopsy of both the pancreas graft and the accompanying duodenum is feasible in BD grafts (*63*). However, the results may be discordant and pancreas biopsy is preferred. A histological grading system has been produced and standardized for pancreas allograft biopsies by Drachenberg et al. (*64*). This system has been validated with serum enzymes, glycemia, and response to treatment (*65*). Rejection can be graded from 0-V and there was a correlation with response to steroids alone. Interestingly, there was an increase in serum lipase from grades I-V (Spearman correlation coefficient $r=0.24$, $P=0.012$) but the variation did not allow one to use the serum lipase to predict the grade of rejection. Furthermore, grade 0 specimens (no rejection) also had elevated lipase for other reasons. Therefore, one can conclude that an elevation of serum lipase alone is not sufficient to diagnose rejection but should raise suspicion. Serum glucose levels and serum amylase levels did not correlate with rejection grade in this study (*65*). The same group has reported on a large experience of pancreas biopsies in 183 patients (50% SPK, 42% PAK, and 8% PTA). All were done percutaneously with ultrasound guidance (*66*). Eighty-eight percent of the biopsies were adequate for diagnosis and there were only 12 (2.8%) complications of which five required surgical interventions. Similar results have been reported from the Mayo Clinic who performed 232 biopsies with ultrasound guidance (*67*). Despite having 73% of the patients on aspirin, only 2.6% had complications and adequate tissue was obtained in 96.1%. Pancreas biopsies can also be safely performed with computer tomography guidance or laparoscopically (*68*, *69*). Percutaneous biopsies can also be utilized to differentiate toxicity to islet cells secondary to calcineurin inhibitors from rejection (*70*).

Several other approaches have been utilized to evaluate long-term function of pancreas allografts. Intravenous glucose tolerance tests (IVGTT) were utilized by Elmer et al. (*71*) to calculate glucose disappearance rate (kG). They determined that there was variation in kG but used a cutoff of 20% change from baseline to monitor 28 patients for 2–36 months. They demonstrated that a decline of kG as a marker for rejection had an 88.7% sensitivity, a 91% specificity, a positive predictive value of 72.3% and a negative predictive value of 96.8%. Battezzati et al. (*72*) utilized IVGTT to study the change in insulin peak as well as the mean fasting glucose to predict which pancreas grafts would fail in the future. They found that a mean fasting glucose >128 mg/dl at one year posttransplant predicted return of the diabetic state within four years with a 93% sensitivity and a 100% specificity. A cutoff value of the change of insulin secretion of <32 uU/ml (peak-baseline) in the IVGTT at one/year posttransplant predicted a return to the diabetic state in four years with a 75% sensitivity and a 75% specificity. The group from Freiburg examined the predictive value of postoperative oral glucose tolerance test and stimulated insulin secretion in 41 patients with systemic venous drainage (*73*). Impaired glucose tolerance and low insulin secretion predicted worse outcome. Whether this could be used for monitoring is unclear.

Conclusions and Recommendations

Abnormal serum lipase is an indicator of pancreas dysfunction and is more sensitive than serum glucose. However, it can be elevated due to nonimmunologic causes and should prompt one to consider a pancreas biopsy. Pancreas biopsy can be performed safely with an excellent of determining the cause of pancreas graft dysfunction. Pancreas biopsy should be considered the gold standard for diagnosing allograft dysfunction. Metabolic monitoring of fasting glucose, glucose disappearance during IVGTT, and insulin secretion during IVGTT may be helpful in identifying patients at risk of having rejection or at risk for long term graft loss. Biopsy may be indicated in those patients who have abnormal results of these tests.

Recurrence of Diabetes

Transplantation of a partial pancreas from one identical twin to another in the absence of immunosuppression has been performed. In these cases, autoimmune recurrence with destruction of beta cells can occur in 6–12 weeks (*74*, *75*). Patients receiving living donor pancreas transplants with minimal immunosuppression can also demonstrate islet cell specific antibodies and recurrence of diabetes (*76*). Furthermore, type I diabetes can recur in cadaveric pancreas allografts (*76–80*). This has been characterized by the recurrence

of islet cell antibodies as well as antibodies directed against glutamic acid decarboxylase (GAD). However, these assays have not been widely utilized in large series of patients. Therefore, the timing of the assays, the clinical utility of their use, or the predictive values of the assays cannot be ascertained. They may be of use in selected patients. Some of the reports of recurrence of type 1 diabetes mellitus commented that several of the patients were on either extremely low amounts of immunosuppression or had stopped their drugs. Regardless of the cause, increased immunosuppression may halt the islet cell destruction.

Type 2 diabetes mellitus can also occur in the pancreas transplant recipient (*81–83*). Smith et al. (*81*) clearly demonstrated normal to elevated C-peptide levels in pancreas transplant patients with hyperglycemia with IVGTT characteristics of a type 2 diabetic. The type 2 diabetes may be secondary to exogenous weight gain or to the toxic effects of tacrolimus on islet cells (*70*). Investigators from Munich examined the difference in glucose metabolism in patients receiving either cyclosporine or tacrolimus following pancreas transplantation (*84*). Early after transplant there were no differences in fasting glucose, HbA_{1C} levels, basal or stimulated insulin secretion. However, at three years posttransplant, the incidence of normal glucose was lower in tacrolimus treated patients and HbA_{1C} levels were higher. Management of these patients consists of alteration of their immunosuppressive protocol or weight loss.

Conclusions and Recommendations

Pancreas transplant recipients presenting with hyperglycemia should have C-peptide measured and undergo pancreas biopsy to distinguish rejection, recurrence of type 1 diabetes, and occurrence of type 2 diabetes. Therapy should then be directed at the appropriate pathologic condition.

Effect of PTA on Native Renal Function

One of the rationales for performing pancreas alone transplant has been protection of renal function in the patient with incipient diabetic nephropathy. Studies of the effect of tight metabolic control in diabetes mellitus have clearly demonstrated that diabetic nephropathy can be halted or controlled with this strategy (*85–87*). A similar effect would be expected in PTA. Only one study has examined this issue. Fioretto et al. (*88*) reported on eight patients who underwent PTA and were studied with pretransplant kidney biopsies followed by biopsies at five and 10 years posttransplant. All patients demonstrated reversal of diabetic nephropathy but this was not evident until at least five years of normoglycemia. These eight patients started with an average creatinine clearance of 108 ± 20 ml/min. Despite the normalization of the biopsies, the average creatinine clearance at 10 years was 74 ± 14 ml/min. This underscores the potential long-term toxicity of calcineurin inhibitors and the need for long-term follow-up. Furthermore, baseline renal function may be important in selecting patients for PTA. Farney et al. (*89*) reported on 97 patients who underwent PTA at the University of Maryland. Nine percent of the patients with functioning grafts developed end-stage renal disease. All 9% of the patients had a pretransplant creatinine clearance of <55 ml/min and in nine other patients, the creatinine clearance declined by $>50\%$. Similarly a recent paper from the Mayo Clinic demonstrated a decline in native GFR from 82 ± 33 ml/min to 52 ± 26 ml/min in 23 recipients of BD PTA recipients (*90*). It is unclear whether the decline was due to immunosuppressive drug therapy, volume contraction and acidosis due to BD, or a combination of both. Patients with lower GFR at the time of transplant had a greater decline of GFR.

Conclusions and Recommendations

More than five years of normoglycemia are necessary for the reversal of diabetic renal lesions in the patient undergoing PTA. Despite the histological improvement, renal function may deteriorate due to the effects of immunosuppressive drug therapy. Care should be taken in evaluating renal function in patients presenting for PTA. Patients with established renal dysfunction may have deterioration postPTA to the point of requiring therapy for stage five chronic kidney disease.

Posttransplant Malignancy in Pancreas Transplant Recipients

As in all immunosuppressed allograft recipients, there is an increased risk of malignancy in pancreas transplant recipients. Careful monitoring and posttransplant surveillance is necessary as in other transplant recipients (*1*). This is particularly important for those tumors reported to occur in a higher incidence in renal transplant patients. These include cancers of the skin and lip, anogenital carcinomas, Kaposi's sarcoma, posttransplant lymphoproliferative disorder, uroepithelial malignancies and renal cell carcinomas, hepatocellular carcinoma, carcinomas of the uterine cervix, and colorectal carcinoma (*1*). In addition, routine screening for breast, lung, and prostate carcinoma is warranted.

There are also some unique characteristics of the pancreas recipient. Martinenghi et al. (*91*) reported on a series of 99 patients who underwent pancreas and/or kidney transplantation. Seventy-three patients had SPK and 26 had kidney alone. There were nine neoplasms in seven patients after SPK and none in the 26 patients with kidney alone. The authors suggested that the greater immunosuppressive therapy given to the combined transplants might have predisposed them to a higher rate of malignancy. Roza et al. (*92*) reported transmission of adenocarcinoma of the pancreas with a graft from a 55-year-old donor. They suggested that care should be taken when accepting a pancreas from an elderly donor. There have been two reported cases of bladder carcinoma arising in SPK recipients with BD (*42*, *43*). It is unclear if this is an increased rate for this population compared to kidney alone transplants. However, as BD pancreas recipients have an increased rate of hematuria, careful monitoring must take place. Finally, Hanaway et al. (*93*) examined posttransplant lymphoproliferative disorder (PTLD) in pancreas recipients from five large centers. Fifty-two recipients were identified. The authors stated that this was a higher incidence than expected, that PTLD occurred sooner after transplant compared to other organ recipients and that PTLD occurring after pancreas recipients had lower survival and shorter time to death.

Conclusions and Recommendations

Patients receiving pancreas transplants may have a higher incidence of malignancy compared to other organ recipients. Careful monitoring is warranted with particular attention to the bladder in bladder drained recipients and careful monitoring for

the appearance of PTLD. Screening recommended for other solid organ recipients should also take place.

Long Term Follow-up of Diabetic Retinopathy after Pancreas Transplantation

Diabetic retinopathy is present in almost all type 1 diabetics by 15 to 20 years of disease (*94*). Retinopathy leading to blindness occurs 25 times more often in patients with diabetes than the general population (*95*). Although mechanisms of disease are not clear, chronic hyperglycemia is a core cause of diabetic retinopathy (*96*). Accordingly, the risk of progression in well-treated diabetic patients (HbA_{1C} less than or equal to 7%) with no or early retinopathy is low at ≤2 per 100 patient-years (*97*). Interestingly, the same study demonstrated a higher incidence of sustained progression of eye disease (22%) in the intensive-therapy group during the first year of improved glycemic control when compared to conventionally treated patients. These findings were confirmed by Wang et al. (*98*) and attributed to development of new soft exudates. In more advanced retinopathy, photocoagulation is effective in stabilizing vision (*99*), but reversal of disease with normoglycemia in these patients is unlikely because of irreversible injury from disease and treatment (*100*). Olsen et al. (*101*) have suggested a "point of no return" may be reached in advanced disease where improvement is unlikely and progression may continue despite normalization of blood sugars.

Many patients undergoing pancreas transplantation have advanced retinopathy treated with photocoagulation therapy. This procedure is designed to stabilize the eye and makes short-term follow-up difficult to interpret (*102*). Available short-term studies have been uncontrolled (*103*) and controlled (*104–107*) with mixed results of the effect of pancreas transplant on eye disease. Ramsay et al. (*107*) did show a trend to greater stabilization of eye disease in pancreas recipients when compared to controls, but only beyond three years. Longer follow-up data is available. Kožnarová et al. (*100*) reported retinopathy outcome of 88 type 1 diabetic patients posttransplant (43 kidney-pancreas transplant versus 35 kidney transplant alone and 10 kidney-pancreas transplant requiring removal of the pancreas early after the surgery). Follow-up of the two groups was 45 and 60 months, respectively. No differences were reported in age, duration of diabetes, or percent with photocoagulation (78% versus 81%). At three years, visual acuity was significantly better in the functioning kidney-pancreas group. That same group had less macular edema, funduscopic progression, and need for laser therapy compared to the control group. Some of these differences were seen only after three years of follow-up. Pearce et al. (*108*) presented results of 17 diabetic patients after kidney-pancreas transplant followed for a mean of 5.1 years (range 1–10). In all, 26 of the 33 eyes studied had received some form of photocoagulation therapy. No regression of retinopathy was seen, but in 32 of 33 eyes examined, the retinopathy remained stable with progression in only one eye. Chow et al. (*109*) report 54 type 1 diabetics with chronic kidney disease, stage five postkidney-pancreas transplant (46 with successful euglycemia and 8 patients who became diabetic after pancreatic graft failure). Mean follow-up in the first group was 4.1 years and 2.3 years in the control arm (lost pancreatic grafts). In all, 83% of the patients had photocoagulation pretransplant. Retinopathy improved in 14% of the functioning pancreatic grafts versus 19% in the nonfunctioning grafts. Disease remained stable in 75% of each group. There were no statistical differences between the groups. They recommend that active proliferative retinopathy be stabilized prior to transplant to reduce postoperative complications. Other studies have failed to show significant differences between recipients with functioning pancreas transplant and controls in long-term follow-up (*110*).

Conclusions and Recommendations

Advanced diabetic retinopathy is common in pancreas transplant candidates. In most patients postpancreas transplant, the degree of retinopathy remains stable over time. Progression of eye disease after transplant occurs in a minority of the patients. Improvement in retinopathy is unlikely to occur with advanced eye disease. Long-term postoperative follow-up should include regular eye exams, with frequency of follow-up determined by a trained ophthalmologist or optometrist (*111*). Potential risk factors of disease progression including hyperlipidemia, hypertension, and proteinuria should be addressed with the patient (*111*).

Long-Term Follow-up of Diabetic Neuropathy after Pancreas Transplantation

Diabetic neuropathy is seen in more than 80% of type 1 diabetics with chronic kidney disease, stage five (*112*). It is difficult to distinguish diabetic neuropathy from uremic neuropathy in these patients (*102*). Lower extremity diabetic neuropathy is a strong predictor of foot ulcers and amputation (*113*). These complications produce disability, death, and significant emotional costs for diabetic patients (*113*). Long-term studies of the effect of pancreas transplantation on diabetic neuropathy have been published. Navarro et al. (*114*) presented up to 10 years follow-up in a cohort of 115 type 1 diabetic patients postkidney-pancreas transplantation. Follow-up at intervals compared to a control group of transplant recipients with kidney only or failed pancreas transplant reveal significantly improved neurological scores and motor/sensory conduction indices at all periods. Improvement in the functioning pancreas transplant group was partial, and the patients did not achieve normal neurological testing even at 10 years. Allen et al. (*115*) described results of electrophysiological studies in 59 patients postkidney-pancreas followed up to eight years. Conduction velocities underwent "a rapid, initial recovery" after six months followed by stabilization without significant change. Action potential amplitudes improved in a "slow, monophasic pattern" for the duration of the study, consistent with axonal recovery. Nerve conduction scores (NCS) improved quicker after six months in recipients with better baseline nerve function, use of nifedipine (upper limbs only), lower body weight, and a longer history of diabetes. Angiotensin-converting enzyme (ACE) inhibitor use was associated with improved NCS in the lower limbs when compared to the upper limbs. Predictors of improved action potential amplitudes include lower body weight, use of nifedipine, worse degree of initial amplitude, and less human leukocyte antigen (HLA) mismatch. NCS did not normalize when evaluated at eight years. Tydén et al. (*7*) showed improved NCS in recipients of kidney-pancreas transplants eight years postsurgery. Interestingly, no significant improvement developed between these patients and the control group of kidney only trans-

plants until after two years. Other reports have confirmed the benefit of pancreas transplant on neuropathy (*116–119*). Early improvement of neuropathy postkidney-pancreas transplant may represent correction of uremic neuropathy (*118*, *119*).

Autonomic dysfunction has also been assessed in long-term pancreas recipients. Autonomic indices were followed at intervals for up to 10 years after pancreas transplant in 115 patients and were significantly improved at all times (*114*). Similarly, parasympathetic autonomic dysfunction improved at eight years postSPK in 14 patients and was significantly better than control group of kidney only recipients (*7*). Both autonomic function and NCS were significantly improved in the patients who lived 10 years as opposed to those that died. This survival advantage is consistent with other studies (*118*, *120*, *121*). Early treatment of orthostatic hypotension in SPK with midodrine shortly after transplant provided improvement in symptoms (*122*). Studies of treatment of orthostatic hypotension in long-term patients were not found. Short-term studies have shown improvement in symptomatic gastroparesis after SKPT (*123–125*).

Conclusions and Recommendations

Neuropathy is common after pancreas transplantation. Clinical improvement occurs with functioning pancreas transplant, but complete reversal of disease is not expected. Confirmatory studies are needed before routine use of nifedipine or ACE inhibitors can be recommended to reverse disease. Because of the risk of ulcers and amputations associated with neuropathy, pancreas transplant recipients with loss of protective sensation, altered biomechanics or peripheral vascular disease should receive an annual foot examination by a trained health care provider (*126*). Visual inspection of high-risk feet should occur with each visit to a health care professional (*126*). Patients with calluses should be regularly seen by a foot specialist and considered for molded orthoses (*127*). Even minor foot ailments should be brought to the attention of a health care professional (*127*). Smoking cessation is recommended to reduce the risk of concomitant peripheral vascular disease (*126*, *127*).

Autonomic dysfunction favorably responds to pancreas transplant in many patients. Its severity appears to be a marker of increased mortality. Treatment of symptomatic orthostasis has not been studied in long-term pancreas recipients. Gastroparesis symptoms may improve early post-SKPT, but long-term results are not available for treatment recommendations.

Long-term Follow-up of Bone Disease after Pancreas Transplantation

Bone disease after SPK has many causes, including common risks such as menopause in women, low testosterone in men, and hyperthyroidism. Low turnover bone disease in type 1 diabetes (*128–130*), secondary hyperparathyroidism (*129*, *131*), or adynamic bone disease (*132*, *133*) in chronic kidney disease, osteopenia with posttransplant use of immunosuppression, particularly glucocorticoids (*131*, *134–136*) and metabolic acidosis (*137*) secondary to persistent parathyroid hormone excess or bladder drainage of the pancreas transplant are other important contributors to bone pathology. Bone disease increases the risk of fractures. Hip and vertebral fractures result in significant morbidity (*138*, *139*) and mortality (*140*) in nontransplant patients. Early improvement in parathyroid hormone levels after SPK is documented along with a decline in bone mass as measured by bone density (*141*). Compared to other solid organ transplants, an increased risk of fractures can be seen early post-SPK (*142*). Long-term studies (more than three years follow-up) in SPK found bone pathology and complications. Smets et al. (*143*) followed 31 type 1 diabetics after SPK with bladder drainage for a mean of 40 ± 23 months. Osteoporosis was described in 25% and gender, age, cumulative glucocorticoid dose, parathyroid hormone (PTH), or glomerular filtration rate (GFR) were not identified as risk factors. In all, 45% of the patients developed fracture. Approximately half of the fractures were vertebral with only 16% in the feet. PTH was significantly higher in those patients with nonvertebral fractures compared to those without nonvertebral fractures. No other risk factors for fracture were identified. Bruce et al. (*144*) also identified a high risk for fracture after SPK. In all, 50 patients with bladder drainage of SPK were evaluated for a mean of 4.3 years. Fractures were found in 36%. The distribution was equal between men and women and over half the fractures were in the feet. These fractures developed at a mean of 2.85 years after the transplant, emphasizing the importance of long-term follow-up. Risk factors were not analyzed. The increased risk of fracture has been confirmed by Chiu et al. (*145*) in 35 patients after SPK (enteric drainage in three) with mean follow-up of 49 months. In all, 49% suffered fractures. They determined the rate of first fracture to be 12.1% per patient year, which equates to a fracture free rate of 48% at five years. Overall, 70% of the fractures were in the feet. Interestingly, none of the 35 control patients with kidney transplant only experienced a fracture during mean 41 months follow-up ($P=0.0002$). Only seven of the control patients were diabetic. The risk of first fracture was increased 9% for each additional 10 mg/kg cumulative prednisone dose ($P=0.031$). The hazard of fracture decreased 62% in men compared to women ($P=0.06$). The number of years with diabetes was not identified as a risk for first fracture. Finally, a study by Kalker (*127*) looking specifically at foot fractures found that about a third of SPK recipients suffered fractures of the feet by five years.

Osteonecrosis after kidney-pancreas transplantation has been reported. Marston et al. (*146*) described 10 SPK recipients in whom osteonecrosis was diagnosed by magnetic resonance (MR) early posttransplant in three patients. None of these patients were screened for the disease preoperatively. Only one of them was symptomatic. Duration of follow-up was not provided. They recommend that all patients undergo screening MR within one year of surgery.

Treatment of bone disease posttransplant includes use of calcium and vitamin D supplements (*147*, *148*), parathyroidectomy (*131*, *149*), biphosphonates (*131*), calcitonin (*131*), estrogen supplement (*150*, *151*) and exercise programs (*152*, *153*). Short-term follow-up of steroid withdrawal in select patients, including a few with bone disease, has been described (*154*). Studies of treatment of bone disease in long-term pancreas recipients are not available.

Conclusions and Recommendations

In long-term recipients of pancreas transplantation, bone disease is common and its consequences increase morbidity and mortality. Risk factors for bone disease and its

complications are being identified for SPK and are ubiquitous in this population. Current literature supports the use of screening tests such as bone mineral density tests, measurement of parathyroid hormone, calcium, and phosphorus (especially in the SPK and PAK recipients). If osteopenia is documented, work-up should include determination of thyroid and sex hormone status in appropriate populations. Specific treatment recommendations for recipients of pancreas transplantation will require further study. Until then, guidelines provided for renal transplantation should provide reference (*1*).

Long-term Follow-up of Cardiovascular Disease after Pancreas Transplantation

According to the American Heart Association, diabetes mellitus is a major independent risk factor for cardiovascular disease (CVD). The prevalence, incidence, and mortality of all forms of CVD are two- to eightfold higher in diabetics (*155*). CVD complications are particularly high in patients with type 1 diabetes and stage five chronic kidney disease (*7*, *156*, *157*). In diabetic kidney transplants, the risk of CVD complications correlates with the degree of preexisting disease. Patients found to have one or more coronaries with narrowing >50% have a 55% risk of CVD morbidity and mortality within three years of renal transplant (*158*). Nearly half of all pancreas transplant recipient deaths in national and single center reports are caused by complications of CVD (*6*, *14*). This important group of diseases has been studied in long-term pancreas recipients. In the University of Minnesota experience, the overall prevalence of one or more manifestations of general vascular disease (coronary artery disease and peripheral vascular disease) was 47% in SPK, 42% in PAK, and 24% in PTA recipients in eras 3 and 4 combined. This group reported a higher incidence of pretransplant MI in SPK (37%) compared to PAK (33%) and PTA (14%) (*5*).

Pancreas transplantation affects coronary circulation in several ways. In an interesting study, Fiorina et al. (*159*) showed changes in atherosclerotic risk factors in SPK recipients that may favorably influence coronary artery disease long-term. SPK patients demonstrated significantly lower levels of triglycerides, homocysteine, and von Willebrand factor when compared to kidney-alone recipients. In a brachial artery model, they also found improved endothelial-dependent dilation and less intima media thickness in SPK compared to kidney-alone. Results were determined with at least one year of follow-up. Jukema et al. (*160*) performed an observational angiographic study determining progression of coronary artery lesions in patients with and without functioning pancreas transplants. Mean follow-up was 3.9 years. In all, 38% of the functioning pancreas group had regression of coronary artery atherosclerosis compared to none in the nonfunctioning pancreas group ($P=0.035$). Clinically, La Rocca et al. (*161*) described a lower incidence of acute myocardial infarction (AMI) in SPK compared to kidney-alone recipients (2.4% versus 17.6%, $P=0.005$). There was a trend to fewer anginas in the SPK group (1.6% versus 8.82%, $P=0.13$). In a follow-up paper, the same group (*162*) confirms their previous findings in a larger cohort of patients with a significantly decreased incidence of AMI in the SPK group when compared to kidney-alone recipients (3% versus 20%, $P=0.01$). No difference between the groups was found for angina. Sollinger et al. (*6*) report AMI as a cause of 23% of the deaths in their long-term follow-up of 500 SPK patients. Additional comparative studies of SPK recipients versus diabetics with kidney-alone describing frequency of coronary artery events are needed.

Left ventricular function has been studied in pancreas transplantation. Secchi et al. (*157*) noted a significant improvement in ejection fraction as measured by radionucleotide left ventriculography when comparing SPK to kidney-alone patients at four years (76.5% versus 64.3%, $P=0.003$). This was later confirmed by the same group (*161*) in 42 SPK and 26 kidney-alone recipients at four-year evaluation. Ejection fraction was significantly higher in the former group ($P=0.02$). Less diastolic dysfunction was seen in the SPK group versus the kidney-alone recipients at four years ($P<0.05$). Gaber et al. (*163*) confirmed these findings with three- to five-year follow-up in SPK patients.

Although short-term studies have found lower blood pressure after pancreas transplant (*157*, *158*, *164*), longer follow-up results are less conclusive. Fiorina et al. (*165*) reported a drop in the hypertension rate in SPK patients, but it was not statistically different than the kidney-alone group at four years (33% versus 77%, respectively). Nãf et al. (*166*) noted a fall in percentage of SPK with hypertension from 88% preoperatively to 49% with an average follow-up of 5 ± 3 years. La Rocca found no statistical difference between SPK and kidney-alone patients at two years (*162*).

Noncoronary atherosclerotic vascular disease contributes to morbidity and mortality in pancreas recipients. Bruce et al. (*144*) reported long-term peripheral complication frequency (amputation, angioplasty, or bypass) of 0.11 events per patient-year. Kalker et al. (*127*) described a 19% incidence of amputation of the lower extremities at five years, without significant differences between SPK and kidney-alone patients. Morrissey et al. (*167*) found no difference in amputation rates between SPK and kidney-alone recipients with mean of 56 months follow-up. Total peripheral vascular complications (amputation, ischemic ulcer, revascularization, or failed bypass) were significantly less in the kidney-alone group ($P=0.005$). Carotid disease appears to progress after pancreas transplant (*168*, *169*). Ojo et al. (*14*) described cerebrovascular events as a cause of death in 7.1% of SPK recipients in long-term monitoring.

Conclusions and Recommendations

CVD is a common cause of mortality in pancreas transplant recipients. Regression of coronary artery lesions has been reported which is clinically supported by a decreased incidence of AMI. Long-term data confirming these relatively early findings are needed. Left ventricular function improves with pancreas transplant. Unfortunately, there is no long-term data demonstrating better blood pressure control in pancreas recipients. Non-coronary vascular disease progresses with studies showing high rates of amputations and mortality from strokes. This outcome difference for non-coronary events when compared to favorable changes in studies of coronary events needs further study.

Additional long-term studies are required before specific recommendations can be made concerning prevention and treatment of CVD in pancreas transplantation. Meanwhile, guidelines specific for diabetes and CVD (*152*) or renal transplant and CVD (*1*) should prove useful in addition to established guidelines to reduce the risk of hypertension (*20*, *21*) and hyperlipidemia (*22*).

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A SCHEMA FOR HISTOLOGIC GRADING OF SMALL INTESTINE ALLOGRAFT ACUTE REJECTION

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Background. **Histologic evaluation of small bowel allograft biopsies is important for the diagnosis of acute rejection. However, a standard histologic schema to grade the severity of intestinal acute rejection is not currently available. The primary goal of this study was to develop a histologic grading system for the diagnosis of small bowel allograft acute rejection.**

Methods. **We evaluated 3268 small bowel allograft biopsies obtained from adult patients who underwent small bowel transplantation at the University of Pittsburgh Medical Center between 1990 and 1999. A histologic grading system was proposed and validated by retrospective correlation with clinical outcomes.**

Results. **Among the 3268 biopsies, 180 acute rejection episodes were diagnosed (88 indeterminate, 74 mild, 14 moderate, and 4 severe). All four histologically diagnosed, severe acute rejection episodes resulted in graft failure before resolution, despite aggressive immunosuppressive therapy. Four of the 14 moderate acute rejection episodes were associated with unfavorable clinical outcomes. In contrast, the 74 mild and 88 indeterminate acute rejection episodes were not associated with unfavorable clinical outcomes. Statistical analysis for trend revealed that grades indicating more severe acute rejection episodes were associated with a greater probability of unfavorable outcomes ($P<0.01$). In addition, there was good overall agreement among different pathologists regarding the diagnosis of acute rejection using the proposed schema, suggesting that this system is practical.**

Conclusions. **This study provides a reliable predictive schema for assessment of the severity of human small bowel acute rejection.**

Small bowel transplantation is being increasingly performed to treat patients with irreversible intestinal failure or short-bowel syndrome (*1*–*7*). Acute cellular rejection (ACR) is the major cause of intestinal graft failure after transplantation (*8*). If not treated early, intestinal ACR can rapidly increase in severity and cause graft failure and death. In fact, despite aggressive immunosuppressive therapy, most patients with histologically diagnosed severe acute rejection experience progression to graft loss or death. Therefore, accurate diagnosis and treatment of acute rejection are critical for posttransplant patient care.

The diagnosis of intestinal ACR requires close correlation of clinical, endoscopic, and pathologic findings. The clinical symptoms of intestinal ACR include fever, nausea, vomiting, increased stomal output, abdominal pain, and distension. In severe cases, acute rejection may manifest as septic shock, with metabolic acidosis, hypotension, and adult respiratory distress syndrome, which likely results from loss of mucosal integrity and bacterial translocation across the intestinal wall. The endoscopic appearances of intestinal ACR range from edema and hyperemia in mild cases to granularity, loss of the fine mucosal vascular pattern, diminished peristalsis, and mucosal ulceration in more severe cases. The final diagnosis depends on histologic analysis of endoscopy-guided mucosal biopsy specimens. The major histopathologic changes of intestinal ACR were documented in previous studies (*8*, *9*) and include varying degrees of (1) infiltration by a mixed but primarily mononuclear inflammatory population, including blastic or activated lymphocytes; (2) crypt injury (characterized by cytoplasmic basophilia, nuclear enlargement and hyperchromasia, decreased cell height, mucin depletion, and loss of Paneth's cells); (3) increases in the number of crypt apoptotic bodies; and (4) distortion of villous and crypt architecture.

The treatment options for intestinal ACR depend on its severity, which is assessed by histologic grading of the rejection with clinical and endoscopic correlation. For example, whereas relatively mild acute rejection usually requires an increase in basal immunosuppressive drug treatment with close clinical observation, more aggressive immunosuppressive therapy should be initiated for moderate or severe episodes of acute rejection. Therefore, accurate grading of acute rejection is extremely important for successful patient treatment. Histopathologic grading of acute rejection has not yet been addressed in detail, however, and no standard criteria are available for assessment of the grade of intestinal ACR. The major goal of this study was to develop a reliable, practical histologic grading system for pathologic evaluation of human intestinal ACR. On the basis of results from animal intestinal transplantation studies (*10*–*12*) and clinical experience in evaluating thousands of small bowel allograft biopsies in our institution (*8*), we proposed a pathologic grading system for the diagnosis of intestinal ACR. This system was used to retrospectively evaluate 3,268 small bowel allograft biopsies from 52 adult patients who underwent intestinal transplantation between 1990 and 1999 at the Thomas E. Starzl Transplant Institute, University of Pittsburgh Medical Center. The histologic grades determined were then correlated with clinical events, including immunosuppressive therapy and graft and patient outcomes. Our results indicate that the proposed grading system is accurate in the diagnosis

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of intestinal ACR and is practical for routine histologic evaluation of intestinal allograft specimens.

MATERIALS AND METHODS

Patient Population

During the 9-year period between May 1990 and June 1999, 52 adult patients (26 male and 26 female patients; age range, 19–58 years) underwent orthotopic intestinal transplantation at the University of Pittsburgh Medical Center. The patient demographic characteristics, types of procedures, and causes of intestinal failure are summarized in Table 1. Baseline immunosuppressive therapy consisted of administration of tacrolimus and corticosteroids (*1*). Details of graft procurement, surgical procedures, tacrolimus-based immunosuppressive therapy, and patient treatment were reported previously (*1*, *2*). Surveillance allograft endoscopy was generally performed once or twice per week for the first 3 months and as clinically indicated thereafter. Multiple random, endoscopy-guided biopsies were routinely obtained from the small intestinal allograft (most often from the ileum) for histologic evaluation. Each biopsy specimen consisted of one to five separate mucosal fragments (median of three). The relevant clinical features and course of each patient were retrieved from our computerized database, and missing data were obtained in reviews of patient flow sheets and medical records. Complete follow-up data were available through the completion of the study (June 30, 1999).

TABLE 1. Demographic summary of patients with small intestine transplants

No. of patients	52
Gender (male:female)	26:26
Age range (yr)	19–58
Types of grafts (55 grafts, with 3 cases of retransplantation)	
Isolated intestine	29 (including colon in 8)
Small bowel/liver	16
Small bowel/pancreas	1
Multivisceral	9 (including colon in 4)
Causes of intestinal failure (no. of cases)	
Vascular thrombosis	17
Crohn's disease	12
Abdominal trauma	7
Mesenteric fibromatosis	5
Volvulus	3
Surgical adhesions	2
Radiation-induced enteritis	2
Familial polyposis	2
Pseudo-obstruction	1
Metastatic gastrinoma	1

Histologic Evaluations

All pathologic specimens from the 55 intestinal allografts were reviewed, including 3268 small intestinal mucosal biopsies. The histologic specimens were routinely fixed in formalin and embedded in paraffin, from which 2 to 18 hematoxylin-eosin–stained sections were obtained, from two or more levels in the blocks. Samples were obtained from deeper levels as indicated. For each specimen, the major histologic features, including architectural distortion (villous blunting, as determined in the best-oriented sections), crypt epithelial injury (characterized by cytoplasmic basophilia, nuclear enlargement and hyperchromasia, decreased cell height, and mucin depletion), inflammatory infiltration of the lamina propria and the constituent cell types, presence and cell type of crypt intraepithelial infiltration (cryptitis), lamina propria fibrosis, granulation tissue, and luminal fibrinopurulent inflammatory exudation (pseudomembrane), were semiquantitatively assessed. In addition, the specimens were carefully examined for viral infections, luminal organisms, and submucosal abnormalities. Apoptotic bodies within the crypt epithelium were identified and quantified. Apoptotic bodies were defined as rounded vacuoles containing fragments of karyorrhectic nuclear debris and cytoplasm and were distinguished from small isolated fragments of nuclear chromatin and intraepithelial neutrophils and eosinophils. These bodies were counted by scanning the specimen at medium power, to identify areas of greatest concentration, and then tallying the total numbers in 10 consecutive crypts (regardless of crypt orientation), including more than one level if necessary.

Slides from all biopsy specimens were reviewed at least twice by at least two pathologists. Histologic features relevant to acute rejection were compiled during the initial review; and a list of biopsy features of rejection was recorded by the second pathologist. Ambiguous or difficult cases were further reviewed using a multihead microscope by three or four pathologists. Attention was focused on changes related to rejection (see later discussion).

Histologic Criteria for Grading of Acute Cellular Rejection

The proposed histologic grading system for small bowel allograft biopsies is based on previous animal studies (*10–12*) and our clinical experience in the evaluation of thousands of small bowel allograft biopsy specimens (*8*). The histologic criteria for grading intestinal ACR are summarized (Table 2).

Indeterminate for acute rejection. Indeterminate for acute rejection is defined by the variable presence of the three main features of acute rejection (infiltration by a mixed but primarily mononuclear inflammatory population, including blastic or activated lymphocytes; crypt injury; and increased numbers of crypt apoptotic bodies), which are usually focal and do not meet the criteria for mild acute rejection. The inflammatory infiltrate is usually minimal and localized. Although the mucosa is intact, crypt epithelial injury is often present. There is a variable increase in crypt epithelial apoptosis but usually with less than 6 apoptotic bodies per 10 crypts (Fig. 1). *Indeterminate for acute rejection* should be used only when the biopsy demonstrates

TABLE 2. Histologic criteria for grading of small bowel allograft acute rejection[a]

Grade	Major Histologic Findings
Indeterminate for ACR	Minimal localized inflammatory infiltrate, minimal crypt epithelial injury, increased crypt epithelial apoptosis (usually with <6 apoptotic bodies/10 crypts), no to minimal architectural distortion, no mucosal ulceration, changes insufficient for the diagnosis of mild acute rejection
Mild ACR	Mild localized inflammatory infiltrate with activated lymphocytes, mild crypt epithelial injury, increased crypt epithelial apoptosis (usually with >6 apoptotic bodies/10 crypts), mild architectural distortion, no mucosal ulceration
Moderate ACR	Widely dispersed inflammatory infiltrate in lamina propria, diffuse crypt epithelial injury, increased crypt apoptosis with focal confluent apoptosis, more prominent architectural distortion; possible mild to moderate intimal arteritis; no mucosal ulceration
Severe ACR	Features of moderate ACR plus mucosal ulceration; possible severe intimal arteritis or transmural arteritis may be seen

[a] ACR, acute cellular rejection.

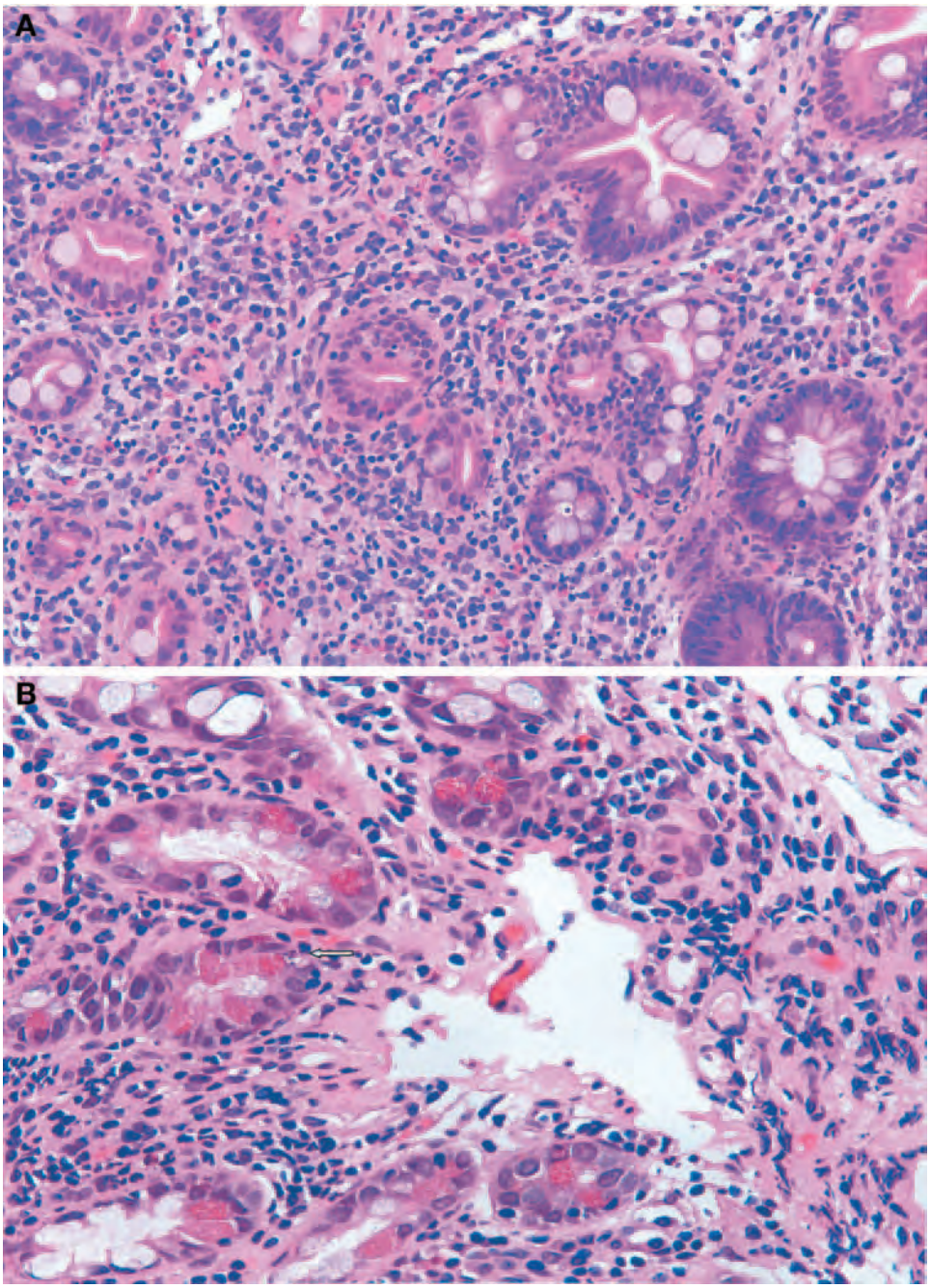

FIGURE 1. Indeterminate for acute rejection. The lamina propria is infiltrated by a heterogeneous population of mononuclear cells composed of blastic and small lymphocytes, plasma cells, and plasmacytoid lymphocytes. There is focal minimal crypt damage and apoptotic bodies (*arrow*) (hematoxylin-eosin; magnification: ×200 in A, ×400 in B). The apoptotic body count is usually less than 6 apoptotic bodies per 10 crypts.

features of acute rejection with degrees of inflammation, epithelial injury, and apoptosis that are lesser than those for mild acute rejection; it should not be applied to nonrejection processes when the diagnosis is not clear.

Mild acute rejection. Mild acute rejection is characterized by a generally mild and localized inflammatory infiltrate, which tends to be concentrated around small venules in the lamina propria. The mucosa is intact, but the crypt epithelium displays evidence of injury, including mucin depletion, cytoplasmic basophilia, decreased cell height, nuclear enlargement and hyperchromasia, and inflammatory infiltration. Crypt epithelial apoptosis is increased, usually with more than 6 apoptotic bodies per 10 crypts. If sampled in the biopsy specimen, preexisting lymphoid aggregates (Peyer's patches) demonstrate an intense accumulation of activated lymphocytes. The villi are variably shortened, and the architectural features may be slightly distorted because of expansion of the lamina propria by inflammatory infiltration (Fig. 2).

Moderate acute rejection. In *moderate acute rejection*, the inflammatory infiltrate is widely dispersed within the lamina propria. Crypt damage is distributed more diffusely than in mild acute rejection, and the villi tend to exhibit a greater degree of flattening. The number of apoptotic bodies is greater than in mild acute rejection, usually with focal "confluent apoptosis." Mild to moderate intimal arteritis may be observed. The mucosa remains intact without ulceration, although focal superficial erosions can be present (Fig. 3).

Severe acute rejection. Severe acute rejection is distinguished by a marked degree of crypt damage and mucosal ulceration. As a consequence of the mucosal destruction, luminal contents gain access to the submucosa, prompting a neutrophil-rich infiltrate and an overlying fibropurulent (pseudomembranous) exudate, with widespread

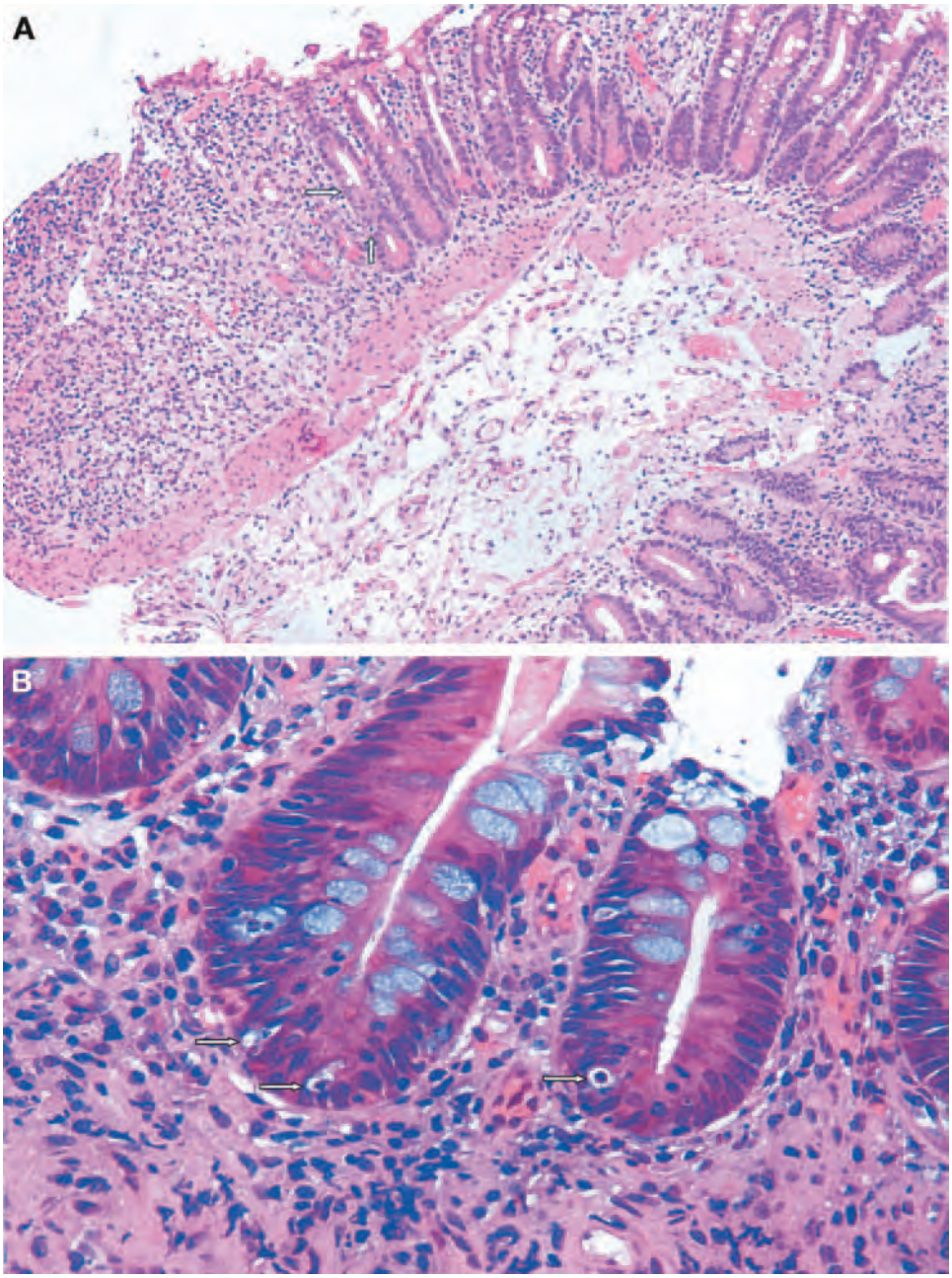

FIGURE 2. Mild acute rejection. (A) The villi are shortened and the architectural features are distorted because of expansion of the lamina propria by the heterogeneous mononuclear cell infiltrate (*left*). The crypts exhibit features of epithelial injury and scattered apoptotic bodies (*arrows*) (hematoxylin-eosin; magnification ×100). (B) Lamina propria mononuclear inflammation, crypt epithelial injury, and apoptotic bodies (*arrows*) (clear spaces with fragmented nuclear debris) (hematoxylin-eosin; magnification ×400). The apoptotic body count in mild acute rejection is usually more than six apoptotic bodies per 10 crypts.

mucosal sloughing as the final result. The adjacent viable epithelium usually exhibits rejection-associated changes, such as crypt epithelial damage and abundant apoptosis (Fig. 4). Severe intimal arteritis or transmural arteritis may be observed.

Prognostic Use of the Grading System

To evaluate the ability of the proposed acute rejection grading system to predict an unfavorable outcome, the histologic diagnoses of acute rejection episodes were retrospectively correlated with the clinical outcomes and treatments. A biopsy was defined as representing an acute rejection episode if the biopsy specimen was the first one to be histologically diagnosed as acute rejection. A new rejection episode was defined by newly developed clinical symptoms and documentation of new histologic features of ACR with at least one normal mucosal biopsy between the rejection episodes. For endpoint analysis, patients were divided into groups with favorable or unfavorable outcomes. Objective unfavorable outcomes were defined by the presence of any one of the following: (1) the rejection resulted in graft failure (death or retransplantation) before resolution; (2) OKT3 or antithymoglobulin was required for the treatment of acute rejection; or (3) complete resolution of the episode failed to occur within 21 days.

Reliability of the Grading System

Sixty-five posttransplantation small bowel biopsy specimens were randomly selected and reviewed by four pathologists. Before reviewing the slides, the pathologists agreed on the histologic grading criteria. Each participating pathologist rendered a final histologic diagnosis on the basis of the standard criteria.

Statistical Analyses

The ability of the grading system to predict an unfavorable outcome was assessed with the chi-square test for trend, using the definitions for unfavorable outcomes. The agreement among pathol-

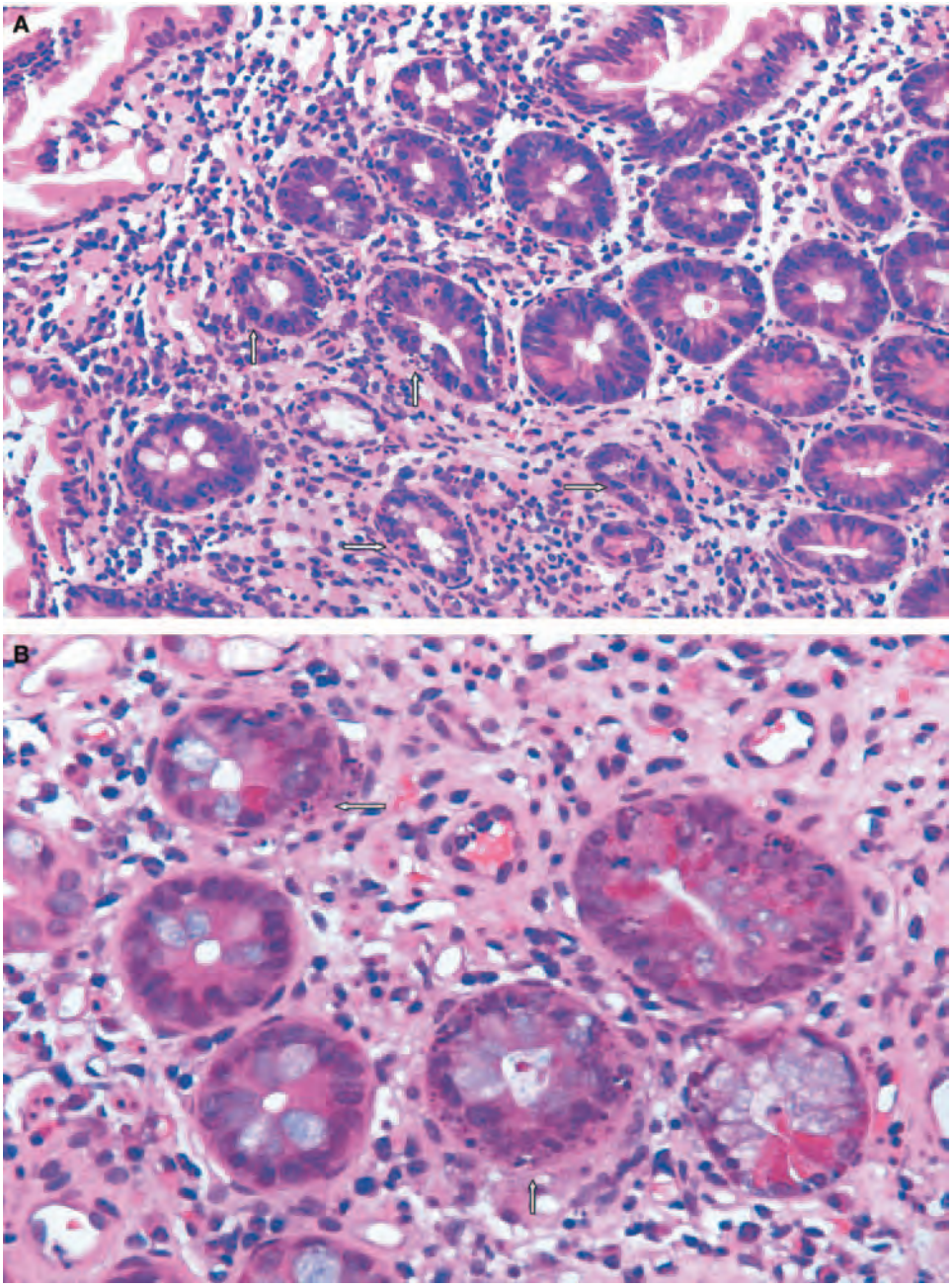

FIGURE 3. Moderate acute rejection. Crypt damage and apoptosis are distributed more diffusely than in mild acute rejection. The number of apoptotic bodies is greater than in mild acute rejection, with focal confluent apoptosis (*arrows*). The mucosa is usually intact, without ulceration (hematoxylin-eosin; magnification ×200).

ogists regarding the histologic diagnosis of ACR was analyzed with multirater kappa analysis.

RESULTS

Histologic Diagnosis of Acute Cellular Rejection

The histologic diagnosis and grading of ACR were performed after careful evaluation of 3268 mucosal biopsies from 55 small intestinal allografts. The initial histologic diagnosis for each biopsy specimen was established by the primary pathologist during the daily signed-out process; each of the biopsies was reevaluated by a separate pathologist (T.W.), and detailed histologic features were recorded. If an ambiguity regarding any histologic feature or a disagreement in diagnoses existed, then the slides were further reviewed under a multihead microscope with two or more additional pathologists, and the consensus opinion was recorded. A biopsy was defined as representing an acute rejection episode if the biopsy specimen was the first one to be histologically diagnosed as acute rejection. A new rejection episode was defined on the basis of newly developed clinical symptoms and documentation of new histologic features of ACR, with at least one normal mucosal biopsy between the rejection episodes. On the basis of the aforementioned criteria, 180 episodes of ACR were histologically diagnosed, among which were 88 (49%) episodes of indeterminate for ACR, 74 (41%) episodes of mild ACR, 14 (8%) episodes of moderate ACR, and 4 (2%) episodes of severe ACR. Among the 180 episodes of histologically diagnosed ACR (including indeterminate for ACR), 85 (47%) episodes occurred within the first 2 months after transplantation, 46 (26%) episodes occurred 2 to 12 months after transplantation, 24 (13%) episodes occurred 1 to 2

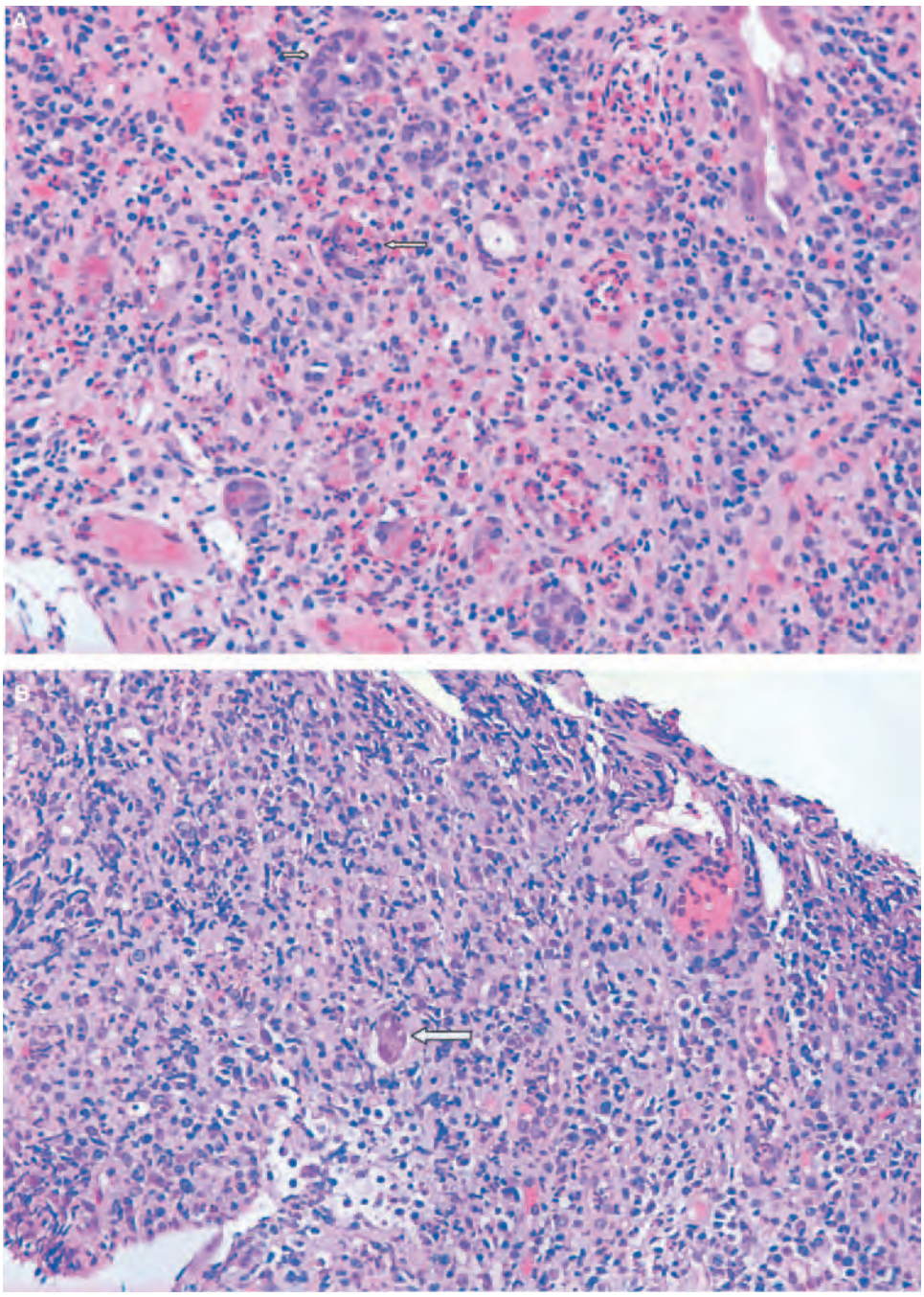

FIGURE 4. Severe acute rejection. There is extensive mucosal destruction, with loss of crypts, mucosal ulceration, and mixed lymphoplasmacytic, eosinophilic, and neutrophilic infiltration. The residual crypts, if present, often exhibit marked epithelial injury and apoptosis (*arrows*) (hematoxylin-eosin; magnification ×200).

years after transplantation, and 25 (14%) episodes occurred more than 2 years after transplantation.

The same histologic grading criteria were used for all biopsies in this study, including biopsies obtained from patients with clinical symptoms and protocol biopsies. The clinical presentations associated with ACR included abdominal pain, nausea, vomiting, diarrhea, fever, and abdominal distention. These symptoms lacked specificity, however, and varied depending on the severity of rejection and the presence of other pathologic conditions, such as acute enteritis, cytomegalovirus (CMV) infection, intestinal obstruction, systemic infection, or posttransplant lymphoproliferative disorder (PTLD). All of the patients with histologic diagnoses of moderate or severe ACR exhibited clinical symptoms, and approximately 95% of the patients with histologic diagnoses of mild or indeterminate acute rejection exhibited symptoms. The remaining 5% of patients with mild or indeterminate acute rejection exhibited no symptoms at the time of the biopsies, and the diagnoses were established with protocol biopsies. Most of the biopsies without histologic evidence of acute rejection demonstrated either normal mucosa or mild nonspecific enteritis; some showed reparative mucosa, CMV infection, Epstein-Barr virus (EBV) infection, or PTLD.

Prognostic Ability of the Grading System

We then wished to analyze the association between acute rejection grades and unfavorable outcomes. For this purpose,

the patients were divided into those with favorable outcomes and those with unfavorable outcomes, according to the aforementioned criteria, and the ability of the grading system to predict an unfavorable outcome was assessed with the chi-square test for trend. The results demonstrated that a grade indicating a more severe rejection episode was associated with a greater probability of an unfavorable outcome ($P<0.01$). In fact, all four of the histologically diagnosed severe acute rejection episodes resulted in graft failure before resolution, despite treatment with OKT3. Of those four grafts, three were removed because of uncontrolled ACR and one patient died as a result of ACR with the graft in place. Of the 14 episodes of moderate acute rejection, 2 episodes required OKT3 treatment and 2 episodes failed to resolve within 21 days with immunosuppressive therapy (other than OKT3). The outcome of one moderate ACR episode could not be determined because of graft removal secondary to chronic rejection, before the resolution of ACR. The remaining nine episodes of histologically diagnosed moderate ACR were not associated with unfavorable outcomes. The outcomes were difficult to assess for 3 of the 74 episodes of mild ACR, because of graft removal in 2 cases (because of chronic rejection and opportunistic infection) and patient death in 1 case (resulting from opportunistic infection) before resolution of the ACR episodes. The remaining 71 mild ACR episodes were not associated with unfavorable outcomes. The 88 indeterminate ACR episodes all resolved within 21 days (spontaneous resolution without treatment, resolution after increased immunosuppressive therapy, or progression to mild ACR that latter resolved with treatment) and were not associated with unfavorable outcomes.

Reliability of the Grading System

A consensus diagnosis was reached by all of the participating pathologists in 60 of the 65 cases (92%), including 4 cases of severe acute rejection, 9 cases of moderate acute rejection, 10 cases of mild acute rejection, 13 cases of indeterminate for ACR, and 24 cases of no acute rejection. Of the five cases for which a uniform diagnosis could not be established, two cases were interpreted as either mild ACR or indeterminate for ACR and three cases were interpreted as either indeterminate or no ACR. There was no disagreement regarding the diagnosis of moderate or severe acute rejection. Multirater kappa analysis demonstrated that there was excellent overall agreement among pathologists regarding the diagnosis and grading of small bowel acute rejection with this grading schema ($P<0.01$). Good intraobserver agreement was noted when the slides were reviewed in a blinded manner by the same pathologist on two separate occasions (with an interval of approximately 6 months).

DISCUSSION

The primary goal of this study was to develop a histologic grading system for the diagnosis of small bowel allograft ACR. To achieve this, we evaluated 3,268 small bowel allograft biopsies obtained from adult patients who underwent small bowel transplantation at our institute during the past decade. On the basis of previously documented major histologic parameters for small bowel allograft acute rejection, the severity of acute rejection was graded as indeterminate, mild, moderate, or severe. This grading system was validated by retrospective correlation with clinical outcomes; more severe rejection episodes were associated with a greater probability of unfavorable clinical outcomes. The excellent overall agreement among different pathologists regarding the histologic diagnosis of acute rejection using the proposed criteria suggests that this system is reliable for the routine pathologic evaluation of small bowel allograft acute rejection. To our knowledge, the criteria in this study represent the first schema for assessment of acute rejection severity in human small bowel allografts.

Several pitfalls in the histologic evaluation of small bowel mucosal biopsies are worth mentioning. We observed that four histologic features are particularly useful for the routine pathologic diagnosis of small bowel allograft ACR, including architectural distortion, crypt apoptosis, crypt epithelial injury, and activated lymphocytic inflammatory infiltration in the lamina propria. These are relatively easily identifiable features that can be reliably quantitatively or semiquantitatively assessed, with a high degree of reproducibility among different pathologists. Because artery sampling is extremely rare in intestinal mucosal biopsies, arteritis has limited diagnostic value in the evaluation of mucosal biopsy specimens, although its presence invariably indicates moderate or severe acute rejection. In this study, arteritis was identified in only 2 of the 3,268 mucosal biopsies. If biopsies are obtained from isolated ulcers or necrotic regions, then an exact histologic diagnosis of acute rejection may be difficult to establish. In such circumstances, careful clinical and endoscopic correlation is particularly important and repeated biopsies from nonulcerated regions are often required.

The quality of the infiltrate (activated lymphocytes mixed with some eosinophils and neutrophils in ACR, compared with nonactivated lymphocytes in nonspecific enteritis) is important in the differentiation of ACR from other conditions. The intensity of the infiltration is generally correlated with the severity of ACR (mild infiltration in mild ACR and intense infiltration in severe ACR). In our experience, the area of infiltration is a less-reliable marker, because the infiltration in low-grade ACR can be diffuse (although less intense). Although eosinophils are frequently observed in intestinal mucosa, significantly increased levels of eosinophils with coexistent activated lymphocytes and crypt apoptosis suggest acute rejection. Peyer's patches are commonly sampled in mucosal biopsies, especially from the ileum. Although localized Peyer's patches without significant lymphoid activation do not indicate acute rejection, Peyer's patches with lymphoid activation (characterized by lymphoid cells with open chromatin, diffuse infiltration into the surrounding mucosa, or mixtures with eosinophils and neutrophils) are frequently associated with acute rejection. The significance of lymphocytic cryptitis (increased numbers of lymphocytes in the crypt epithelium) is unclear. Although cryptitis is present in some cases of acute rejection, it is also observed in biopsy tissues without ACR (such as those exhibiting nonspecific enteritis, viral infections, or PTLD). Statistical analyses in this study failed to demonstrate a correlation between lymphocytic cryptitis and the diagnosis of acute rejection. Acute cryptitis (increased numbers of neutrophils in the crypt epithelium) is usually associated with various causes of acute enteritis and is not a diagnostic criterion for acute rejection.

Adequate tissue sampling is necessary for accurate histologic diagnosis. Because the distribution of acute rejection may be patchy, multiple biopsies (usually three to five) are often required. Biopsies from either the ileum or the jejunum are sufficient for histologic evaluation in most cases, although sampling from both the ileum and the jejunum may be required in some cases with ambiguous diagnoses. The tissue obtained should be fixed in 10% neutral buffered formalin for at least 1 hr before processing, and multiple sections (usually 10–15) should be examined for each biopsy.

Differentiation between indeterminate and mild ACR is important for treatment planning. In our center, most of the histologically diagnosed mild acute rejection episodes were treated with increased immunosuppression (except when rejection occurred in association with opportunistic infections or PTLD), whereas treatment for indeterminate rejections was liberal, based on clinical assessments. A histologic distinction between these two categories can usually be made with this grading system. Among the listed criteria, the number of apoptotic bodies is most helpful (<6 apoptotic bodies per 10 crypts for indeterminate ACR versus >6 apoptotic bodies per 10 crypts for mild ACR), followed by perivenular infiltration (less common for indeterminate ACR and more common for mild ACR). We observed that mild acute rejection was associated with favorable clinical outcomes, which likely reflects successful immunosuppressive therapy. Indeterminate for acute rejection was also associated with favorable clinical outcomes, which likely reflects the minimal activity of acute rejection in this group and the use of immunosuppressive therapy for some of the patients.

Various pathologic conditions must be differentiated from acute rejection, the most common of which include nonspecific enteritis, CMV infection, EBV infection, and PTLD. Acute enteritis is often attributable to bacterial or viral infection and is characterized by neutrophil-rich infiltration in the lamina propria, with acute cryptitis but usually without significantly activated lymphocytes or increased apoptosis. CMV enteritis can sometimes be associated with increased inflammatory infiltration and increased apoptosis, and the diagnosis is made with the identification of characteristic nuclear and cytoplasmic viral inclusions, with confirmatory immunohistochemical staining. EBV infections and PTLD are often associated with significant mononuclear infiltration, and the diagnosis is made with the identification of atypical lymphoid cells on hematoxylin-eosin-stained sections, immunohistochemical staining for T and B cells, in situ hybridization for Epstein-Barr virus-encoded RNA, and clonality analysis. Ischemia-reperfusion injury is generally not a problem in the differential diagnosis, because it usually occurs immediately after reperfusion, with characteristic histologic features that resolve within 2 to 3 days in most cases. For patients with delayed recovery from severe ischemia-reperfusion injury, the diagnosis of early superimposed acute rejection can sometimes be difficult. Under such conditions, the presence of activated lymphocytes and eosinophils, ongoing crypt damage, and significant crypt apoptosis suggests acute rejection.

CONCLUSION

This study provides a reliable, predictive histopathologic schema for assessment of the severity of human small bowel acute rejection. The availability of this grading system should provide important guidance for effective immunosuppressive treatment of patients who undergo small bowel transplantation. It should also facilitate information exchange within and between transplantation centers.

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Current Status of Transplantation of the Small Intestine

Phillip Ruiz,[1,2,3] Tomoaki Kato,[2] and Andreas Tzakis[2]

The evolution of small bowel transplantation has been significant over the past 20 years to the point at which it can now be considered a viable and often successful option in the treatment of many forms of short bowel syndrome. A refinement of surgical techniques, improved immunosuppression, enhanced understanding of gut immunology, and better treatment and prevention of complications have contributed to a marked improvement in graft and patient survival. Whereas this transplant population is still beset with many potential complications after isolated bowel or multivisceral transplantation and long-term graft survival (like with other solid organ transplants) remains a challenge, the future holds promise for a continuation of the current positive trend of improvement in several areas.

Keywords: Small bowel transplantation, total parenteral nutrition, multivisceral transplantation.

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"Nothing endures but change."

Heraclitus, *from Diogenes Laertius, Lives of Eminent Philosophers*

Greek philosopher (540 BC - 480 BC)

The calamitous and potentially deadly development of short bowel syndrome in adults and children had until the last two decades been treated exclusively with parenteral nutrition (PN) supplementation. Although PN remains a therapeutic mainstay for this group of patients, it can be a limiting treatment with potentially devastating complications. In reality, the complications associated with PN, which include catheter-related morbidity (e.g., sepsis, venous thrombosis), metabolic changes (e.g., hepatotoxicity), psychologic strain, and reduced quality of life, all contribute to a five-year survival rate for all patients on PN of approximately 60% (*1*). The successful emergence of small bowel transplantation as a curative alternative has provided many patients with bowel failure to have an improved quality of life, better nutrition, and reduction in PN-associated complications. Although intestinal transplants have customarily been performed when there was danger to the patient's life, usually as a result of the PN-induced development of liver failure secondary to hepatic scarring and cirrhosis or loss of vascular access for PN, there is now an emerging philosophy of earlier intervention. In this regard, reports of transplants performed at an earlier stage (*2*) have shown encouraging results. This earlier approach is justified because patients awaiting combined liver–intestinal transplantation have the highest mortality rates compared with other transplant candidates (*3*). The gamut of underlying diseases causing short bowel syndrome in patients who have been transplanted is extensive and variable between pediatric and adult populations (Table 1). Generally, nonmalignant conditions are the norm for recipients, although occasional tumors such as desmoids (*4*) have been successfully treated with intestinal transplantation. Recurrence of the native disease in the allograft is typically not a significant issue with this form of transplantation.

Since the initial small bowel transplants were first performed in the 1980s (*5*), there have been technical improvements, novel immunosuppressive agents, better understanding of the immune and gastrointestinal physiology, and increased clinical program experience. All of these factors have contributed to a remarkable improvement in bowel transplant one-year graft and patient survival (estimated 80% and 80%, respectively) compared with only several years ago; these numbers are based on Intestinal Transplant Registry (*6*) data presented at the IX International Small Bowel Transplant Symposium in 2005. Figure 1 shows the most recent data provided by the Intestinal Transplant Registry for graft and patient survival for the worldwide experience in small bowel transplantation at the University of Miami. Still, this highly complex transplant continues to be laden with potential complications and to date remains a relatively uncommon procedure with approximately 1300 transplants performed worldwide according to the International registry, 60% of them for children (*6*).

Surgical

Transplantation of the intestine can be performed as an isolated graft or in combination with other abdominal or-

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TABLE 1. Indications for bowel transplantation in children and adults[a]

	Percentage of worldwide cases
Children	
Gastroschisis	22
Volvulus	17
Necrotizing enterocolitis	12
Pseudoobstruction	9
Intestinal atresia	8
Aganglionosis/Hirschsprung	7
Retransplant	7
Microvillous inclusion	6
Other causes	4
Malabsorption	3
Short gut other	3
Tumor	1
Other motility	1
Adults	
Ischemia	25
Crohn disease	13
Trauma	9
Short gut other	9
Volvulus	8
Motility	8
Desmoids	8
Retransplant	6
Miscellaneous	6
Other tumor	5
Gardner's	3

[a] Data obtained from Intestinal Transplant Registry Data, 2005.

gans, because patients with intestinal failure often experience other complex abdominal pathologies that require organ replacement. As a result, there have been several variants of intestinal transplants, all derivatives of the "cluster" concept originally proposed by Starzl et al. (*7*). Isolated intestinal transplantation (ITx) is transplantation of the small intestine with or without the large intestine and is more commonly performed in adults, whereas combined liver–intestinal transplant (LITx), performed en bloc or separately, is more commonly performed in children. The latter scenario occurs when there is concomitant liver failure (typically PN-induced). With ITx, the entire jejunum and ileum has been transplanted in the majority of cases. With ITx from a living donor and in cases in which reduction of the size of the graft is required, a 200-cm segment (*8*) is usually transplanted. In this regard, it is important to match size because of the need for closure of the abdomen. There is maintenance of as much native bowel as possible, particularly with recent data suggesting that increased residual or allograft bowel provides some protection from PN-associated injury. This is particularly relevant because there may be some supplementation of transplanted patients with PN for a period of time.

When ITx is performed en bloc, the duodenum with a segment (or the entire pancreas) (Omaha technique) may be included to avoid the need for biliary reconstruction. In these cases, the graft duodenum represents a second duodenum (in addition to the native duodenum) and is extraneous, serving as a conduit for bile and pancreatic secretions. The upper gastrointestinal continuity is maintained through the native stomach and pancreaticoduodenal complex, which are retained. In LITx, the intestinal transplant is combined with the liver. These organs are transplanted en bloc or separately. When the liver and intestine are transplanted separately, the two organs can be transplanted contemporaneously or sequentially from the same or a different donor. The great majority of the donors for these two forms of intestinal transplantation are from cadaveric donors, although living donors for ITx have been successfully performed without significant donor morbidity (*9*) and may be an important future source, particularly for pediatric recipients.

Multivisceral transplantation (MVTx) is the removal and replacement of both native foregut and midgut (*10*) in which the native abdominal viscera are resected and the composite graft, which includes the stomach, pancreaticoduodenal complex, and small intestine, are transplanted en bloc and form the new gastrointestinal tract. The liver, kidneys, and large intestine of the donor may or may not be included (Fig. 2) depending on the clinical scenario. This latter variant is reserved for the most extensive abdominal catastrophes and organs are only replaced if there is a suspicion of underlying injury from the patient's general condition. This has typically been used as an alternative for small babies who would have ostensibly received a LITx. Evisceration of the native organs is facilitated by early dearterialization. The latter is achieved by mass clamping of the celiac and superior mesenteric arteries. This can be achieved through a cephalad approach after division of the esophagus or proximal stomach or a caudal approach between the inferior surface of the pancreas and left renal vein. Since 2000, the use of MVTx is increasing and despite the fact that the donors for MVTx are exclusively cadaveric, the one-year graft and patient survival is at least as good as the other forms of intestinal transplantation (*6*). As of mid-2005, an isolated intestinal graft has been performed in 44% of the cases, intestine transplanted in combination with the liver (38%) or multivisceral transplant (18%) (*6*). The decision to use one form of intestinal transplantation versus another is typically determined by the individual patient's particular needs. For example, the type of underlying disorder and surgical history of the patient are important considerations in which type of intestinal transplant is performed, the type and size of the donor, and how much abdominal domain is available to the surgeon. The emergence of promising data suggesting improved survival data and long-term sequelae, as well as possible immunologic advantage for MVTx, is allowing the clinical team more options as it determines which form of transplantation will be recommended.

Immunosuppression

With the advent of clinical intestinal transplantation, it was at once apparent that significant immunosuppression (ISP) was to be necessary to attain the goals of engraftment and graft survival of reasonable duration. Many therapies and combinations thereof have been used, but what remains undefined are the optimal immunosuppression regimens to achieve the aforesaid goals while preserving graft function and not predisposing the recipient to increased infections or malignancy. Although

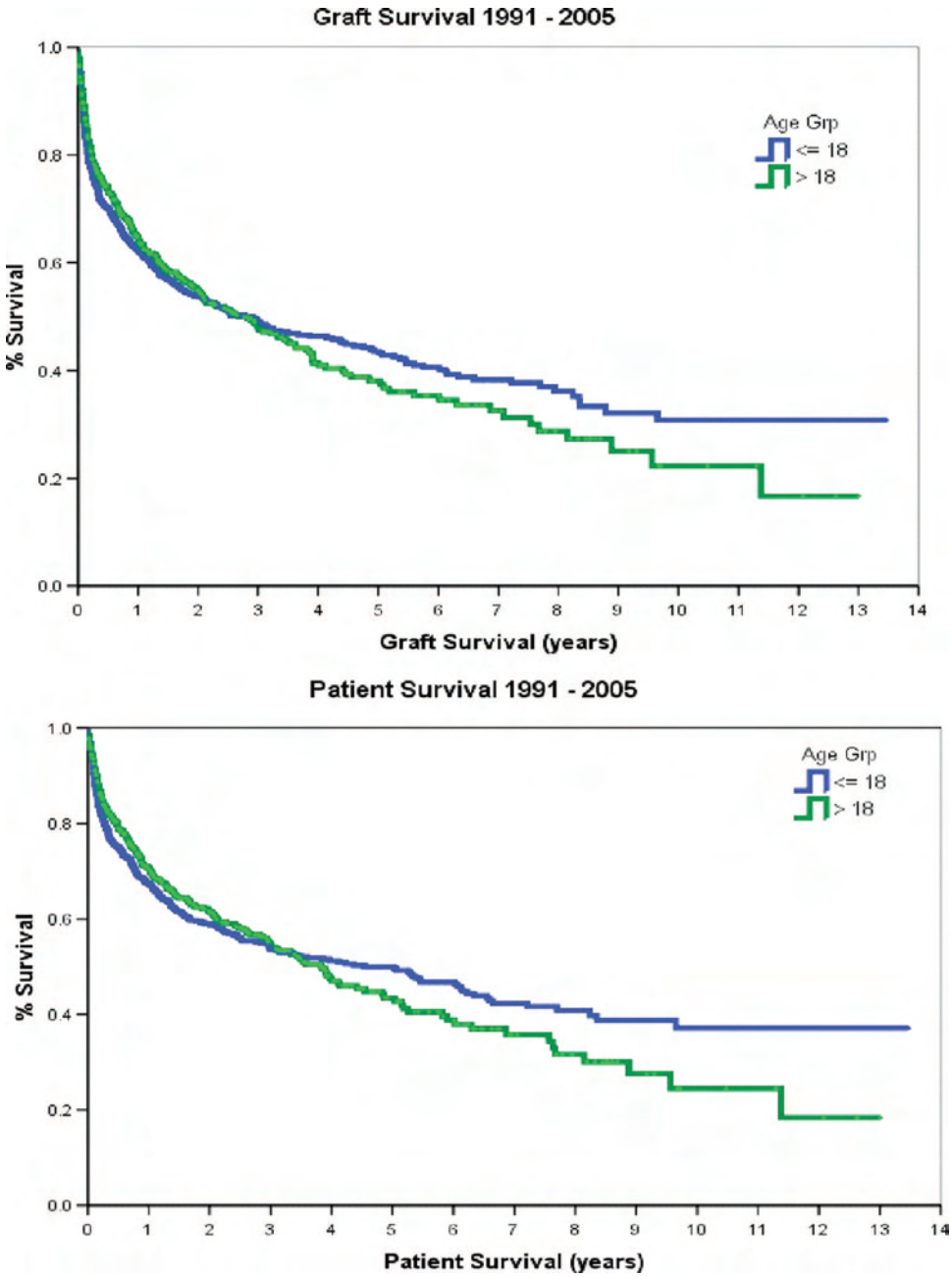

FIGURE 1. Graft and patient survival curves for worldwide adult and pediatric small bowel transplant experience based on data from the Intestinal Transplant Registry.

the first successful cases were reported in the cyclosporine era (*11*), tacrolimus is the drug that allowed development of a consistently successful intestinal transplant series and to date is the maintenance ISP drug of choice. One of the most significant changes to occur with intestinal transplantation is the near ubiquitous use of induction immunosuppression therapy with an estimated 90% of cases now using this as part of the overall regimen. The most common induction ISP agent is anti-IL2-receptor antibody therapy followed by anti-lymphocyte globulin and Campath-1 (*12*, *13*). Their use has been associated with reduction in the incidence and severity of rejection episodes and improvement of survival results, which have allowed maintenance with lower levels of tacrolimus. This latter issue has become important because there is now increasing evidence of calcineurin-inhibitor toxicities in patients receiving nonrenal transplants (*14*). Conversion to noncalcineurin-inhibitor drugs (such as rapamycin), use of steroid-sparing protocols, and a determination as to which ISP therapy best maintains levels of chimeric cells from the donor that promotes graft acceptance remain as new but relatively ill-defined areas in this field of transplantation.

Complications

The technical challenges notwithstanding, one of the most sobering issues continues to be the significant alloimmune response and subsequent rejection of the small intesti-

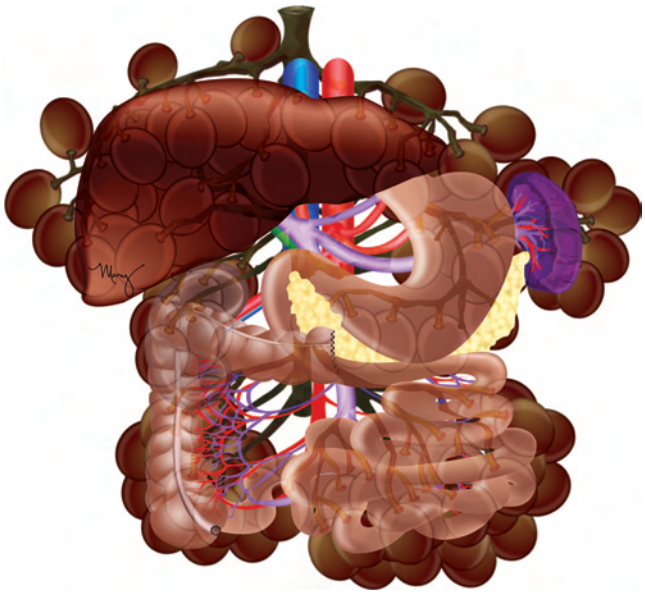

FIGURE 2. Drawing illustrating the organs potentially being transplanted in a multivisceral transplant and how a "cluster of grapes" serves to conceptualize the principal and secondary arterial blood supply to the organ block (Drawing by Mary Campos).

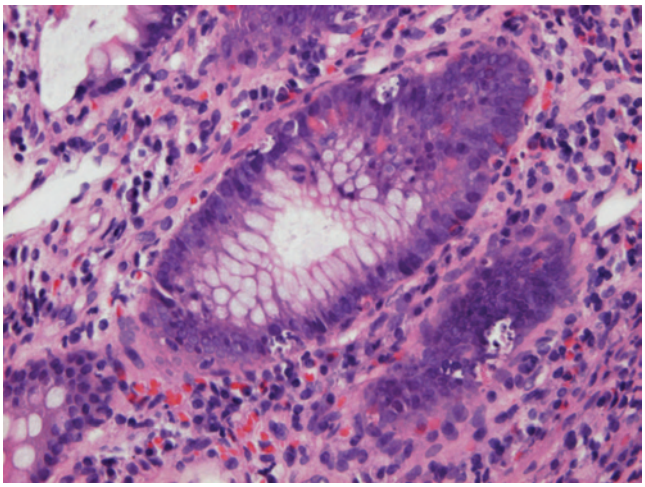

FIGURE 3. Photomicrograph of intestinal allograft from an adult woman approximately 10 years posttransplant. The epithelial structures are undergoing significant apoptosis in the presence of other inflammatory features compatible with acute cellular rejection (hematoxylin & eosin, ×200).

nal graft, an event that occurs more frequently and with greater severity than any other abdominal organ. Indeed, in our experience, rejection of the small intestine occurs with the highest frequency and intensity compared with other transplanted organs; this is best exemplified when comparing rejection rates and severities of the different allograft organs within a given individual who has received a multivisceral transplant (*10*, *15*). The potential reasons for the small intestine to be the nexus of the most vigorous rejection inflammatory response include the heightened immunogenicity and significant donor lymphoid volume in the organ, factors made more significant when considering the large mass of the implanted allograft. For example, small bowel immunogenicity may be enhanced by the type of stimulatory molecules expressed (e.g., major histocompatibility complex class II), the cellular composition (e.g., parenchymal vs. endothelial cells) as well as the donor cell response to injury (e.g., cytokine release).

The attributes and manifestations of the initial wave of alloimmunity to the intestinal graft reveals that the response characterized as "acute rejection" bears some similarities to other solid organ transplants but with unique and poorly understood features. For example, animal and human studies to this point suggest that the archetypical acute rejection response in the bowel is a T-cell-mediated phenomenon (like with other organs) that involves the interstitial and epithelial-lined structures of the organ with kinetics and immune effector cell characteristics that point to a primary immune response. Still, the relationship between the injury that appears to occur in the organ and the clinical manifestations (e.g., fever, increased stool output) remains modestly understood. Acute cellular rejection is now reasonably identifiable by bowel biopsy histology (Fig. 3), and international pathology grading systems have emerged (*16*). In tandem with pathologic changes, improvements in endoscopic monitoring (e.g., magnifying—zoom, capsule endoscopy) help to establish potential sites of rejection (*17*, *18*). For this reason, surveillance endoscopies are now performed two or three times per week or even more frequently during the immediate postoperative period and then at slowly decreasing frequency. A normal endoscopy in the face of pathologic findings suggestive of rejection can simply be repeated the next day and thus avoids overimmunosuppression of the patient.

Disruption in the secretion of products of gastrointestinal and inflammatory cells such as citrulline and calprotectin (*19*, *20*) shows promise as peripheral and adjunctive measurements of altered graft function. Certain molecules such as CD103, like with other bowel diseases (*21*), may be critical cofactors in determining whether immune effector cells can mediate damage to the bowel parenchyma. It is hopeful that measurements of these and other analytes along with biopsy and endoscopy will allow for more efficient screening and identification of acute cellular rejection. The cumulative effect of these advances in prevention, monitoring, and treatment of acute cellular rejection of the intestinal graft has been one of the most important contributors to the significant improvement in patient and graft survival.

Although acute cellular rejection has been reasonably characterized and clinically correlated, other forms of rejection in the bowel remain inadequately defined. In this regard, acute vascular rejection has been recognized infrequently in its most severe form (*22*), although other studies have shown that mild variants and subclinical forms of acute vascular rejection likely exist at a much higher frequency than previously believed (*23*). The role that this humoral-based acute rejection has on long-term graft survival is not known. Chronic rejection (chronic allograft enteropathy) also remains somewhat of an enigma in small bowel transplantation. Diagnosis of this entity is hampered by the lack of specific lesions in the mucosal biopsy, although interstitial fibrosis and other histologic changes considered in the context of the clinical sce-

nario may provide a clue to the presence of chronic allograft enteropathy (*24*, *25*). As patient survival for intestinal transplantation improves, there will likely be better understanding of the frequency of this entity and improved means for its detection and treatment.

Infections in intestinal transplantation, like with other forms of transplantation, have always been a serious problem facing the recipient throughout the posttransplant period. The gamut of viruses, bacteria, and fungi causing morbidity in ITx is similar to other forms of transplant, although occasional unusual microbes (e.g., cryptosporidium) (*26*) involve the allograft. The reemergence and stabilization of the normal bowel flora may have important implications because shifts in the flora toward other atypical microbial residents of the bowel could cause alterations in bowel transit time and may potentiate acute rejection. In our experience, composite organ transplants tend to have fewer infections than isolated bowel (*10*); we suspect that the lower rate of infections (and subsequent less immunosuppression), fewer fistulas, and less complications with arterial anastomosis with MVTx likely all contribute to this finding. However, despite improvements in prophylactic antibiotics, surgical options (e.g., portal venous drainage [*27*]), and earlier identification of infection, sepsis remains the single highest cause of death in this patient population in the short- or long-term posttransplant period (*6*).

Posttransplant lymphoproliferative disease (PTLD) has always been a serious complication in intestinal transplantation (*28*). Interestingly, the rate of PTLD, as defined by the presence of frank malignancy, has remained relatively stable with a frequency of 6% to 8% (although slightly higher in children) despite the introduction of more powerful immunosuppressive agents (*6*). The incidence of the preneoplastic stages of PTLD (e.g., plasmacytic hyperplasia, polyclonal lymphoproliferative changes) remains poorly delineated. PTLD tends to occur approximately at its highest incidence 25 months posttransplantation, but the other precursor forms of PTLD can occur much earlier (*29*). PTLD tends to occur more often with OKT3 or induction therapy, and some forms (e.g., MVTx) of intestinal transplant have a greater risk. Finally, as compared with other solid organ transplants, the gut allograft itself is the most frequent site of early PTLD changes. This causes, at times, a diagnostic dilemma in the allograft biopsy because there are often coexisting inflammatory cells for both acute rejection and early PTLD. Fortunately, current use of rituximab therapy is very useful in the treatment of some forms of PTLD (*30*). The mortality from PTLD has decreased significantly in our experience.

Summary and Future Considerations

Over the past two years, as presented at the 2005 International Bowel Transplant Congress in Brussels, there have been a total of 29 centers performing 323 intestinal transplants. The incidence of 80% one-year graft and patient survival reflects an incredible improvement when compared with results from just several years ago in the year 2000. Positive risk factors for intestinal transplantation include if the center performed greater than 10 transplants and the pretransplant status of the recipient, issues that both reiterate the inherent complexity and morbidity of this procedure (*6*). Unfortunately, most of the gains in patient and graft survival are in the first posttransplant year because long-term survival remains essentially the same as in previous eras of intestinal transplantation. The similarity in the slopes of survival curves with patients undergoing modern-day ITx reflects some of the same problems confronting other solid organ transplants; despite gains in the control of early posttransplant events, there is still a significant decline in graft survival over the subsequent years (*6*). The causes for graft and patient loss over the long term of intestinal transplantation include infections, malignancy, and chronic rejection, similar to other transplants (*6*).

There are numerous areas involving transplantation of the small intestine that hold promise to enhance and improve this procedure so that it will become a cornerstone in the therapy of short bowel syndrome. From a surgical and technical perspective, there continues to be refinement of the three basic techniques (ITx, LITx, and MVTx), but there may be a concomitant role to enhance and lengthen the remaining native bowel (Bianchi procedure, STEP procedure) (*31*). Full-thickness abdominal wall transplantation as an adjunct to small bowel transplantation is now on occasion used to facilitate closure of the abdominal space in certain situations (*32*). Curiously, the skin of the abdominal wall graft shows relatively little acute rejection and is often not synchronized with the changes occurring in the bowel; thus, this graft has had a high rate of success.

Immunologically, the bowel presents an important tool to address the potential relationship between donor cell chimerism (*33*) and immunologic tolerance, because there is a suggestion that the presence of particular cells from the donor may facilitate graft acceptance (*34*). Small bowel allografts may also represent a system to investigate the mechanisms controlling graft-versus-host disease arising from a solid organ allograft, a complication normally in low incidence in bowel transplants (*35*). Little is known regarding the target structures in the bowel for alloimmune cells and the physiological and immunologic restrictions needed for injury to occur. What are the characteristics of the donor lymphoid population mass and repertoire over time and do donor stem cells (*36*) survive long term, possibly serving as a source of chimeric cells?

Will it be possible to supplement bowel allograft surgery with modifications in the native bowel of the host? For example, can intestinal adaptation, a normal phenomenon whereby residual bowel shows compensatory hypertrophy (*37*), be augmented and controlled with the proper growth factors to facilitate engraftment to the transplanted bowel and accelerate healing? Furthermore, as stem cell technology and tissue engineering progress, will there be the capacity to place enterocyte stem cells in matrices on tissue scaffolds (*38*) in the recipient that will eventually generate physiologically capable bowel that will supplement nutrition absorption in the recipient? Modification of intestinal adaptation and gastrointestinal stem cells are among the potential approaches that may assist in recovery from the resection; these areas of investigation share in the fact that they are recipient-derived (thus, not needing immunosuppression) and offering a potentially unlimited supply.

In summary, the field of intestinal transplantation has shown extraordinary growth over the last two decades with a notable level of success so that there is now a realistic alternative for many short bowel syndrome patients over PN.

Although there remain many potential complications and challenges, the upcoming years hold promise of a continuation of our advancement and further improvement with this form of transplantation.

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(updated April 19, 2005)

World Health Organization PTLD Classification of 2001						
Category	**Examples**	**Histopathology**	**Immunophenotype**	**Clonal Status**	**Oncogene, Tumor Suppressor Gene Changes**	**Comments**
"Early" lesions	Reactive plasmacytic hyperplasia (PH) infectious mononucleosis-like PTLD	Some architectural preservation; numerous plasma cells and lymphocytes;variable paracortical expansion; many immunoblasts may be present; atypia slight; some cases may have overlapping features of PH and IM-PTLD	Polyclonal B cells, plasma cells and T cells. Immunoblasts often EBV-positive	Polyclonal; EBV present in most case of PH- IM cases typically EBV positive, may have minor monoclonal or oligoclonal bands	(None)	Often regress with reduced immunosuppression, severe cases may be fatal Examples of posttransplant plasmacytic hyperplasia without EBV should not be considered as PTLD.
Polymorphic PTLD	Polymorphic B cell hyperplasia, Polymorphic B cell lymphoma	Destruction of underlying architecture, full range of B-cell maturation seen, may have necrosis, scattered large bizarre cells (atypical immunoblasts), frequent mitoses, may have monomorphic areas	Mixture of B and T lymphocytes, surface and cytoplasmic Ig polytypic or monotypic; most cases EBV positive	Monoclonal; Rare cases may be polyclonal	None	Overall impression of mixed small and large cell lymphoma or polymorphous immunocytoma; may be multiple; Some cases regress with reduced immunosuppression, others may progress
Monomorphic PTLD	**B-cell neoplasms:** Diffuse large B-cell lymphoma, Burkitt's or Burkitt-like lymphoma, plasma cell myeloma, plasmacytoma-like lesions **T-cell neoplasms:** Peripheral T-cell lymphoma, not otherwise specified; other types	Morphological lymphomas; classify according to current lymphoma categorization; most to all cells transformed, blastic (plasma cell leisons excepted); most look like diffuse large B-cell lymphoma, other types less common; Monomorphic T-cell PTLD probably inlcudes most or all types of T-cell neoplasms	B cell PTLD show CD19, 20, 79a; monotypic Ig expressoin in 50%; Many express CD43, CD45RO (due to upregulation of these T cell markers in B cells harboring EBV); CD30 often postive; most EBV pos. T cell PTLD may express CD4 or 8, CD56, CD30, and alpha-beta or gamma-delta T-cell receptors	Monoclonal Ig genes in B cell PTLD; EBV pos. cases also have clonal EBV; T cell PTLD usu. have clonal T cell receptor; 25% with clonal EBV	Present in some cases	Recommended that these be classified according to standard lymphoma classification, with term " PTLD" added; Monomorphism means that most cells are transformed-cellular monotony may be present but is not required; Regression possible but uncommon compared to early lesions and polymorphic PTLD. Overall mortality 60% solid organ, 80% marrow

						recipients.
Hodgkin lymphoma and Hodgkin lymphoma-like PTLD	Classic HL; Hodgkin-like PTLD	Reed Sternberg cells in appropriate backgound (see comments)	Classic HD CD15, CD30 pos; HD-like PTLD more atypical phenotype, usu B cell antigens expressed; all or almost all cases EBV pos (HD and HD-like)	--	--	Since Reed-Sternberg-like cells can be seen in polymorphic PTLD, diagnosis requires appropriate morphologic and immunophenotypic features

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The Clinicopathologic Spectrum of Posttransplantation Lymphoproliferative Disorders

Lawrence Tsao, MD; Eric D. Hsi, MD

• ***Context.*—Posttransplantation lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphoid proliferations occurring in the setting of solid organ or bone marrow transplantation. They show a clinical, morphologic, and molecular genetic spectrum ranging from reactive polyclonal lesions to frank lymphomas. The close association with Epstein-Barr virus has been established and the pathogenetic role of this virus is becoming better understood. Although they are relatively uncommon, PTLDs are a significant cause of morbidity and mortality in transplant patients.**

***Objective.*—To review the incidence, risk factors, clinical features, pathogenesis, and classification of PTLDs.**

***Data Sources.*—We reviewed relevant articles indexed in PubMed (National Library of Medicine), with emphasis on more recent studies. The classification of PTLDs is based on the most current World Health Organization classification text.**

***Conclusions.*—Posttransplantation lymphoproliferative disorders are a heterogeneous group of disorders showing a wide clinical and morphologic spectrum. Although relatively uncommon, PTLDs represent a serious complication after transplantation. Many risk factors for PTLD are well established, including transplanted organ, age at transplant, and Epstein-Barr virus seronegativity at transplant. However, other factors have been implicated and still require additional examination. Recent studies are shedding some light on the pathogenesis of PTLDs and defining relevant pathways related to Epstein-Barr virus. As the pathogenesis of PTLDs is further elucidated, the classification of PTLDs will most likely evolve.**

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Posttransplantation lymphoproliferative disorders (PTLDs) are a heterogeneous group of lymphoid proliferations occurring in the setting of solid organ or bone marrow transplantation. It has long been known that intact immune systems are required for antitumor surveillance. The occurrence of lymphoma in immunosuppressed transplantation patients was first recognized in 1968 and its close association with Epstein-Barr virus (EBV) infection followed.[1,2] Today we recognize a spectrum of lymphoid proliferations ranging from reactive polyclonal lesions to frank lymphomas. The close association with EBV is well described and the pathogenetic role of this virus is beginning to be understood. However, not all PTLDs are EBV driven, and a significant subset of EBV-negative PTLDs have been identified.[3,4] Although PTLDs represent a relatively uncommon complication in transplant patients, they are a significant cause of morbidity and mortality in these patients. Because of variability in clinical, histopathologic, and immunophenotypic presentations, the diagnosis and classification of PTLDs can be difficult. In this review, we will consider the incidence, risk factors, clinical features, pathogenesis, and histopathology of this group of lymphoproliferative disorders.

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EPIDEMIOLOGY

Incidence

Although there is significant variation in the reported incidences of PTLD after solid organ and bone marrow transplantation, the overall incidence is less than 2% of transplanted patients.[5] There is a clear association between the incidence of PTLD and type of transplantation, with the highest incidence in the first year after transplantation.[6] Among the most commonly transplanted solid organs, cardiac, lung, and hepatic transplantation show the highest incidences of PTLD, ranging from 2% to 5%,[7–9] 2% to 3%,[10,11] and 2% to 5%,[12–14] respectively. The incidence after pancreatic transplantation was recently reported to be 2.1%.[15] Renal transplants show a much lower incidence of PTLD at approximately 1%.[16,17] This may be because of the generally lower intensity of immunosuppression required compared with that of other vital organs. The incidence of PTLD after bone marrow transplantation ranges from 0.5% to 1.0%.[18,19] In recent larger series, the incidence of PTLD appears to be lower than previously cited, possibly the result of better management of immunosuppression (Table 1). Because of the higher risk of PTLD in children (discussion follows), studies examining pediatric populations will generally report incidences 2- to 3-fold higher than in adults.

Risk Factors

Several risk factors for the development of PTLD have been identified (Table 2). These include type of organ

Table 1. Incidence of Posttransplantation Lymphoproliferative Disorders in Organ Transplant in Selected Recent Studies

Organ	Incidence, %	Source, y
Kidney	~1	Caillard et al,[16] 2006
Pancreas	2.1	Paraskevas et al,[15] 2005
Heart	2.3*	Swerdlow et al,[7] 2000
Liver	4.3†	Jain et al,[14] 2002
Lung	2.5	Reams et al,[10] 2003

* Up to 5% in pediatric populations.[8]
† Up to 10% in pediatric populations.[14]

Table 2. Risk Factors for Posttransplantation Lymphoproliferative Disorders

Transplanted organ (multivisceral > lung > liver > heart > kidney)
Pediatric age group
Epstein-Barr virus seronegativity
Immunosuppressive drugs/regimen (OKT3)
Underlying host disease
Cytokine gene polymorphisms

transplanted, immunosuppressive drugs, age of the patient, and EBV status pretransplantation. As previously noted, the incidence of PTLD varies by transplanted organ, with the renal transplants having the lowest incidence, heart transplants having intermediate incidence, and heart-lung or multivisceral transplantation generally having the highest incidence.[20] Large collaborative databases have defined relative risks of PTLD for major organ types.[6] Specific biologic factors may account for these differences. For example, lung and intestinal transplants typically include the highest amount of lymphoid tissue, which may increase the EBV infection rates. Relative ease of mucosal biopsies in these sites may also raise the incidence of early PTLD detection.

The variation in the incidence between the different types of transplanted organs may also be related to varying degrees of immunosuppression necessary for each organ. Specific drugs have been implicated as high-risk factors. In the early days of transplant, use of the potent immunosuppressive OKT3 resulted in a marked increase in PTLDs in cardiac transplant.[21] Use of cyclosporine also increased the incidence of PTLD; however, this could be reduced by careful therapeutic monitoring to avoid overimmunosuppression.[22,23] Although immunosuppression is a major risk factor, it is still unclear whether the contribution is due to the cumulative dose or peak levels of drugs. Some studies, unable to identify any specific agents, have suggested the cumulative immunosuppressive dose to be the contributory factor.[24–27] In bone marrow transplantation, T-cell depletion of the donor bone marrow is a well-known risk factor for PTLD.[28–30] However, studies of newer immunosuppressive agents targeting T cells have not always conclusively demonstrated similar increased risk in solid organ transplantations.[24] Experience with these newer immunosuppressive agents may help define the magnitude of risk for a PTLD associated with their use.

Mismatch of EBV status in the recipient and donor (seronegative recipient with seropositive donor) is another well-known risk factor for PTLD and is intimately associated with the pathogenesis of PTLD.[26–33] In one striking study of a single institution's experience with solid organ transplantation, seronegative patients had a 76-fold risk of PTLD compared with seropositive patients.[34] The higher risk associated with EBV-naive patients also explains, to some extent, the higher incidence of PTLD among pediatric transplant patients.[35] Not surprisingly, EBV-naive patients will frequently present initially with EBV-associated PTLD of the early lesion or polymorphic type, possibly representing an abnormal primary EBV response in these immunosuppressed patients.

A patient's underlying disease has been suggested in some series to be a risk factor for PTLD. Primary immunodeficiency showed a 2.5-fold increased risk in one bone marrow transplant series.[18] Patients with hepatitis C infection,[9,36] autoimmune hepatitis,[37] cystic fibrosis,[38] and Langerhans cell histiocytosis[39] have also been suggested to be at higher risk for PTLD. Other infectious agents including cytomegalovirus,[27,40] human herpes virus 8,[41] and, recently, simian virus 40[42] have all been reported in cases of PTLD and may contribute to increased risk. The number and severity of rejection episodes and degree of HLA mismatching have also been examined as risk factors. However, the magnitude of risk these factors pose is still controversial.

Innate host immune responses may also play a role in the development of PTLD. Cytokine gene polymorphisms associated with regulation of cytokine production during immune responses are being examined. Specifically, there is some evidence that low interferon gamma production may be associated with increased risk of PTLD in liver and renal transplant patients.[43,44]

CLINICAL FEATURES

The clinical presentation of PTLD is highly variable, depending on the type of immunosuppression, type of allograft, and histologic type of PTLD (early lesions, polymorphic PTLD, or monomorphic PTLD). Patients may present with infectious mononucleosis-like symptoms. There is frequent involvement of the tonsillar tissue and Waldeyer ring, especially in pediatric patients. Such PTLDs often have the histology of so-called early lesions. Monomorphic PTLD, like lymphoma, can present with constitutional symptoms, lymphadenopathy, and mass lesions. Up to 25% of patients may present with allograft failure due to involvement by PTLD. In these patients, the clinical presentation can mimic allograft rejection. In bone marrow transplants, widespread involvement is common and may simulate graft-versus-host disease. Bone marrow involvement may present with new-onset or persistent cytopenias. Polymorphic PTLD may present with features overlapping early lesions and monomorphic PTLD. As a result of the variability of presentation, a high index of suspicion must be present in any patient with a history of transplantation.

PATHOGENESIS

Investigations have yielded insight into the pathogenesis of PTLD. Phenotypic and immunoglobulin mutational studies have resulted in a model of histogenesis for PTLD. Molecular studies have supported this model and have identified several genes thought to be important in molecular pathogenesis. Epstein-Barr virus infection, of course, plays a central role in development of PTLD and recent work has also elucidated important mechanisms of oncogenesis relevant to these proliferations.

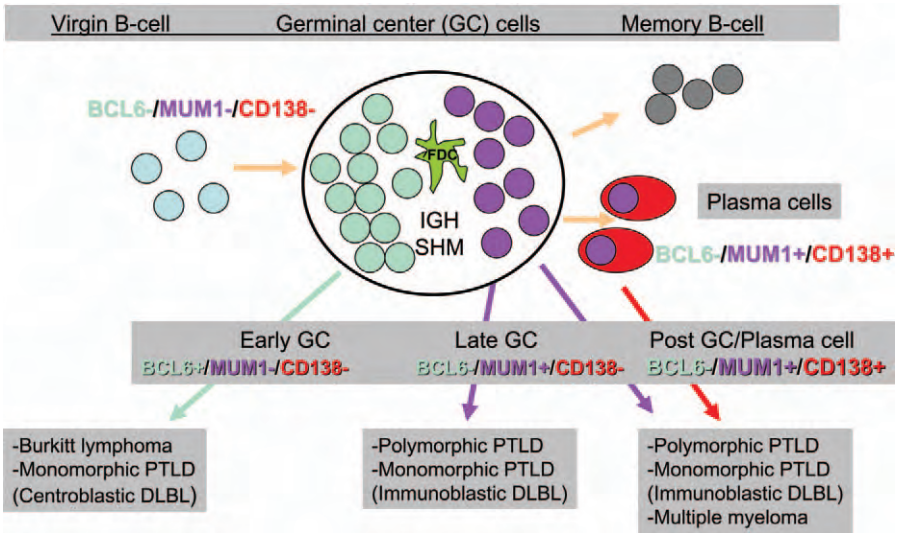

Figure 1. *Model of posttransplantation lymphoproliferative disorder (PTLD) histogenesis. Adapted from Capello et al[46] with permission from John Wiley & Sons Limited, 2005.*

Histogenesis

Like B-cell non-Hodgkin lymphomas, molecular and phenotypic features of PTLD have been compared with normal B-cell counterparts. Analysis of immunoglobulin heavy chain variable (*IGHV*) genes is a powerful tool in determining the maturational state of B cells. According to this model, unmutated (germline) genes represent antigen-naive (pregerminal center or virgin) B cells, and B cells harboring somatic hypermutation have been exposed to the germinal center (GC) microenvironment and thus represent GC or post-GC B cells.[45] Approximately 25% of polymorphic PTLDs and 10% of diffuse large B-cell lymphomas (DLBLs) have unmutated (germline) *IGHV.* Burkitt lymphoma (BL) and 25% of centroblastic DLBL show ongoing somatic hypermutation, consistent with GC B cells. The majority of polymorphic (75%) and monomorphic (65%) PTLDs show somatic hypermutation that is stable among clones, suggesting a late GC or post-GC phenotype. Thus, most PTLDs derive from GC or post-GC cells.[46] The few PTLDs that lack somatic hypermutation appear to arise early after transplantation and are EBV-associated. They may derive from true pre-GC cells or cells that are incapable of undergoing the GC reaction.[46] A recent study that analyzed both immunoglobulin heavy and light chain genes showed that 94% of PTLDs had somatic hypermutation.[47]

Further phenotypic characterization into GC and post-GC stages using Bcl-6 (GC marker), MUM1 (late GC and post-GC), and CD138 (post-GC, terminal differentiation) has resulted in the model shown in Figure 1.[46] The Bcl-6$^+$/MUM1$^-$/CD138$^-$ PTLDs derive from cells experiencing the GC reaction. They harbor ongoing mutations and morphologically correspond often to centroblastic types of DLBL or BL. A Bcl-6$^-$/MUM1$^+$/CD138$^-$ phenotype corresponds to PTLDs that derive from B cells that have completed the GC reaction and include most (65%) polymorphic and some (30%) monomorphic PTLDs, particularly DLBL with immunoblastic features. This phenotype is uncommon in human immunodeficiency virus–related lymphoma.[48,49] A third phenotype, Bcl-6$^-$/MUM1$^+$/CD138$^+$, represents post-GC cells and includes polymorphic or monomorphic PTLDs showing immunoblastic DLBL morphology or plasmacytic differentiation.

Antigen Stimulation and Viral Oncogenesis

Analysis of immunoglobulin gene usage provides evidence that specific antigen stimulation and selection may not play a major role in the pathogenesis. In an analysis of 50 PTLDs, no preferential use of *IGHV* family genes was noted, suggesting a lack of specific pathogenetic antigen. Evidence of antigen selection in tumor cells based on replacement mutations in the complementarity determining regions of the immunoglobulin heavy chain gene (*IGH*) was seen in less than 30% of cases.[47] In fact, up to 50% of PTLDs have lost the ability to express functional immunoglobulin.[47–51]

Epstein-Barr virus infection, in the setting of immunosuppression, has a central role in the pathogenesis of PTLD. Several lines of evidence can be offered. It is present in almost all PTLDs that occur early after transplant.[52,53] It is also frequently clonally integrated in tumor cells of polymorphic and monomorphic PTLDs, indicating that it was present at the time of malignant transformation.[54] Increasing EBV titers can also be detected in the blood of patients prior to development of PTLD and treatment with EBV-specific T cells can result in tumor reduction.[55–59] Finally, EBV latent genes have transforming activity in B cells. In fact, EBV has been found to transform GC cells lacking immunoglobulin.[60] The exact mechanism of viral oncogenesis is yet to be elucidated; however, EBV latent membrane proteins (LMP), LMP-1 and LMP-2A, have been the focus of attention. These oncogenic proteins activate intracellular signaling pathways, mimicking CD40 (a member of the tumor necrosis factor receptor family) and B-cell receptor signals.[61] Latent membrane protein 1 has been shown in PTLD tissue to mimic activated tumor necrosis factor receptor family members through tumor necrosis factor receptor-associated factors. This results in downstream activation of NFκB, an important transcription factor that activates prosurvival genes.[62,63]

Although most PTLDs are EBV related, approximately 20% of patients with PTLD will lack evidence of EBV in their tumors, and the incidence may be increasing.[3] The EBV-positive and EBV-negative PTLDs show differences in clinical course and may represent independent entities.[3,4] Specifically, EBV-negative PTLDs appear to occur late after transplantation, are more often classified as

monomorphic compared with EBV-positive PTLDs, and generally have an aggressive course. However, some will still respond to decreased immunosuppression. Given the relative rarity of these tumors, the pathogenesis of these EBV-negative PTLDs is still poorly understood. Currently, the question of whether these are better considered coincidental lymphomas or part of the heterogeneity of PTLDs remains unanswered.

Genetic Alterations

Several genetic alterations in oncogenes or tumor suppressor genes have been found in PTLDs. These include *MYC, BCL6, NRAS,* and *TP53.*[64–66] Chromosomal translocations involving *MYC* and mutations in *MYC, BCL6, NRAS*, and *TP53* have been described.[64–66] Alterations in *MYC, NRAS*, and *TP53* are uncommon and seen only in monomorphic (immunoblastic lymphoma histology) or multiple myeloma types of PTLDs and are never present in polymorphic lesions.[66] Rearrangement of *BCL6* is very uncommon in PTLD as opposed to DLBL in immunocompetent patients. However, *BCL6* mutations are common (approximately 50%), and have been associated with shorter survival and nonresponsiveness to reduced immunosuppression.[64] Rearrangements of *MYC* have also been associated with more aggressive disease and poor outcome.[67] Microsatellite instability has been described in a higher proportion of PTLDs than in non-Hodgkin lymphoma from immunocompetent hosts, corresponding to the high degree of genetic instability in PTLDs.[68]

Recently, epigenetic alterations have been examined. In particular, hypermethylation of O6-methylguanine-DNA methyltransferase (*MGMT*), a DNA repair gene, has been found in 60% of monomorphic PTLD. Inactivation of *MGMT* has been shown to be lymphomagenic in knockout mice and may promote genetic instability with acquisition of *TP53* and *RAS* mutations.[69,70] Other genes identified as abnormally methylated include death-associated protein kinase (*DAPK1*), a proapoptotic molecule, and *TP73*, a putative tumor suppressor gene related to *TP53.*[69] Much work remains to be done and new tools such as array-based comparative genomic hybridization studies have identified other abnormalities.[71] However, the exact role of these abnormalities in the development of PTLD remains largely unknown.

Donor Versus Host Origin

Studies on the cell of origin of PTLD have shown that at least 90% of PTLDs originate from host B cells in solid organ transplantation.[72] The converse is true for bone marrow transplantation.[73] Although donor-derived PTLDs have been reported with increased incidence in liver and lung transplants, with suggestions of predilection for involving the graft, recent studies have been controversial.[72–76] The prognostic significance of donor versus host-derived PTLD is unclear.[76] In addition, there have been no large-scale studies examining T-cell and natural killer (NK) cell PTLDs.

PATHOLOGIC FEATURES AND CLASSIFICATION

Classification of PTLD is currently based on the World Health Organization (WHO) system for classifying hematopoietic neoplasms.[77] The key morphologic, immunophenotypic, and molecular characteristics of each type of PTLD are listed in Table 3. The WHO divides PTLD into 4 major categories: early lesions, polymorphic PTLD, monomorphic PTLD, and Hodgkin lymphoma (HL) and HL-like PTLD. Early lesions, polymorphic PTLD, and monomorphic PTLD represent a pathologic spectrum that can be observed synchronously or metachronously within a single specimen or within multiple specimens from a single patient.

Early Lesions

Early lesions consist of 2 morphologic types: plasmacytic hyperplasia and infectious mononucleosis–like PTLD. The common defining characteristic of early lesions is some degree of preservation of the underlying architecture of the involved tissue (Figure 2, A). Plasmacytic hyperplasia is a lesion characterized by numerous plasma cells with rare immunoblasts. Infectious mononucleosis–like lesions resemble typical infectious mononucleosis, with marked paracortical expansion by a mixed T-cell and plasma cell infiltrate and a prominent immunoblastic proliferation. Some early lesions may show overlapping features between plasmacytic hyperplasia and infectious mononucleosis–like lesions.

Immunophenotyping of early lesions is of limited diagnostic utility as it will confirm the morphologic impression of variable mixtures of B cells, T cells, and plasma cells with polytypic light chain expression. Immunoblasts will frequently show evidence of EBV infection using in situ hybridization for EBV-encoded RNA (EBER) or EBV LMP-1 immunohistochemical stain. Other EBV-associated nuclear antigens (ie, EBV-encoded nuclear antigen, LMP) are not reliably expressed.[78] As the name implies, early lesions represent the earliest morphologic and genotypic changes of PTLD.[66] These lesions occur early (<1 year) in the course of transplantation and are more common in EBV-naive pediatric and adult transplant recipients. Analysis of *IGH* and episomal EBV genome will frequently yield polyclonal or oligoclonal patterns. Occasionally, a minor clone is seen, but is of no clinical significance. Clonal cytogenetic changes are rare in early lesions.[67,79]

Polymorphic Lesions

Polymorphic PTLD is characterized by a mixed lymphoproliferation consisting of immunoblasts, plasma cells, and intermediate-sized lymphoid cells. In contrast to early lesions, polymorphic PTLD is characterized by destruction of the underlying architecture of the involved tissue (Figure 2, B). However, in contrast to monomorphic PTLD, polymorphic PTLD shows a full spectrum of B cells from small to intermediate-sized lymphocytes to immunoblasts and mature plasma cells (Figure 2, C). Atypia, necrosis, and numerous mitotic figures are all acceptable. In the past, these features of ''malignancy'' were used to distinguish ''polymorphic lymphoma'' from ''polymorphic hyperplasia.''[80] However, subdividing polymorphic PTLD is no longer necessary under the WHO classification because recent findings revealed that morphologic subdivision does not reliably predict clinical behavior.[66,81] Immunophenotyping of polymorphic PTLD will show variable mixtures of B cells and T cells. Analysis of surface or cytoplasmic immunoglobulin expression is useful for identifying monotypic B-cell populations. However, B cells may show polytypic immunoglobulin expression in polymorphic PTLD. Most polymorphic PTLDs will show EBV latency II and III patterns, expressing EBER and EBV–LMP-1 with variable expression of EBV-encoded nuclear antigen 2 and other viral antigens.[78] Although immuno-

Table 3. Summary of Pathologic Features of Posttransplantation Lymphoproliferative Disorders (PTLDs)*

Subtype	Morphology	Immunophenotype	Molecular	EBV Status
Early lesion	Preservation of the underlying architecture	Mixture of B, T, and plasma cells	IgH: polyclonal or oligoclonal EBV: polyclonal or oligoclonal	(+), virtually all
IM-like	Increased numbers of immunoblasts	$CD30^+$ immunoblasts will be present	See early lesion	(+), virtually all immunoblasts $EBER^+$
Plasma cell hyperplasia	Large aggregates and sheets of plasma cells	κ and λ show polytypic plasma cells	See early lesion	(+), majority; occasionally can be (−)
Polymorphic	Some degree of effacement of underlying architecture with a spectrum of lymphoid cells ranging from small lymphocytes to intermediate to immunoblasts	B-cell markers may highlight the spectrum of B cells present CD30 will highlight immunoblasts	IgH: clonal EBV: clonal	(+), majority; variable numbers of $EBER^+$ cells
Monomorphic	Effacement of underlying architecture with cytologic atypia sufficient for a lymphoma	Varies with lineage	Varies with lineage	Varies with lineage
B cell	Majority will resemble diffuse large B-cell lymphoma A subset may resemble Burkitt lymphoma	Positive for B-cell markers, but can show abnormal phenotype (ie, aberrant expression or loss of antigens) Burkitt immunophenotype ($CD20^+$, $CD10^+$, $CD43^+$, Bcl-6^+, Bcl-2^-, Ki-67: ~100%)	IgH: clonal EBV: clonal	(+), majority; large numbers of $EBER^+$ cells
T/NK cell	Varies with type	Varies with type (WHO T-cell lymphomas) Pan–T-cell antigens should be evaluated for aberrant loss	T cell: TCR: clonal NK cell: TCR: germline EBV: clonal (if present)	(−), majority of T cell (+), virtually all NK cell
Plasma cell myeloma Plasmacytoma	Sheets of plasma cells Must be differentiated for early lesion	Positive for plasma cell markers κ and λ show monotypic plasma cells	IgH: clonal EBV: clonal (if present)	Variable
HL and HL-like	RS cells in the classic HL milieu	HL: classic HL immunophenotype ($CD30^+$, $CD15^+$, $CD45^-$, $CD20^{-/+}$, $CD3^-$, weak PAX-5) HL-like: aberrant immunophenotype (ie, $CD20^+$)	IgH: varies EBV: clonal (if present)	(+), majority; RS cells $EBER^+$
MALT-type PTLD	Lymphoid infiltrate of small, mature-appearing lymphocytes expanding underlying mucosa and submucosa Lymphocytes show slightly irregular nuclei with moderate amounts of pale cytoplasm	Similar to MALT-type lymphomas in immunocompetent patients ($CD20^+$, $CD5^-$, $CD10^-$, $CD43^{+/-}$)	IgH: clonal	(−), majority *H pylori* associated

* EBV indicates Epstein-Barr virus; IgH, immunoglobulin heavy chain; IM, infectious mononucleosis; EBER, EBV-encoded RNA; NK, natural killer; WHO, World Health Organization; TCR, T-cell receptor; HL, Hodgkin lymphoma; RS, Reed-Sternberg; MALT, mucosa-associated lymphoid tissue; *H pylori, Helicobacter pylori*.

phenotyping may appear polytypic, molecular analysis of *IGH* or episomal EBV genome will usually show a clonal pattern.[52,81] Clonal cytogenetic changes may be present.[67,79]

Monomorphic Lesions

Monomorphic PTLDs are characterized by architectural and cytologic atypia sufficient to be classified as a lymphoma based on morphologic features.[77] In general, monomorphic PTLDs show invasion and architectural effacement by large aggregates and confluent sheets of transformed cells with large nuclei with prominent nucleoli (Figure 2, D and E). The neoplastic cells can show marked pleomorphism or plasmacytoid/plasma cell differentiation. These cases of monomorphic PTLD generally are not diagnostically problematic. However, occasional cases of PTLD may span the spectrum of polymorphic PTLD and monomorphic PTLD. These cases are difficult to classify within a single category. The presence of areas of monomorphic PTLD, however, should always be clearly indicated.

Monomorphic PTLDs are divided according to B-cell or T-cell lineage and further subclassified according to the WHO classification of lymphomas in the nontransplant population.[77] It is beyond the scope of this review to include a detailed description of the WHO classification of lymphomas, so only a general description will be included with areas of difficulty highlighted.

Monomorphic B-cell PTLD (B-PTLD) is the prototypic monomorphic PTLD. The majority of the B-PTLDs will resemble DLBL in nontransplant patients. Morphologic variants include immunoblastic, centroblastic, and, less commonly, anaplastic morphology. However, as with DLBL, there does not appear to be any clinical significance

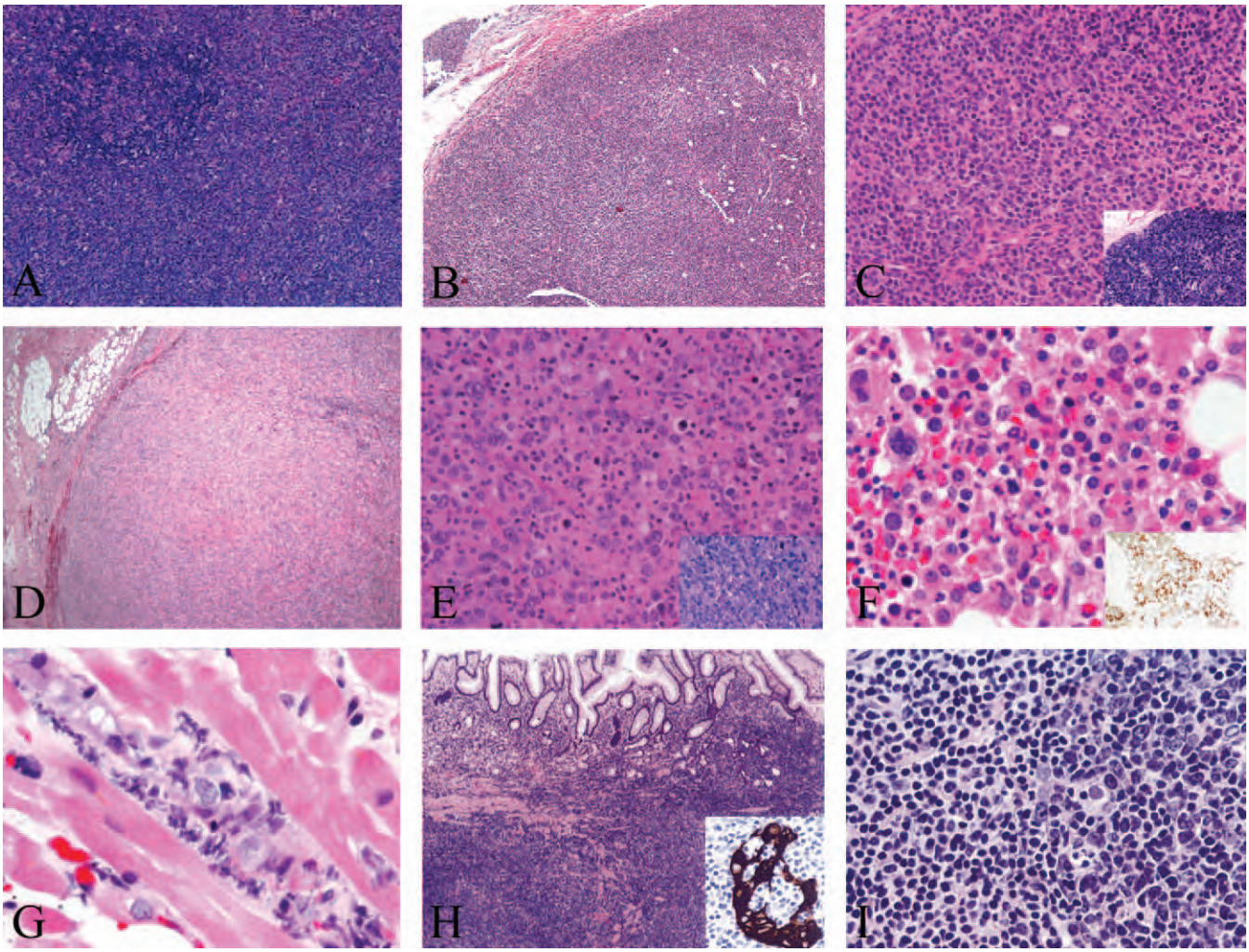

Figure 2. *A, Infectious mononucleosis–like early lesion with marked paracortical expansion surrounding a follicle (hematoxylin-eosin [H&E], original magnification ×20). B, Polymorphic posttransplantation lymphoproliferative disorder (PTLD) showing effacement of the underlying lymph node architecture (H&E, original magnification ×20). C, High magnification of B showing lymphoid cells with minimal cytologic atypia and showing a spectrum of sizes (H&E, original magnification ×200). There are small lymphocytes, scattered immunoblasts, and numerous intermediate-sized cells, which is characteristic of polymorphic PTLDs. The inset shows Epstein-Barr virus (EBV)–encoded RNA in situ hybridization (EBER-ISH) highlighting the numerous EBV-positive cells (original magnification ×200). D, Monomorphic PTLD also showing a destructive pattern, similar to polymorphic PTLDs (H&E, original magnification ×20). E, However, high-power magnification of D shows a predominance of large pleomorphic cells with marked cytologic atypia, characteristic of monomorphic PTLDs (H&E, original magnification ×400). Inset of EBER-ISH also shows EBV in most neoplastic cells (original magnification ×200). F, Plasma cell myeloma PTLD with numerous infiltrating plasma cells in a bone marrow biopsy (H&E, original magnification ×400). Inset of CD138 highlights the infiltrating plasma cells (original magnification ×40). G, T-cell PTLD involving the allograft as a perivascular lymphoid infiltrate with marked cytologic atypia (H&E, original magnification ×400). Immunophenotyping (not shown) is necessary to confirm the T-cell lineage. H, Gastric low-grade muscosa-associated lymphoid tissue type of PTLD expanding the mucosa and submucosa (H&E, original magnification ×20). Inset of a cytokeratin stain highlights characteristic "lymphoepithelial" lesions (H&E, original magnification ×400). I, Marginal zone cells (left) with slightly irregular nuclei and moderate amounts of pale cytoplasm adjacent to and infiltrating a reactive germinal center (right) (H&E, original magnification ×400).*

associated with these morphologic variants. Morphologic resemblance to BL or atypical BL is diagnostically significant and should be confirmed with immunophenotypic and cytogenetic studies. Immunophenotypic analysis will show expression of B-cell antigens or a BL phenotype in cases of BL or atypical BL. Antigens aberrantly expressed by conventional DLBL (ie, CD43, Bcl-2) may be present. Surface immunoglobulin expression may be monotypic or absent. Currently, the evaluation of GC or post-GC phenotype is not required because the clinical and prognostic implications are still uncertain.[45–48] The majority of B-PTLDs show presence of EBV infection within the transformed cells, with variable latency patterns.[82] Virtually all cases show a clonal pattern of *IGH* rearrangement and, if present, episomal EBV genomes. Cytogenetic evaluation will show clonal karyotypic abnormalities, which can include trisomies 9 and/or 11 and abnormalities of 8q24.1, 3q27, and 14q32.[67]

Rare cases of B-PTLD are morphologically and immunophenotypically identical to plasma cell neoplasms (Figure 2, F).[83,84] Plasma cell myeloma and plasmacytoma-like PTLD can also be EBV associated in about 50% of the cases reported.[83,84] Clinically, these can present as rare extramedullary plasmacytic neoplasms similar to plasmacytomas or plasma cell myeloma. Plasma cell PTLDs need to be differentiated from plasmacytic hyperplasia, a non-

destructive early lesion, and DLBL with marked plasmacytic differentiation, a monomorphic PTLD. Because of the rarity of plasma cell PTLD, it is currently unclear if plasma cell directed, B-cell directed, or both, is the most effective therapy. The evaluation for urine and serum M components, serum immunoglobulin levels, and lytic bone lesions, although not always conclusive, can be helpful in the diagnosis of plasma cell myeloma-PTLD.[83] Immunophenotypic evaluation of B-PTLD should include B-cell and plasma cell–associated antigens.

T-cell PTLDs (T-PTLDs) are all classified as monomorphic PTLDs and must show a similar degree of architectural and cytologic atypia required for B-PTLD (Figure 2, G). The T-PTLDs are subclassified according to the WHO classification for T-cell neoplasms in the nontransplant setting. Immunophenotyping is essential for diagnosis and subtyping of T-PTLD. Depending on the subtype, the immunophenotype will vary. Evaluation of pan–T-cell antigens, although not always conclusive, is useful for demonstrating any aberrant losses of expression. CD4 or CD8 expression and αβ or γδ T-cell receptor expression will follow what is generally known for T-cell lymphomas in nonimmunosuppressed patients. Markers of immaturity (CD1a, TdT, and CD34) can be seen in cases of precursor T lymphoblastic lymphoma. CD30 expression can be present, especially in the anaplastic large cell lymphoma subtypes. CD56 and cytotoxic markers can be expressed by T-PTLD. Most (60%–80%) T-PTLDs lack EBV; however, a minor subset may be EBV positive.[85] Molecular analysis of the T-cell receptor (*TCR*) gene should show a clonal pattern. Analysis of episomal EBV genome is usually not indicated, but will show a clonal pattern when EBV is present. True NK-cell PTLD will frequently express CD56 and cytotoxic markers, but must lack surface CD3. Variable expression of pan–T-cell antigens, CD2 and CD7, can be seen. Unlike T-PTLD, the vast majority (80%–90%) of true NK-cell PTLD shows EBV infection with clonal episomal EBV genome.[86] Molecular analysis of *TCR* must show a germline pattern to be diagnosed as a true NK-cell PTLD.

Hodgkin Lymphoma and Hodgkin Lymphoma–like Lesion

Hodgkin lymphoma and HL-like PTLD is a rare category of PTLD that is classified independently from other monomorphic PTLDs. Hodgkin lymphoma PTLD shows the morphologic features characteristic of classic HL in nontransplant patients. These include the proper background inflammatory infiltrate and Reed-Sternberg cells. Hodgkin lymphoma–PTLD must be distinguished from polymorphic PTLD with Reed-Sternberg–like cells. Hodgkin lymphoma–PTLD has Reed-Sternberg cells with the classic HL phenotype ($CD45^-$, $CD3^-$, $CD20^-$/$weak^+$, $CD15^{+/-}$, $CD30^+$). These cases usually arise late in transplantation and frequently show evidence of EBV infection. Although currently HL and HL-like PTLD are considered similar, there is evidence suggesting that HL-like PTLD may be more related clinically and pathologically to a monomorphic B-cell PTLD.[87] The HL-like PTLD frequently shows an atypical immunophenotype for HL such as strong expression of CD20.

Low-Grade B-Cell Lymphoproliferative Disorders

The current WHO classification does not recognize low-grade B-cell lymphoproliferative disorders as PTLD. However, they do occur in the posttransplant setting. Extranodal marginal zone B-cell lymphomas of mucosa-associated lymphoid tissue (MALT) type occurring as PTLDs morphologically and immunophenotypically resemble their counterparts in immuncompetent patients (Figure 2, H and I).[88,89] These MALT lymphomas do not show evidence of EBV, but are frequently associated with *Helicobacter* organisms, especially in gastric sites.[88] Molecular analysis of *IGH* will show a clonal pattern.[89] Other low-grade B-cell lymphoproliferative disorders reported after transplantation include hairy cell leukemia.[90] This is extremely rare. Clinically, morphologically, and immunophenotypically, these cases are identical to those seen in the nontransplant setting and may represent coincidental events.

Clinical Course

The clinical course of PTLD is highly variable and dependent on the type of PTLD, the lineage of the PTLD, and the association with EBV. Virtually all early lesions regress with reduction in immunosuppression and generally show good prognosis, especially in pediatric patients.[81,91] About half of polymorphic PTLDs regress with reduction of immunosuppression; however, some will progress, requiring chemotherapy.[19,81] Of those progressing, more than half will respond to therapy.[81] Some studies have found the presence of *BCL6* gene mutations to predict poor response to reduction of immunosuppression.[64] The majority of monomorphic PTLDs do not regress with reduction of immunosuppression alone. In addition, some monomorphic PTLDs do not show good response even to chemotherapy.[81] Among the monomorphic PTLDs, EBV-associated PTLDs consistently have a better prognosis when compared with EBV-negative PTLDs.[3,4] However, a minor subset, up to one-third, of EBV-negative PTLDs have been reported to regress with reduction of immunotherapy.[3] Monomorphic PTLDs of T-cell/NK-cell lineage almost never regress with reduction of immunosuppression alone and respond poorly to chemotherapy.

Hodgkin lymphoma and HL-like PTLDs usually arise late after transplantation (>1 year). The prognosis of HL and HL-like PTLDs appears relatively good. In one large study, none of 60 patients developing HL and HL-like-PTLD died of PTLD-associated causes.[84]

The low-grade B-cell lymphoproliferative disorders, specifically MALT lymphomas, are also usually seen late after transplantation (>1 year).[88,89] Clinically, the MALT lymphomas behave indolently. The majority of cases can be managed by eradication of the *Helicobacter* organisms and conservative management (localized radiation, surgery, and single-agent chemotherapy).[88,89] The rare cases of hairy cell leukemia reported after transplantation have shown an indolent course and excellent response to conventional hairy cell leukemia therapy.[90]

DIAGNOSIS

The timely and accurate diagnosis of PTLD is essential for early intervention. However, a high clinical index of suspicion is required. Recently, monitoring and quantification of EBV viral load in peripheral blood has been shown to be helpful in predicting the development of PTLDs. Although clear guidelines have yet to be established regarding laboratory procedures and management, the trends are clear. Persistently low EBV viral load has good negative predictive value for development of EBV-positive PTLD. The EBV levels appear to increase prior to PTLD and fall after successful therapy.[92] In an attempt to

define important thresholds, a level of 200 copies/10^5 leukocytes was shown to correlate with symptomatic EBV infection or PTLD in pediatric transplant patients.[93,94] Some investigators have also suggested preemptive therapy with agents such as rituximab.[95,96] However, because not all patients with elevated levels develop PTLD and EBV-negative PTLDs cannot be predicted with such a test, further work is needed to precisely define the role of EBV viral load testing in transplant patient populations.[97,98] Recent studies have suggested using cytokine genotyping in addition to EBV viral loads to increase the predictive value for PTLDs.[99]

PATHOLOGIC EVALUATION: WHAT NEEDS TO BE DONE?

Practically speaking, excisional biopsies of masses or enlarged lymph nodes are preferred because one of the characteristic differentiating features between early lesions and polymorphic and monomorphic PTLD relies on the ability to document preservation of underlying architecture. Extranodal disease is common. Involved sites may include the gastrointestinal tract, liver, lung, and bone marrow. If endoscopic or needle biopsies are used, several biopsies or passes are advised to obtain adequate tissue for ancillary studies.

When multiple sites of involvement are present, sampling of several lesions should be considered as early, polymorphic, and monomorphic lesions can be synchronously present in different sites. In addition, because synchronous lesions may actually represent different clonal proliferations, separate work-up at the genetic level (ie, molecular analysis of *IGH* gene) may be of interest for follow-up purposes.[100] In patients with allograft involvement where rejection enters into the clinical differential diagnosis, allograft biopsies can help differentiate rejection from PTLD. Assessment of EBV can be helpful because PTLDs are often positive, whereas EBV is absent in rejection. Overall, focusing the diagnostic evaluation on the basis of organ dysfunction or a mass lesion provides the highest yield for obtaining adequate tissue for diagnosis. Screening blood or bone marrow evaluations in patients suspected of PTLD is usually of low diagnostic yield.

Immunophenotyping PTLDs is essential because of the significant differences in prognosis and therapy between B-cell and NK/T-cell lymphomas. Evaluation for presence of EBV by immunohistochemical or molecular techniques is also essential because of the differences in prognosis between the EBV-positive and EBV-negative cases. The EBER in situ hybridization is preferred, given its presence in all latency patterns. Although not absolutely required for diagnosis in the majority of cases, testing for clonality (usually by antigen receptor-rearrangement studies) is also helpful for complete characterization and can be used for comparison to simultaneous or future PTLDs. A distinct clone at a later date would suggest a new independent PTLD rather than a relapse. Cytogenetic studies, also not necessary, similarly may be helpful. Assessment of oncogene mutations or translocations, by molecular or cytogenetic techniques, is currently not routinely performed. Diagnostically, the type of PTLD, lineage of the PTLD (if a monomorphic lesion), clonal status, and EBV status should be clearly indicated in the pathology report.

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Conference Report

Tumors and Solid Organ Transplantation: Intersections at Multiple Levels

Highlights from American Society of Transplant Surgeons 3rd Annual Winter Symposium: Tumors and Transplantation; Miami Beach, Florida; January 24-26

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Introduction

Malignancy is a problem that requires careful consideration in the evaluation of organ donors and transplant recipients both before and after transplantation. Utilization of organs from donors with malignancy, allocation of organs to transplant candidates with a history of malignancy, and factors related to development and treatment of recurrent or *de novo* posttransplant neoplasias serve as referential points from which to raise a number of issues. The application of immunotherapy, particularly cell therapy, to both infectious disease and neoplasia in the solid organ transplant population is undergoing a period of rapid development. These and additional topics were discussed and debated at this symposium.

Neoplasia in the Donor

Cancer may be transferred from an organ donor to a recipient as a result of occult malignancy in the donated organ or misdiagnosis, in a known situation such as when a small renal cell carcinoma occurs in the donor kidney and undergoes wide excision, in a low-risk situation such as when the donor has a known skin malignancy or carcinoma in situ of the cervix, or when a known cancer was believed to have been cured. In addition, *de novo* malignancy may arise from either graft parenchymal cells or passenger cells in the graft. Transmission of donor-related malignancies was examined from multiple perspectives, beginning with insight by Joseph Buell, MD,[1] of the University of Cincinnati, from the Israel Penn International Transplant Tumor Registry (IPITTR).[2] This voluntary registry was instituted in 1969 and provides both current and historical perspectives.

In the past, many types of tumors have been considered absolute contraindications to organ donation. Today, however, 2 tumors -- choriocarcinoma (93% transmission, 64% mortality) and melanoma (74% transmission, 60% mortality) -- fall into this category. In addition, since lung cancers and sarcomas, particularly high-grade variants, also behave aggressively, procuring organs from donors with these tumors is not recommended.

Renal Cell Carcinoma

A different picture emerged from the data on renal cell carcinoma (RCC) in the overall IPITTR

series. Recipients had a high (61%) rate of tumor development, but a lower mortality rate (23%), if they received a graft from a patient with renal cell carcinoma. Furthermore, 14 cases of renal transplantation in which tumors in the donors were identified and excised prior to transplantation were reported. All tumors were </= 4 cm in size (median 2 cm) with negative resection margins and Fuhrman grade I-II/VI histology based on rapid permanent histologic sections. No tumor development had been reported at a median follow-up time of 69 months.

Renal cell carcinomas are more frequently detected as small lesions; this allows for the resection of earlier-stage tumors and improved survival. It was also noted that some renal tumors such as oncocytomas or angiomyolipomas are benign, regardless of size, and may be excised, whereupon the remaining kidney can be used for transplantation.[3]

The reason that donors with cancer are even considered is the current organ shortage, according to L.Thomas Chin, MD,[4] of the University of Wisconsin, Madison, and Arthur I. Sagalowsky, MD,[5] of the University of Southwestern Texas, Dallas. The risk of cancer recurrence (in the donor) following partial nephrectomy for tumors < 4 cm in diameter was estimated at 0% to 3%. The incidence of bilateral involvement was estimated at 10% to 20% for familial or papillary RCC, and 2% to 4% for sporadic clear cell RCC (Fuhrman nuclear grade I-II).

Metastatic RCC may occur in tumors < 3 cm in diameter, and may occur in the donor as late as 20 years after the primary disease.[4,5] Most investigators suggest that donors should be disease-free for at least 2-5 years, and potential recipients with prior RCC should be disease-free for at least 2 years before transplantation.

Central Nervous System (CNS) Tumors

CNS tumors accounted for 21% of all cases in the IPITTR. Four risk factors for tumor transmission were identified: high-grade (grade III-IV) histology, previous surgery, radiation therapy, and chemotherapy. The rate of tumor transmission was 7% in the absence of risk factors, but rose to 30% to 40% in the presence of 1 or more of these conditions. Development of CNS tumors in recipients was associated with a high mortality rate; the Cincinnati group advocates limited autopsies of donors with intracerebral hemorrhage of unknown etiology to rule out CNS tumors.

Interpretation of the literature on CNS tumors is difficult for a number of reasons: different pathologic terms may be applied to the same tumors, some series do not report histologic types of CNS tumors, and some tumors may show different grades of differentiation either in a synchronous or metachronous fashion. The spread of glioblastoma multiforme (GBM) recapitulates normal glial cell development, and the tumor invades not by lymphatic or vascular spread, but by migration of individual cells within the CNS, observed Eric C. Holland, MD, PhD,[6] of Memorial Sloan-Kettering Cancer Center, New York, NY. Mass lesions disrupt the blood-brain barrier, which in turn raises the question of spread of tumor cells outside of the CNS even prior to surgery or other forms of therapy. Almost all GBM have Ras activation and 70% have hyperactivity of Akt and downstream activation of mTOR, thought to promote tumor cell survival. Sirolimus and its derivatives have potent mTOR inhibitory activity, and blockade of the Akt-mTOR pathway has antitumor activity in other models. The possibility of a beneficial side effect of sirolimus in this circumstance was raised, although animal models suggest that this approach alone will likely not be curative for these tumors.

The antiangiogenic effects of sirolimus in relation to its mTOR inhibitory function were discussed by Edward K. Geissler, PhD,[7] of the University of Regensburg, Germany.[8] He indicated that this antitumor effect was active at immunosuppressive doses, and suggested that sirolimus may be an exception to the rule that immunosuppressive drugs favor tumor development.

A wide variation (0.5% to 18%) in transmission rates for GBM is reported. The possibility that rates may also vary among different organs was raised as a point worthy of study. The present unfortunate circumstance is that approximately 130,000 patients die annually from GBM in the United States. Continued exploration of specific circumstances associated with minimal risk of tumor transmission may allow some of these individuals to serve as organ donors in the future.

Breast and Colon Cancer

Using recent cancer screening figures[9] and current donation rates, Reid B. Adams, MD,[10] of the University of Virginia, Charlottsville, estimated that inclusion of patients with stage 0-1 breast or colon cancer would result in a mere 9-10 additional donors per year. It was pointed out that a higher proportion of cancers might be detected at early stages in the future, potentially increasing the number of potential donors.

Whether or not organs from such patients are suitable for transplantation is currently unknown, and both historical registry data and data concerning stage-specific cancer survival are starting points from which to estimate the risks of tumor transmission. Older series, comprised mainly of patients with advanced-stage cancers, indicated a transmission rate of 6% for breast cancer and 25% for colon cancer.

On the basis of more recent figures, it was suggested that patients with stage 0 or 1 colon cancer might be considered as organ donors following definitive treatment resolution of their tumors. White males could donate immediately after therapy, whereas an interval of 5 years might be required for females or black males, as predicated by current survival figures.

In the case of breast cancer, patients with stage 0 tumors, excluding those with high-risk features such as extensive carcinoma in situ, might be considered for organ donation at any time following definitive therapy. Patients with stage 1 T1a or T1b tumors could be considered after 10 years of disease-free follow-up. Patients with T1c or higher-stage breast cancer are not considered acceptable donors.

Prostate Cancer

Prostate cancer in the donor was addressed by Stephen C. Jacobs, MD,[3] University of Maryland, Baltimore. The use of widespread prostate specific antigen screening and early biopsy has led to prolonged survival in patients with prostate carcinoma, particularly in the case of low-grade histology. Dr. Jacobs observed that, given the near universal occurrence of this cancer with increased age, many transplants are undoubtedly performed using organs from donors with occult prostate carcinoma. If the patient with prostate cancer is a renal transplant candidate, therapies such as brachytherapy or cryosurgery that spare the bladder from radiation exposure might be considered. There have been no cancer-related deaths at this time in his series of renal transplant recipients who developed prostate carcinoma.

Metastatic Disease

The use of organs from donors with metastatic carcinoma of any type is associated with a high rate of tumor transmission and should be avoided, noted H. Myron Kauffman, MD,[11] consultant for the United Network for Organ Sharing (UNOS), in his perspective on donor malignancy based on UNOS Tumor Registry data.[12] An analysis from this database revealed that the frequency of tumors in donors was 0.04% with an overall transmission rate of 0.016%. It was noted that, even in the case of good-prognosis RCC, the risk for recurrence in the nonimmunosuppressed population was 9.6% after 5 years. This figure has implications for both donors and potential recipients with a history of this tumor. Similarly, the recurrence rate for melanoma in the original

host was up to 1% after 15 years. The risk of cancer transmission must be balanced against the need for life-saving transplantation, but UNOS cautions against using donors with a history of certain types of cancer including choriocarcinoma; melanoma; lymphoma; GBM; medulloblastoma; or cancers of the lung, kidney, breast, or colon.

Recommendations

The risk of tumor transmission must be weighed against the risk of death without transplantation and the benefits of organ transplantation on a case-by-case basis. The patient must be fully informed and involved in the decision to consider use of an organ from a donor with a possible malignancy. Full disclosure to and involvement of the patient at every step of this process was a theme echoed by a number of the speakers.

Neoplasia in the Transplant Candidate and Recipient

Solid organ transplant recipients are at particular risk for the development of cancer (recurrent and *de novo*) after transplantation; a major factor is the effect of immunosuppressive drug therapy necessary to prevent organ rejection. The overall recurrence rate of tumors in transplant recipients with preexistent cancer is 21%, according to data from the IPITTR by E. Steven Woodle, MD, of the University of Cincinnati, Ohio.[13] Cancers with a low (</= 8%) recurrence rate include uterine, cervical, testicular, thyroid, and early RCC. "Incidental" asymptomatic RCC had a recurrence rate of 8% with a 3% mortality rate, whereas symptomatic RCC had a 43% recurrence and 26% mortality rate. Patients with RCC had a significant rate of recurrent disease even after a 5-year disease-free interval prior to transplantation. Patients with prior prostate cancer had a recurrence rate of 18% and a tumor-related mortality rate of 7.8%. The risk of recurrence of stage 3 cancer was more than double the risk of stage 1 or 2 cancer. The recurrence rate for bladder cancer was 18% with a mortality rate of 12%. The overall recurrence rate of breast cancer was 14% with a mortality rate of 8%. Patients with stage 1 or 2 disease had favorable survival compared with patients with stage 3 disease. The recurrence rate of vulvar cancer decreased after a 10-year wait, but the same was not true for cervical cancer. The overall recurrence rate of melanoma was 21%, with lower frequency of recurrence for lower-grade tumors. The tumor recurrence rate in patients with colon cancer (23%) was higher in thoracic vs nonthoracic organ transplant recipients. It was suggested that this might relate to differences in immunosuppression levels.

Data on the increased risk of cancer in transplant recipients from the Surveillance Epidemiology and End Results registry and the Scientific Registry of Transplant Recipients data for Southeast Michigan were presented by Friedrich K. Port, MD, MS,[14] of the University of Michigan, Ann Arbor. The standardized incidence ratio (SIR) was defined as the observed/expected numbers of tumors in transplant recipients compared with age-matched and region-matched controls. By this definition, there was a 2-fold increase in solid tumors (confidence interval [CI] = 1.6-2.4) and a 4.3-fold increase in lymphomas (CI = 2.4-7.0) in transplant recipients compared with lymphomas and leukemia in controls. There were no significant differences in tumor incidences when analyzed by allograft type. Among solid tumors, those arising in the kidney, vulva, or colon appeared to have a high SIR.

Evaluation of Transplant Candidates

UNOS data show that the overall cancer recurrence rates in kidney, liver, and heart transplant recipients are 1.1%, 6.5%, and 2%, respectively. For all organ types, the risk is lower than the risk of developing *de novo* tumors, which is 8%, 5%, and 14%, respectively, in these groups.

Integrating cancer screening into the evaluation of transplant candidates was advocated by

Sundaram Hariharan, MD,[15] of the Medical College of Wisconsin, Milwaukee. While many types of cancer cannot be screened for or detected at an early stage, the major types of cancer encountered after transplantation can be screened for. At this time it is not clear what constitutes optimal screening or the optimal periods of time between cancer treatment and transplantation. However, disease-free delay periods were suggested, taking into consideration probable times of relapse:

- No delay for incidental RCC;
- Delay of 0-2 years for nonmelanoma skin cancer;
- Delay of 2 years for cancers of the bladder, prostate, uterus, melanoma, or Wilms' tumor with appropriate cytogenetic support;
- Delay of 2-5 years for cervical cancer, breast cancer, RCC (2-5 cm in diameter), and lymphoma; and
- Delay of 5 years for colorectal cancer or RCC > 5 cm in diameter.

Despite the availability of reliable and relatively simple and inexpensive screening tests for early detection, advanced cancer represents a major health risk after transplantation.

Skin Cancer

Forty percent to 70% of patients develop skin cancers within 20 years after transplantation, according to Clark C. Otley, MD,[16] of the Mayo Clinic, Rochester, Minnesota. The most common skin cancer, squamous cell carcinoma (SCC), has a 7% metastatic rate and 56% 3-year survival following metastasis. Integration of dermatologic consultation into patient follow-up could aid in identification of high-risk patients, early diagnosis of cancerous and precancerous lesions, and effective patient education regarding preventive measures. A variety of therapies, including topical retinoids, serial chemotherapy, biological response modifiers, and Mohs surgery, can be used in individual cases. Dr. Otley encouraged physicians and other transplant professionals to use the International Transplant Skin Cancer Collaborative as a resource for information and consultation on skin cancer in their patients.[17]

The implications of skin tumors in organ transplant recipients were further elucidated by Stuart J. Salasche, MD,[18] University of Arizona, Tucson. Since transplant recipients have a tendency to develop multiple and aggressive forms of SCC more often than the general population does, a high degree of vigilance was recommended. Fair skin, ultraviolet exposure, duration of immunosuppression, human papillomavirus (HPV) infection, and less common conditions such as osteomyelitis are risk factors for development of skin cancer. Patients with preexistent SCC may be at increased risk for multiple recurrence and metastasis. Delaying transplantation for 2-3 years in patients at high risk for metastatic SCC was suggested, given the poor prognosis associated with this type of cancer.

A similar argument was made in general for melanoma, which is associated with a disproportionately high percentage of deaths. However, early-stage disease is associated with good survival. Stage 1 disease may be curable, but a small percentage of patients have demonstrated recurrent disease up to 15 years after transplantation. Individual prognostic factors need to be weighed when considering the option of organ transplantation in the patient with melanoma. Merkel cell carcinoma is also prone to recurrence and metastasis after transplantation. It was recommended that patients be carefully assessed, preferably with dermatologic consultation. It has been observed that, unfortunately, only 14% of renal transplant recipients receive dermatologic follow-up.

Hepatocellular Carcinoma (HCC)

Assessment of patients with HCC for liver transplantation in the setting of the Model for End-Stage Liver Disease (MELD) criteria[19] was discussed by Richard B. Freeman, MD,[20] of Tufts University, Boston, Massachusetts. This scoring system went into effect in the United States on February 27, 2000, and incorporates measures of bilirubin, INR (international normalized ratio for coagulation testing), and serum creatinine. It results in a score of 6-40, is predictive of death within 3 months, and is modified for pediatric patients (Pediatric End-Stage Liver Disease [PELD]).

In most cases, liver transplantation is preferable to resection of HCC due to underlying cirrhosis, but a significant problem is tumor progression and/or death while awaiting transplantation. Using the Milan criteria,[21] 3-year posttransplant survival was 83% with only 8% recurrence if transplantation was performed for a single HCC < 5 cm in diameter, or for up to 3 separate HCC lesions, each < 3 cm in diameter. In response, the UNOS/Organ Procurement Transplant Network Liver Committee assigned a priority MELD weight of 24-29 points to patients with HCC who met the Milan criteria while awaiting transplantation. Under this system, transplantation in patients with HCC increased 3.5-fold over a corresponding time interval from the prior year; 86% to 91% of patients with HCC received a transplant within 3 months of being issued a priority MELD score based on a combination of liver disease and tumor. There has been no detectable trend toward increased use of priority scores to obtain transplants on a preferential basis. Analysis of the Milan criteria and other systems will continue in an effort to refine criteria for entry of patients with HCC into the liver transplant waiting list. The continuation of studies such as this is necessary to assure the most equitable system of organ distribution possible.

A model for staging HCC based on fraction of allelic loss to define the probability of posttransplant recurrence of HCC in liver transplant candidates with preexistent HCC was described by Wallis Marsh, MD,[22] of the University of Pittsburgh, Pittsburgh, Pennsylvania. Using this approach, Dr. Marsh and colleagues were able to categorize 91 of 103 patients; the model was 100% accurate in 81 evaluable patients. This model has potential to replace the staging system for HCC based on differentiation status. It appears to be an excellent way to determine who will and who will not have recurrent HCC, and this information could be used to decide who and who not to transplant.

Cholangiocarcinoma

Liver transplantation of patients with cholangiocarcinoma has historically been controversial. Charles B. Rosen, MD,[23] of the Mayo Clinic, Rochester, Minnesota, and C. Wright Pinson, MD,[24] of Vanderbilt University, Nashville, Tennessee, debated this issue. Dr. Rosen (pro) reported on a subset of patients with early-stage disease enrolled in a protocol of pretreatment by radiation therapy and chemosensitization between 1993 and 2001. Fifteen of 41 enrolled patients survived 1-9 years after transplantation, 14 of these disease-free. According to Dr. Rosen, hilar cholangiocarcinoma is emerging as an indication for liver transplantation in patients receiving neoadjuvant therapy. Dr. Pinson (con) countered that transplanting patients with cholangiocarcinoma is a misappropriation of an already taxed donor supply, with limited return. Further objections included the high rate of patient dropout from the treatment protocol cited due to advanced disease or toxicity, the similarity of survival rates with those of resection for early-stage cholangiocarcinoma in some series, and the added variable of posttransplant immunosuppression. Despite these reservations, Dr. Rosen and Dr. Pinson concurred that continued development of this investigational protocol in selected expert centers is warranted in efforts to improve the survival for patients with these tumors.

Lung Cancer

The cure rate of resected stage 1 lung cancer is 70% to 80%, but only 15% of patients with lung cancer are diagnosed at this stage, and the overall cure rate is only 12%. Low-dose helical

computed tomography (CT) scan detects early-stage lung tumor nodules at least 3 times more frequently than chest x-ray, noted J. Michael DiMaio, MD,[25] University of Texas/Southwestern Medical School, Dallas. Dr. DiMaio's group performs annual CT scans in heart transplant recipients with a >/= 10 pack-year smoking history. They noted that enforcement of smoking cessation in transplant candidates prior to transplantation is one method of reducing a known risk factor for cancer, and this may have benefit as lung cancer typically arises a number of years after transplantation.

Cancer Screening

Prevention and early detection of cancer in transplant recipients is not a routine part of posttransplant care. Bryce Kiberd, MD,[26] of Dalhousie University, Halifax, Nova Scotia, and William M. Bennett, MD,[27] of Oregon Health Sciences University, Portland, encouraged transplant clinicians to incorporate American Cancer Society guidelines on screening and surveillance for cancer into patient follow-up. Questions were raised, however, regarding the effectiveness and potential harm of cancer screening in transplant recipients with limited life expectancy, since it can take at least 5 years before screening has an impact on survival. Invasive procedures such as colonoscopy might have a higher morbidity in immunocompromised patients, and screening tests such as stool guaiac could have a higher false-positive rate in patients with multiple sources of blood loss. On the basis of these concerns, it was suggested that no screening was required for patients with a life expectancy less than 5-7 years, but that screening be recommended for those with life expectancies exceeding 10-12 years. Patients in between should be informed of the relative risks and benefits of particular screening procedures. Cancer-specific rates of death are required to develop more objective criteria for refining cancer screening recommendations for transplant recipients.

Immunity, Viruses, and Neoplasia

T lymphocytes are a central component of the host immune response to viral infection and some cancers, and viruses are important cofactors in the development of some cancers, particularly in the immunosuppressed patient. Work in the area of T cell-based therapy of cytomegalovirus (CMV) and melanoma was summarized by Philip D. Greenberg, MD,[28] of the University of Washington, Seattle.[29] His group has taken the approach of expanding antigen-specific T-cell lines in vitro and infusing these into immunocompromised patients to provide cellular immunity against specific targets. This therapy has been applied primarily to hematopoietic stem cell transplant recipients, who may develop fatal CMV disease during periods of immunodeficiency.

T cells derived from the original donor are raised against CMV over a 6- to 8-week period and can provide protective immunity against CMV infection when infused into the recipient. Cytotoxic $CD8^+$ cells provide immune effector activity, and long-lived immunity requires support from $CD4^+$ T cells. In Dr. Greenberg's study, side effects were mild. Current studies seek to modify the glucocorticoid receptor of the infused cells, as high-dose corticosteroids (such as might be given for rejection or graft-vs-host disease) normally cause lysis of lymphocytes leading to loss of effector function. Modification of T-cell receptors to introduce antiviral specificities into T cells from CMV-seronegative organ transplant recipients is also developing as a viable strategy to generate a rapid and specific antiviral response.

A modified approach is necessary for antitumor immunity, as tumors may actively downregulate or even destroy invading T cells. Using the melanoma model, this group has shown that CD8+ T-cell activity can be enhanced and prolonged in the setting of concurrent low-dose interleukin (IL)-2 support as a surrogate for CD4+ help. Despite initially effective killing, the tumor undergoes phenotypic evolution to shed the target antigen, leading to an antigen-deficient subclone. One strategy to circumvent this tumor evasion is to identify antigens that are indispensable to the malignant phenotype. Several such potential targets have been identified, but these often have

poor immunogenic capacity. To counter this, modified T-cell receptors exhibiting a higher antigen affinity for these targets, together with modified granulocyte macrophage colony-stimulating factor receptors capable of generating a signal for IL-2 production, have been developed and inserted into CD8+ T cells in vitro. Other changes that lower the energy of activation of the T-cell receptor also lead to endogenous IL-2 production. Such approaches can lead to the development of antitumor T-cell reagents with predefined specificities, and may ultimately result in antitumor reagents that could be produced in advance and administered directly at time of therapy.

James E. Sligh, MD, PhD,[30] of Vanderbilt University, Nashville, reviewed the association of HPV and skin cancer. HPV normally causes cutaneous warts. However, certain conditions, such as epidermodysplasia verruciformis, are associated with an antiviral immune defect and lead to numerous warts that can progress to malignancy, particularly in areas exposed to ultraviolet light. HPV with a high risk for cancer development (most commonly type 16 or 18) integrates its DNA into the host genome, in contrast to low-risk HPV, which persists as separate episomes. This may underlie differences in expression of the viral oncoproteins E6 and E7. These proteins have been shown to immortalize human keratinocytes in vitro and interfere with the tumor suppressor activity of the cell cycle proteins p53 and Rb. E6 protein may also protect against UV-induced apoptosis. Approximately half of all organ transplant recipients will develop warts by 5 years posttransplantation. This patient population also carries high-risk papillomavirus strains in the lower genital tract, more commonly than do nonimmunocompromised individuals. Thus, screening for premalignant lesions of the skin and genital tract was emphasized as a routine component of transplant recipient follow-up.

Donald Ganem, MD,[31] of the University of California, San Francisco, provided insight into the pathogenesis of Kaposi's sarcoma (KS) by contrasting the etiologic agent (KSHV, HHV-8) with other transforming gamma herpes viruses. He noted that KSHV is likely not a fully immortalizing virus and could even be lost from infected cells. He proposed that the lytic portion of the life cycle might contribute to KS by providing paracrine factors to stimulate angiogenesis and inflammation, by recruiting additional infected cells to the lesion to replace apoptotic cells, and by reinfecting cells that had lost their viral episomes.

Murine models of hepatocellular carcinogenesis, particularly transgenic mice with expression of either hepatitis virus proteins or growth factors, were reviewed by Ravi S. Chari, MD,[32] of Vanderbilt University. Numerous strains transgenic for hepatitis B virus (HBV) proteins have been developed, and these animals show variation in tumor characteristics. Tumors are also associated with mice transgenic for expression of hepatitis C virus proteins. Resultant tumors show expression of viral core, but not envelope proteins, suggesting the former as important for carcinogenesis. Mice transgenic for hepatocyte growth factor or transforming growth factor alpha (TGF-alpha) also develop HCC with a high frequency. In the case of TGF-alpha, mice transgenic for both this gene and for either c-myc or HBV surface antigen show increased hepatocarcinogenesis indicating synergism between the genes and likely mimicking the clinical condition more closely. P53 knockout mice develop liver tumors when treated with diethylnitrosamine, but these are largely angiosarcomas.

Posttransplant Lymphoproliferative Disorders (PTLD)

Cliona Rooney, PhD,[33] of Baylor College of Medicine, Houston, Texas, used the biology of the Epstein-Barr virus (EBV) as a foundation for understanding PTLD and other EBV-related neoplasias. This infection normally reaches an asymptomatic steady state in which largely latent viral-infected cells are controlled by a combination of virus-specific T cells and neutralizing antibody. In immunosuppressed transplant recipients, T-cell immunosuppression can allow outgrowth of viral-infected cells that express a wide range of EBV latency-associated proteins. Dr. Rooney's group has used donor-derived EBV-specific T cells to prevent and treat PTLD in bone

marrow and stem cell recipients.[34]

Hodgkin's disease in nonimmunosuppressed patients is an example of an EBV-associated neoplasia in which tumor cells circumvent the immune response by downregulating a number of viral antigens and by producing locally immunosuppressive molecules. Some genetic modifications to counteract this include: engineering T cells to contain an immunodominant TGF-beta receptor to allow function in the presence of normally inhibitory levels of TGF-beta, and the use of IL-12-secreting T cells to counteract the local Th2 microenvironment produced by tumor secretion of IL-13 and the chemokine thymus and activation-regulated chemokine (TARC).

The pathologic classification of PTLD was outlined by Michael A. Nalesnik, MD,[35] of the University of Pittsburgh. The evolution of the classification systems was traced, leading up to the present World Health Organization (WHO) 2001 classification that divides PTLD into 4 categories: (1) early lesions (including infectious mononucleosis-like and reactive plasmacytic hyperplasia lesions), (2) polymorphic PTLD, (3) monomorphic or lymphomatous PTLD (including B- and T-cell neoplasms), and (4) Hodgkin lymphoma/Hodgkin-like PTLD. For additional information on these classifications, go to the Transplant Pathology Internet Services Web site.[36] "PTLD" is a generic term, and subclassification is crucial for appropriate selection of therapy. Evaluation should include histopathologic, phenotypic, clonal, and virologic assessment. The majority of polymorphic PTLDs are clonal proliferations, but may still be capable of regression with reduced immunosuppression, noted Dr. Nalesnik.

There has been an increase in the relative frequency of EBV-negative PTLD in recent years. In some instances, these have been linked to *Helicobacter* infection, and treatment may lead to resolution of the lymphoproliferation. Recurrent PTLD may represent true recurrence or separate tumors, and biopsy is desirable in this setting. Douglas W. Hanto, MD,[37] of the Harvard Medical School, Boston, Massachusetts, focused on the polyclonal-to-monoclonal transition that occurs in the development of PTLD, and classified PTLD into 4 conditions: (1) posttransplant infectious mononucleosis, (2) benign polyclonal B-cell hyperplasia, (3) early malignant transformation in polymorphic B-cell lymphoma, and (4) monoclonal polymorphic B-cell lymphoma; these correspond to the first 2 general categories of the WHO classification. Dr. Hanto stressed the need for a multimodal approach to diagnosis, in particular emphasizing the importance of CD20 and EBV assessment. He noted that mononucleosis might resolve without therapy in some cases, but requires antiviral therapy and reduced immunosuppression in others. Therapy must be individualized, and combinations of reduced immunosuppression, antiviral agents, and intravenous immune globulin in polyclonal disease were suggested. In some cases, CD20 monoclonal antibodies (mAb) and alpha interferon might have a role. He recommended reduced immunosuppression and anti-CD20 mAb or cytotoxic chemotherapy in cases of monoclonal disease, with surgical resection and radiotherapy used as appropriate. Prospective multicenter studies are required for development of optimal treatment algorithms.

Current applications of therapeutic algorithms for PTLD were described by Steven A. Webber, MD,[38] of the University of Pittsburgh. Dr. Webber underscored the role of reduced immunosuppression and pointed out the potential shortcomings such as rebound acute rejection and early chronic rejection. The advocacy of anti-CD20 mAb and/or chemotherapy as initial therapy requires prospective clinical trials for evaluation. Since a proportion of monomorphic PTLD may regress under the proper conditions, there is a need to define markers to separate these from other monomorphic tumors likely to require chemotherapy for resolution.

Two additional therapies that are used without proof of clinical efficacy are antiviral and immune globulin agents. He advocated continued use of these agents based on clinical experience, until objective criteria for their use are established. Questions regarding the extent and length of immunosuppression reduction as well as reinstitution of antirejection therapy are currently based largely on clinical judgment by the individual physician. In the EBV-positive PTLD of pediatric

patients, EBV genomic titers in peripheral blood are a useful marker of disease activity. However, few data exist in the adult population, and this marker is likely of little value in EBV-negative tumors.[39] Given the promising results of anti-CD20 mAb, a prospective multicenter trial addressing the role of this agent at the time of initial diagnosis is being planned.

The use of chemotherapy in the treatment of PTLD was summarized by Thomas G. Gross, MD, PhD,[40] of Ohio State University, Columbus. He observed that in most published series, regimens designed for conventional non-Hodgkin's lymphomas (cyclophosphamide, doxorubicin, vincristine and prednisone [CHOP]; ProMace-CytaBOM; etc.) were used for refractory disease or in patients in whom immunosuppression could not be reduced. He estimated a 50% long-term relapse-free survival using this approach. In order to reduce systemic toxicity and minimize the effect of chemotherapy on antiviral immunity, his group used low-dose cyclophosphamide (600 mg/m^2) and prednisone (2 mg/kg/day) for 5 days in a multicenter pilot study of treatment of refractory PTLD in children.[41] This regimen, termed "CHOP-Lite", led to complete remission in 77%, which rose to 84% if patients with fulminant disseminated disease (which does not respond to any intervention at present) were excluded from analysis. However, the relapse rate was 18% with a 2-year relapse-free survival of 73%. Those patients who did not develop normal anti-EBV immunity appeared to be at greater risk for relapse. He recommended additional studies to optimize this regimen, particularly for fulminant disease, and to evaluate its efficacy in the adult population.

Cellular therapy for the treatment of EBV-associated PTLD[42] was proposed as a treatment by Malcolm K. Brenner, MD,[43] of Baylor College of Medicine. He observed that anti-CD20 antibody appeared to be a significant advance in therapy, but relapses were common in the solid organ transplant population, and in some cases recurrent disease evolved into a CD20-negative phenotype, rendering the antibody treatment ineffective. He reported a greater than 98% success rate in establishing anti-EBV cytotoxic T-cell lines from a series of 300 stem cell or solid organ transplant recipients. He noted that such lines could even be developed from patients who were EBV-seronegative at time of transplantation. Infused cells behave differently in different patient subpopulations, expanding rapidly in stem cell recipients, while growing more slowly in organ transplant recipients. His group has taken the approach of monitoring EBV levels at 2- to 4-week intervals early posttransplantation and utilizing anti-CD20 antibody in cases of PTLD while simultaneously generating cytotoxic T-cell lines, a process that takes 4-6 weeks. The cells are then administered as definitive therapy. With this approach they have had no fatalities from EBV disease in a series of more than 600 solid organ and stem cell transplant recipients.

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Suggested Readings

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