

Understanding the Causes of Kidney Transplant Failure: The Dominant Role of Antibody-Mediated Rejection and Nonadherence

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Abbreviations: ABMR, antibody-mediated rejection; DSA, donor-specific antibodies; GN, glomerulonephritis; MPGN, membranoproliferative glomerulonephritis; PRA, panel reactive antibody; PVN, polyoma virus nephropathy; TCMR, T-cell-mediated rejection.

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Introduction

Late failure of kidney transplants remains an important clinical problem (1,2). In the United States, 5469 kidney transplants developed end-stage kidney failure in 2008 (data provided by Jon Snyder from USRDS), making kidney transplant failure the fourth leading cause of end-stage renal disease. The reasons for failure are not well understood. Some have postulated that late deterioration reflects dysregulated fibrosis, drug toxicity (3) or progressive “chronic allograft nephropathy” (3–5), although this term has been dismissed by the Banff pathology consensus because it does not represent a specific disease entity (6). The more likely explanation is that kidney transplants are essentially stable after recovering from the stress of implantation until specific diseases or conditions develop (7), including antibody-mediated rejection (ABMR) and recurrent renal diseases (8–12).

Recognition of C4d-negative ABMR (12,13) created a new scenario: 50% of ABMR is currently missed, with the biopsy findings and clinical phenotypes wrongfully attributed to other processes such as “chronic allograft nephropathy” or calcineurin inhibitor toxicity (14,15). Thus many patients presenting late with a biopsy for clinical indications have ABMR due to *de novo* donor-specific HLA antibodies, usually anti class II (16), either C4d-positive or C4d-negative. This raises the question of why immunosuppression fails to prevent the development of *de novo* donor-specific antibodies (DSA) and ABMR. One possibility is nonadherence, which is difficult to detect until it presents as rejection, often resistant to therapy (17–21). Another possibility is under-immunosuppression to avoid calcineurin inhibitor-induced failure, assuming that the non-specific hyalinosis and fibrosis in late biopsies reflect progressive calcineurin inhibitor toxicity (3). Thus some

We prospectively studied kidney transplants that progressed to failure after a biopsy for clinical indications, aiming to assign a cause to every failure. We followed 315 allograft recipients who underwent indication biopsies at 6 days to 32 years posttransplant. Sixty kidneys progressed to failure in the follow-up period (median 31.4 months). Failure was rare after T-cell-mediated rejection and acute kidney injury and common after antibody-mediated rejection or glomerulonephritis. We developed rules for using biopsy diagnoses, HLA antibody and clinical data to explain each failure. Excluding four with missing information, 56 failures were attributed to four causes: rejection 36 (64%), glomerulonephritis 10 (18%), polyoma virus nephropathy 4 (7%) and intercurrent events 6 (11%). Every rejection loss had evidence of antibody-mediated rejection by the time of failure. Among rejection losses, 17 of 36 (47%) had been independently identified as nonadherent by attending clinicians. Nonadherence was more frequent in patients who progressed to failure (32%) versus those who survived (3%). Pure T-cell-mediated rejection, acute kidney injury, drug toxicity and unexplained progressive fibrosis were not causes of loss. This prospective cohort indicates that many actual failures after indication biopsies manifest phenotypic features of antibody-mediated or mixed rejection and also underscores the major role of nonadherence.

Key words: Allograft failure, antibody-mediated rejection, kidney transplantation, nonadherence

clinicians “minimize” immunosuppression, exactly the wrong action if a major risk is *de novo* HLA antibody formation and ABMR (16).

Understanding the phenotype of every individual failure is an essential step in transplant progress (7). We previously postulated that progression to failure of a kidney transplant can be assigned to specific disease entities on the basis of such data as biopsy diagnosis and HLA antibody (7). Attribution of causality is not the same as biopsy diagnosis, which predicts future risk to some extent, but actually leaves many failures unexplained. Attribution involves assembling all evidence—biopsy, antibody and clinical—and deciding the best explanation for the clinical course. El-Zoghby et al. (11), in a retrospective review of failures at their center, although with incomplete HLA antibody data, found that glomerular pathologies cause the largest proportion of graft loss, with alloimmune mechanisms playing a major role. In a prospective study of patients followed after indication biopsies, we showed that kidneys diagnosed with ABMR or glomerulonephritis have a high probability of progression to failure (12), but that many cases of ABMR are currently misclassified as calcineurin inhibitor toxicity because they are C4d-negative. The high risk associated with ABMR was confirmed in a second prospective study (15). However, many individual cases are not explained in these studies because failure is related to conditions that begin the future (e.g. nonadherence) but not operating at the time of biopsy. Moreover, the biopsy diagnosis may have a complex relationship to the subsequent failure, which is not obvious. For example, treated T-cell-mediated rejection (TCMR) without DSA *per se* does not predict poorer graft survival (12), but if a late biopsy shows TCMR with no DSA and the graft subsequently fails with DSA, the TCMR may have been an indicator of nonadherence that eventually triggered DSA and ABMR. Thus, attributing causality to failure requires not only the biopsy findings, but also other features.

The present prospective consented study in all patients enrolled at the time of an indication biopsy aimed to attribute a cause to each failure. This required the development of rules for attributing failures, based on biopsy diagnoses, anti-HLA, clinical information, and clinicians’ concerns about nonadherence.

Methods

Patients and sample collection

Written informed consent was obtained from all study patients. Patients were recruited from three centers: the University of Alberta (n = 180), the University of Minnesota (n = 99) and the University of Illinois (n = 35). The study was approved by the University of Alberta Health Research Ethics Board (Issue #5299), by the University of Illinois, Chicago (protocol #2006–0544) and by the University of Minnesota (protocol #0606M87646). Consenting patients undergoing a renal transplant biopsy for clinical indication (deterioration in function, proteinuria or stable but impaired function) as

standard of care between September, 2004, and October, 2008, were included. Patients who received multiorgan transplants were excluded.

Histopathology

Paraffin sections were prepared and graded according to the Banff criteria (22). C4d staining was performed on frozen sections using a monoclonal anti-C4d antibody (Quidel, San Diego, CA, USA) by indirect immunofluorescence. Diffuse linear C4d staining (i.e. in >50% of peritubular capillaries) was interpreted as positive. Biopsies were classified using a modified Banff classification (22) including C4d-negative ABMR and probable ABMR. C4d positive ABMR was defined according to the Banff criteria (22). C4d-negative ABMR was based on our previous description (12): DSA positive, nondiffuse C4d staining (negative or minimal focal or focal) and any of the following microcirculation lesions: peritubular capillaritis (ptc > 0), glomerulitis (g > 0), thromboses and transplant glomerulopathy (cg > 0). Probable ABMR was defined identically with the exception of being panel reactive antibody (PRA) positive but DSA negative (NDSA). Biopsies diagnosed as “no major abnormalities” were defined as having a ci-score (interstitial fibrosis) of <2 and no features of a disease process. Biopsies diagnosed as “atrophy–fibrosis” were defined as having a ci-score of >1 and no features of a disease process.

HLA antibody screening

Of 412 renal allograft biopsies, 372 had available serum at the time of biopsy for HLA antibody testing. HLA antibody testing method varied depending on the transplant center. Antibody specificities were either determined by Luminex single antigen beads for a small subset of samples (n = 15, 4%) or by FlowPRA single antigen I and II beads (One Lambda, Canoga Park, CA, USA) after a positive HLA antibody screening test using FlowPRA beads (n = 357, 86%) (16). Blood samples for HLA antibody testing collected after the biopsy and used for these analyses were acquired before the date of kidney failure. No antibodies to non-HLA antigens were tested.

Allograft failure and nonadherence

Graft failure was either due to patient death or kidney function loss requiring renal replacement therapy and/or retransplantation. Nonadherence was recorded retrospectively by medical chart review, based on records of patient admission or strong clinical suspicion by the attending clinicians, independent of the study. Based on that definition, nonadherence was recorded at the time of biopsy in 22 patients and sometime after the last biopsy in four patients. For 35 patients whose charts were not available for review, we assumed adherence.

Data analysis

Data analyses were performed using the statistical programming and graphics language “R”. Chi-square or Fisher’s exact tests were used for comparisons between groups for categorical data. We calculated conditional density plots using the “R” package “cplot”. Probabilities are derived by applying a smoothing filter to the “R” “density” function. For the diagnostic categories, densities were derived from our dataset of indication biopsies, using the time of biopsy posttransplant. The nonadherence curve was plotted using only one data point per patient (i.e. the time posttransplant when nonadherence was recorded), as otherwise patients with more than one biopsy would create duplicate nonadherence times in the analysis.

Results

Study population and diagnostic classification

We prospectively studied 315 unselected consenting kidney transplant recipients undergoing 412 biopsies for clinical indications, 6 days to 32 years (median 17 months) posttransplant, with a median follow-up postbiopsy of

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Histological diagnosis	Number (%) of biopsies for clinical indications with each diagnosis (The three major groups in each time period are indicated in bold)				Total
	0-6 weeks	6 weeks-6 months	6- 12 months	>12 months	
Antibody-mediated rejection	4 (5%)	1 (2%)	1 (2%)	67 (29%)	73 (18%)
Probable ABMR	1 (1%)	0	1 (2%)	12 (5%)	14 (4%)
Mixed rejection	0	1 (2%)	1 (2%)	23 (10%)	25 (6%)
T cell-mediated rejection	7 (9%)	15 (24%)	5 (12%)	9 (4%)	36 (9%)
Borderline	11 (14%)	9 (14%)	9 (22%)	10 (4%)	39 (9%)
Polyoma virus nephropathy	0	3 (5%)	7 (17%)	2 (1%)	12 (3%)
Glomerular diseases	1 (1%)	2 (3%)	3 (8%)	37 (16%)	43 (10%)
No major abnormalities	49 (62%)	24 (39%)	9 (22%)	37 (16%)	119 (29%)
Atrophy-fibrosis	0	3 (5%)	3 (8%)	25 (11%)	31 (8%)
Other	6 ^a (8%)	4 ^b (6%)	2 ^c (5%)	8 ^d (4%)	20 (5%)
Total	79 (100%)	62 (100%)	41 (100%)	230 (100%)	412 (100%)
Number of patients					Total
Non-adherent ^e	0	1	0	25	26
Adherent or Unknown ^f	59	39	30	161	289

^a Others (n=6): C4d deposition with no pathology (n=4); Thrombotic microangiopathy (n=1); Inadequate (n=1)
^b Others (n=4): C4d deposition with no pathology (n=1); Oxalosis (n=1); Thrombotic microangiopathy (n=2)
^c Others (n=2): Obstruction (n=1); Suspicious viral (n=1)
^d Others (n=8): Transplant glomerulopathy (n=2), Suspicious viral (n=2); Post-transplant lymphoproliferative disorder (n=1); Atrophy-fibrosis with acute tubular necrosis (n=1); Acute pyelonephritis (n=1); Tubulo-interstitial nephritis (n=1)
^e Non-adherence is defined by notes in the records around the time of the biopsy. Patients with multiple biopsies are assigned to the column reflecting their first recorded non-adherence.
^f Patients with multiple biopsies are assigned to the column reflecting their last biopsy.
ABMR, antibody-mediated rejection

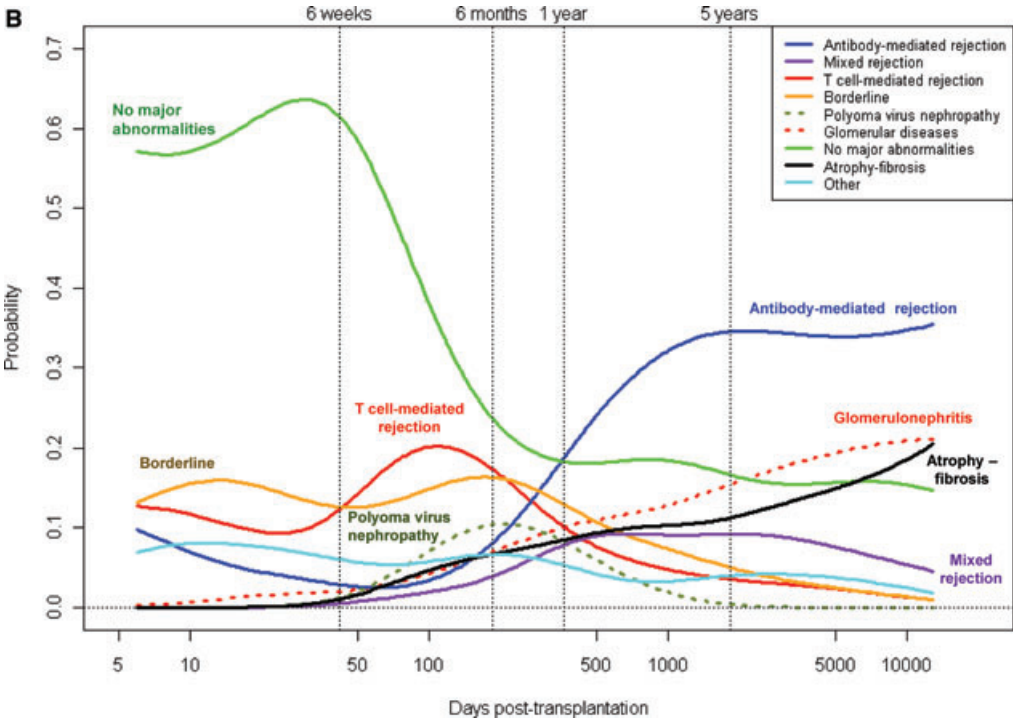


Figure 1: Distribution of histologic diagnoses and nonadherence according to time posttransplantation. (A) Histologic diagnoses and adherence status according to timing of the biopsy posttransplant. (B) Distribution of histopathology diagnoses and adherence status in biopsies expressed as probability plots conditional on the time of biopsy posttransplantation. The ABMR category includes C4d-positive ABMR, C4d-negative ABMR and probable ABMR.

31.4 months. Detailed demographics are published elsewhere (23). Biopsies were classified by the Banff criteria incorporating C4d-negative ABMR (12). The main diagnostic groups were ABMR ($n = 73$) ($n = 17$ C4d-positive and $n = 56$ C4d-negative, all but one of which had chronic active ABMR features), probable ABMR ($n = 14$), mixed rejection ($n = 25$), TCMR ($n = 36$), borderline ($n = 39$), polyoma virus nephropathy (PVN) ($n = 12$), glomerular diseases ($n = 43$), no major abnormalities ($n = 119$), atrophy–fibrosis ($n = 31$) and uncommon entities (“other”) ($n = 20$) (Figure 1A). During follow-up, 74 of 315 (24%) of allografts were lost: 60 due to kidney failure and 14 due to death with functioning kidney.

Time of biopsy for clinical indications posttransplant strongly influences the probable diagnoses

The most frequent diagnoses in 182 early biopsies (<1 year) were acute kidney injury or TCMR/borderline (Figure 1A). In the first 6 weeks posttransplant ($n = 79$), 62% showed no major abnormalities, indicating that the dysfunction that triggered these biopsies mainly reflected acute kidney injury. (Because the centers participating in this study seldom perform transplants with positive cross-matches, early ABMR was rare.) In the 6 weeks to 6 months interval ($n = 62$), most biopsies showed no major abnormalities (39%), TCMR (24%) or borderline (14%). The diagnoses between 6 and 12 months ($n = 41$) were similar, except for more PVN (17%).

The mix was different in the 230 late biopsies, including ABMR (29%), glomerular diseases (16%) or unexplained atrophy–fibrosis (11%). The probability of TCMR declined with time, being rare after a year. Clinical concerns about nonadherence were almost always recorded in relationship to late biopsies (25/26).

Conditional probability plots (Figure 1B) show how the probability of each diagnosis (e.g. TCMR, ABMR) changes with time posttransplant.

Thus the timing of the biopsy strongly influenced the probability of the biopsy diagnosis and the incidence of concerns about nonadherence by the attending clinicians.

The distribution of biopsy diagnoses among grafts that failed versus those that did not fail

Fourteen patients died with a functioning graft (19% of failures): six from malignancies, two from sepsis, two from cardiovascular disease and four in which no cause could be assigned. Two deaths occurred in the first year, seven between years 1 and 5 and five after 5 years.

Failure with return to dialysis or retransplantation occurred in 60 patients. In the last biopsies of kidneys that failed, the most frequent histologic diagnoses were ABMR/mixed (34 [56%]) and glomerulonephritis (12 [20%]). The ABMR/mixed group included 21 C4d-negative

ABMR, seven C4d-positive ABMR, and six mixed rejection. Although the patients presenting for early and late biopsies have been followed for similar times postbiopsy, almost all failures occurred in patients presenting for late biopsies.

Histologic diagnoses were different in the 60 kidneys that subsequently failed, compared to the 255 that had not failed: more ABMR or mixed rejection and fewer borderline or no major abnormalities (Table 1). Glomerular diseases were found both in kidneys that failed and those that did not, but the type differed: kidneys that progressed to failure had more aggressive diseases such as membranoproliferative glomerulonephritis (MPGN) or focal segmental glomerulosclerosis, whereas grafts that did not fail often displayed IgA nephropathy. Four grafts displayed diabetic nephropathy, but none has failed.

Concerns about nonadherence were recorded 10 times more frequently in patients whose graft subsequently failed (32%) than in those whose grafts have not failed (3%, $p = 0.0001$).

Developing rules for attribution of causes of progression to kidney failure

Progression to failure after an indication biopsy does not necessarily mean that the failure was related to the biopsy diagnosis: A patient biopsied for acute kidney injury may later develop unrelated complications, e.g. recurrent disease. We sought to develop rules for attributing causality to each failure, to achieve rigor, transparency and accuracy. While development of rules requires arbitrary choices, these rules are largely based on published associations of failures with time and biopsy diagnosis (Figure 2, Table S1). Table 2 shows the precedence rules for resolving ambiguous situations. A full account of the logic and literature basis of the choices here will be published separately (Selares et al., in preparation).

For example, Banff recognizes only two TCMR phenotypes: TCMR and chronic active TCMR. TCMR seldom progresses to failure in recent clinical trials and biopsy studies (23–25), but if it did, it should do so within a few months (11). If it fails many months or years later, there will often be a new condition operating. Chronic active TCMR may progress slowly but is uncommon, and in fact, was never diagnosed in this series.

The predetermined rules incorporated all available biopsy information for the patient, as well as the clinical parameters such as eGFR, hematuria, proteinuria, HLA antibody status and major medical or surgical events after transplantation.

Attributing causality to each failed kidney

Attribution was possible in 56 of 60 kidneys that progressed to failure (93%), assigning 36 to rejection (64%),

Table 1: Histological diagnosis and HLA antibody status of the transplants that failed during follow-up period versus those that did not fail, in the last biopsy available per patient

	Current status of the graft				p-Values
	Grafts that did not fail in the study period		Failed grafts		
			n	%	
Time of biopsy posttransplant	15.4 ^a (0.2–427) ^b		50 ^a (0.8–381.7) ^b		
Duration of follow-up after biopsy	31.4 ^a (0–60.7) ^b		24.6 ^a (0.3–36.9) ^b		
Histological diagnosis	n	%	n	%	
Antibody-mediated rejection	37	14%	28	47%	4.66E-07
Probable ABMR	9	4%	2	3%	0.26
Mixed rejection	7	3%	6	10%	0.002
T-cell-mediated rejection	17	7%	1	2%	0.13
Borderline	27	10%	1	2%	0.03
Polyoma virus nephropathy	5	2%	1	2%	0.88
Glomerular diseases	26 ^c	10%	12 ^d	20%	0.15
No major abnormalities	92	36%	3	5%	2.04E-06
Atrophy-fibrosis	23	9%	3	5%	0.25
Other	12	5%	3	5%	0.92
Total	255	100%	60	100%	
Patients with donor-specific antibody at time or after the biopsy	66	26%	38	63%	2.85E-08
Patients with recorded non-adherence	7	3%	19	32%	0.0001

^aMedian and ^brange shown in months.

^cIg A nephropathy n = 8; Diabetic nephropathy n = 4; Membrano-proliferative glomerulonephritis (GN) n = 4; Focal and segmental glomerulosclerosis n = 2; Membranous nephropathy n = 1; Immuno-complex GN n = 3; Focal proliferative GN n = 2; Mesangial GN of unknown etiology n = 1; Chronic advanced GN with double contours n = 1.

^dMembrano-proliferative GN n = 3; Focal and segmental glomerulosclerosis n = 4; Fibrillary GN n = 1; Immune complex GN n = 1; Crescentic GN n = 1; Focal proliferative GN n = 1; Severe parenchymal atrophy and fibrosis with one crescent in one glomerulus n = 1. ABMR = antibody-mediated rejection.

10 to glomerulonephritis (18%); four to suspected or proven PVN (7%) and six to intercurrent medical/surgical events (11%) (Figure 3B). Each failure was given a number (KF1–KF60) according to the date of failure (Table S2). Missing clinical information precluded attribution of four failures. No losses were attributed to unexplained fibrosis or calcineurin inhibitor toxicity, in keeping with previous studies (11,12,15).

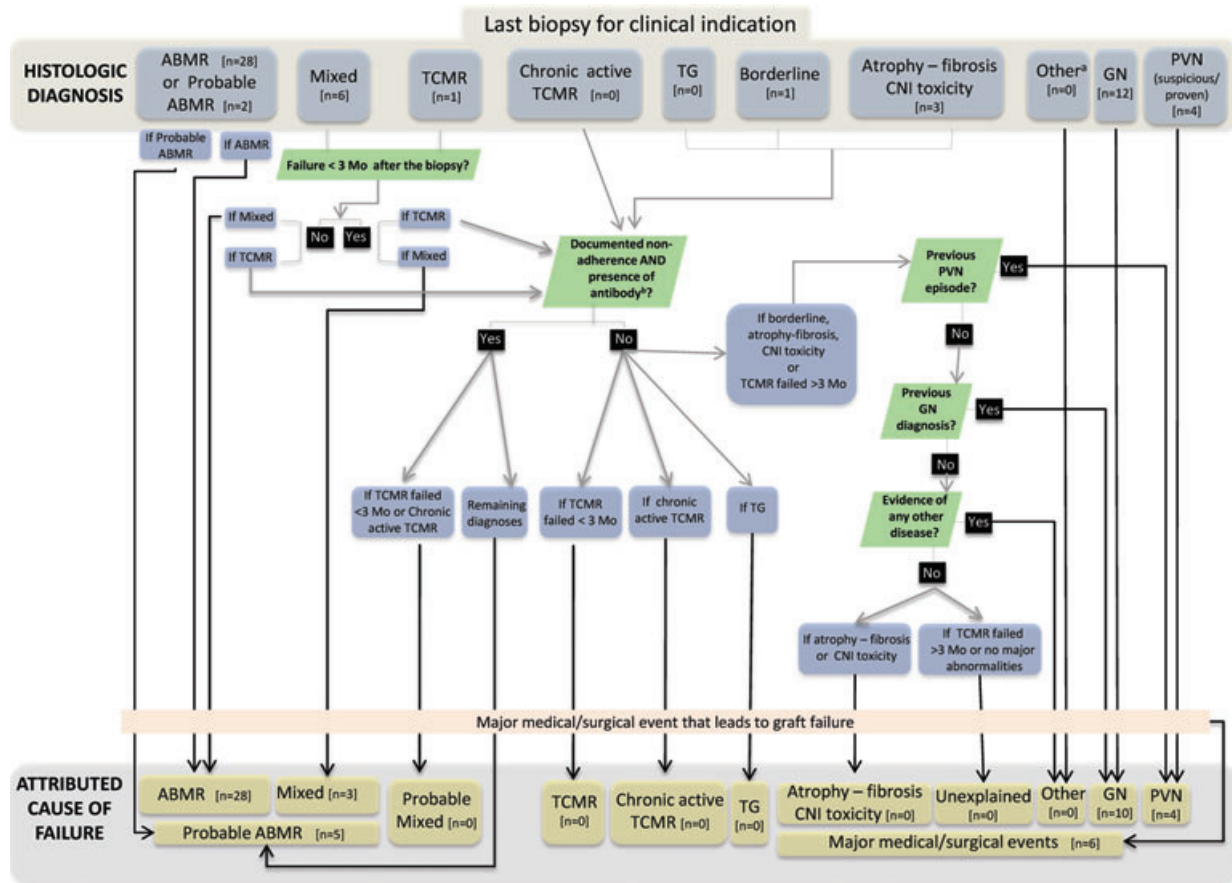
All 36 rejection-related losses involved ABMR: 28 ABMR (all with features of chronic active ABMR, of which seven were C4d-positive), five probable ABMR and three mixed rejection. None was attributable to TCMR alone or chronic active TCMR. Three grafts that failed after a first biopsy diagnosis of TCMR were subsequently lost after a later biopsy that showed mixed rejection (KF30, KF45), ABMR (KF47); two of them were classified as nonadherent (KF45, KF47). Only one graft was lost after a last biopsy diagnosis of TCMR (KF33): The failure occurred 2 years after the biopsy, with *de novo* DSA, proteinuria and admitted non-adherence.

The 10 losses from glomerulonephritis included two MPGN (KF4, KF14), three focal segmental glomeruloscle-

rosis (KF29, KF27, KF22), one fibrillary GN (KF19), one immune-complex GN (KF23) and one crescentic GN of unknown etiology (KF25). Two cases with a history of glomerulonephritis failed with advanced scarring and glomerulosclerosis with small crescents, suggesting recurrent GN (KF1, KF6).

One kidney (KF60) progressed to failure following a biopsy with ambiguous findings of glomerular double contours, compatible either *de novo* MPGN or ABMR. The biopsy showed deposits suggesting MPGN, but the patient was nonadherent, had DSA and had no history of glomerulonephritis. We attributed this failure to probable ABMR, with the deposits presumably being incidental.

Six kidneys failed in the context of major medical or surgical events: sepsis (n = 3), congestive heart failure (n = 2) and posttransplant lymphoproliferative disorder causing obstruction (n = 1). Five of the six kidneys failing due to intercurrent diseases had serious underlying graft disease (ABMR, n = 2; mixed rejection, n = 1; or GN, n = 2), suggesting that the risk of failure during intercurrent illness is increased if there is an underlying progressive kidney disease.



^a Other diagnoses such as no major abnormalities or recurrent/*de novo* systemic diseases

^b Presence of circulating HLA antibody (either, donor specific antibodies (DSA) or PRA positive with no identified DSA)

ABMR, antibody-mediated rejection; TCMR, T cell-mediated-rejection; TG, transplant glomerulopathy; GN, glomerulonephritis; PVN, polyoma virus nephropathy; CNI, calcineurin-inhibitor

Figure 2: Flowchart depicting the attribution system. The gray boxes on the top refer to the histological diagnosis in the last biopsy for clinical indications available before failure. The yellow boxes on the bottom of the chart refer to the attributed causes of allograft failure.

Nonadherence

Concerns about nonadherence were recorded by the attending clinicians in 26 patients, of which 19 experienced kidney failure, 17 of which were due to rejection (47% of rejection-related failures) (Figure 2B). Seven patients recorded as nonadherent at biopsy had functioning kidneys at the censoring date; whether this reflects these patients becoming adherent is not known. The biopsy findings and antibody status in these seven patients were as follows. Two had a diagnosis of C4d-positive ABMR with transplant glomerulopathy (both DSA class I/II), one was suspicious for ABMR (with microcirculation inflammation and C4d-positive, but PRA negative) and the four remaining displayed mild interstitial fibrosis and tubular atrophy (one DSA class II, two PRA negative and one unknown)

In 14 of 19 nonadherent patients whose grafts failed, the last biopsy showed ABMR (n = 12), mixed rejection (n = 2) or TCMR (n = 1). Three showed other findings: one borderline, one glomerulonephritis (*de novo* MPGN, KF60), one atrophy–fibrosis with features suggesting GN (KF6) and one no major abnormalities. In most (14/19) cases the nonadherence was overt and unequivocal and was admitted at the time of failure.

Either at biopsy or by the time of failure, all patients whose kidneys failed due to rejection and who were nonadherent had either DSA (16) or HLA antibody without identified DSA (NDSA) (2) (Table 3). Nonadherent patients had DSA more often than did other patients (Chi-square, $p < 0.001$) (Table S3).

Table 2: Precedence rules for resolving ambiguities in the attribution system

Precedence rules
1. Major medical/surgical events that lead to graft loss take precedence over the remaining attributed causes of failure
2. Ambiguous failures are defined as those with moderate or severe atrophy-fibrosis and sclerotic glomeruli, and which have evidence for two attributions e.g. a nonrejection disease (e.g. recurrent GN, diabetic nephropathy, PVN) and ABMR (e.g. nonadherence, <i>de novo</i> DSA or NDSA). In ambiguous failures, only one cause will be attributed, based on the clinical judgment of the attributing physicians (JS and PFH).

ABMR = antibody-mediated rejection; DSA = donor specific antibodies; NDSA = PRA positive with no identified DSA; GN = glomerulonephritis; PVN = polyoma virus nephropathy.

Detailed analysis of difficult attributions

Attribution was difficult for some failures because of incomplete understanding (Table 4). Probable ABMR was attributed as a cause of failure in five patients, when the failure followed a biopsy with a diagnosis of probable ABMR (transplant glomerulopathy with NDSA, $n = 2$; KF8, KF58), or when the diagnosis plus the clinical features showed a compatible phenotype (KF33, KF5, KF60) and the patient had either subsequent DSA or HLA antibody. Of the five patients whose graft failure was attributed to probable ABMR, four were identified as nonadherent.

Attribution in cases with transplant glomerulopathy

Table 5 shows the distribution of the 26 biopsies that failed with transplant glomerulopathy and the attributed causes of failure. Most were attributed to ABMR ($n = 19$), probable ABMR ($n = 2$) or mixed rejection ($n = 3$), but two failed in the context of intercurrent illness ($n = 2$).

Discussion

In this prospective unselected cohort of patients presenting with indications for a kidney transplant biopsy, we attempted to understand the cause of every kidney that progressed to failure. The failures occurred in an unselected sample of all kidney transplants with dysfunction because these centers biopsy all kidney transplants that are deteriorating, early or late. We developed rules for attributing failures to specific conditions based on histological diagnoses, DSA status and clinical information. Retrospective medical chart review identified those whose clinicians had recorded concerns about nonadherence. Sixty kidneys progressed to failure and 14 patients died with functioning grafts. The majority of kidney failures were attributed to ABMR, probable ABMR or mixed rejection, with nonadherence recorded in nearly half. There was evidence of ABMR in 18 of 19 nonadherent patients who failed. There were also three groups of nonrejection causes of failures: glomerulonephritis, PVN and failure in the context of an intercurrent illness. The results emphasize the

burden of ABMR and mixed rejection and its interaction with nonadherence in observed failures, making these key targets for further progress. The results also illustrate the range of clinical courses leading to failure and the sometimes complex relationships to the indication biopsy findings.

Late rejection in an indication biopsy must always evoke interventions to review and promote adherence. (Inappropriate minimization of immunosuppression by the attending clinicians almost certainly plays a role, but we lack the data to address this in this study.) Defined from the notes of the attending physician, nonadherence presented frequently as late TCMR, ABMR or mixed rejection and often progressed to failure. The phenotype at the time of failure is probably either pure ABMR or mixed because by the time of failure, every nonadherent patient failing with rejection had DSA. The antibody component probably explains why “late acute rejection episodes” (which in the older literature were not distinguished as TCMR vs. ABMR) have such a poor prognosis and response to therapy (26–28). Different strategies to detect adherence would produce different estimates of the prevalence of the nonadherence, which is difficult to define and varies from subtle to intermittent to flagrant. Given the seriousness of the consequences of nonadherence, transplant outpatient follow-up systems should change to address this concern, based on predicted risks and demonstrated risks such as missed clinic visits or unexplained late rejection episodes. In our opinion, certain risk groups should be prospectively identified for extra surveillance, such as teenagers and young adults, certain social and ethnic risk groups and individuals known to be facing financial crises that make drugs unaffordable. The fact that they are in a statistically derived risk group for nonadherence should be disclosed to the patient as objectively as the health providers would discuss a medical risk. Surveillance may include interviews, visits from social workers, records of prescriptions filled at pharmacies and, possibly, additional antibody screening, e.g. annual PRA testing in antibody-negative recipients. However, while we think that screening for DSA in patients in whom nonadherence is suspected will be useful, this and other practices, and the beliefs that underlie them, must be specifically proven in studies. Formal prospective studies of nonadherence should be designed, including social, behavioral and financial issues (29,30) and specifically considering how to engage those patients who are reluctant to reveal and discuss their nonadherence. It will be important to engage researchers in other areas of medicine who are attempting to understand nonadherence.

While attribution of causality to failures is mostly straightforward, certain problem areas require clinician judgment and further development of consensus. One challenge in attributing causality is that current biopsy phenotyping often fails because of diagnostic ambiguity or advanced atrophy-fibrosis. Moreover, the phenotypes produced by

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Histological diagnosis	n	Attributed causes of allograft failure							Non-adherence
		Antibody-mediated rejection	Probable ABMR	Mixed rejection	Polyoma virus nephropathy	Glomerulonephritis	Medical causes	Missing data	
Antibody-mediated rejection	28	26	—	—	—	—	2	—	11
Probable ABMR	2	—	2	—	—	—	—	—	1
Mixed rejection	6	2	—	3	—	—	1	—	2
T cell-mediated rejection	1	—	1	—	—	—	—	—	1
Borderline	1	—	1	—	—	—	—	—	1
Polyoma virus nephropathy	1	—	—	—	1	—	—	—	0
Glomerulonephritis	12	—	1	—	—	9	2	—	2
No major abnormalities	3	—	—	—	—	—	1	2	1
Atrophy-fibrosis	3	—	—	—	—	1	—	2	0
Other	3	—	—	—	3 ^a	—	—	—	0
Total	60	28	5	3	4	10	6	4	19

^a Patients whose biopsies showed histologic changes highly suggestive of polyoma virus nephropathy, although the IC/in situ hybridization was reported either inconclusive (n=1) or negative (n=2)

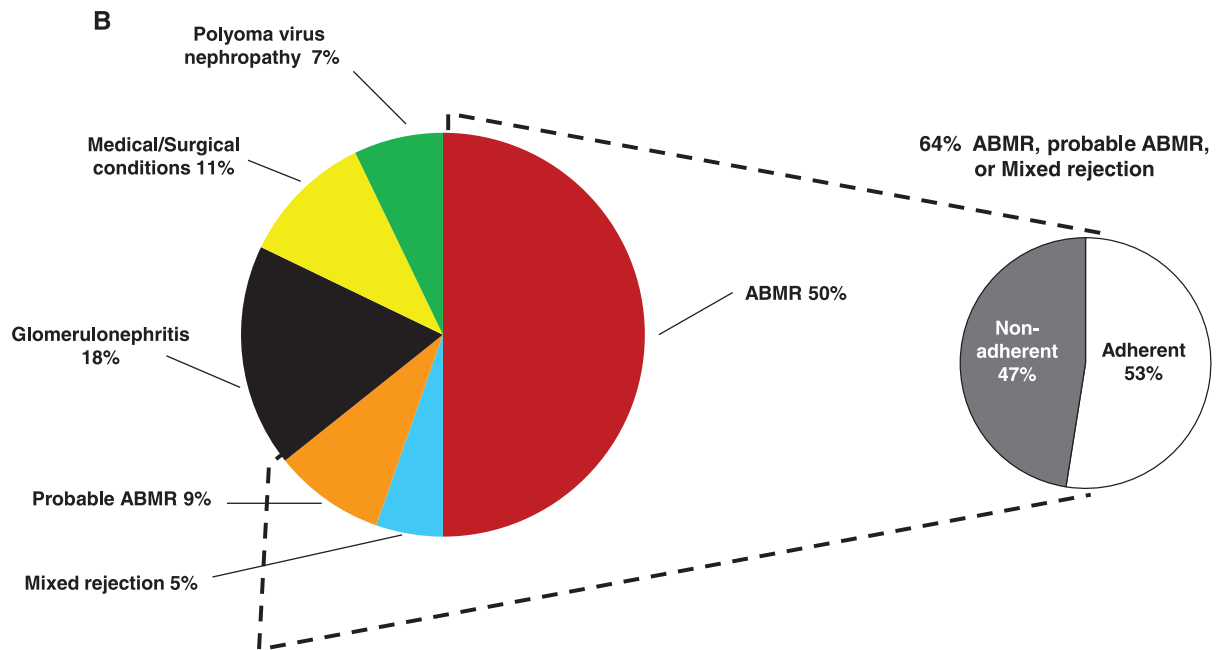


Figure 3: Attributed causes of graft failure in the biopsy-for-cause population. (A) Distribution of the attributed causes of failure (columns) according to the histological diagnosis in the last biopsy available per patient (rows). (B) Distribution of attributed causes of failure. Failures that could not be attributed due to missing clinical information are not represented (n = 4).

DSA and ABMR remains incompletely defined: The knowledge of donor antigens is often imperfect in long-standing transplants, particularly with HLA class II loci, which are now recognized as playing a major role in late ABMR, including HLA-DQB1, DQA1, DPB1 and possibly DPA1. Without donor typing for those loci, DSA assignment and ABMR diagnosis are difficult. Other potential donor targets include MICA (31,32) and HLAC. In terms of our kidney

failure set, these challenges involved three areas: severe scarred kidneys (KF5, KF1, KF6), ambiguous glomerular lesions (KF60) and failure to detect DSA despite diagnostic microcirculation lesions and PRA (KF8, KF58). The contentious cases mainly impact the estimates of the relative frequency of the main two entities—rejection and recurrent disease—but have little impact on the overall conclusions.

Table 3: Biopsy findings and HLA antibody status in non-adherent patients whose kidney failed

	Failure code	Histologic diagnosis (last biopsy)	Antibody status at time of biopsy	Antibody status at time of failure	Attribution of cause of failure
Non-adherence documented before the last biopsy	KF12	C4d+ ABMR	DSA I	DSA I	ABMR
	KF20	C4d+ ABMR	DSA I/I I	DSA I/I I	ABMR
	KF28	C4d+ ABMR	DSA II	DSA II	ABMR
	KF47	C4d+ ABMR	DSA II	DSA II	ABMR
	KF2	C4d+ ABMR	DSA II	DSA II	ABMR
	KF10	C4d+ ABMR	DSA I	DSA I	ABMR
	KF51	C4d- ABMR	DSA II	DSA II	ABMR
	KF53	C4d- ABMR	DSA I/I I	DSA I/I I	ABMR
	KF34	C4d- ABMR	DSA I	DSA I	ABMR
	KF37	C4d- ABMR	DSA II	DSA II	ABMR
	KF43	C4d- ABMR	DSA II	DSA II	ABMR
	KF58	Probable ABMR	NDSA	na	Probable ABMR
	KF45	Mixed rejection	DSA I/I I	DSA I/I I	Mixed rejection
	KF56	Mixed rejection	DSA I/I I	DSA I/I I	Mixed rejection
	KF5	Borderline	DSA II	DSA II	Probable ABMR
	KF60	GN (MPGN)	DSA I	DSA I	Probable ABMR
Non-adherence documented after the last biopsy	KF6	Atrophy-fibrosis / GN suspicious	PRA neg	PRA neg	GN
	KF33	TCMR	PRA neg	DSA I/I I	Probable ABMR
	KF50	No major abnormalities	NDSA	na	Missing data*

ABMR = antibody-mediated rejection; PRA = panel reactive antibody; DSA = donor specific antibodies; NDSA = PRA positive with no identified DSA; TG = transplant glomerulopathy; MPGN = membrano-proliferative glomerulonephritis; GN = glomerulonephritis; na = not available.

*Biopsy performed at day 25 posttransplantation with no rejection. Admitted nonadherence 1 year after transplantation. Graft was lost at 1.6 years posttransplantation and no further clinical information was available.

The time of presenting with indications for biopsy and the original renal disease of the recipient are crucial in understanding the differential diagnoses and the risks of progression. Major time-dependent processes include acute kidney injury due to implantation in the first 6 weeks, TCMR/borderline in the first months, PVN at 6–12 months, progressive diseases such as *de novo* anti-HLA-mediated ABMR and recurrent GN after 1 year, and nonadherence. Using these prior probabilities based on time and the risk of recurrent disease will be particularly useful to clinicians trying to resolve problematic or ambiguous cases.

A new understanding of the role of TCMR in the pathogenesis of kidney transplant failure is emerging from this and other recent studies. Untreated pure TCMR probably can rapidly cause irreversible kidney failure. Early TCMR responds well to treatment (14,23,25,25,33), but may leave irreversible nephron loss, perhaps depending on the severity and time before treatment. Pure late TCMR shares the same lesions as early TCMR and thus is probably as responsive to treatment if ABMR is absent, but because it may remain undiagnosed longer (due to infrequent surveillance), the irreversible damage may be greater. Some believe that pure TCMR triggers a new treatment-resistant disease, chronic active TCMR, which has a characteristic chronic arteriopathy. However, if that were the case, some of our late biopsies should have found this condition, and

we found none (22), in agreement with other recent studies (11). Although larger studies may find occasional cases of chronic active TCMR, we conclude that neither TCMR nor chronic active TCMR are major factors in progression to failure in the current kidney transplant population. However, irreversible injury from unrecognized and untreated TCMR may contribute to failure in nonadherent patients such as KF45, who presented as pure late TCMR with no DSA, initially accepted treatment and responded, but reemerged as mixed with flagrant nonadherence. Thus, TCMR plays a major role as an indicator of that the immunosuppressive prescription is failing, but may also participate in progressive graft damage in some nonadherent patients who eventually develop DSA but differ in the extent of TCMR activity in any one biopsy, from pure TCMR to mixed to pure ABMR. Moreover, the existing Banff criteria underestimate TCMR when atrophy-scarring is advanced because the lesions of TCMR can only be scored in unscarred areas. Thus, TCMR and mixed rejection are probably more frequent than the current Banff system estimates.

The idea that late transplant failures reflect mysterious dysregulated fibrosis is not supported by this data or other recent studies and should be replaced by the understanding that progressive atrophy-fibrosis usually reflects uncontrolled diseases, which may be obscured when the

Table 4: Histological diagnoses and clinical characteristics of failures that were difficult to attribute

Failure code	Primary disease	Histological diagnosis (last biopsy)	Histological description and comments	Antibody status at time of biopsy	Antibody status at time of failure	Documented nonadherence	Attribution of cause of failure	Alternative attribution of cause of failure
KF33	IgA nephropathy	TCMR	Moderate inflammation and tubulitis, moderate parenchymal atrophy and fibrosis. Admitted non-adherence. Follow-up lost and the patient presented 2 years later with end stage renal disease, proteinuria and DSA. No biopsy was performed at that time.	PRA neg	DSA I/I	Yes	Probable ABMR	—
KF5	IgA nephropathy	Borderline	Borderline changes, severe parenchymal atrophy and fibrosis. Mesangial deposits seen by EM. IF positive for IgA but no glomerular changes seen by light microscope. Admitted non-adherence. Follow-up lost during 6 months and the patient presented with kidney failure, DSA and a first biopsy with TCMR. Partial response to anti-rejection treatment. Proteinuria. Graft lost 8 months later.	DSA II	DSA II	Yes	Probable ABMR	Recurrent glomerulonephritis
KF60	Hypertensive/large vessel disease	Glomerulonephritis	Double contours, immune complexes with moderate parenchymal atrophy and fibrosis.	DSA I	DSA I	Yes	Probable ABMR	<i>De novo</i> glomerulonephritis
KF8	Diabetes mellitus type I	Probable ABMR	Transplant glomerulopathy with glomerulitis, peritubular capillaritis and moderate parenchymal atrophy and fibrosis.	NDSA	NDSA	No	Probable ABMR	—
KF58	Acquired obstructive uropathy	Probable ABMR	Transplant glomerulopathy with severe parenchymal atrophy and fibrosis.	NDSA	na	Yes	Probable ABMR	—
KF1	Mesangial proliferative IgM GN	Atrophy – Fibrosis	Extensive parenchymal atrophy and fibrosis with marked arteriolar hyalinosis. Previous biopsy showed high degree of fibrosis with features suggesting glomerulonephritis (epithelial crescent). Hematuria and proteinuria.	DSA I	DSA I	No	Glomerulonephritis	—
KF6	IgA nephropathy	Atrophy – Fibrosis GN suspicious	Severe parenchymal atrophy and fibrosis. Small crescent in one glomerulus partially sclerotic. Hematuria and proteinuria.	PRA neg	PRA neg	Yes	Glomerulonephritis	—

ABMR = antibody-mediated rejection; TCMR = T cell-mediated rejection; PRA = panel reactive antibodies; DSA = donor specific antibodies; NDSA = PRA positive with no identified DSA; EM = electron microscopy; IF = immunofluorescence; na = not available.

Table 5: Distribution of biopsies with transplant glomerulopathy according to the histological diagnosis and attributed causes of failure

Histological diagnosis	Number of biopsies	Number of failures	Attributed cause of failure
C4d positive ABMR	10	6	n = 6 ABMR
C4d negative ABMR	31	12	n = 11 ABMR; n = 1 Medical causes
Probable ABMR	7	2	n = 2 Probable ABMR
Mixed rejection	15	6	n = 3 Mixed rejection; n = 2 ABMR; n = 1 Medical causes
TG with no antibody at the time of biopsy	2	0	n = 0
Total	65	26	

ABMR = antibody-mediated rejection; TG = transplant glomerulopathy.

changes are advanced (23,25,33). Developing criteria for recognizing the probable diseases in these kidneys based on DSA, adherence and the original disease will help to resolve this problem. Atrophy–fibrosis is often stable in kidney transplants: e.g. some atrophy–fibrosis develops in many kidneys due to healing of the injuries sustained in donation–implantation, which are repaired by a wound healing like process, but this is not progressive (14).

Although calcineurin inhibitors are undoubtedly nephrotoxic and induce hyalinosis and fibrosis (3), this seldom progresses to end-stage disease in well-managed kidney transplants because effective options to limit progressive damage are available (11,23,34,35). No recent studies demonstrate that calcineurin inhibitor toxicity is a major cause of late kidney transplant failure (36), and the diagnostic criteria for this condition such as arteriolar hyalinosis and atrophy-scarring are so nonspecific that toxicity will be erroneously assigned as an explanation when other diagnoses are obscured by advanced atrophy–fibrosis, as discussed above (37). However, it remains possible that calcineurin inhibitors play a role as accelerators of progressive diseases such as ABMR or GN, and this should be addressed in new studies.

Because these failures were drawn from an unselected population of patients with clinical indications for biopsy in the participating centers, the mix of attributed causes of failure allows us to estimate the causes of failure across the entire kidney transplant population. On this basis, we propose that the four main groups of kidney failures in the population will emerge as rejection, recurrent disease, PVN, and failure in the context of major medical–surgical stress. Such estimates are not possible from national databases, which lack reliable phenotyping. A study based on indication biopsies has potential for some bias (e.g. subtle choice of which patients to biopsy) and thus could underestimate conditions like recurrent diabetic nephropathy, which progresses so slowly that it may not be biopsied. Nevertheless, the indication-biopsy-based studies are probably the best available for estimating the main drivers of failure across the renal transplant population because they capture the full richness of the phenotype data and include even the long-standing patients from earlier eras. The addition of molecular phenotype will further enhance their utility (14,38).

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Disclosure

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Supporting Information

The following additional supporting information may be found in the online version of this article:

Table S1: Rules for attribution

Table S2: Kidney failure number, last histologic diagnosis and attributed cause of failure

Table S3: Adherence status and donor specific antibodies at the time of biopsy

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