The Natural History of Chronic Allograft Nephropathy


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ABSTRACT

BACKGROUND
With improved immunosuppression and early allograft survival, chronic allograft nephropathy has become the dominant cause of kidney-transplant failure.

METHODS
We evaluated the natural history of chronic allograft nephropathy in a prospective study of 120 recipients with type 1 diabetes, all but 1 of whom had received kidney–pancreas transplants. We obtained 961 kidney-transplant–biopsy specimens taken regularly from the time of transplantation to 10 years thereafter.

RESULTS
Two distinctive phases of injury were evident as chronic allograft nephropathy evolved. An initial phase of early tubulointerstitial damage from ischemic injury (P<0.05), prior severe rejection (P<0.01), and subclinical rejection (P<0.01) predicted mild disease by one year, which was present in 94.2 percent of patients. Early subclinical rejection was common (affecting 45.7 percent of biopsy specimens at three months), and the risk was increased by the occurrence of a prior episode of severe rejection and reduced by tacrolimus and mycophenolate therapy (both P<0.05) and gradually abated after one year. Both subclinical rejection and chronic rejection were associated with increased tubulointerstitial damage (P<0.01). Beyond one year, a later phase of chronic allograft nephropathy was characterized by microvascular and glomerular injury. Chronic rejection (defined as persistent subclinical rejection for two years or longer) was uncommon (5.8 percent). Progressive high-grade arteriolar hyalinosis with luminal narrowing, increasing glomerulosclerosis, and additional tubulointerstitial damage was accompanied by the use of calcineurin inhibitors. Nephrotoxicity, implicated in late ongoing injury, was almost universal at 10 years, even in grafts with excellent early histologic findings. By 10 years, severe chronic allograft nephropathy was present in 58.4 percent of patients, with sclerosis in 37.3 percent of glomeruli. Tubulointerstitial and glomerular damage, once established, was irreversible, resulting in declining renal function and graft failure.

CONCLUSIONS
Chronic allograft nephropathy represents cumulative and incremental damage to nephrons from time-dependent immunologic and nonimmunologic causes.
CHRONIC ALLOGRAFT NEPHROPATHY, characterized by progressive renal dysfunction accompanied by chronic interstitial fibrosis, tubular atrophy, vascular occlusive changes, and glomerulosclerosis, is the chief cause of kidney-transplant failure despite improvements in immunosuppression. Although registry data can be used to define some risk factors, the pathophysiology of chronic allograft nephropathy remains poorly understood. A biopsy of a chronically failing kidney transplant usually shows nonspecific or end-stage changes, so the relative contributions of preexisting disease in the allograft and immunologic and nonimmunologic factors become difficult to distinguish. A major impediment has been the lack of prospective, longitudinal histologic data from studies of humans with chronic graft dysfunction.

We determined the natural history of chronic allograft nephropathy in 120 patients by regularly performing kidney-transplant biopsies for up to 10 years after transplantation. Sequential biopsy specimens were used to pinpoint the onset and evolution of histologic features of chronic allograft nephropathy and their relation to potential risk factors.

METHODS

STUDY POPULATION AND DESIGN

The study group consisted of 119 consecutive patients with type 1 diabetes mellitus who received a kidney–pancreas transplant and 1 such patient who received a kidney transplant alone at Westmead Hospital between 1987 and 2000; three or more sequential kidney-transplant–biopsy specimens were available from all these patients. Written informed consent was obtained from all patients, and the study was approved by the institutional review board and ethics committee. Initially, kidney biopsies were performed according to the protocol at the time of transplantation, at 1 and 2 weeks; at 1, 3, 6, and 12 months; and then yearly for 10 years. Beginning in 1997 this schedule was simplified: biopsy was performed at 0, 1, and 3 months and then 1, 3, 5, 7, and 10 years after transplantation.

Patients were treated with triple-therapy immunosuppression that incorporated cyclosporine (Novartis) in the case of 93 patients or, beginning in 1999, tacrolimus (Fujisawa) in the case of 27. Prednisolone (30 mg per day beginning on the day of transplantation) was also given with either azathioprine (GlaxoSmithKline; 1.5 mg per kilogram of body weight per day) in the case of 74 patients or mycophenolate mofetil (Roche; 3 g per day initially) in the case of 46. Induction antilymphocyte therapy was used in the first 13 patients and in 2 in whom the allograft was initially nonfunctional.

This study formed part of a prospective evaluation of diabetic complications after kidney–pancreas transplantation. However, sustained euglycemia rendered the original raison d'être redundant, and recurrent diabetic nephropathy was not seen. Instead, we observed the evolution of chronic allograft nephropathy over the decade after transplantation, noting that it occurred in kidneys that were essentially normal at implantation.

PER-PROTOCOL BIOPSY STUDY AND DEFINITIONS

Needle-core biopsies were obtained according to the protocol with use of a Biopry gun (Bard) with an 18-gauge needle. Renal histopathological analysis was performed in a blinded fashion by two observers using the Banff working classification, as described previously. This semiquantitative scoring system is used for the classification and grading of short- and long-term changes that occur in the glomeruli, tubules, interstitium, arteries, and arterioles of a renal transplant. Scores range from 0 to 3, with higher scores indicating more severe abnormalities. Chronic allograft nephropathy was defined as chronic interstitial fibrosis and tubular atrophy, with or without fibrointimal vascular thickening, and was graded according to the proportion of cortical area affected: grade 0, less than 6 percent of the cortical area affected; grade I, 6 to 25 percent affected; grade II, 26 to 50 percent affected; and grade III, more than 50 percent affected.

Subclinical rejection was defined by histologic findings consistent with the occurrence of acute rejection without immediate functional deterioration and was classified as acute when histologic evidence of acute rejection was present (Banff type 1A or higher) or borderline (tubulitis score of 1 and mononuclear-cell–infiltration score of 1 or 2, without arteritis). True chronic rejection was arbitrarily defined as subclinical rejection that occurred beyond one year, and was present in 50 percent or more of biopsy specimens from a given patient or in the last two biopsy specimens obtained more than one year after transplantation or evidence of subclinical rejection for two or more years in sequential biopsy specimens. Calcineurin-inhibitor nephrotoxicity was defined by striped cortical fibrosis or new-onset arteriolar hyalinosis (not from renal ischemia or preexisting hyalinosis in the allograft) supported by...
tubular microcalcification (without preceding acute tubular necrosis). We excluded alternative explanations for arteriolar hyalinosis, including preexisting disease in the allograft (by comparing the specimens with those obtained at transplantation), hypertension (by means of survival analysis), and dyslipidemia or hyperglycemia (by comparing lipid levels measured after an overnight fast and oral glucose-tolerance data, respectively).

**STATISTICAL ANALYSIS**

Cox regression was used for actuarial analysis of survival, and logistic regression was used for dichotomous data, preceded by backward elimination. A generalized estimating equation was used for repeated measurements. Data are expressed as means ± SD unless otherwise stated. All P values were two-sided, and a value of less than 0.05 was considered to indicate statistical significance.

**RESULTS**

**STUDY GROUP AND CLINICAL OUTCOMES**

Recipients were a mean of 38.2 ± 7.0 years old at the time of transplantation, 58.3 percent were male, and all but one were white. The mean number of HLA mismatches was 4.4 ± 1.4, donors were a mean of 25.5 ± 9.8 years old, and hemodialysis was required after transplantation in three patients. The numbers of episodes of acute cellular and vascular rejection were 1.1 ± 1.1 and 0.14 ± 0.4 per patient, respectively, and only 28.6 percent of patients had no episodes of rejection. The 1-, 5-, and 10-year survival rates were 96.7, 94.0, and 84.4 percent, respectively. Cardiovascular events caused 64.3 percent of all deaths.

Diabetes mellitus was present for a mean of 24.9 ± 6.9 years before combined transplantation, and after transplantation the mean glycosylated hemoglobin level was 5.6 ± 1.3 percent and was accompanied by sustained euglycemia. The cyclosporine doses at 1, 5, and 10 years were 5.1 ± 1.5, 4.7 ± 1.5, and 4.1 ± 1.0 mg per kilogram per day, respectively. The 1-, 5- and 10-year rates of graft survival with a functioning allograft (data on patients who died were censored) were 99.2, 98.2, and 95.2 percent, respectively, for kidney allografts and 87.4, 86.5, and 86.5 percent, respectively, for pancreas transplants. Early thrombosis caused 84.6 percent of the losses of pancreas transplants. Chronic allograft nephropathy combined with cyclosporine nephrotoxicity caused three kidney-transplant failures over a period of 10 years. The median follow-up was 7.0 years (range, 0.21 to 15.7).

**RENAL-TRANSPLANT BIOPSIES**

Of 961 biopsy specimens, 2 were unusable because there was insufficient tissue; thus, 959 biopsy specimens were analyzed; 808 were obtained according to the protocol and 151 were clinically indicated and were performed at or near the times indicated in the protocol. The mean number of biopsy specimens obtained according to the protocol was 8.0 ± 4.4 per patient (range, 3 to 18), in addition to diagnostic biopsies. A total of 67 biopsy specimens were obtained at or near transplantation, 68 at 1 week, 78 at 2 weeks, 118 at 1 month, 138 at 3 months, 87 at 6 months, 101 at 1 year, 67 at 2 years, 69 at 3 years, 35 at 4 years, 52 at 5 years, 26 at 6 years, 18 at 7 years, 12 at 8 years, 9 at 9 years, and 16 at 10 years.

The median histologic follow-up (as measured from the date of the last biopsy) was 3.9 ± 3.3 years. Biopsy specimens that could be assessed histologically were scored by two observers (1 assessed 820 and the other 703), and a mean of 13.8 ± 8.9 glomeruli and 2.3 ± 1.2 arteries were present per biopsy. A total of 15.7 percent had fewer than seven glomeruli or no artery. In biopsy specimens obtained at implantation or up to one week after transplantation, 1.7 percent of glomeruli were sclerosed and there was no or minimal preexisting damage (Table 1).

The Banff grade of chronic allograft nephropathy inversely correlated with the glomerular filtration rate, measured with use of isotopic [Tc99m] diethyleneetriamine pentaacetic acid (r = −0.31, P < 0.001), but the use of renal-transplant function to determine the degree of histologic damage resulted in an underestimate. The mean glomerular filtration rate was 66.3 ± 15.8 ml per minute in the absence of chronic allograft nephropathy, 59.5 ± 17.9 ml per minute in the presence of grade I nephropathy, and 49.8 ± 21.8 ml per minute in the presence of chronic allograft nephropathy of grade II or higher. The histologic findings in the limited number of renal–allograft–biopsy specimens (59) from patients with pancreatic thrombosis during follow-up were similar to those in the other biopsy specimens.

**EARLY TUBULOINTERSTITIAL DAMAGE**

The natural history of chronic allograft nephropathy could be divided into two distinct phases. The first year was characterized by new-onset tubulointerstitial damage and rapidly increasing Banff scores for interstitial fibrosis and tubular atrophy (Fig. 1A).
Grade I chronic allograft nephropathy occurred a median of three months (interquartile range, one to six) after transplantation and was present in 94.2 percent of patients by one year (Fig. 1B) as a result of both immunologic and ischemic insults. Acute tubular necrosis was present in 22.7 percent of biopsy specimens at the time of transplantation and was subsequently associated with an increased prevalence of chronic allograft nephropathy (55.3 percent among those with acute tubular necrosis, as compared with 28.1 percent among those without acute tubular necrosis; P<0.001) and increased fibrointimal vascular thickening (Banff score, 0.28±0.46, as compared with 0.06±0.22; P<0.001) at one month.

Severe acute rejection, defined by the need for antilymphocyte therapy, increased the Banff scores for chronic allograft nephropathy at three months (0.91±0.47, as compared with 0.56±0.48 in the absence of such therapy; P=0.001). Acute cellular rejection without the need for antilymphocyte therapy had no direct effect on chronic allograft nephropathy. Cox regression indicated that prior severe rejection (hazard ratio, 2.03; 95 percent confidence interval, 1.36 to 3.04; P<0.001) and evidence of acute tubular necrosis on biopsy (hazard ratio, 1.80; 95 percent confidence interval, 1.04 to 2.99; P<0.05) predicted chronic allograft nephropathy, after adjustment for the upper quartile of donor age of 32.5 years (hazard ratio, 1.52; 95 percent confidence interval, 0.97 to 2.38; P=0.06). Chronic glomerulopathy scores, glomerulosclerosis, and fibrointimal vascular thickening were minimal in the first year after transplantation (Fig. 1 and 2 and Table 1). Chronic allograft nephropathy gradually increased in severity and persisted, once established (Fig. 1).

Clinically significant acute lymphocytic infiltration, tubulitis, and subclinical rejection occurred in 60.8 percent of patients one month after transplantation, with the prevalence falling to 45.7 percent at three months, 25.8 percent at one year, and an average of 17.7 percent thereafter (Fig. 3A). The three-month risk of subclinical rejection was increased by cyclosporine therapy, as compared with tacrolimus use (hazard ratio, 4.34; 95 percent confidence interval, 1.30 to 14.44; P<0.05), and a previous episode of severe rejection (hazard ratio, 2.46; 95 percent confidence interval, 1.09 to 5.59; P<0.05). Tacrolimus and mycophenolate mofetil, individually and in combination, reduced the prevalence of subclinical rejection (P<0.05 to P<0.001). Patients who had acute and borderline subclinical rejection had subsequent biopsy specimens showing higher grades of chronic allograft nephropathy (2.48±0.66, P<0.001, and 0.75±0.25, P=0.07, respectively), as compared with those without subclinical rejection (0.32±0.09).

Among 114 patients who had two biopsies between 1 and 12 months after transplantation, the
initial biopsy showing acute subclinical rejection, borderline subclinical rejection, or no evidence of subclinical rejection was followed by a second biopsy showing increased grades of chronic allograft nephropathy (in 44.4, 34.1, and 29.6 percent, respectively; P<0.05) or a final grade of II or III (in 13.5, 12.7, and 2.2 percent, respectively; P<0.001). By one year, moderate chronic allograft nephropathy was evident in 25.6 percent of biopsy specimens with evidence of any type of subclinical rejection (as compared with 7.5 percent of those without such evidence, P<0.05). A generalized estimating equation showed that predictors of the grade of chronic allograft nephropathy 1 to 12 months after transplantation were acute tubular necrosis (P<0.01) and a previous episode of acute subclinical rejection (P<0.01), with mycophenolate therapy having a protective effect (P<0.01). Hence, subclinical rejection exacerbated chronic allograft nephropathy.

**Late Arteriolar Hyalinosis and Glomerulosclerosis**

Beyond one year, the patterns of allograft injury changed, reflecting microvascular and glomerular damage (Fig. 1 and 2). Early arteriolar hyalinosis was common and often transient, resolving in 45.8 percent of serial biopsy specimens by one year after transplantation. This abnormality was followed...
by a pattern of persistent arteriolar hyalinosis accompanied by high-grade vascular narrowing, progressive ischemic glomerulosclerosis, additional tubulointerstitial damage, and late renal functional decline (Table 1 and Fig. 1 and 2). By 10 years after transplantation, 37.3 percent of glomeruli were sclerosed and partial glomerulosclerosis and periglomerular fibrosis were increasingly prevalent. Severe chronic allograft nephropathy was present in 58.4 percent of patients by 10 years. Biopsy specimens with chronic allograft nephropathy were primarily (93.2 percent) Banff type a (reflecting nonspecific tubulointerstitial damage, 93.2 percent), and a minority (5.4 percent) were Banff type b (reflecting specific vascular and glomerular alterations suggestive of the presence of chronic rejection) or had unclassifiable changes (1.4 percent). Acute inflammatory activity was usually minimal or absent (below the Banff cutoff value of 10 percent of cortical area) (Fig. 3A) after one year, and vascular fibrointimal thickening and chronic glomerulopathy scores indicated the presence of only relatively minor abnormalities (Table 1 and Fig. 1A). True chronic rejection was found in only seven patients (5.8 percent) and significantly increased chronic allograft nephropathy scores (P<0.05). The presence of hypertension or the degree of HLA mismatching did not appear to have any independent effect on the risk of chronic allograft nephropathy. Calcineurin-inhibitor nephrotoxicity became increasingly prevalent (Fig. 3B and Table 2), becoming virtually universal by 10 years after transplantation and progressing despite mild-to-moderate reductions in calcineurin doses. Calcineurin-inhibitor nephrotoxicity was the chief cause of late histologic injury and ongoing decline in renal function.

### Discussion

This longitudinal analysis provides new insights into the pathophysiology and natural history of chronic allograft nephropathy. This type of nephropathy appears to consist of two distinctive phases of injury occurring at different times after transplantation within different histologic compartments. Early tubulointerstitial damage correlates with immunologic factors, including severe acute rejection and persistent subclinical rejection with the addition of ischemia–reperfusion injury. Later damage is characterized by progressive arteriolar hyalinosis, ischemic glomerulosclerosis, and further interstitial fibrosis associated with long-term calcineurin-inhibitor nephrotoxicity. Chronic allograft nephropathy thus represents the histologic sequelae of a series of pathologic insults that result in incremental and cumulative damage to nephrons within the transplanted kidney.

Our data, derived from diabetic recipients of a kidney–pancreas transplant, demonstrate that chronic allograft nephropathy is a sequential multifactorial process caused by a series of time-dependent insults. These transplantations were characterized by a young donor age, minimal periods of renal ischemia and incidence of delayed graft function, high rates of acute rejection, and excellent treatment compliance among recipients, as compared with a broader population of kidney-transplant recipients, thus limiting our ability to extrapolate data to transplantations affected by issues such as recurrent glomerulonephritis, marginal donor kidneys, and noncompliance. However, the pristine condition of the kidneys transplanted allowed us to observe the evolution of chronic allograft nephropathy without the confounding influences of preexisting damage in the donor organ.

Immunologic factors including acute rejection have previously been associated with graft loss. Acute vascular rejection results in immediate and extensive histologic damage, initiation of chronic allograft nephropathy, and reduced graft survival. In contrast, acute cellular rejection caused minimal direct damage, unless it was severe (and usually corticosteroid resistant) or heralded persistent subclinical rejection. Subclinical rejection was common early after transplantation and was followed by chronic...

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**Figure 2. Prevalence of Glomerular Damage and Failure after Transplantation.**

The point-prevalence percentages of glomeruli with complete and partial sclerosis and periglomerular sclerosis progressively increased with time.

<table>
<thead>
<tr>
<th>Years after Transplantation</th>
<th>Biopsy Specimens (%)</th>
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<tbody>
<tr>
<td>0</td>
<td>Totaly sclerosed glomeruli</td>
</tr>
<tr>
<td>1</td>
<td>Periglomerular fibrosis</td>
</tr>
<tr>
<td>5</td>
<td>Partial glomerulosclerosis</td>
</tr>
<tr>
<td>10</td>
<td>Periglomerular fibrosis</td>
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</tbody>
</table>

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interstitial fibrosis, tubular atrophy, and nephron loss, contributing to chronic allograft nephropathy especially between 3 and 12 months after transplantation. Persistent graft inflammation led to evidence of chronic tubulointerstitial damage on subsequent biopsy specimens in this and other studies. 12-14 Because subclinical rejection and tubulitis are patchy processes, the uninvolved nephrons can maintain stable serum creatinine levels by means of compensatory hyperfiltration; consequently, the immunologic injury is clinically silent. When rejection remains undetected, as occurs with subclinical rejection or late acute rejection, there may be substantial damage, exacerbating chronic allograft nephropathy. 5,15 Hence, the effects of rejection-related injury causing chronic allograft nephropathy are dependent on the type, persistence, timing, and severity of episodes of rejection.

Progressive functional failure of kidney grafts has been assumed to result from a low-grade process of persistent subclinical or chronic rejection, a hypothesis never properly validated by studies in humans. Although early subclinical rejection was common in our study, it usually resolved with calcineurin-based maintenance immunosuppression. True chronic rejection, defined by sequential histologic abnormalities with implied continuous immunologic injury, occurred in only 5.8 percent of patients and thus appears uncommon. The absence of subacute cellular inflammation in most biopsy specimens obtained long after transplantation alters our conception of the pathophysiology of late chronic allograft nephropathy. Although immunologic factors result in a substantial burden of early tubulointerstitial injury, later damage appears to be predominantly associated with the histologic pattern of calcineurin-inhibitor nephrotoxicity.

Figure 3. Point Prevalence of Histologically Defined Subclinical Rejection in Sequential Biopsy Specimens (Panel A) and Calcineurin-Induced Nephrotoxic Effects (Panel B).

In Panel A, solid portions of bars represent episodes of clinical acute rejection that occurred at or near the time of biopsy. The prevalence of this complication contrasts with the prevalence of calcineurin-inhibitor nephrotoxicity, which was substantial and increased in concert with the time after transplantation (Panel B).
gest that calcineurin inhibitors are unsuitable as long-term immunosuppressive agents for kidney transplantation.16-21

The same immunosuppressive agents are often continued for the life span of the kidney transplant, with modification of the dose as the only adjustment. Our data suggest that a two-stage treatment may be preferable, optimizing therapy according to the individual risks during each period after transplantation. An initial stage of powerful therapy incorporating a calcineurin inhibitor might minimize early immunologic injury and its attendant nephron loss. Despite the concern that early calcineurin blockade during organ engraftment may limit the development of T-cell tolerance,24 our data showed maximal preservation of nephrons with the use of potent calcineurin inhibitors in the early period after transplantation. Once subclinical rejection is controlled and possibly verified by biopsy, a second stage of therapy incorporating long-term non-nephrotoxic immunosuppression might be undertaken. All but one of the renal-transplant recipients in our study were recipients of a kidney–pancreas transplant, which might limit the general applicability of our results. Formal testing of the optimal clinical approach to prevent chronic allograft nephropathy will require a controlled trial.

Dr. O’Connell reports having received consulting fees from Wyeth and lecture fees from Janssen–Cilag. Dr. Chapman reports having received consulting and lecture fees from Novartis, Roche, Fujisawa, and Wyeth; lecture fees from Janssen–Cilag; and a grant from Novartis.

CHRONIC ALLOGRAFT NEPHROPATHY

Table 2. Cumulative Kaplan–Meier Estimates of the Prevalence of Histologic Diagnoses, According to the Time after Transplantation.

<table>
<thead>
<tr>
<th>Histologic Diagnosis</th>
<th>1 Yr</th>
<th>5 Yr</th>
<th>10 Yr</th>
</tr>
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<tbody>
<tr>
<td>Chronic allograft nephropathy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Banff grade I</td>
<td>94.2</td>
<td>100.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Banff grade II or III</td>
<td>24.7</td>
<td>65.9</td>
<td>89.8</td>
</tr>
<tr>
<td>Calcineurin-inhibitor nephrotoxicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arteriolar hyalinosis</td>
<td>76.4</td>
<td>93.5</td>
<td>96.8</td>
</tr>
<tr>
<td>Stripped fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tubular microcalcification</td>
<td>62.0</td>
<td>90.3</td>
<td>100.0</td>
</tr>
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REFERENCES