Protocol biopsies in renal transplantation: Prognostic value of structural monitoring

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The natural history of renal allograft damage has been characterized in serial protocol biopsies. The prevalence of subclinical rejection (SCR) is maximal during the first months and it is associated with the progression of interstitial fibrosis/tubular atrophy (IF/TA) and a decreased graft survival. IF/TA rapidly progress during the first months and constitutes an independent predictor of graft survival. IF/TA associated with transplant vasculopathy, SCR, or transplant glomerulopathy implies a poorer prognosis than IF/TA without additional lesions. These observations suggest that protocol biopsies could be considered a surrogate of graft survival. Preliminary data suggest that the predictive value of protocol biopsies is not inferior to acute rejection or renal function. Additionally, protocol biopsies have been employed as a secondary efficacy variable in clinical trials. This strategy has been useful to demonstrate a decrease in the progression of IF/TA in some calcineurin-free regimens. Quantification of renal damage is associated with graft survival suggesting that quantitative parameters might improve the predictive value of protocol biopsies. Validation of protocol biopsies as a surrogate of graft survival is actively pursued, as the utility of classical surrogates of graft outcome such as acute rejection has become less useful because of its decreased prevalence with actual immunosuppression.

Kidney International (2007) **72,** 690–697; doi:10.1038/sj.ki.5002396; published online 27 June 2007

KEYWORDS: renal transplantation; epidemiology and outcomes; kidney biopsy; interstitial fibrosis/tubular atrophy

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Received 27 March 2007; revised 24 April 2007; accepted 8 May 2007; published online 27 June 2007

In the late 70s and early 80s, the interest on renal allograft histology was mainly focused on acute rejection. In many studies, the phenotype of infiltrating cells was characterized as an attempt to grade the severity of rejection or to distinguish rejection from acute cyclosporine nephrotoxicity. The first protocol biopsy studies were performed during this period of time, mainly to explore whether acute rejection episodes could be predicted by histological lesions appearing before renal function deterioration.[1] It is worth to remark that, at this time, the information on the histology of stable grafts was really scarce. In fact, the aim of one of these pioneering studies was just to explore whether graft histology was normal in stable grafts.[2] Many of these studies used monoclonal antibodies to characterize interstitial infiltrating cells, and it was observed that a proportion of stable patients displayed different degrees of acute tubulointerstitial inflammation.[3,4] These findings stimulated some centers to start the first systematic protocol biopsy programs in order to study whether early graft lesions were associated with prognosis. Despite the characterization of acute lesion-stimulated protocol biopsy studies, the interest rapidly shifted to the study of chronic lesions, as the first associations between renal damage and outcome were observed with tubulointerstitial chronic but not acute damage. The introduction of Banff criteria[5–7] to evaluate indication biopsies facilitated the use of a common classification system for renal allograft pathology that was also applied in protocol biopsy studies. The recent Banff meeting report has replaced the term chronic allograft nephropathy for interstitial fibrosis/tubular atrophy (IF/TA) in cases were no definitive underlying cause for graft damage can be identified.[8] In the present revision, the expression IF/TA will be used throughout the text.

TEMPORAL EVOLUTION OF ACUTE AND CHRONIC RENAL ALLOGRAFT DAMAGE

The presence of interstitial inflammation and tubulitis in protocol biopsies obtained in stable grafts is a frequent finding during the first months after transplantation. During the 90s, Rush *et al.*[9] introduced the term subclinical rejection (SCR) to refer to stable allografts displaying an interstitial infiltrate and tubulitis. In studies of serial protocol biopsies, it has been demonstrated that the prevalence of SCR is maximal during the initial 3 months, progressively decreases until the first year, and persists in a small number of patients

after the first year.[10] Approximately 2/3 of SCR episodes are classified as borderline changes and 1/3 as interstitial acute rejection grade I, according to Banff criteria.[10] The presence of vascular acute rejection grade II is exceptional in protocol biopsies.

The presence of IF/TA, referred as chronic allograft nephropathy in the 1997 Banff criteria, rapidly progresses during the first few months following an exponential curve and slower thereafter. The prevalence of IF/TA in patients treated with a calcineurin inhibitor-based regimen is approximately 40, 60, and 90% at 3 months, 2, and 5 years, respectively.[11–13] Glomerular and vascular chronic lesions progress at a slower rate than IF/TA.[13]

REPRODUCIBILITY OF HISTOLOGICAL DAMAGE IN PROTOCOL BIOPSIES

Studies evaluating the reproducibility of renal transplant biopsy scoring among centers showed that the interobserver variability using the Banff 1991 schema[5] was rather large,[14,15] especially in the evaluation and grading of acute rejection. Later on, a new classification system for acute rejection that was shown to be more reproducible[16] was incorporated in the 1997 Banff schema.[6] In protocol biopsies, the interobserver variability of the Banff schema has been evaluated in different studies showing that SCR is a reproducible diagnosis ($\kappa = 0.6$–0.8). However, these studies demonstrated that the reproducibility of SCR grade I was superior to borderline changes. Rating chronic changes also gave an acceptable interobserver agreement ($\kappa = 0.5$–0.6 for IF/TA).[17,18]

SCR AND THE PROGRESSION OF IF/TA

Risk factors associated with SCR are the number of human leukocyte antigen mismatches, degree of sensitization, retransplantation, the presence of a previous clinical acute rejection episode, and the immunosuppressive regimen.[10,19] In this regard, it has been reported that patients treated with tacrolimus and mycophenolate mofetil display the lowest prevalence of SCR.[10,20,21]

The presence of SCR in protocol biopsies has been consistently associated with the progression of IF/TA, supporting that persistent interstitial inflammation is harmful for the allograft.[13,19,22] Even very-low-grade inflammation has been associated with progression of chronic tubulointerstitial damage.[23] Thus, it has been proposed that IF/TA will progress in parallel to the intensity and duration of SCR and, consequently, a chronological sequence of events initiated by subclinical inflammation may lead to graft failure. This hypothesis is supported by the association between SCR at 2-week and 10-year graft survival in a large series of recipients of a living donor kidney.[24] However, in other studies with a shorter follow-up and a lower statistical power, this association has not been observed.[25,26] Recently, different studies have shown that the concomitant existence of SCR, either 'borderline changes' or acute rejection grade I, with IF/TA is associated with a lower graft survival than the presence of isolated chronic lesions,[22,27,28] further supporting the deleterious effect of SCR on graft outcome.

As SCR may have a deleterious effect on long-term graft outcome, different approaches for its prevention and treatment have been proposed. Treatment of SCR has been only evaluated in one prospective randomized trial. In the treatment group, patients were biopsied at 1, 2, 3, 6, and 12 months, and SCR was treated during the first 3 months with methylprednisolone boluses, whereas the control group was only biopsied at 6 and 12 months. Treatment of SCR was associated with a reduced progression of IF/TA at 6 months and a better graft function at 2 years.[29] It is important to remark that in this study patients were treated with cyclosporine, azathioprine, and steroids, and the prevalence of SCR was over 30% during the initial 3 months. As immunosuppressive schedules based on tacrolimus, mycophenolate mofetil, and steroids have reduced the prevalence of SCR to very low levels, new prospective randomized studies are needed to confirm whether this approach may still be useful. Thus, additional trials will be necessary to clarify whether treatment of SCR modifies renal graft outcome and to evaluate the risk–benefit balance of protocol biopsy programs conducted to treat SCR.[30]

THE SILENT PROGRESSION OF CHRONIC ALLOGRAFT LESIONS

In studies of serial protocol biopsies, chronic damage progress, whereas serum creatinine remains stable, suggesting that histological damage precedes renal functional deterioration.[13] In a study of paired 4- and 14-month protocol biopsies, chronic lesions significantly increased in all renal compartments, whereas mean serum creatinine remained unchanged.[31] Even when renal function was studied with more precise methods at 4 months in stable grafts classified according to the presence or absence of IF/TA, glomerular filtration rate, effective renal plasma flow, or renal functional reserve were not different between diagnostic categories.[32] In a study of serial protocol biopsies conducted in pediatric recipients, acute and chronic lesions were also not associated with renal function deterioration or proteinuria.[33]

The progression of IF/TA has been associated with a large number of risk factors including pre-existing donor damage, degree of sensitization, cold ischemia time, clinical and subclinical acute rejection, cyclosporine exposure, or renal calcifications.[11–13,34,35]

CHRONIC ALLOGRAFT DAMAGE AND ITS PREDICTIVE VALUE ON OUTCOME

In a study of 2-year protocol biopsies, Isoniemi *et al.*[11] observed that patients displaying chronic lesions had a higher probability for renal function deterioration and/or graft failure, pointing out, for the first time, that lesions evaluated by means of protocol biopsies were associated with outcome. This study inspired the definition of the so-called 'chronic allograft damage index' consisting on the sum of scores of those lesions that correlated with serum creatinine at the time of biopsy. Later on, Dimény *et al.*[36] described an association

between chronic histological lesions in protocol biopsies obtained at 6 months and graft survival. This finding introduced the notion that the information obtained from protocol biopsies was relevant to identify patients at risk for graft failure. Thereafter, in a 3-month protocol biopsy study using the Banff criteria, it was again described an association between IF/TA and graft survival. In this study, the predictive value on outcome of IF/TA was independent from clinical and analytical parameters such as serum creatinine, acute rejection, or proteinuria.[12] Furthermore, the chronic allograft damage index evaluated in 1-year protocol biopsies obtained from two pivotal mycophenolate mofetil trials was once more an independent predictor of 3-year graft survival.[37] Altogether, these findings suggest that protocol biopsies contain relevant information to predict graft outcome that is not present in clinical or analytical data.

RECOGNITION OF HISTOLOGICAL PATTERNS THAT IMPROVE THE PREDICTIVE VALUE OF HISTOLOGY ON OUTCOME

The presence of isolated IF/TA is only weakly associated with graft survival, whereas there are some specific lesions associated with IF/TA that improve its predictive value on outcome. In a 3-month protocol biopsy study in which biopsies were classified according to the presence or absence of arterial intimal thickening, graft survival was significantly reduced in patients with transplant vasculopathy.[38] In another 3-month protocol biopsy study, histology was classified according to Banff criteria as normal, IF/TA without transplant vasculopathy, and IF/TA with transplant vasculopathy. Ten-year graft survival was 95% in patients with normal histology, 82% in patients with IF/TA without transplant vasculopathy, and 41% in patients with IF/TA and transplant vasculopathy.[39] These observations support that the recognition of histological patterns characterized by certain groupings of lesions in different renal compartments allow a more precise identification of patients at risk for graft loss. This hypothesis was recently extended by the description of a deleterious effect of the concomitant association of SCR and IF/TA. In a pediatric population biopsied at 1 year, it was noticed that survival was very poor in patients with the simultaneous presence of SCR and IF/TA in comparison with patients displaying IF/TA without SCR.[27] This observation was confirmed in the adult population and extended by the observation that also patients with SCR without IF/TA have a much better outcome than patients with both diagnoses.[22,28] It is not clearly understood why the simultaneous presence of SCR and IF/TA implies such a poor prognosis. It could be argued that the inflammatory infiltrate might be more severe in patients with SCR and IF/TA than in patients with SCR without IF/TA. In this regard, we have observed that the number of infiltrating B cells is increased in patients with the simultaneous presence of SCR and IF/TA (unpublished observation). Alternatively, we may speculate that the repair capacity of an already damaged tissue in the presence of low-grade inflammation may be reduced.[40]

Finally, in a study of 1-year protocol biopsies, it has been observed that the simultaneous presence of IF/TA and incipient transplant glomerulopathy implies a shorter graft survival than the presence of IF/TA without transplant glomerulopathy.[28] This last observation raises the question whether the recently described chronic antibody-mediated rejection, which is characterized by specific histological lesions, C4d deposition in peritubular capillaries, and donor-specific antibodies,[7,8] might be early diagnosed by means of protocol biopsies. Nevertheless, information on the utility of protocol biopsies to early detect chronic antibody-mediated rejection is scarce. In a large multicentric study,[41] the prevalence of C4d-positive protocol biopsies was 4%, and there was no association between C4d deposition and graft survival. Conversely, in a protocol biopsy study comparing the progression of chronic allograft lesions in patients receiving a living donor kidney with a positive cross-match after plasmapheresis treatment, IF/TA progressed more rapidly in patients with a positive C4d staining.[42]

Altogether, these data suggest that appropriate grouping of histologic lesions allow to increase the predictive value of protocol biopsy on graft survival. These observations raise the question whether a more precise recognition of damage patterns, for example, the coexistence of three or four of these lesions in a biopsy may further increase the predictive value of histological lesions.

HISTOLOGICAL LESIONS IN PROTOCOL BIOPSIES AS A SURROGATE OF GRAFT LOSS

The gold standard outcome variable in renal transplantation is patient and/or graft survival. The first clinical trials performed in renal transplant patients relied on graft survival to demonstrate differences between treatment groups.[43,44] Once graft survival at 1 year was over 80%, minimum sample size to detect a difference between groups was too large to consider the gold standard outcome variable as a feasible approach to differentiate between treatments. For this reason, acute rejection was later employed as a surrogate of survival in clinical trials until its prevalence felt below 20%.[45] Nowadays, many trials are designed to test the non-inferiority hypothesis between treatments for acute rejection.[46] This situation has stimulated the search for new surrogates of graft survival.[47,48]

Chronic tubulointerstitial damage, chronic vascular damage, and, more recently, the presence of SCR evaluated in protocol biopsies are independent predictors of graft survival. These observations, confirmed at different centers, have raised the question whether protocol biopsies could be considered as a surrogate of graft survival, that is, a measure that may be used instead of graft survival.[49] It is important to remark that the description of an association between histological lesions in protocol biopsies and graft survival is not a sufficient condition to accept histological lesions as a surrogate of survival. There are many examples in the literature showing that the modification by treatment of a predictor of survival in epidemiological studies has no effect

on survival in clinical trials. For example, although increased levels of plasma homocysteine are associated with an increased cardiovascular risk, supplementation with folic acid and B vitamins had no effect on major cardiac events despite a significant reduction of homocysteine levels.[50]

The validation of histological damage in protocol biopsies as a surrogate of graft survival implies the demonstration that changes in the prevalence of histological lesions due to treatment modification are associated with changes in renal allograft survival. This sequence of events has been only described in one clinical trial evaluating early cyclosporine withdrawal from a sirolimus- and prednisone-based regimen.[51] The severity of chronic allograft lesions evaluated according to the chronic allograft damage index at 3 years was reduced and 4-year graft survival was significantly improved in the cyclosporine withdrawal group.[52] This observation suggests that reducing the severity of chronic lesions in protocol biopsies by treatment partly explains improved allograft survival. However in this study, only low-risk patients were included and the control group received cyclosporine and sirolimus, a combination that is especially nephrotoxic. Thus, this observation suggesting that chronic damage in protocol biopsies constitutes a surrogate of graft survival should be confirmed in other clinical trials evaluating different risk populations. In the other hand, co-stimulation blockade with belatacept is also associated with reduced severity of chronic lesions at 1 year in comparison to a cyclosporine-based regimen. Longer follow-up of this trial may add valuable information to further characterize the potential value of protocol biopsies as a surrogate of graft survival.[46]

ACCURACY OF PROTOCOL BIOPSIES TO PREDICT GRAFT SURVIVAL

Despite additional information is necessary to accept that histological damage evaluated in protocol biopsies constitutes a surrogate of graft survival, it should be taken into consideration that the interest of any surrogate depends on its accuracy to predict the main outcome variable. Serum creatinine has been considered a reasonable candidate to be accepted as a surrogate of graft survival. This variable is an independent predictor of outcome, is easy to measure and can be obtained early after transplantation. Moreover, it has been also shown that cyclosporine withdrawal from a sirolimus- and prednisone-based immunosuppressive schedule is associated with a lower 1-year serum creatinine and a superior 4-year graft survival than cyclosporine maintenance.[51,52] Nevertheless, Kaplan *et al.*[53] evaluated the predictive value of serum creatinine on graft survival at 2 and 7 years and showed that it is rather low. The same was true for estimated creatinine clearance or for the deterioration of renal function between the 6th and 12th month. As an example, the authors transformed 1-year serum creatinine into a binary variable using 1.8 mg/dl as the cutoff to estimate its sensitivity and specificity to predict 7-year graft failure. The sensitivity, that is, the proportion of patients with a serum creatinine higher than 1.8 mg/dl who lost their graft at 7 years, was 48%. The specificity, that is, the proportion of patients with a 1-year serum creatinine below 1.8 mg/dl who retained their graft at 7 years, was 71%. Furthermore, they represented this information by means of receiver operator curves just to confirm that the predictive value of renal function on outcome was rather low.[53]

The predictive value of the different histological patterns evaluated by means of protocol biopsies on graft survival has not been evaluated using the same approach as Kaplan *et al.*[53] In order to obtain an approximation of the predictive value of protocol biopsies on graft survival, we revisited a previous publication including 435 protocol biopsies performed during the first 6 months.[22] There were 361 patients followed for at least 7 years and 57 of them lost their graft before the 7th year. The sensitivity and specificity to predict graft loss at 7 years was calculated for different histological patterns: IF/TA, IF/TA associated with transplant vasculopathy, or IF/TA associated with SCR. In order to compare the predictive value of histological lesions on 7-year graft survival with classical clinical predictors of outcome, we also estimated accuracy, sensitivity, and specificity for acute rejection and serum creatinine over 1.8 mg/dl at the time of biopsy. As shown in Table 1, the accuracy, sensitivity, and specificity of histology was not inferior to acute rejection or serum creatinine. The interpretation of these results is complex, as the transformation of histological patterns into a dichotomous variable is an oversimplification. Some lesions such as IF/TA associated with transplant vasculopathy or SCR are highly specific for graft failure, that is, graft survival is excellent in patients without the condition during a long period of time. In the other hand, the sensitivity rapidly decreases, as patients not showing the condition in the protocol biopsy will also loose their graft because of the conditions not detected in the protocol biopsy such as, recurrence of primary disease, hepatitis C-related glomerulonephritis, polyoma virus nephropathy, or treatment non-compliance. The only way to overcome this problem is to perform iterative biopsies to detect new patients at risk. Alternatively, validation of composite end points considering histological and clinical/

Table 1 | Predictive value of clinical variables and different histological patterns during the initial 6 months on 7-year death-censored graft survival

Surrogate	Category	Accuracy (%)	Sensitivity (%)	Specificity (%)
Acute rejection	Yes	72	30	80
3-month SCr	>1.8 mg/dl	73	58	76
Protocol biopsy	IF/TA	67	65	67
Protocol biopsy	IF/TA+cv-score $\geqslant 1$	81	21	92
Protocol biopsy	IF/TA+SCR	78	31	86

cv-score, chronic vascular score according to Banff criteria; IF/TA, interstitial fibrosis/tubular atrophy; SCr, serum creatinine; SCR, subclinical rejection.

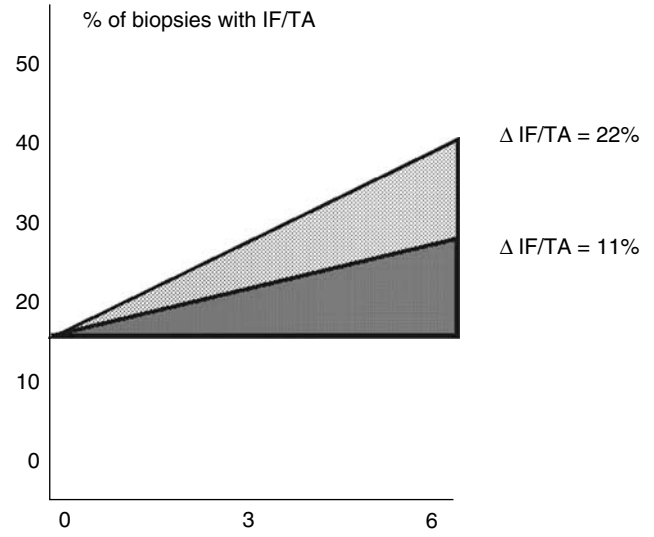

Figure 1 | Estimation of minimum sample size in an hypothetical clinical trial to detect a 50% reduction in the incidence of IF/TA. For power calculation, it was assumed a 16% prevalence of IF/TA in the preimplantation biopsy, 38% in the protocol biopsy of the control group, and 27% in the protocol biopsy of the treatment group. It was estimated that 285 cases per group would be necessary to detect a difference between groups ($\alpha = 0.05$ and $\beta = 0.20$).

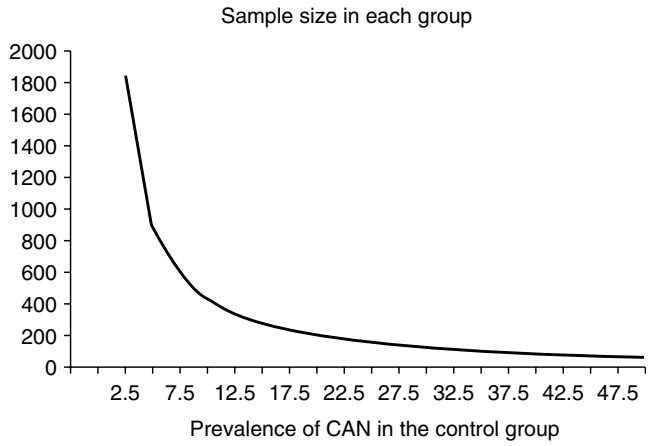

Figure 2 | Relationship between the prevalence of IF/TA in the control and minimum sample size to detect a 50% reduction in the prevalence of IF/TA in the study group.

analytical variables together may improve the predictive value of biopsies on outcome.[54]

PROTOCOL BIOPSIES AS AN EFFICACY VARIABLE IN CLINICAL TRIALS

The lack of a trustful primary efficacy variable in clinical trials favored the introduction of protocol biopsies as a secondary efficacy variable in order to evaluate its potential utility to detect differences between treatments. The first large trial using this strategy compared cyclosporine and tacrolimus and failed to observe a difference in the prevalence of IF/TA between groups.[55] Some years ago, the minimum sample size to detect a difference between treatment groups in an hypothetical trial aimed to prevent the progression of IF/TA was estimated.[39] As the incidence of IF/TA rapidly increases during the first few months, we evaluated whether a trial with a short-term follow-up was feasible. Thus, we performed power calculations assuming a 6-month follow-up and a 50% difference in the incidence of IF/TA between treatment and control groups. For this estimation, we considered that the mean prevalence of IF/TA in a donor and a 6-month protocol biopsy were 16 and 38%, respectively; and accordingly, the incidence of IF/TA was 22% (Figure 1). We estimated that minimum sample size to detect a 50% reduction in the incidence of IF/TA from 22 to 11% was 285 patients per group ($\alpha = 0.05$, $\beta = 0.20$). This estimation was performed before the introduction of calcineurin-free regimens, and soon we realized that our proposal was too conservative. In a single-center study comparing two different immunosuppressive treatments based on cyclosporine or sirolimus associated with mycophenolate mofetil and prednisone, the prevalence of IF/TA at 2 years was 79% in the cyclosporine group and 33% in the sirolimus group.[56] In this study, only 24 cases per group were necessary to demonstrate a significant difference in the prevalence of IF/TA. Similarly, in the belatacept trial, in which patients treated with basiliximab, mycophenolate mofetil, and prednisone were randomized to receive an intensive or less intensive belatacept regimen or cyclosporine, a protocol biopsy was performed at 1 year.[46] Only 50 cases

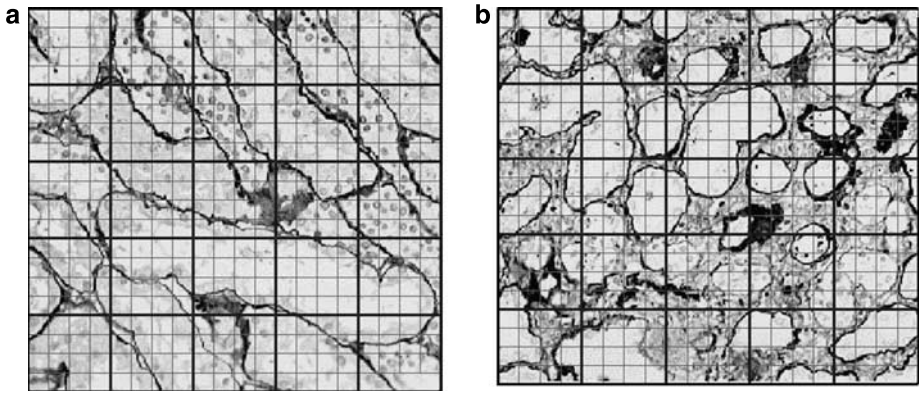

Figure 3 | Quantification of cortical interstitial volume fraction in a normal and diseased renal allograft biopsies by means of a point counting technique (silver methenamine stain, original magnification × 200).

per group were necessary to observe a significant difference in the prevalence of IF/TA at 1 year. The prevalence of IF/TA was 29% in the intensive belatacept group, 20% in the less intensive belatacept group, and 44% in the cyclosporine group. However, not in all studies evaluating calcineurin-free regimens the difference in the prevalence of chronic tubulointerstitial damage was so evident as in the previous mentioned trials. In patients treated with thymoglobulin, mycophenolate mofetil, and prednisone and randomized to receive sirolimus or tacrolimus, IF/TA at 1 year was not different.[57] These data show that in some clinical trials evaluating calcineurin-free regimens, IF/TA has been significantly reduced. Thus, we can foresee that if the prevalence of IF/TA consistently falls below 20%, this variable will not be any more useful to discriminate the effect of immunosuppressive treatments. This argument is based on the exponential relationship between the prevalence of the efficacy variable and the minimum sample size as shown in Figure 2.

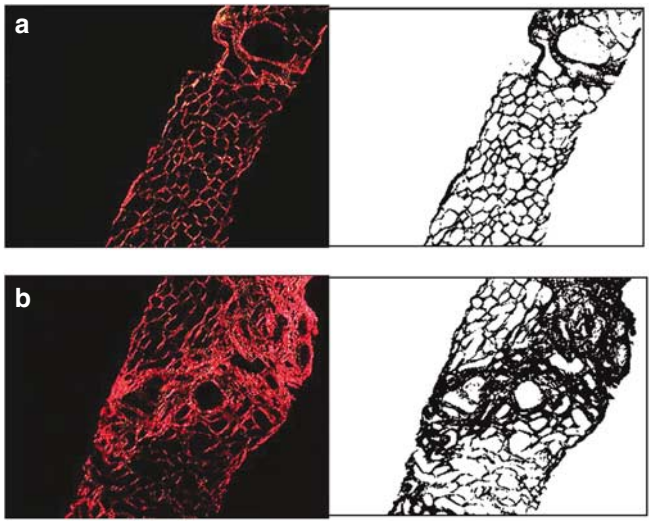

Figure 4 | Quantification of sirius red-positive area in a normal and diseased renal allograft biopsy by means of image analysis technique (original magnification × 100).

QUANTITATIVE EVALUATION OF HISTOLOGICAL DAMAGE IN PROTOCOL BIOPSIES

As an attempt to improve the predictive value of protocol biopsies on outcome, renal damage has been evaluated with quantitative parameters based on morphometry, image analysis, immunohistochemistry, or molecular biology techniques. In protocol biopsies, the quantification of interstitial damage by means of cortical interstitial volume fraction with morphometry (Figure 3) or the sirius red-positive cortical area with image analysis (Figure 4) is associated with graft survival.[58–61] Even in a clinical trial comparing cyclosporine- versus tacrolimus-based immunosuppression, sirius red-stained positive area was employed as an efficacy variable.[62] Additionally, the quantification of intimal arterial volume fraction (Figure 5) and mean glomerular volume has been also associated with graft survival.[63,64] These results suggest that quantitative parameters may provide an early surrogate for time to graft failure. To further explore this hypothesis, the progression of cortical interstitial volume fraction and intimal arterial volume fraction was estimated in serial protocol biopsies, and power calculation was performed to estimate minimum sample size in an hypothetical trial aimed to prevent the progression of interstitial or vascular damage.[65] The minimum sample size to detect a 50% reduction in the 1-year cortical interstitial volume fraction was 50 cases per group and to detect a 50% reduction in the 1-year intimal arterial volume fraction was 42 cases per group ($\alpha = 0.05$, $\beta = 0.20$). However, these results have to be considered with caution, as quantitative parameters have not been properly validated as surrogates of survival. Moreover, there is scarce information even on intra- and interobserver variability. As an example, we compared the interobserver variability for the estimation of mean glomerular volume and mean intimal arterial volume fraction in a set of protocol biopsies. As shown in Figure 6, the agreement was better for mean glomerular volume than for intimal thickening (unpublished observation), supporting that the reproducibility of quantitative data depends on the evaluated renal lesion. The low reproducibility of some

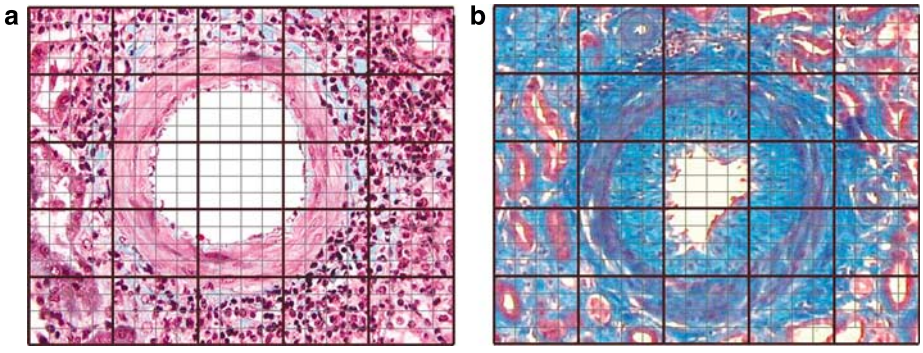

Figure 5 | Quantification of intimal arterial volume fraction by means of a point counting technique in a normal artery and an artery with transplant vasculopathy (Masson's tricrome, original magnification × 400).

quantitative parameters may explain the paradox that, in

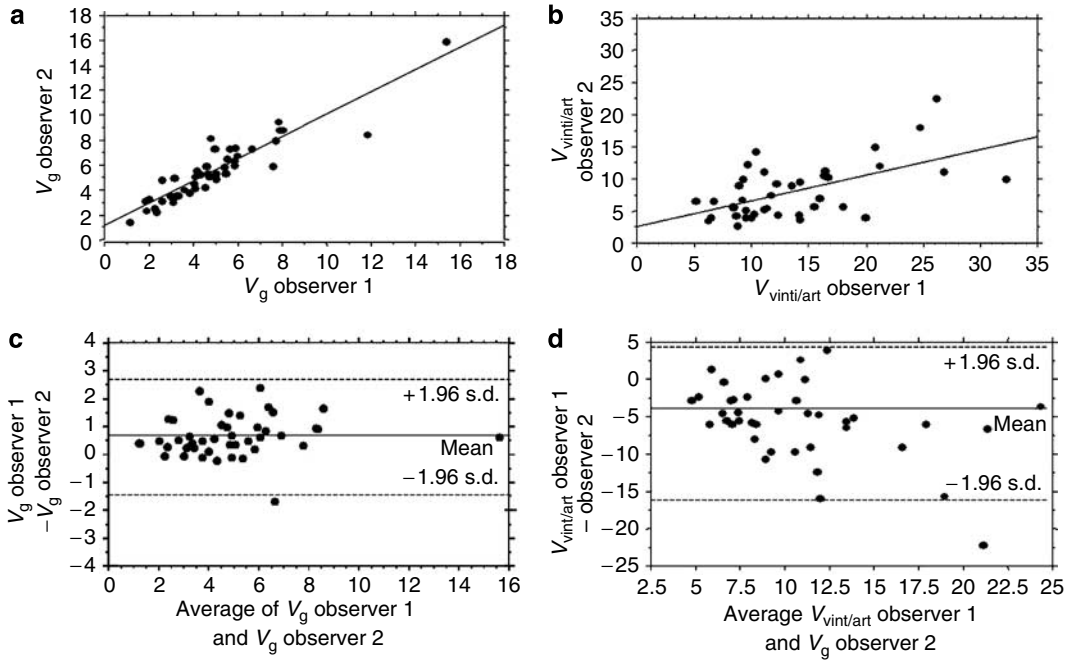

Figure 6 | Correlation and Bland-Altman plots showing interobserver variability for the estimation of mean glomerular volume (Vg) and mean intimal arterial volume fraction (Vvint/art).

some settings, ordinal measures may be superior to quantitative parameters to predict graft outcome. As an example, in a study evaluating the predictive value of histologic lesions in preimplantation biopsies, we showed that chronic vascular lesions graded according to Banff criteria better predicted graft outcome than quantification of intimal thickening.[66] Thus, only quantitative parameters that are highly reproducible could offer an advantage over classical histology.

CONCLUSION

The presence of IF/TA in protocol renal allograft biopsies is an independent predictor of graft survival. Recently, it has been shown that IF/TA associated with transplant vasculopathy, SCR, and transplant glomerulopathy implies a poorer outcome than IF/TA without additional lesions. These findings raise the question whether protocol biopsies could be employed as a surrogate of graft survival and, accordingly, as an efficacy variable in clinical trials. Although histological lesions predict graft survival, its accuracy has been poorly characterized. Preliminary data suggest that its predictive value on graft survival is at least not inferior to acute rejection or renal function. Additionally, quantitative evaluation of histological lesions has been performed as an attempt to provide a better surrogate for time to graft failure. Both morphometry and image analysis have been employed to quantify interstitial fibrosis and transplant vasculopathy, but none of these parameters has been properly validated. An alternative strategy to increase the predictive value of protocol biopsies on outcome is the validation of composite end points containing histological, clinical, biological, and/or analytical parameters.

ACKNOWLEDGMENTS

Studies on protocol biopsies have been partly supported by Fondo Investigación Sanitaria (FIS) Grants PI040086 and PI040177.

REFERENCES

1. Matas AJ, Sibley R, Mauer SM *et al.* Pre-discharge, post-transplant kidney biopsies does not predict rejection. *J Surg Res* 1982; **32**: 269–274.
2. Burdick JF, Beschorner WE, Smith WJ *et al.* Characteristics of early routine renal allograft biopsies. *Transplantation* 1984; **38**: 679–683.
3. McWhinnie DL, Thompson JF, Taylor HM *et al.* Morphometric analysis of cellular infiltration assessed by monoclonal antibody labelling in sequential human renal allograft biopsies. *Transplantation* 1986; **42**: 352–358.
4. Seron D, Diaz Gallo C, Grino JM *et al.* Characterization of interstitial infiltrate in early renal allograft biopsies in patients with stable renal function. *Transplant Proc* 1991; **23**: 1267–1269.
5. Solez K, Axelsen RA, Benediktsson H *et al.* International standardization of criteria for the histologic diagnosis of renal allograft rejection: the Banff working classification of kidney transplant pathology. *Kidney Int* 1993; **44**: 411–422.
6. Racusen LC, Solez K, Colvin RB *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713–723.
7. Racusen LC, Colvin RB, Solez K *et al.* Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003; **3**: 708–714.
8. Solez K, Colvin RB, Racusen LC *et al.* Banff' 05 meeting report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007; **7**: 518–526.
9. Rush DN, Jeffery JR, Gough J. Sequential protocol biopsies in renal transplant patients. *Transplantation* 1995; **59**: 511–514.
10. Nankivell BJ, Borrows RJ, Fung CLS *et al.* Natural history, risk factors, and impact of subclinical rejection in kidney transplantation. *Transplantation* 2004; **78**: 242–249.
11. Isoniemi HM, Krogerus L, von Willebrand E *et al.* Histopathological findings in well functioning long term renal allografts. *Kidney Int* 1992; **41**: 155–160.
12. Serón D, Moreso F, Bover J *et al.* Early protocol renal allograft biopsies and graft outcome. *Kidney Int* 1997; **51**: 310–316.
13. Nankivell BJ, Borrows RJ, Fung CLS *et al.* The natural history of chronic allograft nephropathy. *N Eng J Med* 2003; **349**: 2326–2333.
14. Marcusen N, Olsen TS, Benediktsson H *et al.* Reproducibility of the Banff classification of renal allograft pathology. *Transplantation* 1995; **60**: 1083–1089.
15. Furness PN, Taub N. Convergence of European renal transplant pathology assessment procedures. International variation in the interpretation of

renal transplant biopsies: report of the CERTPAP project. *Kidney Int* 2001; **60**: 1198–2010.
16. Colvin RB, Cohen AH, Saiontz C *et al.* Evaluation of pathologic criteria for acute renal allograft rejection: reproducibility, sensitivity, and clinical correlation. *J Am Soc Nephrol* 1997; **8**: 1930–1941.
17. Gough J, Rush D, Jeffery J *et al.* Reproducibility of the Banff schema in reporting protocol biopsies of stable renal allografts. *Nephrol Dial Transplant* 2002; **17**: 1081–1084.
18. Veronese FV, Manfro RC, Roman FR *et al.* Reproducibility of the Banff classification in subclinical kidney transplant rejection. *Clin Transplant* 2005; **19**: 518–521.
19. Legendre C, Thervet E, Skhiri H *et al.* Histologic features of chronic allograft nephropathy revealed by protocol biopsies in kidney transplant recipients. *Transplantation* 1998; **65**: 1506–1509.
20. Gloor JM, Cohen AJ, Lager DJ *et al.* Subclinical rejection in tacrolimus treated renal transplant recipients. *Transplantation* 2002; **72**: 1965–1968.
21. Moreso F, Seron D, Carrera M *et al.* Baseline inmunosuppression is associated with histological findings in early protocol biopsies. *Transplantation* 2004; **78**: 1064–1068.
22. Moreso F, Ibernon M, Goma M *et al.* Subclinical rejection associated with chronic allograft nephropathy in protocol biopsies as a risk factor for late graft loss. *Am J Transplant* 2006; **6**: 747–752.
23. Mengel M, Gwinner W, Schwarz A *et al.* Infiltrates in protocol biopsies from renal allografts. *Am J Transplant* 2007; **7**: 356–365.
24. Choi BS, Shin MJ, Sjin SJ *et al.* Clinical significance of an early protocol biopsy in living-donor renal transplantation: ten-year experience at a single center. *Am J Transplant* 2005; **5**: 1354–1360.
25. Roberts ISD, Reddy S, Russell C *et al.* Subclinical rejection and borderline changes in early protocol biopsy specimens after renal transplantation. *Transplantation* 2004; **77**: 1194–1198.
26. Sholten EM, Rowshani A, Cremers S *et al*. Untreated rejection in 6-month protocol biopsies is not associated with fibrosis in serial protocol biopsies or with loss of graft function. *J Am Soc Nephrol* 2006; **17**: 881–888.
27. Shishido S, Asanuma H, Nakai H *et al.* The impact of repeated subclinical acute rejection on the progression of chronic allograft nephropathy. *J Am Soc Nephrol* 2003; **14**: 1046–1052.
28. Cosio FG, Grande JP, Wadei H *et al.* Predicting subsequent decline in kidney allograft function from early surveillance biopsies. *Am J Transplant* 2005; **5**: 2464–2472.
29. Rush DN, Nickerson P, Gough J *et al.* Beneficial effects of treatment of early subclinical rejection: a randomized study. *J Am Soc Nephrol* 1998; **9**: 2129–2134.
30. Nankivell BJ, Chapman JR. The significance of subclinical rejection and the value of protocol biopsies. *Am J Transplant* 2006; **9**: 2006–2012.
31. Seron D, Moreso F, Fulladosa X *et al.* Reliability of chronic allograft nephropathy diagnosis in sequential protocol biopsies. *Kidney Int* 2002; **61**: 727–733.
32. Fulladosa X, Moreso F, Torras J *et al.* Structural and functional correlations in stable renal allografts. *Am J Kidney Dis* 2003; **41**: 1065–1073.
33. Birk PE, Stannard KM, Konrad HB *et al.* Surveillance biopsies are superior to functional studies for the diagnosis of acute and chronic renal allograft pathology in children. *Pediatr Transplant* 2004; **8**: 29–38.
34. Bosmans JL, Woestenburg A, Ysebaert DK *et al.* Fibrous intimal thickening at implantation as a risk factor for the outcome of cadaveric renal allografts. *Transplantation* 2000; **69**: 2388–2394.
35. Schwarz A, Mengel M, Gwinner W *et al.* Risk factors for chronic allograft nephropathy after renal transplantation: a protocol biopsy study. *Kidney Int* 2005; **67**: 341–348.
36. Dimény E, Wahlberg J, Larsson E, Fellström B. Can histopathological findings in early renal allograft biopsies identify patients at risk for chronic vascular rejection? *Clin Transplant* 1995; **9**: 79–84.
37. Yilmaz S, Tomlanovich S, Mathew T *et al.* Protocol core needle biopsy and histologic chronic allograft damage index (CADI) as surrogate end-point for long-term graft survival in multicenter studies. *J Am Soc Nephrol* 2003; **14**: 773–779.
38. Nankivell BJ, Fenton-Lee CA, Kuypers DRJ *et al.* Effect of histological damage on long-term kidney transplant outcome. *Transplantation* 2001; **71**: 515–523.
39. Seron D, Moreso F, Ramon JM *et al.* Protocol renal allograft biopsies and the design of clinical trials aimed to prevent or treat chronic allograft nephropathy. *Transplantation* 2000; **69**: 1849–1855.
40. Melk A, Schmidt BM, Vongwiwatana A *et al.* Increased expression of senescence-associated cell cycle inhibitor p16INK4a in deteriorating renal transplants and diseased native kidneys. *Am J Transplant* 2005; **5**: 1375–1382.
41. Mengel M, Bogers J, Bosmans JL *et al.* Incidence of C4d stain in protocol biopsies from renal allografts: results from a multicenter trial. *Am J Transplant* 2005; **5**: 1050–1056.
42. Haas M, Montgomery RA, Segev DL *et al.* Subclinical acute antibody-mediated rejection in positive crossmatch renal allografts. *Am J Transplant* 2006; **6**: 1–10.
43. Cyclosporine A as sole immunosuppressive agent in recipients of kidney allografts from cadaver donors. Preliminary result of a European Multicenter trial. *Lancet* 1982; **2**: 57–60.
44. A randomized clinical trial of cyclosporine in cadaveric renal transplantation. *N Engl J Med* 1983; **309**: 809–815.
45. Halloran P, Mathew T, Tomlanovich S *et al.* Mycophenolate mofetil in renal allograft recipients: a pooled efficacy analysis of three randomized, double-blind, clinical studies in prevention of rejection. The International Mycophenolate Mofetil Renal Transplant Study Groups. *Transplantation* 1997; **63**: 39–47.
46. Vincenti F, Larsen C, Durrbach A *et al.* Costimulation blockade with belatacept in renal transplantation. *N Engl J Med* 2005; **353**: 770–781.
47. Hariharan S, McBride MA, Cohen EP. Evolution of endpoint for renal transplant outcome. *Am J Transplant* 2003; **3**: 933–941.
48. Hariharan S, Kasiske B, Matas A *et al.* Surrogate markers for long-term renal allograft survival. *Am J Transplant* 2004; **4**: 1179–1183.
49. Lachenbruch PA, Rosenberg AS, Bonvini EE *et al.* Biomarkers and surrogate end-points in renal transplantation: present status and considerations for clinical trial design. *Am J Transplant* 2004; **4**: 451–457.
50. The Heart Outcomes Prevention Evaluation (HOPE) 2 investigators. Homocysteine lowering with acid folic and B vitamins in vascular disease. *N Engl J Med* 2006; **354**: 1567–1577.
51. Mota A, Arias M, Taskinen EI *et al.* Sirolimus-based therapy following early cyclosporine withdrawal provides significantly improved renal histology and function at 3 years. *Am J Transplant* 2004; **4**: 953–961.
52. Oberbauer R, Segoloni G, Campistol JM *et al.* Early cyclosporine withdrawal from a cyclosporine-based regimen results in better renal allograft survival and renal function at forty eight months after transplantation. *Transpl Int* 2005; **18**: 22–28.
53. Kaplan B, Schold J, Meier-Kriesche HU. Poor predictive value of serum creatinine for renal allograft loss. *Am J transplant* 2003; **3**: 1560–1565.
54. Kasiske BL. Endpoint or turning point? *Am J Transplant* 2003; **3**: 1463–1464.
55. Solez K, Vincenti F, Filo RS. Histopathological findings from 2 year protocol biopsies from a US multicenter kidney transplant trial comparing tacrolimus versus cyclosporine. *Transplantation* 1998; **66**: 1736–1740.
56. Flechner SM, Kurlan SM, Solez K *et al. De novo* kidney transplantation without use of calcineurin inhibitors preserves renal structure and function at two years. *Am J Transplant* 2004; **4**: 1776–1785.
57. Larson TS, Dean PG, Stegall MD *et al.* Complete avoidance of calcineurin inhibitors in renal transplantation: a randomized trial comparing sirolimus and tacrolimus. *Am J Transplant* 2006; **6**: 514–522.
58. Nicholson ML, McCulloch TA, Harper SJ *et al.* Early measurement of interstitial fibrosis predicts long-term renal function and graft survival in renal transplantation. *Br J Surg* 1996; **83**: 1082–1085.
59. Nicholson ML, Bailey E, Williams S *et al.* Computerized histomorphometric assessment of protocol renal transplant biopsy specimens for surrogate markers of chronic rejection. *Transplantation* 1999; **68**: 236–241.
60. Grimm PC, Nickerson P, Gough J *et al.* Computerized image analysis of sirius red-stained renal allograft biopsies as a surrogate marker to predict long-term allograft function. *J Am Soc Nephrol* 2003; **14**: 1662–1668.
61. Pape L, Henne T, Offner G *et al.* Computer-assisted quantification of fibrosis in chronic allograft nephropathy by picrosirius red-staining: a new tool for predicting long-term graft function. *Transplantation* 2003; **76**: 955–958.
62. Rowshani AT, Scholten EM, Bemelman F *et al.* No difference in degree of interstitial sirius red-stained area in serial biopsies from area under concentration-over-time curves-guided cyclosporine versus tacrolimus-treated renal transplant recipients at one year. *J Am Soc Nephrol* 2006; **17**: 305–312.
63. Bosmans JL, Holvoet P, Dauwe EH *et al.* Oxidative modification of low-density lipoproteins and the outcome of renal allografts at 1½ years. *Kidney Int* 2001; **59**: 2346–2356.
64. Azevedo F, Alperovich G, Moreso F *et al.* Glomerular size in early protocol biopsies is associated with graft outcome. *Am J Transplant* 2005; **5**: 2877–2882.
65. Moreso F, Lopez M, Vallejos A *et al.* Serial protocol biopsies to quantify the progression of chronic transplant nephropathy in stable renal allografts. *Am J Transplant* 2001; **1**: 82–88.
66. Lopes JA, Moreso F, Riera L *et al.* Evaluation of pre-implantation kidney biopsies: comparison of Banff criteria to a morphometric approach. *Kidney Int* 2005; **67**: 1595–1600.