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ORIGINAL ARTICLE

Impact of immunosuppression on the incidence of early subclinical renal allograft rejection: implications for protocol biopsy policy

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Summary

In order to determine the impact of immunosuppression (IS) on the incidence of early subclinical rejection (SCR), we studied two groups of patients receiving different immunosuppressive regimens. Patients received cyclosporin (CsA), azathioprine and prednisolone (group 1; $n = 304$) or IS according to immunological risk (group 2; $n = 150$). The highest-risk patients received basiliximab induction, tacrolimus, mycophenolate mofetil (MMF) and prednisolone; medium-risk patients CsA, MMF and prednisolone; low-risk CsA, azathioprine and prednisolone. Protocol biopsies were performed in all patients, irrespective of graft function, on days 7 and 28 post-transplantation. Only patients with good stable function at the time of biopsy were included for assessment of SCR. Group 2 patients showed significant reductions in total rejection frequency (32.6% vs. 57.2%, $P = <0.0001$) and SCR frequency in day 7 protocol biopsies (2% vs. 13%, $P = <0.05$). In group 2 patients, all SCRs, but not borderline changes, were treated. Untreated borderline changes did not have an adverse impact on graft function at 1 year post-transplantation. New immunosuppressive regimens may reduce subclinical in addition to clinical rejection-frequency, suggesting that the relative benefit of early protocol biopsies in detecting SCR is also reduced.

Introduction

Post-transplantation protocol biopsies, performed at predetermined time points irrespective of graft function may be used to detect and monitor subclinical rejection (SCR) and to detect and quantify graft fibrosis as an early marker of chronic injury. The potential benefit to an individual patient of performing a protocol biopsy to diagnose SCR is a product of the frequency of SCR in the patient population at the time of biopsy and the benefit to be gained in terms of long-term graft function from treatment of SCR. The incidence of SCR is therefore a major factor in making the decision to include protocol biopsies in routine patient management.

The reported frequency of SCR varies greatly between units from 1% to 60% [1–10]. Possible explanations for these variations include timing of biopsy, human leucocyte antigen (HLA) mismatch and level of baseline immunosuppression (IS). Most previous reports are based on data from the last decade, with patients having received cyclosporin (CsA)-based triple therapy. More recently, however, there have been reports of patients receiving newer, more potent, IS regimens. In a study of 119 recipients of simultaneous kidney-pancreas transplants, Nankivell *et al.* [10] reported a SCR frequency of 60% at 1 month and 45.7% at 3 months. IS regimen had no impact on the frequency of SCR at 1 month but in protocol biopsies from 3 to 12 months, the combination of tacrolimus and mycophenolate mofetil (MMF) was associated with a 20-fold reduction in SCR when compared with patients receiving CsA and azathioprine. Similarly, in a case-control study of 98 patients, Moreso *et al.* [11]

found that in protocol biopsies at 4 months, the relative risk of acute inflammation of patients receiving tacrolimus was 0.3, compared with CsA-treated patients.

In this report, we aimed to investigate the impact of baseline IS on SCR in very early protocol biopsies (1 week and 1 month post-transplant). We compared protocol biopsy findings in 150 consecutive renal transplant recipients receiving IS according to immunological risk, including basiliximab induction, tacrolimus and MMF in high-risk patients, with a historical control group of 304 patients who received CsA, azathioprine and prednisolone. We report that the increase in IS is associated with a reduction in both overall rejection frequency and early SCR in day 7 protocol biopsies. The potential benefit of early protocol biopsies is therefore reduced by the introduction of newer, more potent, immunosuppressive regimens.

Patients and methods

Patient groups

Two groups of patients were studied. A historical control group of 304 consecutive transplant recipients, transplanted in our unit during the period between 1992 and 1995 (group 1) and 150 consecutive recipients, transplanted over a 2-year period between 2001 and 2003 (group 2). Data on the protocol biopsy findings in the group 1 patients has been previously reported [9]. All these patients received baseline IS with CsA, azathioprine and prednisolone. All patients in group 2 received baseline IS according to a protocol introduced in 2001. In this protocol, patients were stratified into three groups at the time of transplantation, according to immunological risk (Table 1). The 1992–1995 group was selected for comparison purposes as immunosuppressive protocols were unchanged during this period. Apart from an increase in baseline IS between the two groups, all other clinical protocols were unchanged, including the policy of protocol biopsies at days 7 and 28 post-transplant.

Clinical and HLA matching data were collected on all patients in order to confirm that the two groups were matched in all respects other than IS received. The total rejection incidence was expressed as the percentage of patients treated for at least one episode of biopsy-proven rejection, clinical or subclinical.

Protocol biopsies

Unit protocol for both groups was to perform protocol biopsies in all patients on days 7 and 28 post-transplantation. Only patients with good, stable graft function [defined as serum creatinine (sCr) <200 μmol/l and less

Table 1. Immunosuppressive protocol for group 2 patients.

Risk group	Immediate function	DGF	DGF > 7days
High risk			
PRA > 85%	Basiliximab (Simulect®)	$^1/_2$ Level tacrolimus	Stop tacrolimus*
Serious cross-match concern	Tacrolimus		Weekly biopsy
5–6 HLA mismatches	MMF		
	Prednisolone†		
Medium risk			
Previously rejected graft	CyA (Neoral®)	$^1/_2$ Level CyA	Stop CyA*, start ATG§
3–4 HLA mismatches or 2	MMF 3–6 months‡		Biopsy at end of ATG course
DR mismatches	Prednisolone†		then weekly until function improves
Low risk			
All others	CyA (Neoral®)	$^1/_2$ Level CyA	Stop CyA*, start ATG§
	Azathioprine	Stop azathioprine and	Biopsy at end of ATG course then
	Prednisolone†	start MMF 3–6 months‡	weekly until function improves

DGF, delayed graft function; PRA, panel reactive antibodies; ATG, anti-thymocyte-globulin; CyA, cyclosporin; MMF, mycophenolate mofetil.
*Provided renal function is restored and patient is off dialysis, reintroduce calcineurin inhibitor at full dose (aiming for CyA levels 150–300 ng/ml or tacrolimus levels 10–15 ng/ml), 3 days before stopping ATG. If patient is still dialysis-dependent at the end of the ATG course, then continue on dual therapy (MMF and steroids). Monitor closely with FNA every 3 days and weekly tru-cut biopsies. Reintroduce calcineurin inhibitor at full dose (aiming for CyA levels 150–300 ng/ml or tacrolimus levels 10–15 ng/ml) only when renal function is restored and patient is off dialysis.
†Prednisolone dose: 20 mg od (patients > 60 kg) or 15 mg od (patients < 60 kg). This dose is used for the first 2 months post-transplant, then steroid reduction is commenced, unless there have been major rejection episodes in that time. Steroid reduction program reduces the dose at 2 months post-transplant by 2.5 mg every 4 weeks until 5 mg od is achieved. The patient then remains on this dose until seen in the medical transplant clinic at 1 year, at this time a decision will be made whether to continue with steroid withdrawal or leave the patient on 5 mg.
‡Continue MMF for 3 of 12 months, then convert to azathioprine (1.5 mg/kg od). However, if patient has experienced a rejection episode during these first 3 months then the course of MMF must be extended to 6 months, and at 6 months convert to azathioprine.
§Start ATG 2 mg/kg, 10–14 days course (depending on patient response). Give ATG dose when absolute T-cell count > 50.

than a 15% increase in sCr in the 2 days prior to biopsy] at the time of protocol biopsies were considered potential candidates for subclinical (rather than clinical) rejection. Therefore, only these patients were included in the analysis of SCR frequency ($n = 115$ in group 1, 88 in group 2). IS treatment received at the time of transplantation in group 2 was as follows: CsA, azathioprine and prednisolone ($n = 30$); CsA, MMF and prednisolone ($n = 41$); tacrolimus, MMF and prednisolone associated with basiliximab ($n = 17$). Biopsies were performed using an 18-gauge needle. Adequacy of biopsy was defined using Banff criteria. The Banff 97 classification [12] was used for the assessment of the biopsies in the 2001–2003 cohort and was performed by two renal pathologists. Biopsies in group 1 were classified initially, at the time of the biopsies, according to Banff 93 criteria. These biopsies were subsequently reviewed and classified according to Banff 97, blind of clinical data, as previously described. Minor complications of protocol biopsies were not analysed. There was no mortality and no graft loss relating to protocol biopsies during the period of this study.

Although there was no clear unit protocol in group 1 patients, the majority of SCRs were treated with pulse methylprednisolone. Group 1 patients were followed up for a minimum of 6 years. The impact of SCR on graft outcome in this group has been previously reported [9]. For group 2 patients, data was collected prospectively and follow-up was for a minimum of 1 year. Clinical intervention was made on the basis of the protocol biopsy diagnosis; unit protocol for group 2 patients was to treat clinical rejection, clinical borderline changes and SCR, but not subclinical borderline changes. Variance from this protocol occurred in only one patient following the days 7 and 28 protocol biopsies, with treatment for subclinical borderline changes. Treatment of rejection in both groups was with pulsed i.v. steroid therapy (methylprednisolone 0.5 mg daily for 3 days).

Statistical analysis (chi-squared test, unpaired *t*-test and Mann–Whitney *U*-test) was performed using SPSS software (SPSS, Chicago, Illinois, USA).

Results

Details of donor type, delayed graft function (DGF), HLA-matching and total rejection frequency are shown in Table 2. There was a significantly higher incidence of DGF in group 2 than group 1 (37.3% vs. 24.6%). This is believed to be as a result of the introduction of a 'donation-after-cardiac death' programme between 2001 and 2003 that was initially associated with a high frequency of severe acute tubular necrosis and DGF. In group 2, the number of patients in each subgroup was: low risk, 53 patients; medium risk, 58 patients; high risk, 39 patients.

The change in IS regimen was associated with a highly significant reduction in the percentage of patients suffering at least one biopsy-proven rejection episode (57.2% in group 1 vs. 32.6% in group 2). In group 2 patients, the total rejection frequencies in low-, medium- and high-risk subgroups were 23%, 41% and 28% respectively.

Details of the SCR frequency in group 1 and group 2 patients who had good stable graft function at the time of protocol biopsies is shown in Table 3. The change in IS was associated with a significant reduction in SCR incidence in the day 7 protocol biopsy (13% in group 1 vs. 2% in group 2). Of the 16 SCR in group 1, 10 were Banff type IA, four, type IB and two, type IIA. Of the five SCR in group 2, four were Banff type IA and one type IB.

All five patients with SCR in group 2 were treated immediately with pulsed steroids, as were one out of 11

Table 2. Comparison of all patients in groups 1 and 2.

	Group 1 (1992–1995)	Group 2 (2001–2003)	*P*-value
Number of patients	304	150	
Donor: LRD/DD/DACD	20/284/0	28/104/18	
Delayed graft function	75 (24.6%)	56 (37.3%)	<0.01
Total mismatch	2.68 ± 1.38	2.26 ± 1.28	NS
DR mismatch	0.48 ± 1.38	0.50 ± 0.54	NS
Rejection, clinical + SCR	174 (57.2%)	49 (32.6%)	<0.0001
Cold ischaemic time (h)	NA	13.8 ± 7.4	NA
Hypersensitization (PRA > 50%)	NA	9 (6%)	NA
2nd or subsequent transplant	33 (10.9%)	16 (10.6%)	NS
Pre-emptive transplants	53 (17.5%)	21 (14%)	NS
Cr 1 year	158.34 ± 61.24	175.5 ± 126.9	NS
Graft survival 1 year	274/304 (90.1%)	135/150 (90%)	NS
Pt survival 1 year	285/304 (93.75%)	141/150 (94%)	NS

LRD, living related donor; DD, deceased donor; DACD, donor after cardiac death; SCR, subclinical rejection; NA, not assessed.

Table 3. Comparison of subclinical rejection incidence in groups 1 and 2.

	Group 1 (1992–1995)	Group 2 (2001–2003)	*P*-value
Number of patients, days 7 and 28	115	88	
Day 7: adequate biopsies	76	50	
Day 7: SCR	10 (13)	1 (2)	<0.05
Day 7: borderline changes	9 (12)	11 (22)	NS
Day 28: adequate biopsies	79	71	
Day 28: SCR	6 (8)	4 (6)	NS
Day 28: borderline changes	13 (16)	7 (10)	NS

Values in parentheses are percentages.
SCR, subclinical rejection.

Table 4. Outcome of patients with untreated subclinical borderline changes.

Group 2 (2001–2003)	Subsequent rejection (%)	1 year graft survival/median sCr (range)
Day 7: borderline changes (untreated)	4/10 (40)	
Day 7: no rejection	12/38 (32)	
Day 28: borderline changes (untreated)	0/6 (0)	100%/149 (128–163)
Day 28: no rejection	12/60 (20)	97%/139 (81–353)
P-value	NS	NS

sCr, serum creatinine.

patients with subclinical borderline changes at day 7 and one out of seven patients with subclinical borderline changes at day 28. The outcome of those patients with untreated subclinical borderline changes was compared with the patients who had no evidence of rejection or borderline changes in their protocol biopsies (Table 4). There was no significant difference in the incidence of subsequent rejection, graft survival or sCr at 1 year post-transplantation.

Discussion

The decision as to whether to include protocol biopsies in routine patient management and if so when to perform them are issues that should be based on maximization of patient benefit. Early protocol biopsies, in the first month post-transplant, are primarily performed to detect SCR, although they also provide information on the extent of chronic damage secondary to donor- and peri-transplant injury. Using conventional immunosuppressive regimens, the frequency of SCR is highest in the first month post-transplantation, as is clinical rejection. The frequency and timing of SCR varies greatly between units and baseline IS is likely to be a major factor responsible for these differences. Each unit should therefore perform a risk-benefit analysis for their patients and this should be repeated with each change in IS protocol.

The principal finding of this study is that an increase in baseline IS in a single centre is associated with a reduced frequency of SCR in addition to clinical rejection. The impact was highest in 1-week protocol biopsies which revealed a SCR incidence of 2% in group 2 patients. At this frequency, the potential benefit to an individual patient of diagnosing and treating SCR is low, and this biopsy has now been dropped from our unit protocol. This latest finding is supported by a recent randomized, multicentre study which indicated that the procurement of early protocol biopsies may be less useful in renal transplant patients treated with tacrolimus, MMF and prednisolone, at least in the first 6 months of follow-up. SCR was infrequent, occurring in 4.6% of the biopsies overall in the biopsy arm patients, reaching up to 9% at 6 months. Borderline rejection was infrequent (1.4% at 1 month) [13]. Another open-label prospective, randomized trial showed that the prevalence of SCR at 6 months was significantly higher in patients who received CsA therapy, as compared with tacrolimus [14].

Similarly uncontrolled studies have reported a reduced incidence of early SCR in patients who received tacrolimus, either with [7,8,10,11,15] or without [16] MMF. Some authors [17] have shown that the use of tacrolimus and MMF, either individually or in combination, reduces the prevalence of SCR. Other two recent studies reported a significant decline in the incidence of SCR when MMF was used as the primary immunosuppressant compared with patients without such treatment (biopsy performed at day 14 after transplantation in renal transplant recipients from living donors) [18] or when MMF was increased by 50% in paediatric renal transplant recipients [19]. On the contrary, Nickerson *et al.* [20] showed that increasing IS reduces the frequency of clinical rejections but not SCR (Neoral and MMF versus Sandimmune and azathioprine). However, in general these data suggest that SCR can be treated with increased baseline IS. Overall, subclinical cellular infiltration is more effectively suppressed by tacrolimus compared with CSA, and MMF with azathioprine, or the combination.

The follow-up of group 1 patients revealed that many cases of early SCR are either early or resolving clinical rejections. It is therefore probable that the frequency of true SCR (histological rejections not associated with acute graft dysfunction) is lower than published series suggest. Our conclusions were essentially the same as those of Hoffman *et al.* [21], to the effect that subclinical and

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clinical rejections likely are different stages of the same potentially damaging alloimmune process. Furthermore, several reports include all rejections diagnosed in protocol biopsies as SCR, irrespective of graft function at the time of biopsy. The impact of SCR on subsequent graft function cannot be assessed without careful follow-up data and taking into account the impact of clinical rejection. For example, Nankivell *et al.* [17] reported that SCR in a third month protocol biopsy was associated with an increased risk of chronic allograft nephropathy. However, in the same study, severe clinical rejection was strongly associated with an increased risk of subsequent SCR, and it may be that the injury following the severe clinical rejections, rather than subclinical infiltrates, were responsible for the worse long-term outcome.

The immediate management of SCR will also clearly have its impact on long-term graft function, but this data is omitted from many reports on protocol biopsies, adding further difficulty to the interpretation of follow-up data. In our group 1 patients, there was no clear unit protocol, although the majority of SCRs were treated with pulse methylprednisolone. A unit protocol was introduced in 2001 and in group 2, patients with SCR were treated as clinical rejection with 3 days of pulse methylprednisolone, whilst patients with subclinical Banff 97 borderline changes, unlike clinical borderline changes, were not treated for rejection. It is essential that before embarking on a protocol biopsy programme, there should be firm protocols for management of subclinical infiltrates and that these should be evidence-based. Our decision not to treat subclinical borderline changes was based on our experience of group 1 patients in whom untreated subclinical borderline changes was not an adverse prognostic factor for long-term function after a follow-up of 6 years.

Optimum protocol biopsy practice continues to change. In this study it was evident that the total rejection frequency in group 2 patients remained unacceptably high (32.6%), largely because of a high rejection rate in medium-risk patients. Although the explanation for this is likely to be the absence of induction therapy, we hypothesize that this is primarily because of the lower threshold used to suspect clinical rejection (i.e. ≥15% change from the baseline sCr). In most centres, and in clinical trials, a ≥20% rise in sCr is required before a diagnosis of acute rejection is considered. In the light of this, there has been a further increase in baseline IS in our patients, including the use of antibody induction in all patients (anti-CD25, Simulect or anti-CD52, Campath). This may be expected to result in a further reduction in SCR as antibody-induction therapies reduce the incidence of early clinical rejection [22,23]. Our recent experience in patients receiving Campath indicates that, with this agent, rejection is rare in the first 3 months post-transplantation and the value of protocol biopsies at month 1 is likely to be low.

With both clinical rejections and SCR affecting only a small minority of patients, the focus of protocol biopsies is moving away from SCR to the detection of early chronic damage, secondary to various factors including calcineurin inhibitor toxicity. Several studies have recently demonstrated that subclinical chronic damage is common, even in the first year post-transplantation, and in many patients is progressive [6,24–26]. There is only one randomized study done to date that showed that treatment of SCR in months 1, 2, and 3 was associated with a reduction in interstitial fibrosis and tubular atrophy at month 6 and with the preservation of graft function at 2 years, as compared with a control group in whom protocol biopsies were not done [3]. Others have shown that interstitial fibrosis and tubular atrophy develop in patients in whom SCR is diagnosed but not treated [6,17].

The most important role of protocol biopsies in future may be the early detection of graft fibrosis at a stage before the onset of irreversible loss of graft function, allowing changes in IS that may slow the progression of chronic allograft nephropathy. The optimum timing of these biopsies is later than those aimed to detect early SCR; at least two biopsies are required to demonstrate progression of chronic injury with the second biopsy not earlier than 6–12 months, when chronic damage resulting from various acute pathologies in the early post-transplant period can be assessed. As for early protocol biopsies performed to detect SCR, a risk-benefit analysis should be performed, and protocols introduced for acting on the biopsy findings. The use of protocol biopsies for monitoring chronic damage is valid only if changes are made to the immunosuppressive regimen on the basis of histological lesions, even in the presence of good stable graft function.

Authorship

ISDR: designed and performed the study and wrote the paper. CS: collected and analyzed the data and wrote the paper. SR and MZ: collected and analyzed the data. PJF: designed and performed the study.

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