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Sub-clinical acute rejection detected using protocol biopsies in patients with delayed graft function

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Abstract Acute rejection in renal transplants is difficult to diagnose when patients have delayed graft function (DGF) in the early post-transplant period. In this study protocol, renal transplant biopsies were performed in an attempt to detect sub-clinical acute rejection episodes. Eighty-three patients were eligible for the study, of whom 33 had DGF. All had protocol renal transplant biopsies performed under ultrasound control at 7 days post-transplant, and those with DGF had further biopsies weekly until the graft functioned. All histologically confirmed acute rejection episodes were treated. Sub-clinical acute rejection was detected in 6/33 (18%) patients with DGF compared to 2/50 (4%) in the other patients ($P < 0.05$). Borderline rejection was present in 4/33 (12%) and 4/50 (8%) patients, respectively. Because of the high detection rate of sub-clinical acute rejection and the low morbidity of renal transplant biopsies, their use is recommended in patients with DGF.

Key words Kidney transplantation · Delayed graft function · Acute rejection · Biopsy

Introduction

If renal transplant patients with delayed graft function also suffer an episode of acute rejection (AR), there is a strong association with poor graft outcome [5, 6]. Unfortunately, because of their dependence on dialysis renal function, measurements are of no value and monitoring of graft function can be difficult in these patients. In this situation, there is a real danger that diagnosis of acute rejection episodes may be delayed or even missed altogether. Early treatment of AR is likely to be of vital importance in preventing adverse long-term effects [15] and therefore methods to aid prompt diagnosis of AR in patients with DGF are of interest. One of these methods is the use of protocol biopsies, which have been shown in previous studies to be able to detect sub-clinical acute rejection [10]. In this paper, the diagnostic usefulness of protocol biopsies in the early post-transplant period has been assessed.

Materials and methods

Patients

All patients undergoing renal transplantation between January 96 and August 98 were eligible for the study. Excluded were those patients who refused consent and those who for clinical indications underwent a renal transplant biopsy before the protocol biopsy was due. Patients were classified as initial function (IF) or delayed graft function (DGF) according to the need for dialysis in the post-transplant period.

Immunosuppression

All patients during the period of the study were involved in a randomised controlled trial with immunosuppression based on either Neoral cyclosporine or tacrolimus (Prograf). The standard dosing protocol was as follows: cyclosporine 15 mg/kg per day initially reducing to 5 mg/kg per day by 6 weeks or tacrolimus 0.2 mg/kg per day. Patients with delayed graft function received lower doses of calcineurin inhibitors: either cyclosporine 7 mg/kg per day reduced to 5 mg/kg per day at 2 weeks post-transplant or tacrolimus 0.1 mg/kg per day. All patients received prednisolone 20 mg/kg per day for

Table 1 Patient details

	Initial function	Delayed graft function	*P*
Age*	41 (14)	50 (10)	0.001
Sex (M : F)	19 : 31	8 : 25	0.190
Donor age*	40 (15)	49 (11)	0.005
Donor type			
Cadaveric	32/50 (64%)	7/33 (21%)	
Non-heart beating	4/50 (8%)	24/33 (73%)	< 0.001
Living related	14/50 (28%)	2/33 (6%)	
HLA DR mismatch	25/50 (50%)	23/33 (70%)	0.075
Warm ischaemic time*	1 (6)	18 (13)	< 0.001
Cold ischaemic time*	13 (9)	16 (6)	0.191
Drug therapy (CyA : Tac)	23 : 27	16 : 17	0.824

* Values given as mean (SD)

3 months with a tapered reduction to 10 mg on alternate days by 6 months post-transplant. Recipients of kidneys from non-heart beating donors also received azathioprine 1–2 mg/kg per day.

Biopsies

Protocol renal transplant biopsies were taken under ultrasound guidance using a 16G Tru-Cut needle mounted in a spring-loaded biopsy gun. All patients included in the study had a biopsy at 7 days post-transplant, and those with delayed graft function had further biopsies weekly until the graft functioned. Presence of renal cortex was ensured by checking under a low power stereomicroscope prior to sending tissue for processing. Sections from the biopsy were examined by an experienced histopathologist (P.N.F.), and graded according to the Banff classification [13]. The biopsy result was normally available to clinicians on the same day as the biopsy.

Treatment of rejection

Biopsies showing acute rejection were treated even in the absence of symptoms or deteriorating renal function. Standard treatment was with intravenous methylprednisolone 500 mg/day × 3. Steroid resistant rejection was treated with anti-thymocyte globulin (ATG) at a dose of 2.5–5 mg/kg per day for 10–14 days adjusted by peripheral CD3 count. If a patient had a borderline biopsy, they were monitored closely, and treated if there was any clinical suspicion such as a subsequent rise in serum creatinine.

Results

Of 109 patients eligible for the study, 26 were excluded (five declined biopsy and 21 required early biopsy for clinical indications). The remaining patients were split as IF ($n = 50$) and DGF ($n = 33$).

Characteristics of the two groups are shown in Table 1. Factors associated with DGF included increased donor and recipient age, non-heart beating donor and increased warm ischaemic time. Median duration of DGF was 18 days (range 7–75) and six patients had primary non-function.

Table 2 Drug therapy at 7 days post-transplant. All results expressed as mean (SD)

	Initial function	Delayed graft function	*P*
Cyclosporine A			
Mean dose (mg/day)	702 (235)	408 (222)	< 0.001
Mean level (ng/ml)	447 (189)	370 (241)	NS
Tacrolimus			
Mean dose (mg/day)	11.2 (3.2)	8.6 (4.7)	< 0.05
Mean level (ng/ml)	14.0 (6.1)	17.3 (12.0)	NS

Table 3 Sub-clinical rejection episodes in the two groups

	Number of patients	Acute rejection	Borderline changes
Immediate function	50	2 (4%)	4 (8%)
Delayed graft function	33	6 (18%)	4 (12%)

The patients in the DGF group were on a lower dose of immunosuppression at 7 days post-transplant, but interestingly levels at the same point in time were not significantly different (Table 2). The overall rate of acute rejection within the first month post-transplant in patients with IF and DGF was similar at 17/50 (34%) and 11/33 (33%), respectively.

Sub-clinical acute rejection was detected significantly more commonly in patients with DGF ($P < 0.05$), and sub-clinical borderline rejection was also commoner (Table 3). Graft survival was poorer in the patients with DGF (Fig. 1), and most of this difference was due to graft losses in the first year.

Complications after renal transplant biopsy were minimal and comprised clot retention ($n = 2$) and haematoma ($n = 2$). No patient required blood transfusion or surgical intervention.

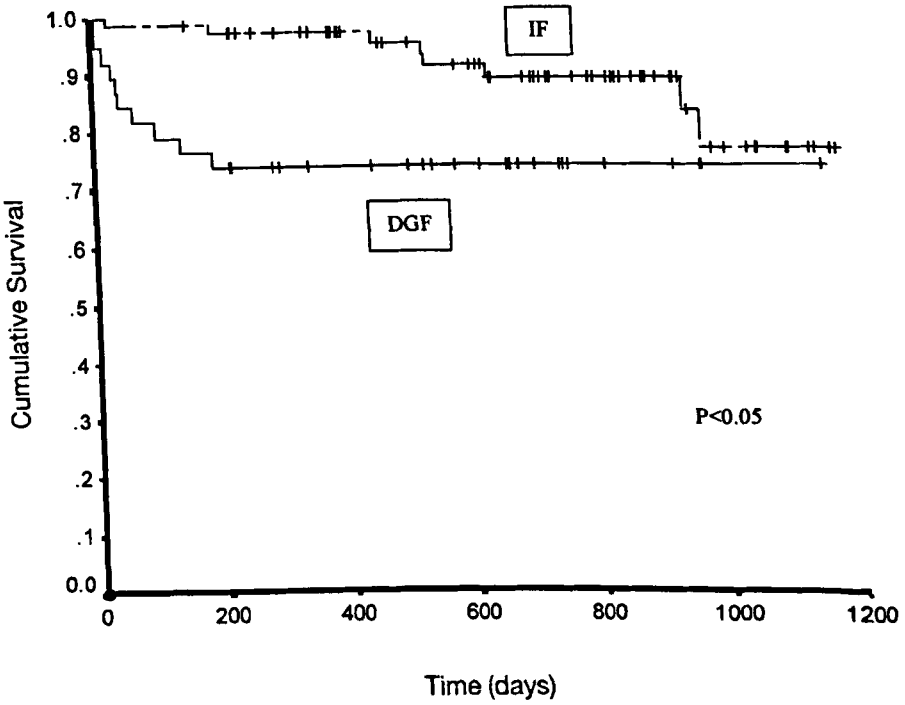

Fig. 1 Comparative graft survival between IF and DGF groups

Discussion

This study has demonstrated that acute rejection in the first month post-transplant has a similar incidence in patients with early and delayed graft function. However, over half the AR episodes that occurred in patients with DGF were not diagnosed clinically but were picked up only on protocol biopsies and indeed no episodes of rejection were diagnosed while patients remained on dialysis. This suggests that if protocol biopsies are not performed, diagnosis of sub-clinical acute rejection may be delayed or even missed completely in this group of patients.

The patients in the DGF group received a lower dosage of primary immunosuppressant (cyclosporine A or tacrolimus) than those with IF. It has been suggested that inadequate immunosuppression in patients with DGF may put them at increased risk of acute rejection and that in view of this, they should be given induction therapy with ATG or OKT3 [2]. As our patients did not receive this, it could be suggested as a reason for the high rate of sub-clinical rejection. However, the drug levels (albeit one-off measurements) suggest that the DGF patients were not inadequately immunosuppressed, and the overall rate of AR by 1 month was not higher in this group. Indeed, another study found a rate of sub-clinical rejection of 35% in patients with DGF despite induction therapy [1].

Although the significance of abnormal renal transplant histology in the absence of clinical correlates has been questioned [4], there is increasing evidence that sub-clinical AR is an important entity. The molecular features of biopsies showing sub-clinical AR are in keeping with an active inflammatory process [3, 7], and clinically stable patients with immune activation on urine flow cytometry have been shown to have impaired prognosis [8]. The first randomised study of the use of protocol biopsies in treating sub-clinical rejection was published recently and showed an improved functional and histological outcome in the biopsied group [9]. There remains controversy over the management of Banff borderline rejection [12]. A benefit for treating this in the setting of graft dysfunction has been shown [11], but its meaning in protocol biopsies is not clear, and at our centre these cases are managed on an individual basis. Recent studies on the underlying molecular changes in rejection may help to clarify the situation [14].

The development of acute rejection in association with DGF has been linked with the progression to primary non-function (PNF) [2]. In our study, PNF occurred in two of the six patients who had acute rejection on protocol biopsy (33%). In a previous study, a similar proportion (38%) of patients with AR on a 1-week protocol biopsy went on to have PNF [1]. It seems in these cases that even though sub-clinical rejection is being detected, treatment is futile and these are likely to be grafts that have sustained so much damage that they are never going to function.

There were no major complications from the renal transplant biopsies in this study, and only minor morbidity. This is in concordance with modern experience of

performing biopsies under ultrasound control. Because of the high incidence of sub-clinical acute rejection in patients with DGF it is therefore justifiable to perform protocol biopsies in this group, although they are probably not worthwhile if the graft functions immediately.

With the increasing use of marginal donors in renal transplantation, particularly non-heart beating donors as in this study, there is a possibility that DGF will be seen more frequently, and if this is the case the importance of early protocol biopsies will increase. Further studies are required to confirm that the treatment of sub-clinical acute rejection in patients with DGF has a positive influence on long-term outcome. These will need to be prospective and include large numbers of patients. It will be particularly important to ensure standard definitions of acute rejection (e.g. according to the Banff criteria), if such trials are to have external validity.

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