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ORIGINAL ARTICLE

Early subclinical rejection treated with low dose i.v. steroids is not associated to graft survival impairment: 13-years' experience at a single center

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Abstract Subclinical rejection (SCR) has been variably associated with reduced graft survival, development and progression of interstitial fibrosis/tubular atrophy and chronic allograft nephropathy, but data are controversial concerning SCR treatment in terms of graft survival improvement. In this single-center retrospective study, we enrolled 174 adult kidney transplant recipients with a protocol biopsy performed at 30 days after transplantation to evaluate the incidence rate and risk factors for early SCR and its impact on 10-year graft survival. Five patients showed primary non function and were excluded. Among 159/169 (94.08 %) patients with stable graft function who underwent protocol biopsy, 17 (10.7 %) showed signs of SCR and were treated with low-dose intravenous (i.v.) steroids. Ten patients showed functional impairment, 8 (4.73 %) resulting as acute rejection. At multivariate analysis, donor age [odds ratio (OR) 1.04, 95 % confidence interval (CI) 1.01–1.09], and delayed graft function (DGF) (OR 1.08, 95 % CI 1.03–1.12) were significantly associated with SCR. The 10-year graft survival rate in the SCR group was similar to that in the normal-findings group (76.5 vs. 74.9 % respectively; p = 0.61). At multivariate Cox regression, acute [hazard ratio (HR) 5.22, 95 % CI 1.70–16.01], but not sub-clinical, rejection was independently associated with long-term graft failure. In conclusion, early protocol biopsy is a useful and safe tool to detect early SCR which seems not to affect the long-term survival. We suggest that this could be, probably, linked to early SCR treatment with low dose i.v. steroids.

Keywords Graft survival · Kidney transplantation · Steroids · Subclinical rejection

Introduction

Subclinical rejection (SCR) is histologically defined as an acute rejection [1] characterized by tubulointerstitial mononuclear infiltration identified from a biopsy specimen without the presence of a functional deterioration [2, 3]. SCR is therefore, by definition, diagnosed only on biopsies taken as *per protocol* at a fixed time after transplantation, rather than driven by clinical indication.

SCR peaks during the two initial months of post-transplantation and declines thereafter [4]. The averaged prevalence of SCR in the first year post renal transplantation depends on protocol biopsy strategy variation between nephrology units [4], ranging from 29 % at 1 month to 17 % at 1 year [2, 5].

The long-term impact of SCR on renal graft function remains poorly understood, notwithstanding it was previously suggested as having a significant impact on allograft outcome [6–9]. Indeed, SCR has been variably associated with impaired renal function at 6 and 12 months after transplantation [8, 10], reduced graft survival [8, 9, 11–13], development and progression of interstitial fibrosis and tubular atrophy (IF/TA) [14], and chronic allograft nephropathy (CAN) [2, 8–10, 15], which is the most common cause of late renal allograft failure. Nevertheless, biopsies with SCR as early as 2 weeks can distinguish survival differences when followed to 10 years [11].

Therefore, early detection and treatment of SCR should reduce the incidence of CAN and increase graft survival, but controversial data exist about SCR treatment efficacy [16, 17] even if the kidney disease/improving global outcomes (KDIGO) guidelines recommend, with a low grade of evidence, to treat subclinical and borderline acute rejection [18].

The aims of our study were, first, to evaluate the incidence of early SCR revealed by protocol biopsy at 1 month post kidney transplantation and the factors potentially involved in this manifestation; and, secondly, to evaluate the association between SCR precociously treated with low-dose intravenous (i.v.) steroids and long-term graft survival.

Materials and methods

This was a single-center retrospective study performed at our Renal Transplant Unit at Annunziata Hospital in Cosenza, Italy. Between July 2000 and December 2013, 174 adult patients received a primary kidney transplant from deceased donors. A protocol biopsy was performed on patients with stable graft function at day 30 after transplantation. Stable graft function in the recipients was defined as an increase between 0 and 15 % in serum creatinine in the 5 days before protocol biopsy. Core renal biopsies were taken using an 18-gauge biopsy gun using ultrasound localization. Primary non function was diagnosed in 5 patients, who were excluded from the analysis. Of the 169 remaining, 159 patients underwent protocol biopsy while 10 patients presented, within the first month post transplantation, a clinically suspected acute rejection episode that was confirmed by a percutaneous graft biopsy in 8 patients, while the other two showed acute cyclosporine toxicity.

Renal biopsy slides were scored by two independent figures (a pathologist and a nephrologist) according to the Banff 97 working classification [1]. To minimize inter-observer variability, every 10th biopsy was scored by both doctors and the findings discussed. All biopsy specimens included five or more glomeruli. Presence of SCR was based on the absence of functional deterioration and histologic findings indicative of rejection on the basis of the tubulitis ('t') and mononuclear cell infiltration ('i') scores. All findings of 't' score ≥ 1 and 'i' score >0 in the absence of functional deterioration were classified as SCR.

Patients with SCR were treated with i.v. steroid pulse therapy (methylprednisolone (Solu-Medrol, Pharmacia, The Netherlands) 250 mg/day for three consecutive days. An acute rejection episode was treated with methylprednisolone 500 mg/d for three consecutive days or anti-thymocyte globulin as clinically and histologically indicated.

All participants received induction treatment based on basiliximab, steroids, mycophenolate mofetil (MMF) followed by maintenance with calcineurin inhibitors (after day 2 post transplantation), steroids and MMF. Tacrolimus was used in 113 patients and cyclosporine (CsA) in 61. We used the dose of tacrolimus achieving 12-h trough levels of 10 (5–15) ng/ml [18, 19]. Standard-dose CsA may be defined as the dose achieving C0 of 200 (150–300) ng/ml or C2 1400–1800 ng/ml early and 800–1200 ng/ml later after transplantation [20, 21]. The initial dose of MMF was 1.5 g/day, and the dose was modified to minimize adverse effects such as diarrhea or leukopenia.

Delayed graft function (DGF) was defined as necessity for dialysis in the first week after surgery. Cytomegalovirus (CMV) infection was defined as detection of viremia >650 copies/ml of whole blood. All transplant patients were treated according to a preemptive strategy, consisting of the initiation of antiviral treatment upon detection of a viral load (CMV-DNAemia) above 5,000 copies/ml. Anti-CMV preemptive treatment included i.v. administration of ganciclovir (5 mg/kg daily) adjusted for renal function. Antiviral therapy was considered successful when two sequential negative CMV-DNAemia tests were obtained.

Our 174 patients were divided into normal, SCR and acute rejection groups according to the Banff classification. The clinical characteristics observed among three groups were compared with respect to the age and sex of recipients and donors, the duration of dialysis before transplantation, the total number of human leukocyte antigen (HLA) mismatches, types of immunosuppressant used, presence of DGF, incidence of CMV infection and the cold ischemia time. Clinical outcomes of each group were compared with respect to graft survival (10 years). Graft survival was calculated using the Kaplan–Meier method and compared for statistical significance using the log-rank method. Cox proportional hazards regression models were used to evaluate factors associated with graft survival. Chi-Square, Student's t tests or logistic regression were used for other comparisons. A p value <0.05 was assumed statistically significant.

The design of the study was approved by the local ethical committee. All patients enrolled provided written informed consent, and the study was conducted in conformity with the declaration of Helsinki and guidelines of the local ethical committee.

Results

The main demographic and baseline characteristics at 30 days post transplantation (time point for protocol biopsy) of all 174 participants are shown in Table 1. Five patients showed primary non function due to venous

thrombosis in three cases, arterial thrombosis in one case and in one patient both vessels were thrombosed. DGF was diagnosed in 69 (39.7 %) cases. The majority of transplant recipients (127, 74.1 %) exhibited three or four mismatches at the time of transplantation.

Among the 159 out of 169 patients (94.08 %) who showed stable graft function, protocol biopsy revealed normal findings in 142 patients (89.30 %), while 17 patients (10.7 %) showed SCR. Among those with functional alterations, 8 patients (4.73 %) showed acute rejection according to the Banff classification while 2 patients presented acute cyclosporine toxicity and were added to the normal findings group. The comparison of the selective mean Banff scores according to the status of the graft (normal, SCR or acute rejection) is shown in Table 2. The Banff component scores were significantly higher in the groups with SCR and acute rejection than in the normal

findings group, without a significant difference between the subclinical and acute rejection groups. Nevertheless, there was no correlation between elementary lesions and renal function at day 30. Incidence of SCR, as well as of acute rejection, did not differ significantly between tacrolimus and cyclosporine treated patients. In terms of complications after the biopsy procedure, we registered only three cases of peri-renal hematoma and one fistula.

A comparison between the clinical characteristics of normal and SCR patients is shown in Table 3. There were no differences between the recipients in terms of age or sex. Serum creatinine levels at the time of graft biopsy did not differ between groups. The duration of dialysis before transplantation, cold ischemia time, number of mismatches, type of calcineurin inhibitors, CMV infection and date of transplantation did not affect the histological findings. However, patients with subclinical rejection showed more DGF events and higher donor age than the normal findings group ($p < 0.05$).

Table 4 shows the results of the multivariate analysis of factors associated with subclinical rejection. Factors significantly associated with SCR were donor age with an odds ratio (OR) of 1.04 [95 % confidence interval (CI) 1.01–1.09], and DGF with an OR of 1.08 (95 % CI 1.03–1.12).

The 10-year graft survival rate in the subclinical rejection group was similar to that of the normal findings group (68.8 vs. 74.9 % respectively; $p = 0.27$), while the acute rejection group had a significantly lower 10-year graft survival (31.1 %, $p < 0.001$) (Fig. 1). At multivariate Cox regression, donor age [hazard ratio (HR) 1.03, 95 % CI 1.01–1.05], DGF (1.57, 1.04–2.22) and acute (5.22, 1.70–16.01), but not sub-clinical, rejection were independently associated with 10-year graft failure (Table 5).

We observed 13 (7.5 %) cases of patient death due to cardiovascular accident (61.5 %), neoplasia (23.1 %) and infection (15.4 %).

Discussion

Our study suggests that early protocol biopsy is a useful and safe tool to detect early SCR which seems not to affect the long-term survival, in contrast to acute rejection.

Table 1 Main demographic and baseline characteristics of the entire study group (174 kidney transplant recipients)

Variable	
Gender (male)	104 (59.8 %)
Time on dialysis (months)	73.73 ± 53.6
eGFR (ml/min)	59.36 ± 12.6
Donor age (years)	44.98 ± 15
Recipient age (years)	46.15 ± 12.2
Cold ischemia time (h)	12.04 ± 4.1
Delayed graft function	
Primary non function	5 (2.9 %)
0 days	100 (57.5 %)
1–5 days	21 (12.1 %)
6–10 days	17 (9.8 %)
>10 days	31 (17.8 %)
Mismatches (MM)	
0–2	33 (19 %)
3–4	127 (74.1 %)
5–6	14 (7 %)
Body mass index (kg/m^2)	23.7 ± 3.8
Cyclosporine/tacrolimus	61/113
Cytomegalovirus infection (yes/no)	61/113

eGFR estimated glomerular filtration rate

Table 2 Comparison of the selective mean Banff score according to graft status: normal histological findings vs. subclinical rejection (SCR) vs. acute rejection

Banff component score	Normal (n. 144)	SCR (n. 17)	Acute rejection (n. 8)	p
i (interstitial inflammation)	0.6 ± 0.7	1.7 ± 0.5[a]	2.1 ± 0.8[a]	<0.01
t (tubulitis)	0.4 ± 0.8	1.6 ± 0.7[a]	1.5 ± 0.9[a]	<0.01

[a] vs. normal

Table 3 Comparison of the clinical characteristics of patients with normal histological findings and SCR patients

	No acute rejection (n. 144)	Subclinical rejection (n. 17)	p value
Gender (M:F)	84:60	10:7	0.983
Time on dialysis (months)	71.63 ± 53.27	70.35 ± 45.65	0.797
eGFR (ml/min)	61.22 ± 11.9	58.87 ± 19.5	0.611
Donor age (years)	42.89 ± 15.13	53.16 ± 12.88	0.003
Recipient age (years)	46.09 ± 12.3	46.24 ± 13.18	0.398
Cold ischemia time (h)	12.15 ± 4.33	11.6 ± 3.74	0.830
Delayed graft function (days)			
0	96 (66.7 %)	4 (24 %)	<0.001
1–5	18 (12.5 %)	3 (18 %)	
6–10	15 (10.4 %)	3 (18 %)	
>10	15 (10.4 %)	7 (40 %)	
Mismatches (MM)			
0–2	26 (18.1 %)	4 (23.5 %)	0.418
3–4	106 (73.6 %)	11 (64.7 %)	
5–6	12 (8.3 %)	2 (11.8 %)	
Body mass index (kg/m^2)	23.41 ± 3.75	24.43 ± 3.89	0.291
Cyclosporine/tacrolimus	51/93	6/11	0.202
Cytomegalovirus infection (yes/no)	51/93	10/7	0.069

eGFR estimated glomerular filtration rate

Table 4 Multivariate logistic regression to identify risk factors for subclinical rejection

	OR (95 % CI)	p
Donor age (years)	1.04 (1.01, 1.09)	0.025
Recipient age (years)	0.98 (0.94, 1.02)	0.332
Cold ischemia time (h)	0.96 (0.85, 1.09)	0.576
Delayed graft function (days)	1.08 (1.03, 1.12)	<0.001
Mismatches (number)	0.89 (0.52, 1.54)	0.686
Cytomegalovirus infection	1.23 (0.36, 2.01)	0.496

OR odds ratio, *CI* confidence interval

Indeed, we did not find any survival difference at 10 years between the steroid-treated SCR group and the normal findings group while the acute rejection group had a significantly lower 10-year graft survival and acute rejection was independently associated with graft survival. We speculate, based on the current literature, that this could be probably linked to our strategy of early SCR treatment with low-dose pulse i.v. methylprednisolone therapy.

Evidence linking SCR with reduced graft survival in children and adults is supported by findings from long-term studies [8, 9, 11–13]. Choi et al. showed a marked 10-year graft survival difference between patients with untreated SCR (obtained from biopsy performed at day 14 after transplantation) and patients with normal histological findings. They clearly demonstrated that the incidence of acute rejection increased and graft survival rate decreased in patients showing untreated subclinical rejection [11].

Thierry et al. reported a similar difference in terms of estimated glomerular filtration rate (eGFR) at 30 months post transplantation [22]. Only a few studies have focused on SCR treatment in terms of graft survival improvement, and in the published literature, to our knowledge, there are few randomized controlled trials (RCTs), reporting conflicting data. In one of these, Rush and colleagues randomized 72 patients to protocol biopsies at 1, 2, 3, 6 and 12 months (biopsy group) or to 6- and 12-month biopsies only (control group), and followed them for a minimum of 2 years. They concluded that the detection and treatment of subclinical acute rejection resulted in better graft function [16]. Conversely, in a multicenter RCT of 218 patients, protocol biopsies and treatment of subclinical acute rejection were not beneficial [23]. In our retrospective cohort, independently of histological score and immunosuppressive strategy, the choice to treat SCR with i.v. pulse steroid could have led to a graft survival improvement in SCR groups, possibly explaining the absence of any significant difference in SCR group graft survival with respect to the normal findings group.

Early SCR treatment could prevent the acute rejection onset and so ameliorate the graft survival. Indeed, Roberts et al. [24] reported that most patients whose day 7 biopsy specimens showed SCR go on to develop clinical rejection if left untreated, and this was confirmed by Park et al. who reported that 20 % of recipients with borderline changes experienced acute rejection within 6 months [25]. Schweitzer et al. [26] reported that borderline changes frequently represent acute rejection, and that anti-rejection

Fig. 1 Graft survival rate in patients with normal histological findings, subclinical rejection and acute rejection at 30 days protocol biopsy
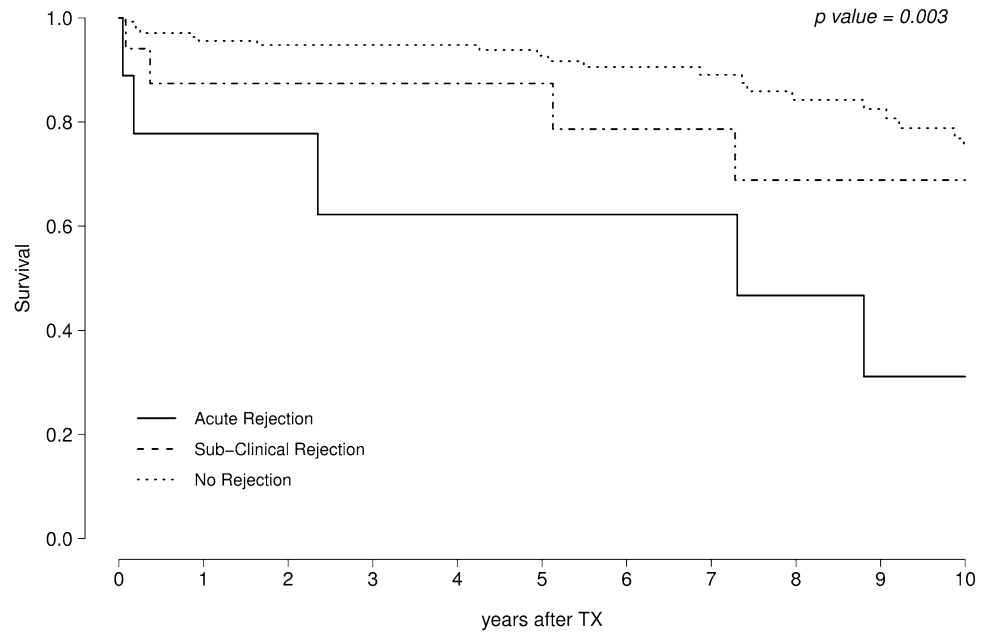
Table 5 Cox proportional hazard models of transplant failure

	HR	95 % CI
Recipient age (years)	1.004	0.969–1.041
Donor age (years)	1.027*	1.006–1.049
DGF (vs. no DGF)	1.568*	1.039–2.223
Sub-clinical rejection (vs. no rejection)	1.609	0.464–5.581
Acute rejection (vs. no rejection)	5.221*	1.703–16.008

* $p < 0.05$

HR hazard ratio, *CI* confidence interval, *DGF* delayed graft function

treatment is therefore appropriate in most cases. Our results also are in line with previous data reporting that recipients with SCR who were treated with anti-rejection therapy showed significant improvements in graft function compared to patients without anti-rejection treatment [27]. Moreover, in our renal transplantation unit we chose, as therapeutic protocol of SCR, i.v. pulse 250 mg of methylprednisolone which represents a relatively low dose of steroids. This strategy appeared useful to ameliorate long-term graft survival in the SCR group, probably preventing acute rejection onset, without exposing patients to the side effects of a high dose of steroids.

Our findings underlie the usefulness of establishing an early time-point for graft biopsy as part of the protocol biopsy strategy to ameliorate late graft survival. One of the concerns of protocol biopsies is that they may lead to a significant risk of graft failure and that this risk is greater than the potential benefit for the patients. However, several studies [28, 29] have shown that the benefits of protocol biopsy could exceed the risks, and that biopsy is the most

accurate tool for detecting subclinical rejection. According to previous reports, in our study we found no major problems caused by biopsy-related complications [11]. With regard to biopsy timing, we chose 1 month after transplantation because graft function is generally normalized within this time-frame and usually at day 30 patients begin the in-hospital follow-up after discharge.

We observed an incidence for SCR of 10.7, and 4.73 % for acute rejection after 1 month post transplantation, data similar to those reported in the literature even if there are few epidemiological reports on the incidence of early acute rejection.

SCR can be sub-classified into 'acute' SCR (Banff i2 and t2 or worse) or milder 'borderline' SCR (e.g. i1 and t1), synonymously designated as 'suspicious for acute rejection' [1]. We chose not to sub-classify SCR because we think that this histological difference should not influence the therapeutic approach. Indeed, also borderline changes should not be regarded as harmless, since previous studies have shown that their presence is associated with an incidence of clinical acute rejection double that of patients without these changes [11] and a worse renal function 5 years after transplantation [6]. In addition, the presence of SCR in protocol biopsies has been consistently associated with the progression of IF/TA, supporting the notion that persistent interstitial inflammation is harmful for the allograft [14]. Even very low-grade inflammation has been associated with progression of chronic tubulointerstitial damage. 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.

At multivariate regression analysis, we found donor age and DGF to be predictors of early SCR development. Previous studies also associate HLA mismatches (in particular HLA DR mismatch) with cold ischemia time to SCR development [11, 30]. Rush et al. [30] performed protocol biopsies at 1, 2, and 3 months after renal transplantation, and found that the incidence of subclinical rejection in the first protocol biopsy was 20, 30 and 63 % in patients with zero, one and two HLA-DR mismatches, respectively. Choi et al. reported that, in living donor transplantation, the incidence of subclinical rejection increased with the increase in the number of HLA-DR mismatches (2.7, 15.4 and 20.8 % in patients with zero, one and two HLA-DR mismatches, respectively) [11]. Probably this association is lacking in our cohort because the majority of our patients presented 3–4 mismatches and a short average cold ischemia time.

Our study has several limitations, the chief one being the lack of an SRC group without steroid treatment as control. To overcome this, we used previous study reports even if it is difficult to compare studies of protocol biopsy findings due to the wide variations in patient demographics, utilization of induction therapy, timing of the biopsy and selection of calcineurin inhibitor.

In conclusion, an early protocol biopsy performed 1 month after renal transplantation is a useful tool to detect subclinical rejection and we suggest that anti-rejection treatment for SCR, with low-dose i.v. pulse steroids, could be an appropriate strategy to improve kidney transplant graft survival in the long term even if this therapeutic approach needs to be confirmed through a clinical trial.

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