

# Risk Factors for Steroid-Resistant T-Cell-Mediated Acute Cellular Rejection and Their Effect on Kidney Graft and Patient Outcome

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## Abstract

**Objectives:** Acute rejection in renal transplant is considered a risk factor for short-term and long-term allograft survival. The expected reversal rate for the first acute cellular rejection, by steroid pulse, ranges between 60% and 100%, and lack of improvement within 1 week of treatment is defined as *steroid-resistant rejection*. This work sought to evaluate factors that lead to steroid-resistant acute cellular rejection among patients with first live-donor renal allotransplant and its effect on graft and patient survival.

**Materials and Methods:** Patients with an improvement in serum creatinine levels were considered controls (group 1; n=100); while the others were considered an early steroid-resistant group (group 2; n=99). Both groups were matched demographically.

**Results:** Patients with a target cyclosporine level below accepted therapeutic levels were significantly higher in group 2 ( $P = .02$ ). We found no significant differences between the groups regarding posttransplant complications ( $P > .05$ ). Mean hospital stay was longer in group 2 ( $P = .021$ ). Living patients with functioning graft were more prevalent in group 1, while those alive on dialysis were more prevalent in group 2. The groups were comparable regarding long-term patient and graft survival despite significantly lower creatinine values in patients of group 1 at 6 months' follow-up ( $P \leq .001$ ).

**Conclusions:** Prebiopsy low cyclosporine trough levels and associated chronic changes among patients who were maintained on calcineurin inhibitor-based regimens represented the most-important risk factors for the early steroid-resistant group. Rescue therapies improve short-term graft outcome; however, they did not affect either patient or long-term graft survival after 5 years' follow-up.

**Key words:** Steroid resistant, Acute rejection, Long-term graft outcome, Risk factors

## Introduction

Renal transplant is the criterion standard for treating end stage renal disease and has the greatest potential for restoring a healthy and productive life.<sup>1</sup> Immunologic rejection is one of the most-important complications affecting a transplanted kidney. Three major forms of rejection are recognized: hyperacute, acute, and chronic. Each had its own distinctive changes.<sup>2</sup>

The most-common form of immunologic rejection in the early posttransplant period is acute cellular rejection, which is mediated predominantly by host lymphocytes responding to the allogeneic donor kidney. Acute rejection typically occurs 5 to 7 days after the transplant, but it could occur at virtually anytime afterwards, with the highest incidence in the first 3 months. Overall rates of rejection varied from 10% to 50% within the first 6 months depending on human leukocyte antigen (HLA) matching and immunosuppressive protocol.<sup>3</sup>

Acute rejection in renal transplant is considered a risk factor for short-term and long-term allograft survival. Since the introduction of new immunosuppressive agents, progressive decrease in the incidence of rejection episodes, and subsequent

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improvement in graft survival have been observed. However, these beneficial effects on early graft survival have less effect on late attrition rate, due mainly to chronic transplant nephropathy and patient death with functioning grafts.<sup>4,5</sup>

The incidence of chronic rejection is less than 1% in those patients with no episodes of acute rejection, but it has reached 20% among living-related transplants if acute rejection occurs within 60 days of the transplant, and 43% if acute rejection occurs later than 60 days of transplant.<sup>6</sup>

The expected reversal rate for the first episode of acute cellular rejection, by steroid pulse, ranges between 60% and 100%,<sup>7,8</sup> and lack of improvement in urine output or the plasma creatinine concentration within 5 to 7 days after treatment is defined as *steroid-resistant rejection*.<sup>9</sup> Rabbit anti-thymocyte globulin has been found to be more effective than horse anti-thymocyte globulin in reversing rejection (88% vs 76%) with a lower rate of recurrent rejection (17% vs 36%).<sup>10</sup> In another study,<sup>11</sup> low-dose anti-thymocyte globulin and low-dose orthoclone were found to be equally effective in reversing steroid-resistant acute rejection. Mycophenolate mofetil has been successfully used to treat steroid-resistant rejection, but only of the interstitial (cellular) type. Switching from cyclosporine to tacrolimus to treat recurrent or antibody-resistant rejection was successful in approximately 60% of cases. Plasmapheresis and intravenous immunoglobulin have been used in some cases, with much success.<sup>12-14</sup>

### **Aim of the work**

This work sought to evaluate factors that lead to steroid-resistant acute cellular rejection among patients with first live-donor renal allotransplant and its effect on graft and patient survival.

### **Patients**

All protocols were approved by the ethics committee of the institution before the study began, and the protocols conformed with the ethical guidelines of the 1975 Helsinki Declaration. Written, informed consent was obtained from all patients. Of 1691 live, renal transplanted patients at Mansoura Urology and Nephrology Center, Egypt, between March nineteen seventy-six and December two thousand four, 199 patients (154 males, 48 females; aged, 28.8-29 ± 9 y) who experienced 1 episode of acute

allograft rejection were selected for this retrospective study.

All patients experienced at least 1 episode of acute cellular rejection; received 500 mg intravenous pulse steroid for at least 5 days without any adjuvant therapy or increased basal immunosuppression. Patients with improvement of serum creatinine level to basal level or above level by 10% were considered controls (group 1; n=100), while those without improvement were considered early steroid-resistance (group 2; n=99). We excluded high-risk patients, retransplant recipients, pediatric transplants (younger than 15 years), and cases with different mechanisms of rejection as acute antibody-mediated rejection.

Patients in both groups were subdivided according to the primary immunosuppressive regimens into 2 subgroups: Noncalcineurin-based and calcineurin-inhibitor-based. All renal transplants received their grafts from live donors, warm ischemia ranged from 1 to 2 minutes, and mean cold ischemia was 40 minutes.

### **Immunosuppression protocols**

Patients before 1983 received corticosteroid and azathioprine (2 mg/kg/d) with subsequent tapering of the dosage. After 1983, other protocols evolved overtime. Cyclosporine was introduced with 2 main protocols: Cyclosporine 12 mg/kg/d and prednisolone, or triple therapy including cyclosporine 10 mg/kg/d, prednisolone, and azathioprine (1 mg/kg/d). Cyclosporine dosage was adjusted to keep the cyclosporine trough level between 200 and 400 ng/mL during the first 2 months of transplant and between 125 and 175 ng/mL, thereafter. Cyclosporine trough level was measured at first using radioimmunoassay kits (Sandoz AG, Basel, Switzerland) and then using a monoclonal specific antibody (Abbott Laboratories, Abbott Park, IL, USA).

In the 1990s, tacrolimus was introduced in a dosage of 0.15 mg/kg/d and/or mycophenolate mofetil in a dosage of 2 g/d. The tacrolimus dosage was adjusted to achieve a trough level between 5 and 10 ng/mL using the MIA technique (Abbott Diagnostics). All acute rejection episodes (control group) were documented by histopathologic examination of graft biopsies and treated by methylprednisolone pulses 750 mg/d for azathioprine group and 500 mg/d for cyclosporine

or tacrolimus-treated group. All biopsies were re-scored retrospectively according Banff 2007. Cases with morphologic criteria suggesting antibody-mediated rejection (eg, acute tubular necrosis, vasculitis, and those with borderline suspicious findings) were confirmed by either C4d immunoperoxidase staining (Figure 1) and/or anti-HLA antibodies by enzyme-linked immunosorbent assay, and all were all excluded from the study. Pulses were given for 5 days and then were tapered to the basal maintenance prednisolone dose. Steroid-resistant rejections were treated by monoclonal antibody therapy, anti-thymocyte globulin, or orthoclone (Figure 2). From June 1998, induction therapy was introduced in the form of basiliximab, daclizumab, or anti-thymocyte globulin according to the immunosuppressive protocol adopted at that time. Sirolimus was recently introduced to immunosuppressive protocols since 2001 with a starting dose of 5 to 10 mg/d, then the dosage was adjusted according to the blood level.

Secondary and tertiary immunosuppression was defined as *the first and second modification of maintenance (primary) immunosuppressive protocol*

Figure 1. C4d Positive Vs Negative Staining of the Peritubular Capillaries

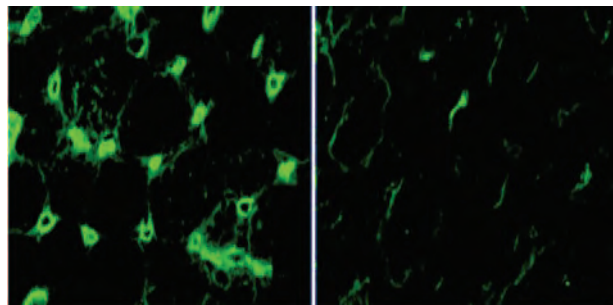
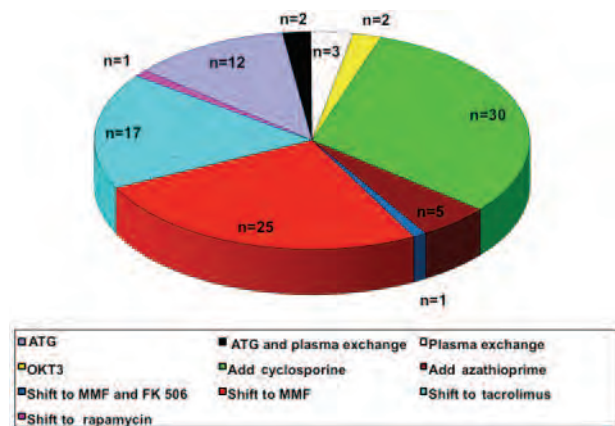


Figure 2. Management of Steroid-Resistant Rejection After Pulse Steroid



Abbreviations: ATG, anti-thymocyte globulin; FK 506, tacrolimus; MMF, mycophenolate mofetil; OKT3, Muromonab-CD3

owing either to the need to increase the level of immunosuppression or drug toxicity.

## Methods

Clinical records of all kidney transplant recipients were reviewed for demographic data including recipient age and sex, donor age and sex, causes of end-stage renal failure, HLA mismatching, pretransplant hypertension and schistosomiasis, and type of immunosuppression. All episodes of rejection were verified by biopsy and graded using the BANFF classification 2007.<sup>15</sup> Severity and response to treatment of acute rejection, and other medical complications (eg, hypertension, serious infections, malignancies, and diabetes mellitus) were evaluated. (Diagnoses were based on clinical suspicious and confirmed by biopsy.)

Follow-up data were recorded, especially date of transplant, basal serum creatinine, peak serum creatinine during rejection episode, serum creatinine at 3, 7, 14, and 30 days after pulse steroid, and 3, 6, and 12 months after the transplant.

## Patient follow-up

In each follow-up visit, each patient was given a clinical examination, with special emphasis on blood pressure, organomegaly, and neurologic evaluation. Laboratory investigations included creatinine and creatinine clearance using Cockcroft and Gault formula, tacrolimus whole blood trough level, liver function tests, and fasting and postprandial venous plasma glucose levels.

## Statistical analyses

Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 11.5, IBM Corporation, Armonk, New York, USA). Qualitative data are presented as number and percentage, while quantitative data are presented as mean and standard deviation. The *t* test was used to compare means and standard deviations of the 2 groups. Categorical data were compared using the chi-square test. Graft and patient survival were computed using the Kaplan-Meier technique. The difference in intensity of histologic changes of more than 2 groups was tested with Kruskal-Wallis nonparametric test, and for 2 groups with the Mann-Whitney *U* test. Multivariate logistic stepwise regression analysis was performed. Values for *P* less than .05 were considered significant.

## Results

Table 1 illustrates the donors' and the recipients' characteristics. The majority of recipients were men in their 20s, while the majority of the donors were women in their 30s. Also, the 2 groups were homogenous in terms of donor's age, sex, recipient age, prior blood transfusion, HCV status, original kidney diseases, tissue typing, and pretransplant hypertension. No preformed antibodies against donor antigens were detected in the pretransplant crossmatch of any of the studied patients. Moreover, the 2 groups were matched regarding ischemia time. Primary immunosuppression was maintained with calcineurin inhibitor-based triple regimen.

We observed that cases with proteinuria (as detected by a dipstick test; mg/dL) and hemoglobin (g/L) were comparable between the groups

**Table 1.** Demographic Data of Patients

	Group 1 (n=100) n (%)	Group 2 (n=99) n (%)	P Value
Recipient sex (male/female)	76/24	75/24	.905
Recipient age (mean ± SD)	29.04 ± 9.8	28.8 ± 10.3	.873
Donor sex (male/female)	41/59	46/53	.466
Donor age (mean ± SD)	36.09 ± 9.6	36.1 ± 10.3	.963
Related donor (frequency, %)	91 (85.8)	91 (90.1)	.431
Preemptive cases (frequency, %)	9 (8.5)	7 (16.6)	.52
Pretransplant hypertension	57/100 (62.5)	57/99 (59.5)	.688
Same blood group (frequency, %)	75 (70.8)	83 (82.2)	.532
HLA matching			
0 Match	44	5 (5.1)	
1 Match	22 (22)	19 (19.1)	
2 Matches	63 (63)	60 (60.6)	
3 Matches	99	10 (10.1)	
4 Matches	2 (2)	5 (5.1)	.772
DR match			
1 Match	14 (14)	93 (94)	
2 Matches	1 (1)	6 (6)	.46
Original kidney disease			
Glomerulonephritis	9 (9)	14 (14)	
Tubulo-interstitial disease	14 (14)	19 (18.8)	
Adult polycystic kidney disease	0 (0)	3 (3)	
Idiopathic	77 (77)	64 (63.2)	.431
Immunosuppression protocols			
Induction vs no induction	19/100	16/99	.35
Noncalcineurin inhibitor-based	24 (24)	27 (27.2)	.682
Calcineurin inhibitor-based	76 (76)	72 (71.8)	.682
Ischemia time (min)	45.9	45.6	.99
Time to diuresis			
< 2 min	88 (88)	94 (96)	
> 2 min	12 (12)	4 (4)	.81

**Abbreviations:** DR, HLA-DR locus; HLA, human leucocyte antigen

( $P > .05$ ). However, although mean hemoglobin in patients early steroid resistance in group 1 was lower than that detected in the control group, this difference was not significant ( $P > .05$ ). Patients with below target cyclosporine levels were significantly higher in group 2 (Table 2;  $P = .02$ ). We found no significant differences between the 2 groups regarding posttransplant malignancies, hepatic impairment, new onset diabetes posttransplant, hypertension, bacterial infections, or previous acute rejections (all were steroid sensitive;  $P > .05$ ). However, mean hospital stay (mo) was significantly longer in group 2 (Table 3;  $P = .021$ ). Late chronic rejection was more prevalent in patients in group 2, but this was not significant ( $P > .05$ ).

We found that living patients with a functioning graft were more prevalent in group 1, while those maintained on dialysis were more prevalent in group 2 (Table 4). However, patients who died, regardless the graft function, were more prevalent in group 2, but none of these was significant.

We observed that patient survival at 5 years was 88.2% ± 3.3% and 85.4% ± 3.7% in group 1 and

**Table 2.** Number of Patients With Significant Laboratory Findings

	Group 1 (n=100) n (%)	Group 2 (n=99) n (%)	P Value
Proteinuria (mg/dL)	17 (17)	14 (14)	.081
Hemoglobin (g/L)	85 g	92	.71
	(n=76)	(n=72)	
Cyclosporine level			
Above target window	8 (10.0)	4 (4.2)	.159
Within the target	53 (67.7)	39 (55.6)	.148
Below the target	18 (22.8)	29 (40.3)	.02

**Table 3.** Posttransplant Medical Complications

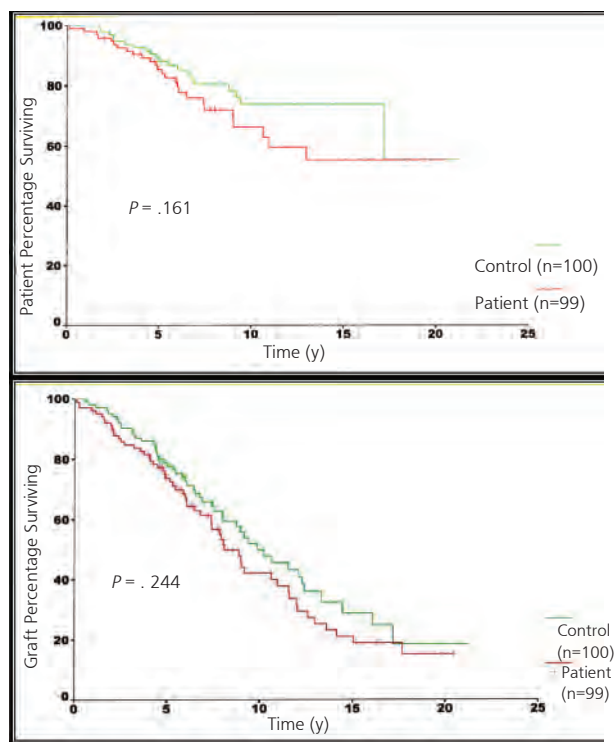
	Group 1 (n=100) n (%)	Group 2 (n=99) n (%)	P Value
Hypertension	79 (79)	84 (83.1)	.14
Diabetes mellitus	14 (14)	17 (16.8)	.5
Hepatic	89 (89)	93 (92)	.27
Serious infections	94 (94)	95 (94)	.81
Malignancy	7 (7)	4 (3.9)	.27
Hospital stay (mo)	0.6	1.4	.021
Rejection data			
Before resistant rejection			
No rejection	76 (76)	89 (89.8)	.119
1 rejection	23 (23)	15 (15.1)	.109
2 rejections	2 (2)	1 (1)	.53
3 or more rejections	0 (0)	1 (1)	
At the time of the study			
Borderline changes	29 (27.4)	33 (32.7)	.49
Acute T-cell-mediated rejection	76 (71.6)	61 (60.4)	.56
Mixed (ACR and CAN)	1 (0.94)	7 (6.9)	.025
Late chronic rejection	34 (32)	41 (40.5)	.323

**Abbreviations:** ACR, T-cell mediated acute cellular rejection; CAN, calcineurin inhibitor

**Table 4.** Patient Condition at the Final Follow-Up

	Group 1 (n=100) n (%)	Group 2 (n=99) n (%)	P Value
Living patients with function graft	53 (53)	42 (41.6)	.35
Living patients on dialysis	31 (31)	32 (31.7)	.70
Dead patients with function graft	6 (6)	10 (9.9)	.37
Dead patients on dialysis	16 (16)	17 (16.8)	.73

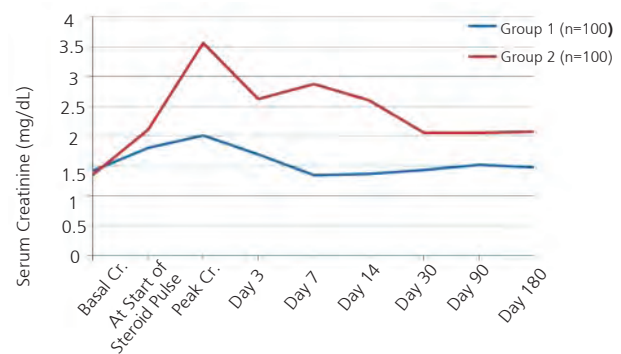
group 2, but this was not significant at 20 years ( $P = .224$ ). Similarly, graft survival at 5 years was  $77.7\% \pm 4.2\%$  and  $73.6\% \pm 4.5\%$  in groups 1 and 2, and this declined gradually until 20 years without a significant difference (Figure 3;  $P = .161$ ).

**Figure 3.** Patient and Graft Survival in the Recipients

We observed that graft function, represented by serum creatinine (Figure 3) was comparable in both groups regarding basal creatinine until the onset of rejection and its management. Thereafter, mean serum creatinine levels were significantly lower in patients of group 1 only until the end of 6 months (Figure 4;  $P \leq .001$ ).

## Discussion

Successful kidney transplant improves the quality of life and reduces mortality for most patients when

**Figure 4.** Follow-Up Serum Creatinine Levels

compared with maintenance dialysis.<sup>16</sup> The incidence of acute rejection and the time it occurs varies with the immunosuppressive therapy. Many centers achieve acute rejection rates below 15%; some below 10%.<sup>17</sup> Acute rejection episodes also have a negative effect on long-term graft survival, being the major predictor of chronic rejection, which is responsible for most death-censored graft loss after the first year posttransplant.<sup>18</sup> Steroid-resistant acute cellular rejection is considered when there is a lack of improvement in urine output or the plasma creatinine concentration within 5 to 7 days after starting pulse steroids.<sup>19</sup>

In this retrospective study, we sought to assess factors responsible for steroid-resistant acute cellular rejection among live-donor renal allotransplants compared with a well-matched steroid-sensitive group. Our incidence of acute cellular rejection was 38.9%, and 15.3% of them were defined as *steroid-resistant episodes*. The relatively high incidence of rejection might be due to genotypic heterogeneity of Egyptian patients. Moreover, the difference in pharmacokinetics of cyclosporine among our patients might be due to endemic hepatic disorders (eg, schistosomiasis and hepatitis C) in addition to the nephrotoxic effects of earlier high levels of cyclosporine.

Although mismatching at the rhesus blood group antigen (Rh) was not considered a risk factor for allograft rejection, a multivariate analysis of UNOS data did find that Rh incompatibility might confer worse long-term survival.<sup>20</sup> Moreover, most of our patients received their renal allografts from donors with the same or matched blood group and therefore, we found no significant effect of blood group on acute rejection episodes or their steroid responsiveness among the groups ( $P = .532$ ).

Over the last 10 years, most transplant centers do not routinely give pretransplant transfusions because of the widespread use of erythropoietin, concerns about the risk of transfusion-related infections, and sensitization.<sup>21</sup> We found that most of patients (> 70%) who received pretransplant blood transfusions developed early steroid resistance ( $P = .02$ ). Moreover, more than 70% of early steroid resistance received frequent pretransplant blood transfusions (> 5 times) and developed chronic late rejection ( $P = .004$ ). On the other hand, more than 60% of the patients who did not receive blood transfusion enjoyed a functioning graft at last follow-up ( $P = .004$ ). This negative effect of pretransplant blood transfusion was matched with a Dutch national study reported by Aalten and associates,<sup>22</sup> who showed that prekidney-transplant blood transfusions did not improve transplant outcome.

Gjertson<sup>23</sup> reported that older patients expressed decreased graft survival which was attributed to higher mortality. On the other hand, de Fijter and associates<sup>24</sup> found that old and young donors had relatively decreased numbers of functioning nephrons and survived less when transplanted. In our study, we found that neither age nor sex of the recipient or the donor had significant effect on the development of early steroid resistance ( $P = .905$  and  $0.466$ ; Table 1). The discrepancy between these results may be due to differences in the mean ages of the different studies, and the fact that most of our donors were young females.

Gjertson<sup>25</sup> found that donor-recipient relationship was associated with the greatest variation in risk of graft loss. Live-related and unrelated transplants have a 15% better 1-year graft survival rate than deceased-donor kidneys. As all our patients received their grafts from living donors (> 90% related donors), we found that the donor-recipient relationship did not correlate significantly with the development of early steroid resistance ( $P > .05$ ). Similarly, we observed that pretransplant hemodialysis had no significant effect on the development of early steroid resistance. This finding was matched with our first report by el-Agroudy and associates<sup>26</sup> who concluded that preemptive transplant did not affect the incidence of acute rejection.

Previous sensitization could affect long-term graft outcome. The number of performed anti-HLA

antibodies not only exposes the recipient to an increased risk of acute rejection,<sup>27</sup> but also, is associated with a decline in half-life in well-matched and mismatched recipients. Anti-human leukocyte antibodies play a central role in graft survival, particularly in kidney transplant.<sup>29</sup> The contribution of both humoral and cell-mediated alloimmune responses against mismatched donor histocompatibility antigens in the pathogenesis of chronic rejection is well established.<sup>30</sup> However, in our study HLA, A-B, and DR had no effect on developing early steroid resistance, which maybe at low immunologic risk (with 50% mismatches in most of our cases). We found that more than 50% of early steroid resistance patients were maintained on a noncalcineurin inhibitor-based regimen, while those who were maintained on cyclosporine-based regimen developed late chronic rejection ( $P = .05$ ). This was matched with that reported by Smak Gregoor and associates<sup>31</sup> who reported a higher incidence of acute rejection with azathioprine-treated cases.

Poor compliance represents a frequent cause of late graft failure and a cogent argument supporting the immunologic nature of rejection.<sup>32</sup> The use of dosages of cyclosporine that are too low could expose one to the risk of chronic rejection.<sup>33</sup> In a previous report, we found that our kidney transplant patients had good compliance with immunosuppressive medications, but not with other recommended lifestyle behaviors.<sup>34</sup>

Delayed graft function could initiate acute rejection in renal transplant patients,<sup>35</sup> and predicted poor long-term graft survival only when associated with acute rejection<sup>36</sup>; moreover, prolonged cold ischemia time and azathioprine, steroid, and cyclosporine combinations were risk factors for acute rejection. Meanwhile, anti-CD25 with mycophenolate mofetil, steroid, and cyclosporine combinations were protective factors for acute rejection in deceased-donor kidney transplants.<sup>37</sup> In our study, the 2 groups were comparable regarding short ischemia time and primary maintenance immunosuppression. Neither showed any significant effect on developing early steroid resistance.

The presence of proteinuria 1 year after transplant was considered a marker of poor prognosis in the long term rather than a cause for chronic graft dysfunction.<sup>38</sup> Our proteinuria patients were comparable in both groups without significant effect on graft and graft survival.

From a multivariate analysis, we found that prebiopsy low cyclosporine level ( $P = .04$ ) and biopsy-associated chronic changes in the graft ( $P = .02$ ) were significantly affecting the response to steroid pulse. However, steroid resistance did not affect both long-term patient and graft survival, possibly owing to aggressive intervention by rescue therapy.

Mycophenolate mofetil has been used successfully to treat steroid-resistant rejection, but only of the interstitial (cellular) type. Switching from cyclosporine to tacrolimus to treat recurrent or antibody-resistant rejection was successful in approximately 60% of cases. Plasmapheresis and intravenously administered immunoglobulin have been used in some cases. Sirolimus was used as rescue treatment for steroid-resistant rejection not responding to ordinary pulse steroids.<sup>12, 13</sup>

Different rescue immunosuppressive therapies outlined in Figure 2 gave poorer graft outcomes compared with the control group after 6 months of rejection (serum creatinine  $< 125 \mu\text{mol/L}$ ,  $P = .004$ , Figure 3). Therefore, renal grafts with steroid-resistant rejection must be rescued by more-potent maintenance immunosuppression after termination of the acute attack. However, we observed that the 2 groups were comparable regarding 5-year graft survival; similarly, patient survival was comparable in both groups (Figure 2;  $P = .224$  and  $P = .161$ ). It is worthwhile that cases with early steroid resistance experience significantly longer hospital stays ( $P = .021$ ), more anemia ( $P = .71$ ), and more-frequent late chronic changes than would the control group ( $P = .32$ ).

## Conclusions

Prebiopsy low cyclosporine trough level and associated chronic changes among patients who were maintained on calcineurin inhibitor-based regimens represent the most-important risk factors for early steroid resistance. Rescue therapies improve short-term graft outcome; however, they did not affect either patient or long-term graft survival at 5-year follow-up.

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