

Original Article

Histological isolated endarteritis benefits glomerular filtration rate and allograft survival after anti-rejection treatment

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Abstract: Isolate endarteritis after renal transplantation has been regarded as T cell-mediated acute rejection in Banff classification. Recently, results from DNA microarrays suggested that isolate endarteritis did not represent T cell-mediated acute rejection exactly and its effect on allograft outcomes was unclear. A total 192 cases of biopsy-proved acute cellular rejection had been recorded between September 2001 to December 2014. There were 111 cases in endarteritis negative group and 81 cases in endarteritis positive group. The baseline information, serum creatinine and glomerular filtration rate (GFR) before and after anti-rejection treatment, steroid resistance, rejection reversal rate, graft survival were compared in two groups. The rejection reversal rate of endarteritis positive group was 90.1% (73/81), significantly higher than 77.5% (86/111) of endarteritis negative group ($P = 0.022$). GFR of endarteritis negative group was considerably higher before and at biopsy but significantly lower after biopsy compared with endarteritis positive group. The graft loss rate of endarteritis positive group was 17.3% (14/81) and 28.8% (32/111) of endarteritis negative group ($P = 0.064$). Although the graft survival since the time after kidney transplant was similar between two groups, the death-censored or death-uncensored survival since the time after anti-rejection treatment in endarteritis positive group was significantly higher than that in endarteritis negative group. Isolated endarteritis benefits the patients from better GFR and allograft survival after rejection treatment.

Keywords: Kidney transplantation, isolated endarteritis, acute cellular rejection, allograft survival, prognosis

Introduction

Renal allograft vascular rejection is defined as endarteritis, manifested as infiltration of immune cells under the endothelium, and this process is considered T cell-mediated process [1]. Patients with transmittal arteritis or arterial fibrinoid change or vascular smooth muscle cell necrosis were diagnosed as type III acute rejection. Some were cell-mediated and others were antibody-mediated rejection [2, 3]. Intimal arteritis is graded according to severity (v1-v3). V1 and v2 lesions are classified as type IIA and IIB, respectively, while patients with type IIA and IIB were diagnosed as T cell-mediated rejection. Lefaucheur proposed the concept of antibody-mediated vascular rejection in patients with v1 or v2 vasculitis based on the presence of donor-specific antibodies (DSA) [4].

But some studies indicated that the prognosis of v1 or v2 vasculitis was better than that of non vascular intima acute rejection [3]. Interestingly, three large sample research using

DNA microarray technology found that T lymphocytes, cytokines and chemokines transcriptional activity in these renal tissues were fairly low in 700 renal transplant patients with pure endarteritis. This performance was not accordance with T-cell-mediated acute rejection at all [5-7]. Pure endarteritis without interstitial inflammation has appeared in many patients since it was first mentioned since 2007 [2, 5-10]. Intimal vascular lesions which are termed isolated 'v' lesions are considered features of acute T-cell-mediated rejection yet can occur in the absence of tubulointerstitial inflammation. The clinical significance of these lesions is not certain [4, 11, 12]. We now concentrate on the result of our patients with pure endarteritis in kidney transplantation.

Materials and methods

Patients

The patients who received the kidney transplant operation between September 2001 and

Isolated endarteritis benefits graft survival

Table 1. Characteristics of baseline clinical information

	Enderteritis negative group (n = 111)	Enderteritis positive group (n = 81)	p-value
Sex (male/female)	73/38	58/23	0.391
Age (year)	37.8 ± 11.4	38.6 ± 12.2	0.639
Warm ischemic time (min)	5.5 ± 2.1	5.6 ± 2.3	0.699
Cold ischemic time (min)	441 ± 213	442 ± 200	0.977
Donor type (living vs. cadaveric)	22/89	17/64	0.843
HLA mismatch	3.06 ± 1.31	2.98 ± 1.19	0.682
Pre-transplant induction			0.694
CD25 mono-antibody	44 (39.6%)	31 (38.3%)	
ATG	5 (4.5%)	6 (7.4%)	
No induction	62 (55.9%)	44 (54.3%)	

December 2014 were studied in this retrospective study. Acute rejection was identified clinically by the presence of fever and a tender and swollen graft. Allograft biopsy was performed in recipients with an increasing serum creatinine (Cr) level, analyzed according to the Banff classification. A total of 217 recipients were diagnosed with biopsy-proven acute cellular rejection (grade I or II) according to Banff 2013 criteria. All patients were regularly followed up to June 30, 2016. If the patient loss to follow-up, the related data would not be included into the study. One author recorded the related clinical data and another author separately collected the pathology result. The development of the research question and outcome measures were not informed by patients' priorities, experience, and preferences because there were no specific intervention applied to the patients. The patients were included in the design of this study according to the clinical record during the hospitalization and the regular follow-up. There were no patients involved in the recruitment to and conduct of the study.

Immunosuppressive agent

The maintenance immunosuppressive regimen included the calcineurin inhibitor (CNI) (Cyclosporine or Tacrolimus) in combination with Mycophenolate Mofetil (MMF) and steroids in most recipients, while rapamycin instead of CNI in some patients. Cyclosporine (CsA) was initiated at 5 mg/kg/d and Tacrolimus (FK506) at 0.10-0.15 mg/kg/d. The drug dosage was adjusted according to the plasma trough concentration. The target plasma trough concentration for CsA and FK506 was 250-300 µg/L and 8-12 µg/L, respectively, during the first

month after transplantation; 200-250 µg/L and 6-10 µg/L, respectively, from the second to the third month; 150-200 µg/L and 4-8 µg/L, respectively, from the fourth to the sixth month; and around 150 µg/L and 3-6 µg/L, respectively, after the sixth month. MMF was begun at 1.5-2 g/d for half a month and maintain-

ed at 1 g/d thereafter. Methylprednisolone was given at 6 mg/kg on the third postoperative day. Since then, starting from 80 mg/d, with a daily reduction of 10 mg, prednisone was maintained at 10-15 mg/d.

Anti-rejection therapy

Once acute rejection was proven by allograft biopsy, intravenous methylprednisolone was administered at 6-10 mg/kg daily for 3 days as pulse therapy. If the serum Cr level decreased more than 50% or went back to the baseline level within 1-2 weeks, the treatment was considered effective. If not, ACR was further treated with OKT3 in 5-10 mg/d or ATG at 100-200 mg/d for 5-7 days. Response to therapy was determined by comparing the serum Cr level measured two weeks after completion of the anti-rejection treatment of the baseline serum Cr level measured pre-rejection [13]. Response was considered complete if a decrease in serum Cr level to maximally 125% of baseline, partial if the Cr was 125%~175% of baseline, and no response if Cr was still more than 175% of the baseline or graft loss (back to dialysis or nephrectomy).

Statistical analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median. Categorical variables were presented as numbers (frequencies). Normally distributed continuous variables were analyzed using student's t test, and non-normally distributed continuous variables were analyzed using Mann-Whitney test. A chi-square test was used for categorical variables. The graft survival and patient survival of both groups calculated by the Kaplan-Meier

Isolated endarteritis benefits graft survival

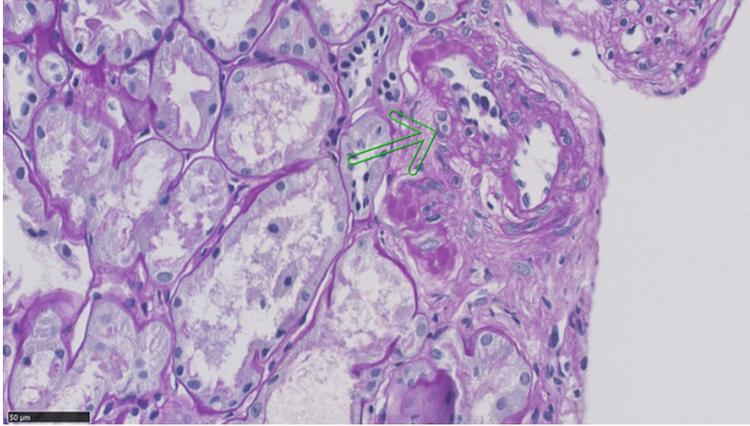


Figure 1. The green arrow indicated endometritis when acute rejection occurred. The length of black column was scaled as 50 μm .

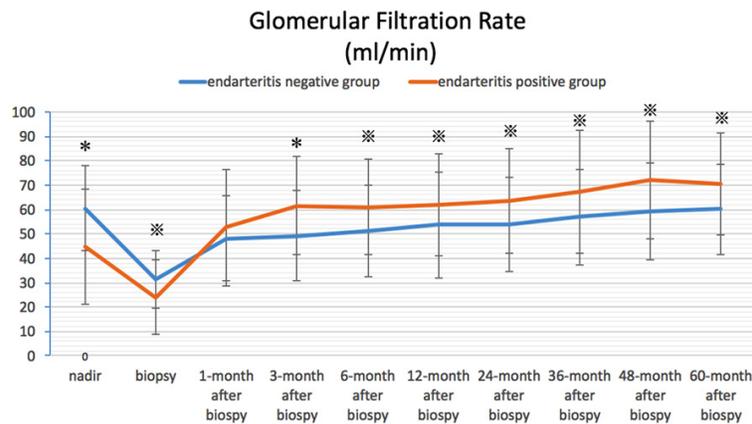


Figure 2. Curve of the GFR before and after biopsy between endarteritis negative group and endarteritis positive group. * $P < 0.001$, ** $P < 0.05$.

method was compared with a log-rank test. Statistical analyses were performed using SPSS20.0 for Windows computer software (SPSS Inc., Chicago, IL, USA). A two sided $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics

A total of 217 recipients were diagnosed with biopsy-proven acute cellular rejection (grade I or II) according to Banff 2013 criteria. Excluding of one patient for loss of follow-up, 16 patients with grade IIA + IA acute cellular rejection (ACR), 6 patients with grade IIA + IB ACR and 2 patients with grade IIB + IA ACR, a total of 192 were included finally in this analysis. According to the presence of endarteritis in the biopsy specimen, 111 patients were classified

into endarteritis negative group and 81 patients were classified into endarteritis positive group. The mean follow-up time was 63.4 ± 23.5 months. No significant differences were found between two groups focusing on sex, age, cold/warm ischemia time, donor type, HLA-mismatch, induction regimen, panel reactive antibody (PRA) $> 10\%$ pre-rejection or primary disease (**Table 1**). The histological changes of isolated endarteritis was showed in **Figure 5**. Acute rejection was diagnosed at 659 ± 856 days post-transplantation in endarteritis negative group, which was delayed significantly than endarteritis positive group (291 ± 740 days) ($P = 0.002$). More patients in the endarteritis positive group (32/81, 39.5%) were observed graft rejection within 10 days after transplantation, compared with the endarteritis negative group (5/111, 4.5%) ($P < 0.001$).

Anti-rejection therapy

There were no significant differences in maintenance immunosuppressive regimen post-rejection, anti-rejection therapy and response to anti-rejection treatment. The immunosuppressive regimen pre-rejection differed in two groups. More patients in the endarteritis positive group (59/81, 72.8%) take orally FK-506 in combination with MMF and steroids as maintenance immunosuppressive regimen compared with endarteritis negative group (55/111, 49.5%; $P = 0.001$). Complete and partial response to anti-rejection treatment in endarteritis positive group was significantly greater than that in endarteritis negative group, 90.1% (73/81) vs. 77.5% (86/111, $P = 0.022$; **Table 2**).

Serum creatinine and GFR before and after biopsy

The endarteritis negative group was presented with significantly lower creatinine at nadir and at the time of biopsy, compared with endarteri-

Isolated endarteritis benefits graft survival

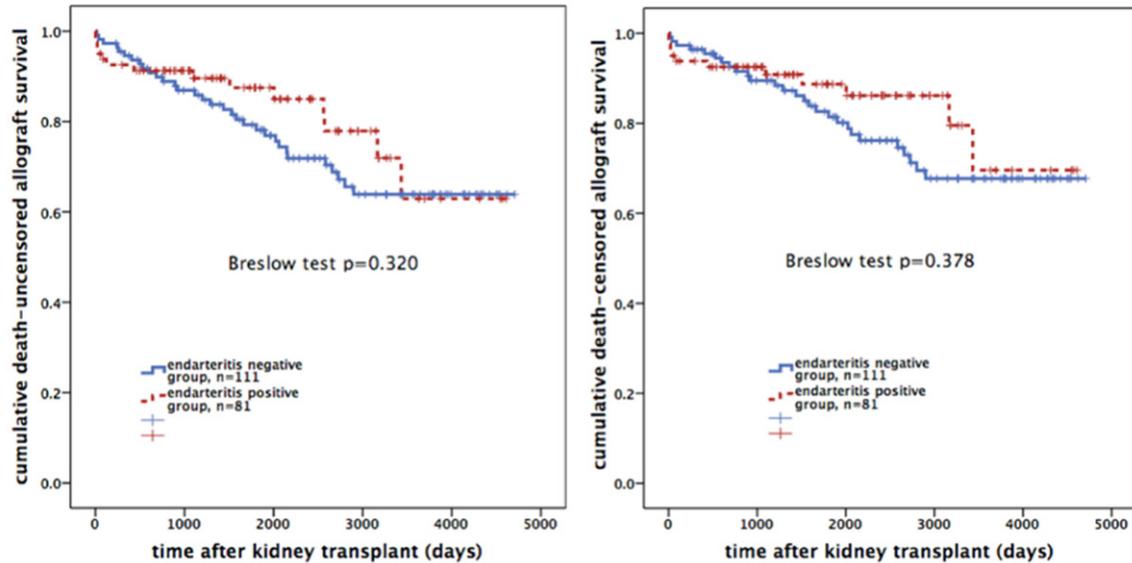


Figure 3. Allograft survival since the time after kidney transplant.

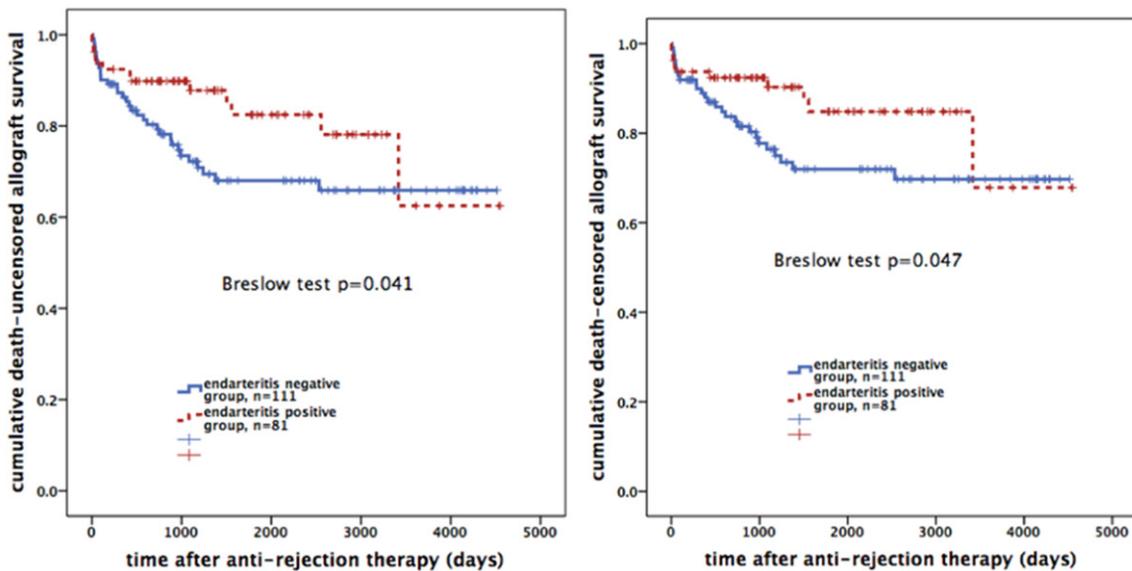


Figure 4. Allograft survival since the time after anti-rejection treatment.

tis positive group. After biopsy, serum creatinine was significantly higher at the third month, the sixth month and the twelfth month post-rejection in the endarteritis negative group. No significant difference was found at any other time during follow up (Table 3). Better GFR was observed before and at the time of rejection in endarteritis negative group, significantly. However, GFR was significantly worse after the third month post-rejection in endarteritis negative group (Figure 1).

Allograft and patient survival

There was significant difference in Kaplan-Meier death-censored and death-uncensored allograft survival since the time after kidney transplant between endarteritis positive group and endarteritis a negative group (Figure 2). In addition, we found allograft survival significantly was higher in endarteritis positive group than that in endarteritis negative group since the time after anti-rejection treatment whether

Isolated endarteritis benefits graft survival

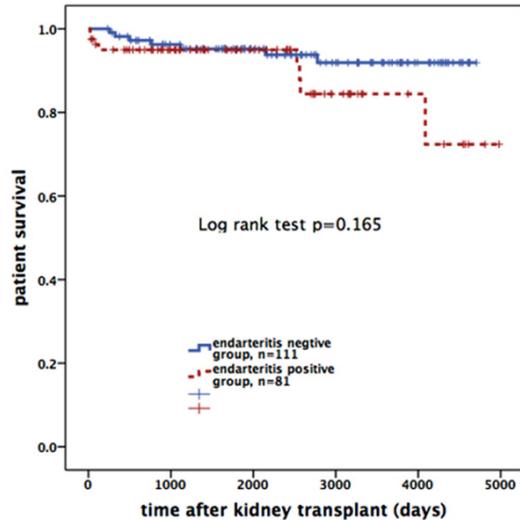


Figure 5. Patient survival between endarteritis negative group and endarteritis positive group.

death-censored or death-uncensored (**Figure 3**). There were 32 patients (28.8%) in the endarteritis negative group who experienced graft loss during follow-up, compared with 14 (17.3%) in endarteritis positive group ($P = 0.064$). There was also no significant difference in patient survival between two groups (**Figure 4**). Seven patients died during follow-up in the endarteritis negative group, while eight patients found death in the endarteritis positive group ($P = 0.363$).

Discussion

Our study showed that patients with isolated endarteritis with better GFR and graft survival rate after the anti-rejection therapy. As we know, acute T-cell-mediated rejection (ATCMR) is classified by the Banff schema according to the presence of significant interstitial inflammation with tubulitis (type I) or the presence of intimal arteritis (type II/III) [2]. Intimal arteritis is also graded according to severity (v1-v3). The v1 and v2 lesions are classified as ATCMR type IIA and IIB, respectively. The v3 lesions can be classified either with ATCMR or acute antibody-mediated rejection, if combined, with C4d deposition in the peritubular capillaries and the presence of DSA [2]. As arteritis can be present in the absence of significant tubulointerstitial inflammation, so it is called isolated endarteritis [2]. The clinical significance of apparently isolated endarteritis remains uncertain and it

is also the current focus of Banff Working Group [2, 13].

Some reports suggest that endarteritis has a poor response to corticosteroid pulse treatment and may require more intensive immunosuppression [14]. Sis analyzed the renal biopsy data from seven kidney transplant centers in the United States and Canada in 1999-2011, excluding those with blood group mismatch, cross-matching positive and C4d positive in the tissue [11]. Patients were divided into three groups: isolated endarteritis group ($n = 103$), positive control group with type I acute cellular rejection and isolated endarteritis ($n = 101$), negative control group with no rejection ($n = 103$). During a follow-up of 3.2 years, the survival of renal allograft in isolated endarteritis group was 79%, positive control was 79%, and negative control was 91%. Multivariate regression analysis showed that the risk of graft failure was increased by 2.51 times in patients with isolated endarteritis. These findings suggested that isolated endarteritis was an independent risk factors for renal allograft outcome. On the other hand, some authors issued that isolated endarteritis had no significant difference with renal allograft survival [8-11].

However, recent genetic microarray technology discovered that T-lymphocytes, cytokines and chemokines transcriptional activity were fairly low in renal tissue with isolated endarteritis. So, acute rejection with isolated endarteritis were not seemed as true T-cell-mediated rejection [5, 7, 12]. Transcriptome analysis of biopsy samples with isolated endarteritis also demonstrated lower T-cell transcripts. Thus, these lesions were may obviously distinct from 'typical' T cell-mediated rejection [5]. A publication proposed isolated endarteritis especially occurred within the first year, 75% (9/12) were re-diagnosed as non-acute rejection [12].

In contrast, some other studies showed that isolated endarteritis without significant turbulence and interstitial inflammation were correlating with a better response to treatment [15]. Some isolated v1-lesions occurred early post-transplant in both compatible and incompatible grafts and were regarded as ischemic changes rather than vascular rejection [8]. It appeared to be more likely that these early v-lesions reflected as endothelial injuries during the

Isolated endarteritis benefits graft survival

Table 2. Immunosuppressive regiment and anti-rejection treatment

	Endarteritis negative group (n = 111)	Endarteritis positive group (n = 81)	p-value
Baseline immunosuppressive regiment			0.013
FMP	55 (49.5%)	59 (72.8%)	
CMP	50 (45.0%)	21 (25.9%)	
RMP	3 (2.7%)	0 (0)	
CRP	2 (1.8%)	0 (0)	
CAP	1 (0.9%)	1 (1.2%)	
Anti-rejection regimen			0.077
Steroid pulse	65 (58.6%)	37 (45.7%)	
Steroid + antibody	46 (41.4%)	44 (54.3%)	
Anti-rejection response			0.050
Complete	66 (59.5%)	60 (74.1%)	
Partial	20 (18.0%)	13 (16.0%)	
No	25 (22.5%)	8 (9.9%)	

Note: FMP: FK506 + MMF + Pred; CMP: CSA + MMF + Pred; RMP: Rapamycin + MMF + Pred; CRP: CSA + Rapamycin + Pred; CAP: CSA + AZA + Pred.

Table 3. Serum creatinine level at different time point

	Endarteritis negative group (n = 111)	Endarteritis positive group (n = 81)	p-value
Nadir	118.7 ± 31.2 (111)	202.1 ± 140.9 (81)	< 0.001
Biopsy time	236.4 ± 139.3 (111)	378.4 ± 253.1 (81)	< 0.001
Month after biopsy			
One	154.9 ± 81.1 (108)	172.5 ± 136.5 (80)	0.306
Three	149.8 ± 72.8 (86)	124.9 ± 50.9 (69)	0.006
Six	149.8 ± 72.8 (97)	124.1 ± 42.6 (71)	0.005
Twelve	148.3 ± 88.9 (86)	123.3 ± 44.8 (70)	0.024
Twenty-four	143.8 ± 89.8 (71)	116.7 ± 48.4 (62)	0.060
Thirty-six	133.5 ± 62.8 (53)	112.7 ± 74.2 (32)	0.143
Forty-eight	126.8 ± 55.5 (43)	104.1 ± 40.9 (35)	0.349
Sixty	125.7 ± 55.9 (38)	102.9 ± 40.5 (32)	0.089

assessment of renal biopsies would contribute to validate histopathological diagnostic criteria.

Vascular rejection was more frequently classified as antibody-mediated rejection rather than T-cell-mediated rejection. A large study conducted by Lefaucheur, in which a retrospective analysis of various histological variables along with DSA was performed in cases of acute biopsy-proven rejection, a distinct subgroup of patients with antibody-mediated vascular rejection was identified, occurring in 21% of cases [4].

transplantation process distinct from injury caused by alloimmunity. It could be observed in acute tubular necrosis, glomerulonephritis, and TCMR, as documented in the study that 19% of the biopsy samples with micro vascular inflammation ≥ 2 did not show histologic findings of rejection [16]. Our result revealed that more patients in the endarteritis positive group (32/81, 39.5%) suffered rejection within 10 days after transplantation and our early published data showed more less late rejection when early (< 1 month) completely reversed vascular rejection happened [17]. In some degree, our results supported the concept that early v-lesions of endothelial injury may be caused by non-immune reaction. Molecular as-

sessment of renal biopsies would contribute to validate histopathological diagnostic criteria. Vascular rejection was more frequently classified as antibody-mediated rejection rather than T-cell-mediated rejection. A large study conducted by Lefaucheur, in which a retrospective analysis of various histological variables along with DSA was performed in cases of acute biopsy-proven rejection, a distinct subgroup of patients with antibody-mediated vascular rejection was identified, occurring in 21% of cases [4]. The authors also drew attention to the poorer prognosis of patients with antibody-mediated vascular rejection. Transplantation time, DSAs and interstitial inflammation should be considered in the diagnosis of isolated endarteritis as acute rejection [12]. In our study, Banff type III rejection and isolated endarteritis accompanied with type I rejection were excluded, if patients with positive PRA in the biopsy. DSA was done to rule out antibody-mediated vascular rejection. Moreover, the time of rejection in isolated endarteritis patients occurred much earlier than endarteritis negative patients. GFR before and during rejection in endarteritis positive group was also significantly lower than that in endarteritis neg-

ative group. In summary, though we observed the benefit effects of isolated endarteritis on graft kidney, the immunological mechanism of isolated endarteritis needs to be further studied in the future.

Disclosure of conflict of interest

None.

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Isolated endarteritis benefits graft survival

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