TRANSPLANT INTERNATIONAL

Transplant International ISSN 0934-0874

ORIGINAL ARTICLE

Clinical significance of isolated v lesions in paediatric renal transplant biopsies: muscular arteries required to refute the diagnosis of acute rejection

Chrysothemis C. Brown,[1] Neil J. Sebire,[2,3] Per Wittenhagen,[1] Olivia Shaw[4] and Stephen D. Marks[1,3]

1 Department of Paediatric Nephrology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
2 Department of Paediatric Pathology, Great Ormond Street Hospital for Children NHS Foundation Trust, London, UK
3 Institute of Child Health, University College London, London, UK
4 Clinical Transplantation Laboratory, Guy's and St. Thomas' NHS Foundation Trust, London, UK

Keywords
allograft rejection, Banff schema, vascular rejection.

Correspondence
Dr Stephen D Marks, Department of Paediatric Nephrology, Great Ormond Street Hospital for Children NHS Foundation Trust, Great Ormond Street, London, WC1N 3JH, UK.
Tel.: +44 207 405 9200, ext. 0292;
fax: +44 207 829 8841;
e-mail: stephen.marks@gosh.nhs.uk

Conflicts of interest
The authors have declared no conflicts of interest.

Received: 23 April 2013
Revision requested: 14 June 2013
Accepted: 28 October 2013
Published online: 16 December 2013

doi:10.1111/tri.12227

Summary

Intimal vascular lesions are considered features of acute T-cell-mediated rejection yet can occur in the absence of tubulointerstitial inflammation, termed isolated 'v' lesions. The clinical significance of these lesions is unclear. The diagnosis requires a biopsy with the presence of arteries. The frequency of adequate biopsies was analysed in 89 renal transplant biopsies from 57 paediatric renal allograft recipients, and the incidence of isolated endarteritis was determined. 60 (67%) biopsies contained an artery and of these, isolated 'v' lesions occurred in 6 (10%). 5 (83%) biopsies with isolated 'v' lesions were associated with positive DSA, suggesting that these lesions may represent acute antibody-mediated rejection. Patients with vessel-negative biopsies had an increased decline in eGFR (median −20.5, IQR −24.4 to 1.2 ml/min/1.73 m^2 vs. −9.6, IQR −78.7 to −6.8 ml/min/1.73 m^2; $P = 0.01$). Patients with vessel-negative biopsies were more likely to have repeat biopsy for ongoing allograft dysfunction, (25.0% vs. 2.4%; $P < 0.01$). The data suggest that isolated 'v' lesions are more common than previously thought. A significant proportion of biopsies classified as 'normal' or 'borderline change' in the absence of a large vessel may represent undiagnosed acute rejection. This may result in suboptimal therapy with possible adverse effects on renal outcome.

Introduction

The current gold standard for determining the cause of renal allograft dysfunction is the histological evaluation of a renal allograft biopsy. The Banff schema, as revised in 2009, classifies acute T-cell-mediated rejection (ATCMR) either by the presence of significant interstitial inflammation with tubulitis (Grade I) or by the presence of intimal arteritis (Grade II/III) [1]. Intimal arteritis is graded according to severity (v1-v3). v1 and v2 lesions are classified as ATCMR Grade IIA and IIB, respectively, whereas v3 lesions can be classified either with ATCMR or, if combined, with C4d deposition in the peritubular capillaries and the presence of donor-specific antibodies (DSA), acute antibody-mediated rejection (AAMR) [1].

Demonstration of endarteritis clearly requires the examination of such vessels and therefore requires the presence of muscular arteries in the biopsy sample. As arteritis can occur in the absence of significant tubulointerstitial inflammation, so-called isolated 'v' lesions [1], the absence of an appropriate artery in the biopsy specimen may lead to the erroneously reporting of 'no evidence of acute TCMR'. We hypothesised that a proportion of biopsies reported as normal or showing borderline change but in the absence of a large artery in the biopsy may have been affected by isolated 'v' lesions and, if untreated, might lead to worse renal outcomes.

The developing immune system differs between children and adults and therefore studies need to be conducted in paediatric practice to determine whether different factors are important in the development of tolerance and

reducing ATCMR and AAMR. The principal objectives of this study were to establish the frequency of isolated arteritis in paediatric renal transplant patients with acute allograft dysfunction and to estimate the frequency of possible undetected ATCMR due to biopsies with the absence both of large arteries and of tubulointerstitial changes.

Patients and methods

We retrospectively identified all paediatric patients who had undergone a renal transplant biopsy for suspected acute or acute chronic allograft dysfunction as part of their routine clinical care from November 2008 to December 2010 from a clinical database. Percutaneous renal transplant biopsies were obtained by the interventional radiology team using at least two passes and two cores for every biopsy sample (younger patients underwent general anaesthesia and older patients underwent local anaesthesia with the use of Entonox).

Renal transplant biopsies were examined and rejection graded at the time of biopsy by paediatric pathologists and the reports retrospectively reviewed. The 2009 Banff criteria were used to grade rejection [2,3]. Briefly, Grade I ATCMR was defined as significant interstitial infiltration (>25% parenchyma) and foci of moderate tubulitis (IA) or severe tubulitis (IB). Grade II ATCMR was defined as mild-to-moderate arteritis (IIA) or severe (>25% luminal area) intimal arteritis (IIB) (Fig. 1). Grade III ATCMR was defined as transmural arteritis and/or arterial fibrinoid change and necrosis of medial smooth muscle cells with accompanying lymphocytic infiltration. Where no intimal arteritis was present, but foci of tubulitis with minor tubulo- or interstitial infiltration +/− tubulitis were seen, biopsies were considered 'suspicious' for ATCMR and classified as 'borderline change'.

The presence of at least one 'large' artery (>approximately 200 μm diameter, assessed subjectively by the reporting pathologist) was determined either from the original report or, where this was not explicitly stated, the histological sections were reviewed. For biopsies reported as Grade II or III ATCMR, the presence of co-existent interstitial infiltration and extent of tubulitis were noted to ascertain whether these biopsies would have met the criteria for Grade I ATCMR in the absence of arteritis.

For the purpose of this study, for patients who had more than one biopsy for acute allograft dysfunction during the study time period, only the first biopsy was included for the analysis of renal outcome.

Clinical records for each patient were reviewed from the unit database, including age at transplant, gender, primary diagnosis, prebiopsy baseline serum creatinine and creatinine at follow-up, renal allograft loss, treatment of the acute rejection episode and the need for repeat renal transplant biopsy for the same indication. The follow-up endpoint was renal allograft loss or last recorded status as at May 2011. Ethical approval was obtained for this study from the Institute of Child Health and Great Ormond Street Hospital for Children NHS Trust Research Ethics Committee.

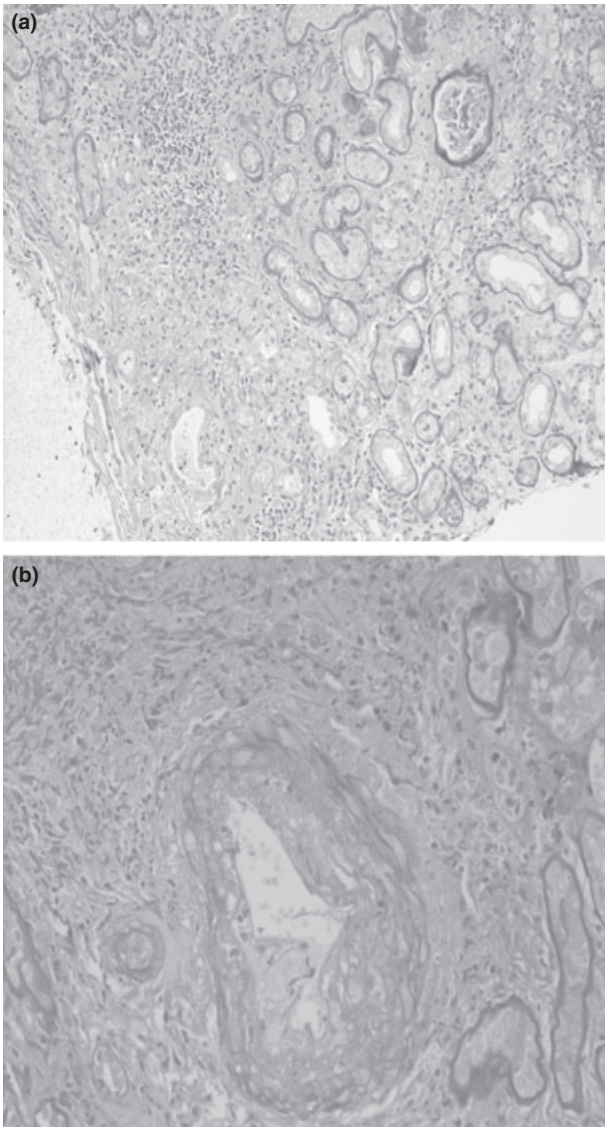

Figure 1 (a) Low-power photomicrograph of a renal transplant biopsy showing mild tubular atrophy and interstitial fibrosis but no evidence of acute T-cell-mediated rejection, however no large vessels present on biopsy tissue. (b) High-power photomicrograph of a subsequent renal transplant biopsy performed in the same patient for ongoing allograft dysfunction, containing a large vessel showing evidence of severe arteritis (Grade IIb acute T-cell-mediated rejection).

Statistical methods

Renal allograft survival was analysed by Kaplan–Meier estimates for survival distribution and compared using the

log-rank test. For comparison of clinical and laboratory data, proportional differences were tested with Pearson's chi-square test. Mann–Whitney U test was used for non-parametric unpaired comparisons of distributions between groups. eGFR was estimated using the modified Schwartz formula which has been validated for renal transplant recipients in our institution [4]. P values < 0.05 were considered statistically significant. Statistical analyses were performed using SPSS, version 20 (SPSS, Inc., Chicago, IL, USA).

Results

89 consecutive unselected renal transplant biopsies were performed in 57 patients for acute allograft dysfunction between November 2008 and December 2010 (median age, 12.3 years; range, 1.6 to 18.3 years). Clinical characteristics at the time of first biopsy are summarized in Table 1 and according to the presence or absence of an appropriate artery on biopsy. There were no significant baseline differences in variables between these groups.

Table 2 shows the histopathological diagnosis according to the presence of a large artery. Sixty (67%) of the paediatric renal transplant biopsies contained at least one large artery. Of these, eleven (18%) had evidence of arteritis fulfilling the criteria for Grade II ATCMR. In six (55%) of the biopsies with Grade II ATCMR, arteritis was present without coexisting features of Grade I rejection, so-called isolated 'v'-lesions. Of the six biopsies with isolated intimal arteritis, five (83%) had alloantibody on DSA testing and 1 (16%) showed C4d deposits on immunostaining (Table 3). In the three biopsies in which arteritis occurred in conjunction with tubulointersititial inflammation, C4d staining and DSA were absent.

Table 1. Patient characteristics at the time of biopsy.

Patient characteristic	All patients $n = 57$ no. (%)	Vessel + $n = 41$ no. (%)	Vessel – $n = 16$ no. (%)	P value
Age at biopsy – months				
Median	168.0	160.0	172.0	0.41
Interquartile range	107.0–191.0	98.0–188.0	126.3–195.8	
Sex				
Male	38 (66.7)	12 (29.3)	7 (43.8)	0.30
Female	19 (33.3)	29 (70.7)	9 (56.2)	
Primary diagnosis				
CAKUT – no. (%)	33 (57.9)	25 (70.0)	8 (50.0)	0.61
Glomerulopathy – no. (%)	16 (28.0)	10 (24.4)	6 (37.5)	
Other – no. (%)	8 (14.0)	6 (14.6)	2 (12.5)	
Donor type				
Living related – no. (%)	28 (49.1)	20 (48.7)	8 (50.0)	1.00
Deceased donor – no. (%)	29 (50.9)	21 (51.3)	8 (50.0)	
Regraft				
First	55 (96.5)	40 (97.6)	15 (93.8)	0.48
Second	2 (3.5)	1 (2.4)	1 (6.3)	
HLA mismatch – no. antigens				
Median	2.0	2.0	2.0	0.72
Interquartile range	2.0–3.0	2.0–3.0	1.3–3.0	
Immunosuppression				
CNI	51 (89.5)	35 (85.3)	16 (100.0)	0.27
MMF/Aza	46 (80.7)	35 (85.3)	11 (68.8)	0.21
Prednisolone	55 (96.5)	39 (95.1)	16 (100.0)	0.67
Graft age (months)				
Median	8.0	8.0	9.5	0.48
Interquartile range	2.0–56.5	2.0–70.5	3.0–23.8	
eGFR prebiopsy (ml/min/1.73 m^2)				
Median	57.6	54.5	65.0	0.37
Interquartile range	45.6–78.9	43.8–82.0	49.7–79.0	
Prednisolone prior to biopsy	14 (24.6)	8 (19.5)	6 (37.5)	0.34
Donor-specific antibodies (DSA)*	18 (38.3)	15 (46.9)	3 (20.0)	0.07

CAKUT, congenital anomaly of the kidney or urinary tract; CNI, calcineurin inhibitor; MMF mycophenolate mofetil; Aza, Azathioprine.
P value calculated for vessel + vs. vessel–. Analysis performed using Mann–Whitney test or chi-square.
*Data available for 47 patients.

Table 2. Histopathological diagnosis according to the presence of large vessel on biopsy specimen.

Histopathological diagnosis	Vessel – $n = 29$ no. (%)	Vessel + $n = 60$ no. (%)
Normal	7 (24.1)	12 (20)
ABMR	0 (0.0)	1 (1.7)
Borderline changes	7 (24.1)	5 (8.3)
ATCMR Grade I	5 (17.2)	6 (10.0)
ATCMR Grade II		
Isolated 'v' lesion	0 (0.0)	6 (10.0)
'v' lesion + tubulitis	0 (0.0)	5 (8.3)
Chronic changes	6 (20.7)	21 (35)
Other	4 (13.8)	4 (6.7)

ABMR, Antibody-mediated rejection; ATCMR, Acute T-cell-mediated rejection.

Table 3. The relationship between histopathological lesion, donor-specific antibody and C4d deposition in vessel-positive biopsies.

Histopathological diagnosis	Donor-specific antibody – no. (%)*	C4d deposition – no. (%)†
Normal	2/9 (22.2)	0/8 (0.0)
ABMR	1/1 (100)	1/1 (100)
Borderline changes	2/4 (50.0)	0/4 (0.0)
ATCMR Grade I	2/5 (40.0)	1/5 (20.0)
ATCMR Grade II		
Isolated 'v' lesion	5/6 (83.3)	1/3 (33.3)
'v' lesion + tubulitis	0/3 (0.0)	0/2 (0.0)
Chronic changes	5/11 (45.5)	2/12 (16.7)
Other	0/2 (0.0)	0/3 (0.0)

ABMR, Antibody-mediated rejection; ATCMR, Acute T-cell-mediated rejection.
*Data available for 41 patients.
†Data available for 38 patients.

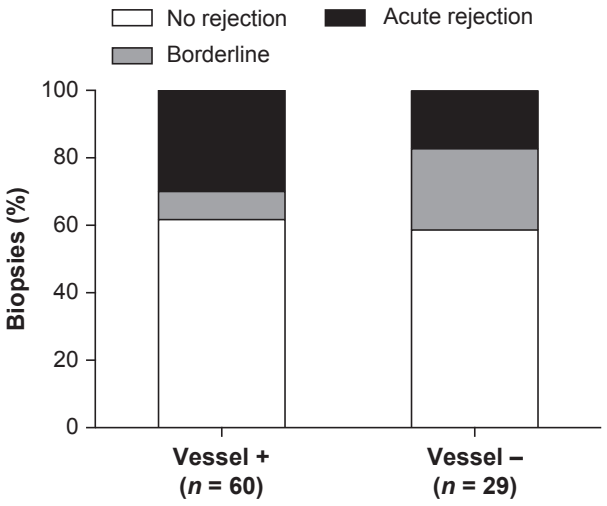

Figure 2 Relationship of histopathological findings with the presence of large vessel in biopsy specimen. Acute rejection = acute T-cell-mediated rejection or acute antibody-mediated rejection.

Table 4. Clinical outcome: relationship with the presence of large vessel on biopsy.

	Vessel + $n = 41$	Vessel – $n = 16$	*P* value
Repeat biopsy – no. (%)	1 (2.4)	4 (25.0)	<0.01
Prednisolone prior to biopsy – no. (%)	8 (19.5)	6 (37.5)	0.34
Treatment for ARE – no. (%)	18 (43.9)	9 (56.2)	0.40
Number of steroid courses			
Median	1.0	1.0	0.45
Interquartile range	1.0–2.0	1.0–2.0	
Follow-up – months			
Median	11.0	8.5	0.07
Interquartile range	6.5–20.0	1.75–10.0	
Change in eGFR (ml/min/1.73 m^2/yr)			
Median	−20.5	−9.6	0.01
Interquartile range	−24.4–1.2	−78.7 to −6.8	
Graft survival – months*	26.1 (1.3)	21.7 (3.5)	0.12

*Analysed by Kaplan–Meier estimator for survival distribution and compared using the log-rank test. Values shown are mean (sem).

There were more episodes of biopsy-confirmed acute rejection in biopsies containing arteries than those that did not: 30.0 vs. 17.2% (Fig. 2; $P = 0.09$). There was no significant difference in the numbers of patients in each group who had received treatment with high-dose prednisolone for presumed acute rejection prior to the biopsy: 8 (19.5%) vessel-positive *vs.* 6 (37.5%) vessel-negative, $P = 0.34$.

Table 4 summarizes the clinical management and renal outcome of patients according to the presence of an artery on biopsy. Patients with biopsies in which an artery was absent were more likely to have a repeat biopsy for ongoing allograft dysfunction: 4 of the 16 patients with vessel-negative biopsies (25%) had a repeat biopsy during the same episode of acute allograft dysfunction, as compared to 1 of the 41 patients (2.4%) with vessel-positive biopsies ($P < 0.01$). One of the 4 vessel-negative patients who underwent repeat biopsy was found to have isolated 'v' lesions on the second biopsy, 2 patients were found to have chronic changes and 1 of the patients had evidence of vascular infarction. Patients in whom an artery was absent on their biopsy had a significantly greater decline in eGFR compared with patients with vessel-positive biopsies, (median −20.5, IQR −24.4 to 1.2 ml/min/1.73 m^2 vs. median −9.6, IQR −78.7 to −6.8 ml/min/1.73 m^2), ($P = 0.01$). Patients with an initial vessel-positive biopsy had a 1-year renal allograft survival of 77.4 ± 15.2% as compared to 90.3 ± 5.4% among patients in the vessel-negative group

($P = 0.32$). There was no significant difference in the number of patients who received prebiopsy high-dose steroid treatment between the two groups and no significant difference in the number of patients who had donor-specific antibodies at the time of biopsy.

Discussion

The findings of this study suggest that isolated 'v' lesions are more common than previously reported in paediatric renal transplant recipients, with around half of biopsies with Grade II rejection having apparently isolated arteritis without significant associated tubulointerstitial inflammation. In the absence of an appropriate artery being present, these biopsy specimens would not have met the criteria for Grade I ATCMR and would be graded as otherwise 'normal' or borderline change. In this cohort of patients, vessel-negative biopsies occurred in a third of patients, suggesting that around 3% of all patients undergoing clinically indicated transplant, biopsy may have acute rejection present which remains undetected. The reduced frequency of biopsy-confirmed rejection episodes in 'vessel-negative biopsy' patients provides further evidence for probable under detection of ATCMR in such 'inadequate' biopsies.

The greater decline in renal function in patients with vessel-negative biopsies furthermore suggests that failing to identify isolated 'v' lesions may lead to delay in treatment or suboptimal therapy. Reports suggest that endarteritis has a poorer response to pulsed corticosteroid treatment and may require more intensive immunosuppression [5]. Therefore, if patients are treated only with pulsed corticosteroid therapy on clinical suspicion of acute rejection with a normal but 'vessel-negative' biopsy, there may not be a response to therapy.

The clinical significance of apparently isolated 'v' lesions remains uncertain and is currently the focus of the most recent Banff Working Group [1,6]. Although the current Banff criteria classes 'v1' and 'v2' lesions as features of ATCMR rather than antibody-mediated rejection (AMR), this concept has been challenged by recent studies [7–9]. The presence of 'v' lesions was found to correlate with many features of AAMR, and isolated 'v' lesions may indeed represent AAMR in the absence of classical DSA or C4d staining [7]. In addition, a high proportion of biopsies from patients with DSA have other features suggestive of AAMR such as glomerulitis and/or capillaritis but are C4d negative [10]; therefore, C4d staining may not be sufficiently sensitive to allow the diagnosis of AAMR. Interestingly in a much larger study by Lefaucheur *et al.* 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 [9]. Vascular rejection was more frequently classified as antibody-mediated rather than T-cell-mediated. These authors also drew attention to the poorer prognosis of patients with antibody-mediated vascular rejection. The clinical significance of isolated 'v' lesions is supported by the observation that presensitized patients with subclinical AAMR (microcirculation inflammation but no C4d deposition) on 3-month biopsies have worse renal outcome than those with no evidence of microcirculation inflammation [11]. It has therefore been argued that biopsies with microcirculatory inflammation in association with DSA should be considered as AAMR. The apparent co-existence, in our study, of isolated 'v' lesions and DSA (in 5 of the 6 cases) is consistent with a previous study in which DSA was present in 56% of biopsies with isolated v lesions [7]. Our findings are also consistent with the previous reports, demonstrating that the presence of microcirculation inflammation was found to correlate with DSA more closely than C4d staining, as well as being an independent prognostic indicator for renal outcome [12]. The incorporation of glomerulitis and peritubular capillaritis into the Banff schema might aid the interpretation of isolated 'v' lesions as either ATCMR or AAMR.

Transcriptome analysis of biopsy samples with isolated 'v' lesions also demonstrated lower T-cell transcripts further supporting the notion that these lesions may represent pathology distinct from 'typical' T-cell-mediated rejection [13]. Immunophenotyping and gene expression analysis of biopsies with AAMR biopsies (either C4d– or C4d+) have identified a natural killer cell signature [14]; therefore, gene expression analysis of biopsy tissue may be able to identify the underlying pathology in biopsy specimens with 'v' lesions in the absence of other ATCMR or AAMR features.

The Banff classification is reliant on an adequate biopsy specimen. In paediatric patients, the acquisition of adequate specimens poses special problems. Nevertheless, the risks associated with obtaining multiple cores, to ensure adequate sampling of vessels, may not outweigh the benefits.

The limitations of the present study include the small sample size, which prevents analysis of clinical outcome by histopathological subgroup, in particular patients with isolated 'v' lesions.

In conclusion, our study demonstrates the importance of adequate sampling of renal biopsies for histopathological analysis and provides further insight into the frequency and clinical significance of isolated 'v' lesions. Larger prospective studies utilising newer techniques such as gene expression analysis of renal biopsy tissue as well as urine metabolomics and proteomics alongside histopathological classification are required to determine the clinical significance of isolated 'v' lesions.

Authorship

CCB: participated in research design, collection, analysis and interpretation of the data, and participated in the writing of the paper. NS: participated in research design, performed evaluation of histology material and participated in the writing of the manuscript. PW and OS: participated in data collection. SDM: participated in research design and writing of the manuscript.

Funding

The authors are grateful for support from the Great Ormond Street Hospital National Institute for Health Research Biomedical Research Center and Great Ormond Street Hospital Children's Charity.

References

1. Sis B, Mengel M, Haas M, *et al.* Banff '09 meeting report: antibody mediated graft deterioration and implementation of Banff working groups. *Am J Transplant* 2010; **10**: 464.
2. Solez K, Colvin RB, Racusen LC, *et al.* Banff 07 classification of renal allograft pathology: updates and future directions. *Am J Transplant* 2008; **8**: 753.
3. Racusen LC, Solez K, Colvin RB, *et al.* The Banff 97 working classification of renal allograft pathology. *Kidney Int* 1999; **55**: 713.
4. Kausman JY, Patel B, Marks SD. Standard dosing of tacrolimus leads to overexposure in pediatric renal transplantation recipients. *Pediatr Transplant* 2008; **12**: 329.
5. Nickeleit V, Vamvakas EC, Pascual M, Poletti BJ, Colvin RB. The prognostic significance of specific arterial lesions in acute renal allograft rejection. *J Am Soc Nephrol* 1998; **9**: 1301.
6. Mengel M, Sis B, Haas M, *et al.* Banff 2011 Meeting report: new concepts in antibody-mediated rejection. *Am J Transplant* 2012; **12**: 563.
7. Shimizu T, Tanabe T, Shirakawa H, Omoto K, Ishida H, Tanabe K. Acute vascular rejection after renal transplantation and isolated v-lesion. *Clin Transplant* 2012; **26**(Suppl 24): 2.
8. Sis B, Einecke G, Chang J, *et al.* Cluster analysis of lesions in nonselected kidney transplant biopsies: microcirculation changes, tubulointerstitial inflammation and scarring. *Am J Transplant* 2010; **10**: 421.
9. Lefaucheur C, Loupy A, Vernerey D, *et al.* Antibody-mediated vascular rejection of kidney allografts: a population-based study. *Lancet* 2013; **381**: 313.
10. Cosio FG, Lager DJ, Lorenz EC, Amer H, Gloor JM, Stegall MD. Significance and implications of capillaritis during acute rejection of kidney allografts. *Transplantation* 2010; **89**: 1088.
11. Loupy A, Suberbielle-Boissel C, Hill GS, *et al.* Outcome of subclinical antibody-mediated rejection in kidney transplant recipients with preformed donor-specific antibodies. *Am J Transplant* 2009; **9**: 2561.
12. Sis B, Jhangri GS, Riopel J, *et al.* A new diagnostic algorithm for antibody-mediated microcirculation inflammation in kidney transplants. *Am J Transplant* 2012; **12**: 1168.
13. Mueller TF, Einecke G, Reeve J, *et al.* Microarray analysis of rejection in human kidney transplants using pathogenesis-based transcript sets. *Am J Transplant* 2007; **7**: 2712.
14. Hidalgo LG, Sis B, Sellares J, *et al.* NK cell transcripts and NK cells in kidney biopsies from patients with donor-specific antibodies: evidence for NK cell involvement in antibody-mediated rejection. *Am J Transplant* 2010; **10**: 1812.

