The prognostic values of Caveolin-1 immunoreactivity in peritubular capillaries in patients with kidney transplantation

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Abstract

Nakada Y, Yamamoto I, Horita S, Kobayashi A, Mafune A, Katsumata H, Yamakawa T, Katsuma A, Kawabe M, Tanno Y, Ohkido I, Tsuboi N, Yamamoto H, Okumi M, Ishida H, Yokoo T, Tanabe K; Japan Academic Consortium of Kidney Transplantation (JACK) The prognostic values of Caveolin-1 immunoreactivity in peritubular capillaries in patients with kidney transplantation. Clin Transplant The low sensitivity of C4d immunoreactivity in peritubular capillaries (PTCs) hinders its use in the diagnosis of chronic active antibody-mediated rejection (CAAMR). C4d-negative CAAMR was defined in the 2013 Banff classification, which included on the expression of endothelial-associated transcripts (ENDATs). We previously showed that the ENDAT caveolin-1 (CAV-1) is a distinct feature of CAAMR. In this study, we investigated the prognostic value of CAV-1 immunoreactivity in PTCs in kidney transplant patients. Ninety eight kidney transplant recipients were included in this study. The prognostic value of CAV-1 immunoreactivity in PTCs was evaluated by double-immunostaining for CAV-1 and pathologische Anatomie Leiden endothelium (PAL-E, a PTC marker) in the PTCs of kidney allograft biopsy samples. The patients were divided into two groups: CAV-1/PAL-E <50% and CAV-1/PAL-E Ó50%. Kaplan-Meier curves showed that CAV-1/PAL-E Ó50% patients had a significantly worse prognosis than that of CAV-1/PAL-E <50% patients (log-rank; $p < 0.001$). C4d staining of PTCs was not associated with the development of graft failure (log-rank; $p = 0.345$), whereas in a multivariate Cox regression analysis, CAV-1 immunoreactivity in PTCs was independently associated with graft failure (hazard ratio: 11.1; $p = 0.0324$). CAV-1

immunoreactivity in PTCs may serve as a prognostic marker for kidney allograft survival.

Key word: Caveolae, Caveolin-1, transplant glomerulopathy, chronic active antibody-mediated rejection, kidney graft survival

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Introduction

The rate of kidney allograft survival improved dramatically following the introduction of effective immunosuppressive agents, including calcineurin inhibitors (tacrolimus, cyclosporine) and mycophenolate mofetil. Nonetheless, chronic rejection remains a major cause of allograft dysfunction and failure. In a 2001 report, chronic rejection accounted for 19.7% of all allograft failures (1). Of the various forms of rejection, chronic active antibody-mediated rejection (CAAMR) is more difficult to treat and is closely associated with allograft survival (2). Its diagnosis therefore requires accurate histopathological, serological, and immunological examinations. C4d, a complement component detected in peritubular capillaries (PTCs), is a widely used diagnostic marker for antibody-mediated rejection. In fact, the diagnostic definition of CAAMR provided by the 2007 Banff classification consisted of the following: (1) histopathological findings, (2) detection of donor-specific antibodies (DSA), and (3) C4d staining of PTCs. Of these three criteria, however, C4d staining was questioned because of its low sensitivity. Thus, at the 2013 Banff classification, C4d-negative CAAMR was included in the groupôs final report (3, 4).

Sis et al. (5) suggested the use of endothelial cell (EC)-associated transcripts (ENDATs) as a diagnostic marker of kidney transplant rejection, based on the hypothesis that allograft rejection alters the degree of gene expression in ECs (5). The same authors found that ENDATs have a higher sensitivity and slightly lower specificity than those of C4d staining in the diagnosis of allograft rejection (77% vs. 31% and 71% vs. 94%, respectively). ENDATs include caveolin-1 (CAV-1), the

expression of which is increased significantly in CAAMR, but not in T-cell-mediated rejection (5).

CAV-1 is a primary component of the 50- to 100-nm flask-shaped invaginations of the plasma membrane referred to as caveolae (6). Caveolae are present in most cells, including fibroblasts, muscle cells, adipocytes (in which they account for 20% of the cell membrane), and ECs, but not in neurons or lymphocytes (6). Their functions have not been fully determined, but so far they include macromolecular transcytosis, vascular permeability, the regulation of endothelial nitric oxide synthase (eNOS), angiogenesis, and endothelial regeneration (7-12).

We previously reported that caveolae and CAV-1 expression levels in PTCs are distinct markers of chronic-rejection-induced transplant glomerulopathy (TG) and capillaropathy. CAV-1 immunoreactivity in PTCs is higher in patients with TG than in those with interstitial fibrosis or tubular atrophy (11). TG is a distinct histological finding of CAAMR in the Banff classification, which suggests the potential of CAV-1 immunoreactivity as a diagnostic tool in CAAMR (11). However, the association between CAV-1 immunoreactivity in PTCs and graft survival has yet to be evaluated. Therefore, in this study we explored whether CAV-1 immunoreactivity in PTCs correlates with graft survival in kidney transplant recipients.

Materials and Methods

From a pool of 3574 kidney indication and protocol biopsies in 1362 cases reported at the Tokyo Womenôs Medical University (TWMU) between July 2004 and May 2013, we selected the following

cases with: 1) long-term post-transplant period, 2) no missing values of his or her clinical and prognostic data, 3) no ABO-incompatible cases, 4) specimens with both CAV-1 and PAL-E immunostaining at the time of survey. We evaluated 98 biopsies obtained 8.29±5.46 years after kidney transplantation, which was possible to evaluate clinical and prognostic data. The average allograft survival after allograft biopsy was 2.98±1.79 years. Approval was obtained from the Institutional Ethics Committee of Tokyo Women's Medical University (Identifier: 3336-R), and written informed consent was obtained from patients. We identified sampling data from the electronic database of the TWMUôs Kidney Center and reviewed the patientsô clinical characteristics, including sex, age at transplantation, biopsy date after transplantation, follow-up period after diagnosis, C4d immunoreactivity in PTCs, and Banff 2013 classification as a pathological finding.

The pathological assessment was based on the Banff 2013 classification and was used to diagnose rejection. Formalin-fixed paraffin sections were stained with hematoxylin and eosin, periodic acid-Schiff, Massonôs trichrome, and periodic-acid methenamine silver. Pathological findings were assessed by more than two pathologists.

Immunohistochemistry was performed as described previously, using cold acetone-fixed frozen sections reacted with a monoclonal antibody (mAb) against Pathologische Anatomie Leiden endothelium (PAL-E; Progen, Heidelberg, Germany), a CAV-1 polyclonal antibody (pAb) (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and C4d mAb (Quidel, Alkmaar, The Netherlands) (11). The bound primary antibody was detected using Alexa-488-conjugated goat anti-mouse IgG

antibody (1:500; Molecular Probes, Eugene, OR, USA) for both the PAL-E and C4d mAbs and Alexa 568-conjugated goat anti-rabbit IgG antibody (1:1000; Molecular Probes) for the CAV-1 pAb. To examine the degree of CAV-1 immunoreactivity in PTCs, biopsy samples were double-immunostained for CAV-1 and PAL-E. PAL-E have been used as a specific endothelial marker, and PAL-E antigen is identical with plasmalemmal vesicle associated protein-1 that is a component of endothelial fenestral diaphragms (FDs). PV-1 is abundantly expressed in the endothelium of peritubular capillaries and vasa recta, while is absent in glomerular and arterial endothelium due to absence of FDs. Therefore, we used PAL-E as a marker of peritubular capillaries in this study. Renal histopathology was photographed under a light microscope (DP-70, DP-73, Olympus, Tokyo, Japan). CAV-1 immunoreactivity in PTCs was defined as the ratio of CAV-1- to PAL-E-positive PTCs in the whole specimen (CAV-1/PAL-E; Fig. 1). To evaluate CAV-1/PAL-E, we count all PTCs stained with PAL-E and CAV-1 in a whole biopsy section, and provide percentage/ratio for CAV-1 positivity.

C4d immunoreactivity in PTCs was evaluated according to the Banff 2013 classification. To assess the significance of CAV-1 in PTCs, the patients were divided into two groups: CAV-1/PAL-E Ó50% (n=42) and CAV-1/PAL-E <50% (n=56).

Continuous data are expressed as the mean ± standard deviation and discontinuous and ordinal data as the rate and 95% confidence interval (CI). The Mann-Whitney test was used for comparisons of the two groups of patients (CAV-1/PAL-E Ó50% vs. CAV-1/PAL-E <50%). Allograft survival

failure was defined as the return to dialysis. Kaplan-Meier curves were used to estimate death-censored allograft survival; the results were compared with those obtained using the log-rank statistic. The influence of clinical and pathological variables on graft survival was evaluated by univariate and multivariate Cox regression analyses. In the multivariate analysis, the Banff g (transplant glomerulitis) and ptc (peritubular capillaritis) scores were excluded because of their linear combination with the microvascular inflammation (MVI) score, which was also determined in this study. The Banff cv (fibrous intimal thickening) score was also excluded because only five patients were positive. In all analyses, a p-value <0.05 was considered to indicate statistical significance.

Results

Clinical characteristics of the patients

The kidney allograft biopsy results of 98 recipients were analyzed. The characteristics of the patients are listed in Table 1. The mean age of the patients was 44.7±14.7 years, and 66.3% were male. Fifty two percentages of specimens were obtained by induction biopsies (51 of 98 specimens). Living kidney grafts accounted for 93.3% of the transplant procedures (91 of 98 patients)

The patients were divided into two distinct cohorts, CAV-1/PAL-E <50% and CAV-1/PAL-E Ó50%, to evaluate the influence on graft survival of CAV-1 immunoreactivity in PTCs. The clinical characteristics of the two groups are shown in Table 2 and their Banff 2013 classifications in Table 3. The age, sex, and follow-up period (months) at transplantation were similar between the

CAV-1/PAL-E <50% and CAV-1/PAL-E Ó50% patients. The latter group had a higher serum creatinine level at diagnosis, later time to diagnosis, longer follow-up period after diagnosis, and higher rate of graft failure.

CAV-1 immunoreactivity and histopathological characteristics

As previously reported (11), higher CAV-1 expression was strongly associated with a higher rate of TG (CAV-1/PAL-E <50% vs. CAV-1/PAL-E Ó50%, 12.5% vs. 59.5%, $p < 0.0001$; Table 2). All Banff 2013 classification scores, except tubulitis (t), and arteritis (v) were significantly higher in the CAV-1/PAL-E Ó50% group than in the CAV-1/PAL-E <50 group (Table 3).

CAV-1 immunoreactivity in PTCs could be a useful diagnostic marker for Transplant Glomerulopathy.

Our data demonstrated that PTCs C4d positivity had low sensitivity and high specificity for the diagnosis of TG (53.1% and 87.9%), whereas, CAV-1 immunoreactivity (CAV-1/PAL-EÓ50%) in PTCs had higher sensitivity and slightly lower specificity than PTCs C4d positivity (78.1% and 74.2%).

Association between graft survival and CAV-1 immunoreactivity, or C4d staining in PTCs

The cumulative incidence of graft failure was higher in the CAV-1/PAL-E Ó50% group (21.4%, 47.6%, and 54.1% at 1, 3, and 5 years, respectively) than in the CAV-1/PAL-E <50% group (0.0%,

5.5%, and 12.1% at 1, 3, and 5 years, respectively) (log-rank, $p < 0.001$; Fig. 2). There was no significant correlation between C4d immunoreactivity in PTCs and the development of graft failure (log-rank, $p = 0.345$; Fig. 3).

CAV-1 immunoreactivity as an independent risk factor for graft survival

Putative determinants of graft failure development were analyzed using univariate and multivariate logistic regression models. In the univariate Cox regression analysis, most variables were significantly associated with graft survival, except recipient age at diagnosis, C4d staining in PTCs, and the Banff i and v scores. In the multivariate analysis, CAV-1 immunoreactivity in PTCs, which was superior to the pathological CAAMR, ci (interstitial fibrosis) score and the Banff cg (duplication or ñdouble contoursò in glomerular basement membrane) score, was independently associated with graft survival (Table 4).

Discussion

This study showed that CAV-1 immunoreactivity levels in PTCs, derived from semi-quantitative determinations, may be a useful prognostic marker for kidney transplant graft survival. It also demonstrated that CAV-1 immunoreactivity in PTCs reflected pathological findings, including ñcgò and ñciò score, and was an independent factor for kidney graft survival. An additional finding was that C4d immunoreactivity in PTCs was not associated with kidney graft survival.

Caveolae, and thus CAV-1 expression, are a distinct feature of chronic-rejection-induced transplant capillaropathy (11). However, the association between CAV-1 immunoreactivity in PTCs and graft survival is not fully understood. One of the main findings of the present study was the relationship between semi-quantitatively determined CAV-1 immunoreactivity levels in PTCs and kidney graft survival. As expected, the CAV-1/PAL-E Ó50% group had a lower graft survival rate because of a higher rate of TG expression, which has been reported to be strongly correlated with lower graft survival. This result is, however, in contrast to the findings of a molecular microscopy study (5), which showed that the expression of CAV-1 mRNA was not significantly associated with the survival of the transplanted kidney. The discrepancy to our own observations may be due to the different methodologies: semi-quantitative CAV-1 immunoreactivity in PTCs versus CAV-1 mRNA collected from a core biopsy (5). As shown in Fig. 1, the normal distribution of CAV-1 immunoreactivity in the stable kidney allograft is mainly in the medial (smooth muscle cell) layer of interlobular arteries, with additional expression in Bowmanôs capsule and the vasa recta. Therefore, the measurement of CAV-1 mRNA in a core biopsy could dilute the effect of CAV-1 expression in PTCs.

Since the discovery of CAV-1 in 1992, much has been learned regarding the functions of caveolae and CAV-1 in ECs, such as macromolecular transcytosis, vascular permeability, regulation of eNOS, atherosclerosis, ion channel regulation, angiogenesis, and endothelial regeneration (7-12). In the PTCs of kidney allografts, the expression of CAV-1 may be related to endothelial regeneration following active endothelial damage by antibody-mediated mechanisms. Evidence supporting this

hypothesis comes from developmental studies of rat glomerular capillary ECs, in which caveolae are present during the immature stage but disappear from S-shaped bodies in the maturing kidney. In addition, in a model of Thy-1.1 nephritis used in the study of glomerular endothelium regeneration, caveolae were transiently expressed during the regeneration stage and disappeared after its completion (12). However, these findings are not from PTCs but from glomerular capillaries, such that the significance of CAV-1 expression in PTCs in kidney allografts remains to be definitively demonstrated. CAV-1 immunoreactivity in the endothelial cells of glomerular capillaries (GCs) is segmental and weaker pattern than that in PTCs in the case of transplant glomerulopathy as shown in Fig. 1B and 1C. We have no convincing reason to this discrepancy, however, one possible explanation is the difference of GCs and PTCs. For example, endothelial cells in GCs have at least four complement inhibitors (decay accelerating factor, membrane cofactor protein, complement receptor 1 and protectin), in contrast, endothelial cells in PTCs have only one (protectin). These difference often explain the difference of C4d immunoreactivity between GCs and PTCs in the case of transplant glomerulopathy (13). Of note, in human glomerulus, glomerular CAV-1 expression is normally recognized in mesangial cells, podocytes and Bowman epithelial cells. Therefore, the evaluation of glomerular endothelial CAV-1 has potential limitation using only immunohistochemistry (14).

The pathophysiological significance of caveolae expression is also unclear. In CAAMR, leukocytes migrate and adhere to ECs in PTCs, in response to increased expression of the leukocyte adhesion

molecules ICAM (intracellular adhesion molecule)-1, E-selectin, and VCAM-1 by capillary ECs. The activated leukocytes adhere to and thereby damage the endothelium (15). In this study, higher CAV-1 immunoreactivity (CAV-1/PAL-E≥50%) in PTCs was associated with higher microvascular inflammation ($p < 0.0001$ compared with CAV-1/PAL-E<50% by the Mann-Whitney test, Table 3). These results did not clarify whether CAV-1 expression was harmful or protective to endothelial injury because endothelial injury and repair/regeneration could occur at the same time in continuous endothelial injury such as CAAMR. Powter et al. showed the anti-inflammatory effect of caveolae *in vitro* using senescent ECs expressing high levels of caveolae and its components (CAV-1, Cavin-1 and Cavin-2). These cells inhibited neutrophil recruitment by decreasing the expression of VCAM-1 and E-selectin and by suppressing NF-əB (nuclear factor kappa B) activity; the effect was reversed by CAV-1 knockout in senescent ECs. The results suggested the anti-inflammatory effect of caveolae and its components, which may have implications in the protection of kidney transplants (16).

CAV-1 has a unique morphology in that both its C- and N-termini face the cytoplasm. The protein binds to the caveolae membrane via its hydrophobic domain. The scaffolding domain has been mapped to amino acids 61ï 101 and comprises the oligomerization domain (amino acids 81ï 101), which binds to and regulates the activity of signaling proteins. Moreover, the scaffolding domain of CAV-1 interacts with eNOS to suppress the enzymeôs activation (17). This interaction is also suggested by observations in CAV-$1^{\bar{I}/\bar{I}}$ mice, which have elevated basal levels of endothelial NO (18). In ECs, NO exhibits anti-aggregation effects and suppresses the expression of leukocyte adhesion

molecules (19). Therefore, increased CAV-1 expression in PTCs could be harmful to ECs via inhibition of eNOS activities. However, an opposite relationship between CAV-1 and eNOS has also been reported. For example, an increase in vascular endothelial growth factor-induced eNOS stimulation in response to CAV-1 was reported (20). Further studies are needed to clarify the role of CAV-1 expression in eNOS activity in kidney allografts.

In our multivariable regression model, CAV-1 immunoreactivity in PTCs was an independent risk factor for kidney graft survival, and was superior to pathological findings such as TG and interstitial fibrosis for graft survival risk. Previous reports showed direct roles for TG and interstitial fibrosis / tubular atrophy (IF/TA) in kidney graft failure (21-23). Kieran et al. reported a graft failure rate of 68% (13 of 19) in transplant patients with TG compared with 12% (7 of 59) in those without TG ($p < 0.0001$) (21). In addition, 30.7% and 15.0% of the graft failures were caused by IF/TA and TG, respectively (22). Moreover, moderate or severe IF/TA and TG were independently associated with death-censored graft survival in the recipients who had allograft biopsies after one-year post-transplant (23). These results suggest that CAV-1 immunoreactivity in PTCs, which may be related to endothelial regeneration after chronic allograft damage, is a significant risk factor for allograft survival along with other chronic histopathological damage, including IF/TA and TG.

Another main finding of this study was the lack of an association between C4d immunoreactivity in PTCs and graft survival. In 1996, Feucht et al. proposed C4d immunoreactivity in PTCs as a footprint of the classical activation of complement, induced by the interaction between ECs and DSA,

and the association with kidney graft survival (24). Since then, many studies have suggested the importance of C4d immunoreactivity in the diagnosis of CAAMR and in poor graft survival. Consequently, C4d immunoreactivity in PTCs was introduced into the 2007 Banff classification as one of the diagnostic criteria for CAAMR (24-27). However, C4d-negative CAAMR was added to the 2013 Banff classification because of the low diagnostic sensitivity of C4d immunoreactivity (3). Moreover, in recent years, several studies have failed to find an association between C4d immunoreactivity and graft survival, similar to our own results. Loupy et al. described C4d as an insufficient indicator of CAAMR activity and proposed the MVI score and positivity for class II DSA at the early post-transplantation phase as superior substitute markers (28). Also, Sis et al. showed that the concomitance of ENDATs and DSA is associated with kidney allograft survival, but that the latter was not further influenced by the additional presence of C4d immunoreactivity (5). Alloantibodies themselves can upregulate the expression of leukocyte adhesion molecules, including VCAM-1 and ICAM-1, which can induce leukocyte recruitment to ECs (29, 30) and cause their injury via interactions with NK (natural killer) cells and macrophages (31, 32). In fact, recent transcriptional analyses of kidney transplant specimens showed that transcripts associated with NK cells and their signaling pathways were increased in CAAMR (33, 34). These complement-independent mechanisms of alloantibody induction might be significantly associated with graft survival, without C4d immunoreactivity in ECs. In addition, in PTCs, C4d immunoreactivity is easily altered by immunosuppressive therapies for CAAMR and can rapidly (4ï

8 days) diminish, which can complicate evaluation of the degree of histopathological injury due to CAAMR (35). In the present study, CAV-1 immunoreactivity (CAV-1/PAL-E≥50%) in PTCs was higher sensitivity than PTCs C4d positivity for the diagnosis of transplant glomerulopathy, which is a major histopathological finding of chronic endothelial cell injury, suggesting CAV-1 could be a useful diagnostic marker for transplant glomerulopathy.

This study has several important limitations. First, detailed information on DSA was lacking, and the follow-up period was relatively short. Second, it was retrospective in its design and based on a small sample size. Additional studies are needed to assess whether the increased expression of caveolae and CAV-1 in PTCs is beneficial or injurious to the kidney graft. Third, we used cut-off level at 50% in CAV-1 immunoreactivity provisionally. Given clinical usefulness, we try to adopt cut-off levels at 10%, 25%, and 50% for CAV-1 immunoreactivity according to 2013 Banff classification (3). However, cases with CAV-1 immunoreactivity 10%>CAV-1/PAL-E were only two cases and that in 25%>CAV-1/PAL-E were 16 cases, respectively. Therefore, we cannot avoid adopting the cut-off level at 50% in CAV-1 immunoreactivity in this study. Fourth, this study was performed in a single transplant institution and followed until May 2013, therefore, we could not evaluate validity and a reproducibility using a validation set in this study. Thus, further studies are needed to confirm these results clearer in multi-institutional research with long-term follow-up. Nonetheless, our results suggest that CAV-1 immunoreactivity in PTCs, as determined by a

semi-quantitative evaluation, is a useful prognostic marker and, compared with other conventional predictive factors, a superior independent risk factor for kidney graft survival. Investigation of the function of caveolae and their components will shed light on their relationships with kidney transplant survival and on the underlying pathophysiologic mechanisms. This should facilitate the development of new treatment strategies to prevent allograft rejection and failure.

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Author contributions

YN: designed research/study, performed research/study, collected data, analyzed data, and wrote the paper. IY: designed research/study, performed research/study, and wrote the paper. SH:

performed/analyzed histological data. AK-HY: performed research/study, MO: performed research/study, collected data, and manuscript review. HI, TY, and KT: performed research/study, and manuscript review

Figure legends

Figure 1: Representative CAV-1 and PAL-E immunoreactivities in the peritubular capillaries of transplant biopsy specimens with (Aï C) and without transplant glomerulopathy (Dï F). The expression of PAL-E, a marker of peritubular capillaries, was seen in both sets of specimens (compare A and D). CAV-1 immunoreactivity was detected in the walls of peritubular and glomerular capillaries in transplant glomerulopathy specimens (B), but was not detected when there was no rejection (E). CAV-1 staining in the medial smooth muscle cells and Bowman capsules of rejected and non-rejected specimens was the same as that in the normal kidney (B, E). CAV-1 and PAL-E staining in the transplant glomerulopathy (C) and non-rejection (F) images were merged.

Figure 2: Graft survival rate of the CAV-1/PAL-E < 50% and CAV-1/PAL-E Ó50% groups.

Figure 3: Survival rate of C4d (+) and C4d (ī) grafts.

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【Table 1】Patient characteristics

	Mean±SD
N	98
Age (years)	44.7±14.7
Male sex	65 (66.3%)
Serum creatinine at diagnosis (mg/dl)	1.63±1.02
Post-transplant time to biopsy (years)	8.29±5.46
Follow-up period after biopsy (years)	2.98±1.79
Induction biopsies	51 (52.0%)
Number of graft failures	24 (24.5%)
Rate of C4d positivity on PTCs	24 (24.5%)
Caveolin-1 immunoreactivity (%)	47.7±22.3
Living kidney transplantation	91 (92.9%)
TG cases	32 (32.7%)

PTCs: peritubular capillaries

TG: transplant glomerulopathy

Caveolin-1 immunoreactivity: the mean percentage of PTCs staining for Caveolin-1 in PTCs

【Table 2】Clinical characteristics of the CAV-1/PAL-E <50% and CAV-1/PAL-E Ó50% groups

	CAV-1/PAL-E <50%	CAV-1/PAL-E Ó50%	*p*-value
N	56	42	
Age	43.68±12.73	46.0±12.8	0.3026
Male sex	35 (62.5%)	30 (71.4%)	0.3571
Serum creatinine at diagnosis	1.33±0.43	2.03±1.40	0.0013
Post-transplant time to biopsy (year)	7.39±5.02	9.36±5.86	0.0958
Follow-up period (year)	3.47±1.87	2.34±1.45	0.0916
Induction biopsies	18 (32.1%)	33 (78.6%)	<0.0001
Number of graft failures	5 (8.9%)	19 (45.2%)	<0.0001
Rate of C4d positivity on PTCs	6 (10.7%)	18 (42.9%)	0.0003
Caveolin-1 immunoreactivity (%)	31.4±10.3	69.3±14.0	<0.0001
TG cases	7 (12.5%)	25 (59.5%)	<0.0001

PTCs: peritubular capillaries TG: transplant glomerulopathy

Caveolin-1 immunoreactivity: the mean percentage of PTCs staining for Caveolin-1 in PTCs

【Table 3】Renal histopathology according to the Banff scoring system in the CAV-1/PAL-E <50% and CAV-1/PAL-E Ó50% groups

	CAV-1/PAL-E <50%	CAV-1/PAL-E Ó50%	*p*-value
i	0.054±0.227	0.381±0.623	0.0006
t	0.018±0.134	0.095±0.484	0.3931
g	0.125±0.429	0.881±1.087	<0.0001
v	0.000±0.000	0.048±0.216	0.1007
ci	0.893±0.802	1.452±0.942	0.0028
ct	0.857±0.796	1.452±0.942	0.0015
cg	0.196±0.553	1.357±1.265	<0.0001
cv	0.000±0.000	0.214±0.606	0.0084
ptc	0.196±0.585	1.262±0.964	<0.0001
ah	1.536±1.078	1.976±1.047	0.0395
MVI	0.321±0.936	2.143±1.775	<0.0001

MVI: microvascular inflammation (g + ptc according to the 2013 Banff classification).

【Table 4】Cox regression hazard analysis of graft survival correlations[a]

Univariate analysis			
Variable	χ^2 score	HR (95% CI)	*p*-value
Clinical Paramaters			
Age at diagnosis	1.9504	1.0221 (0.9912-1.0539)	0.1625
Post-transplant time to biopsy	3.9217	1.0064 (1.0001-1.0128)	0.0477
Pathological Paramaters			
i	1.4165	1.5198 (0.7628-3.0278)	0.2340
g	19.6898	2.1243 (1.5230-2.9632)	<0.0001
v	1.6695	3.7795 (0.5029-28.4024)	0.1963
ci	9.3445	2.0463 (1.2930-3.2386)	0.0022
ct	9.7431	2.0606 (1.3087-3.2445)	0.0018
cg	23.1929	2.2827 (1.6314-3.1940)	<0.0001
cv	15.8175	3.3071 (1.8343-5.9627)	0.0001
ptc	10.9146	1.7885 (1.2668-2.5251)	0.0010
ah	6.7668	1.8647 (1.1661-2.9820)	0.0093
MVI	17.2736	1.5133 (1.2447-1.8398)	<0.0001
Pathological CAAMR	15.1505	5.8270 (2.3989-14.1540)	<0.0001
CAV-1/PAL-E	19.2812	64.4592 (10.039-413.88)	<0.0001
C4d staining	0.8870	1.5049 (0.6428-3.5230)	0.3463
Multivariate analysis model 1			
ci	3.6718	1.6697 (0.9884-2.8206)	0.0553
cg	6.8054	2.0696 (1.1983-3.5746)	0.0091
Pathological CAAMR	0.0335	1.1427 (0.2742-4.7616)	0.8547
Multivariate analysis model 2			
CAV-1/PAL-E	4.5852	11.017 (1.2253-99.061)	0.0322
ci	3.4098	1.6368 (0.9702-2.7614)	0.0648
cg	2.9705	1.5972 (0.9378-2.7202)	0.0848
Pathological CAAMR	0.0522	1.1665 (0.3110-4.3753)	0.8193

MVI: microvascular inflammation (g + ptc according to the 2013 Banff classification). HR: hazard ratio, CI: confidence interval.

Pathological CAAMR: meeting both criteria 1 and 2 of the Banff 2013 classification for CAAMR

[a] The ñtò score in the Banff classification could not be calculated because of the very low number of positive samples in the univariate analysis.

[b] Hazard ratios in each Banff score are per unit increase

Figure 1

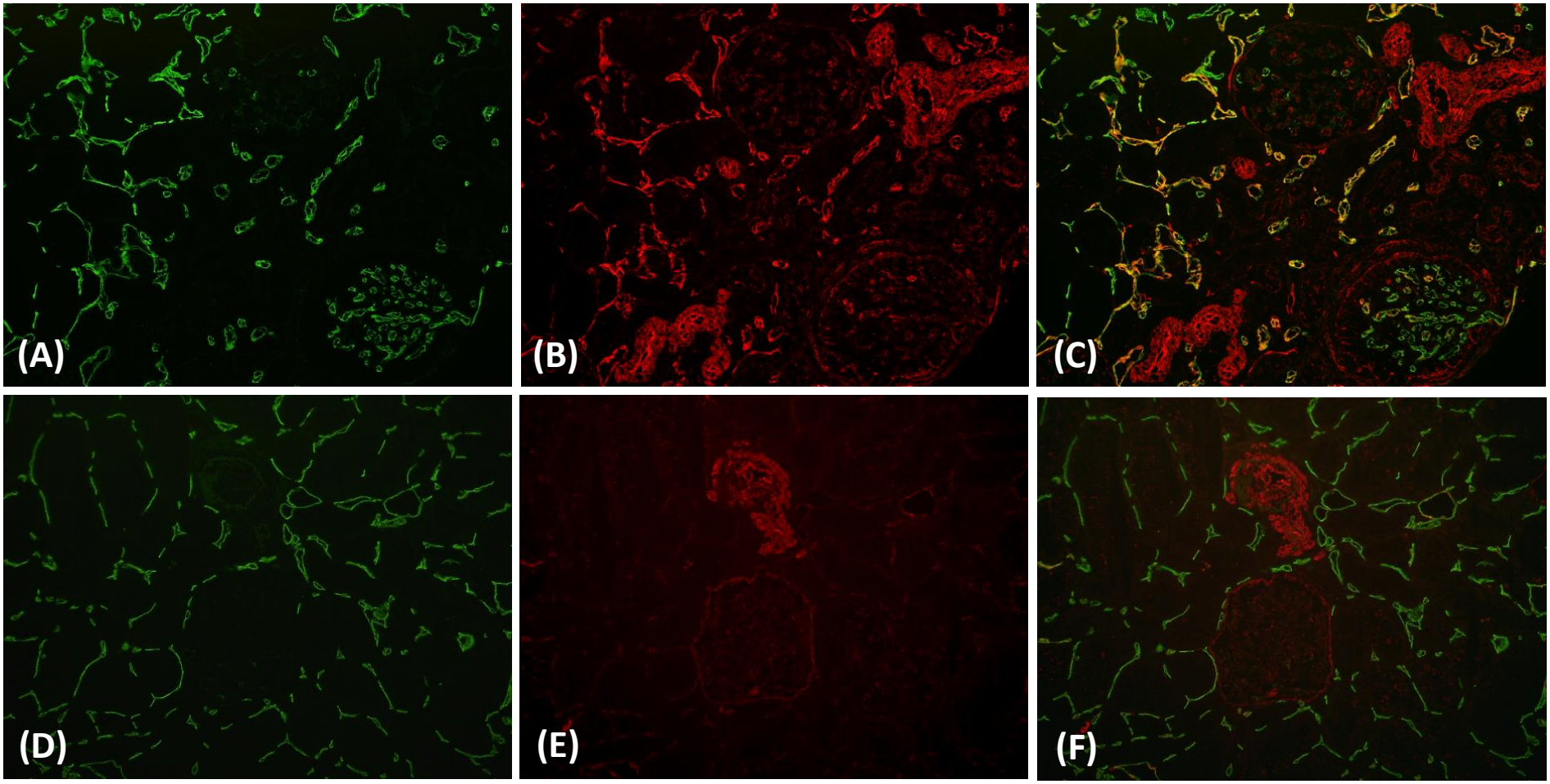
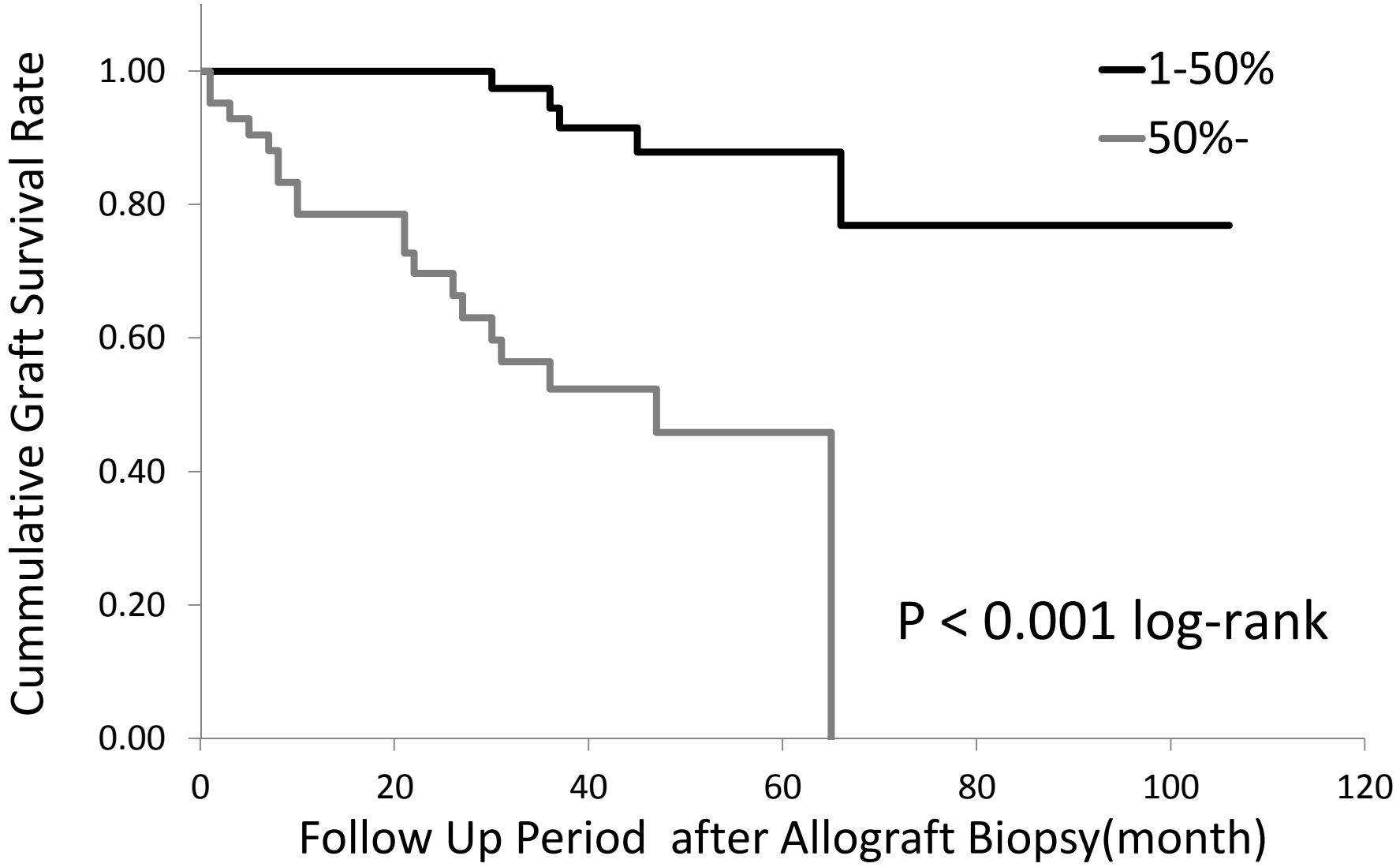
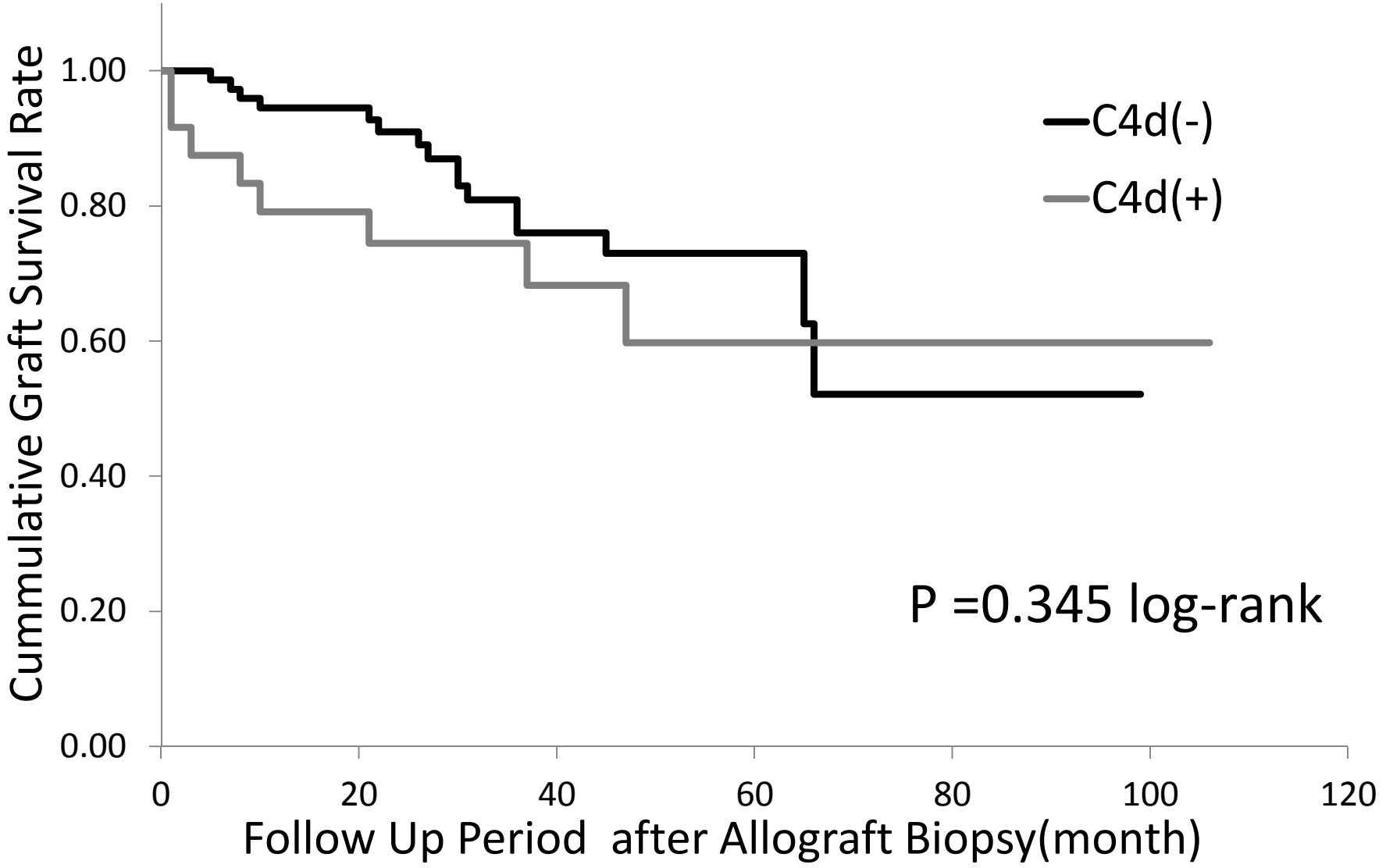


Figure 2



No. at risk						
1-50%	56	44	29	12	5	2
50-%	42	28	14	2	0	0

Figure 3



No. at risk

C4d(-)	74	54	29	10	3	0
C4d(+)	24	18	12	4	3	1