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Significance of Multinucleated Polyploidization of Tubular Epithelial Cells in Kidney Allografts

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Keywords
Kidney transplantation · T-cell-mediated rejection · Multinucleated polyploidization

Abstract

Introduction: Multinucleated polyploidization (MNP) of tubular epithelial cells is occasionally observed in kidney allografts. The present study aimed to clarify the clinical and pathological significance of MNP of tubular epithelial cells in kidney allografts. ***Methods:*** Fifty-eight 1-year biopsies from 58 patients who underwent kidney transplantation at our hospital from January 2016 to December 2017 were included. MNP was counted in each specimen, and the specimens were divided into two groups by the median value. The differences in clinical and pathological characteristics were compared. Ki67-positive cells were counted among tubular epithelial cells to explore the association between cell cycle and MNP. In an additional cohort, MNP was compared between biopsies after precedent T-cell-mediated rejection and precedent medullary ray injury. ***Results:*** The 58 cases were divided into two groups by the median total amount of MNP: group A (MNP > 3) and group B (MNP ≤ 3). Maximum t-score before the 1-year biopsy was significantly higher in group A compared with group B. Other clinical or histological characteristics did not differ significantly. Total amount of Ki67-positive tubular epithelial cells was significantly correlated with total amount of MNP. Significantly higher amount of MNP was observed in cases with precedent T-cell-mediated rejection compared with precedent medullary ray injury. On receiver operating characteristic curve analysis, the cut-off value of MNP to predict precedent T-cell-mediated rejection was 8.5. ***Conclusions:*** MNP in tubular epithelial cells reflects prior tubular inflammation in kidney allografts. High amount of MNP indicates precedent T-cell-mediated rejection rather than precedent medullary ray injury caused by nonimmune etiologies.

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Introduction

Multinucleated polyploidization (MNP) of tubular epithelial cells, defined as three or more nuclei, is occasionally observed in kidney allograft biopsies based on expert experience [1]. However, the clinical and pathological significance has not been determined in previous studies. Polyploidy describes three or more copies of the genome [2] and polyploidization makes cells capable of growth [3]. Polyploidization has been observed after injury to the heart [4] and liver [5]. During repair of an injured kidney, surviving intrinsic epithelial cells have an important responsibility for tissue recovery [6]. Therefore, tubular epithelial cells are suggested to have high regenerative capacity for replacing injured tissue. However, it was previously demonstrated that progenitor cells, as a subset of tubular epithelial cells, have the capacity to differentiate and proliferate, while other cells lack that capacity and cause polyploidization instead of normal mitosis [7].

We hypothesized that MNP results from regenerative changes caused by tubular injuries in kidney allografts. To investigate the association between MNP and tubular injuries in allografts, we performed a retrospective study using biopsies obtained at 1 year after kidney transplantation.

Materials and Methods

Population, Histopathology, and Study Design

We selected patients who underwent kidney transplantation at Toho University Omori Medical Center from January 2016 to December 2017 and had an allograft biopsy at 1 year after transplantation. We excluded patients who lacked a 1-year biopsy and were aged <20 years. Finally, 58 biopsies from 58 patients were eligible in the study. Of these specimens, 57 were protocol biopsies and 1 was an episode biopsy. Corticosteroids, tacrolimus or cyclosporin, and mycophenolate mofetil were used in combination as maintenance immunosuppressants at the time of the 1-year biopsy. Everolimus was also used in some cases. Before the 1-year biopsy, a 1-h implantation biopsy was performed in all cases and a 3-month protocol biopsy was performed in 57 cases in addition to the indication biopsy.

MNP in tubular epithelial cells was defined as three or more nuclei that were adjacent to or overlapped each other in a proximal tubule without severe atrophy. Cells with MNP were identified and their total amounts were counted in periodic acid Schiff-stained samples. A representative image of MNP is shown in Figure 1. The 58 patients were assigned to two groups (groups A and B) by the median total amount of MNP. Clinical and pathological characteristics were extracted from the medical records, and the clinical and histological characteristics were compared between the two groups. Factors that were independently correlated with MNP were further investigated. MNP may be affected by acute tubular necrosis (ATN), considering that ATN was shown to cause endoreplication, which can lead to multinucleated polyploidy [7]. MNP was also occasionally observed in cytomegalovirus (CMV) infection [1]. Therefore, as covariates, we selected ATN, CMV viremia, and factors significantly correlated with MNP. ATN was evaluated by the renogram pattern immediately after transplantation as previously described [8]. CMV C7HRP (pp65 antigenemia assay) was evaluated by the maximum number of positive cells in 50,000 white blood cells. We defined one or more positive cells in the C7HRP test as CMV viremia. Histological findings were evaluated according to the Banff classification [9–12].

To explore the association between MNP and cell cycle, Ki67 staining of the 1-year biopsies was performed. All proximal tubular epithelial cells positive for Ki67 staining were counted. A representative image of Ki67-positive findings is shown in Figure 2.

Statistical Analysis

All statistical analyses were performed with SPSS 23.0 software (IBM, Tokyo, Japan). Data were presented as mean ± standard deviation, number (percentage), or number. Comparisons between two groups were performed using the t test or Mann-Whitney U test for continuous variables and the Pearson χ^2 test or Fisher's exact test for dichotomized variables. In the multivariate analysis, ATN grade and prior CMV viremia, considered possible causes of MNP, and significant factors in univariate analyses of characteristics between the two groups were included as covariates. Correlation analysis was performed using Spearman's correlation analysis. We defined statistical significance as $p < 0.05$.

Results

Among the total 58 cases, MNP was observed in 54 cases (93%). The median total amount of MNP was 3 (range, 0–71). The cases were divided into two groups by the median total amount of MNP: group A (MNP > 3) and group B (MNP ≤ 3). Table 1 shows the clinical characteristics of the patients in the total cohort and the two groups. There were no significant differences between group A and group B. Table 2 presents the histological characteristics in the total cohort and the two groups. Maximum t-score before the 1-year biopsy was significantly higher in group A than in group B (0.61 ± 0.84 vs. 0.24 ± 0.61, $p = 0.031$), while t-score at the 1-year biopsy did not differ significantly. Only one case with precedent T-cell-mediated rejection (TCMR) before the 1-year biopsy was observed in group B. There were no differences in the length of renal tissue and other histological characteristics between the two groups. In the multivariate analysis, maximum t-score before the 1-year biopsy was the only significant factor that was independently correlated with MNP (odds ratio: 2.43, 95% CI: 1.02–5.79, $p = 0.045$), as shown in Table 3.

Regarding Ki67 staining, the median total count of Ki67-positive cells in the 58 cases was 13.5 (range: 0–142). In the correlation analysis, total amount of Ki67-positive

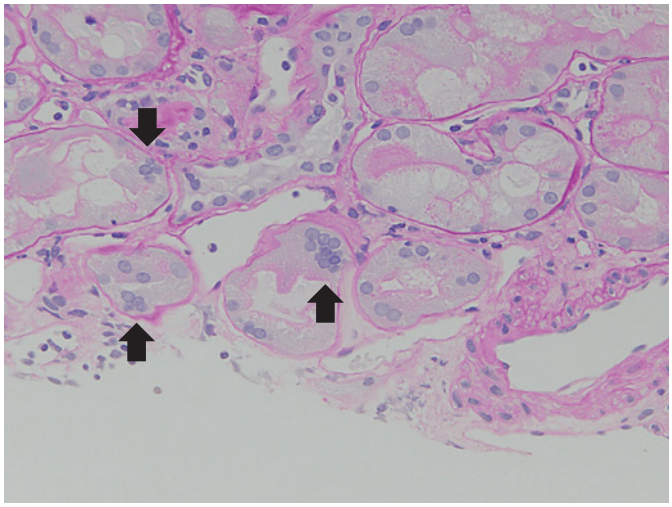

Fig. 1. Representative image of tubular epithelial cells with MNP (periodic acid Schiff staining; original magnification, ×400). The arrows indicate cells with MNP.

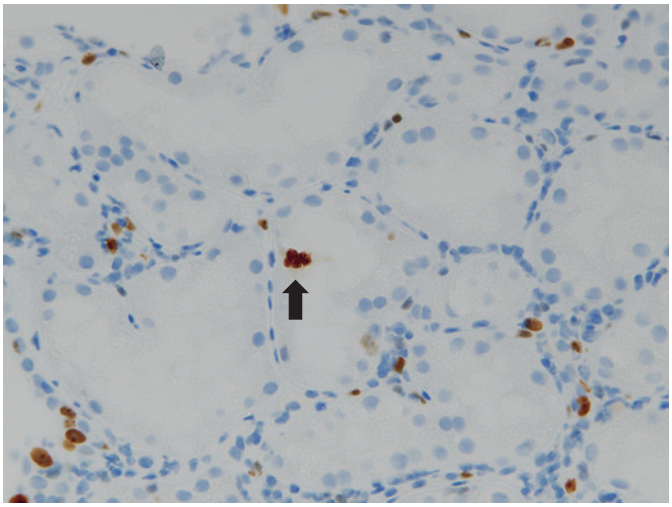

Fig. 2. Representative image of Ki67-positive epithelial cells in a proximal tubule. The arrow indicates Ki67-positive multinucleated polyploid cells.

Table 1. Clinical characteristics of the patients

	Total (n = 58)	Group A (n = 24)	Group B (n = 34)	p value
Recipient age, years	47.8±14.0	48.5±13.2	47.3±14.7	0.735
Recipient sex (male/female), n	35/23	14/10	21/13	0.792
Donor age, years	57.9±11.0	60.4±12.0	56.2±10.1	0.088
Donor sex (male/female), n	25/33	11/13	14/20	0.724
ATN grade, 1–4	1.75±0.85	1.57±0.79	1.88±0.88	0.171
CMV C7HRP (<1 year, maximum)*	25.3±77.5	27.7±61.6	23.6±87.9	0.447
Warm ischemic time, min	3.7±1.5	3.7±1.3	3.6±1.7	0.314
Cold ischemic time, min	103.3±122.2	90.3±48.2	112.4±154.4	0.443
Deceased donor, n (%)	3 (5.2)	1 (4.2)	2 (5.9)	1.000
Serum creatinine (1-year biopsy), mg/dL	1.29±0.37	1.34±0.41	1.25±0.34	0.331
eGFR (1-year biopsy), mL/min/1.73 m^2	47.0±13.5	45.2±15.2	48.3±12.2	0.182
Tacrolimus trough concentration (n = 32), ng/mL	6.8±1.6	6.4±1.3	7.1±1.8	0.336
Cyclosporine trough concentration (n = 26), ng/mL	120.7±51.8	145.3±68.2	109.7±40.2	0.067
MMF use, n (%)	58 (100)	24 (100)	34 (100)	
Everolimus use, n (%)	34 (58.6)	12 (50.0)	22 (64.7)	0.263
Urinary inclusion body (<1-year biopsy), n (%)	13 (22.4)	6 (25.0)	7 (20.6)	0.692
Urinary inclusion body (1-year biopsy), n (%)	3 (5.2)	1 (4.2)	2 (5.9)	1.000

Data are shown as mean ± standard deviation unless otherwise indicated. Group A, total amount of MNP >3; group B, total amount of MNP ≤3; ATN, acute tubular necrosis; CMV, cytomegalovirus; WBCs, white blood cells; eGFR, estimated glomerular filtration rate; MMF, mycophenolate mofetil. *C7HRP is shown as number of positive cells among 50,000 WBCs.

tubular epithelial cells was significantly correlated with total amount of MNP (r = 0.344, p = 0.008), as shown in Figure 3.

Additional Study

The multivariate analysis demonstrated that maximum t-score before the 1-year biopsy was a significant factor for MNP, but the study cohort included only 1 case with precedent TCMR. Therefore, an additional cohort analysis was performed to investigate whether precedent TCMR was associated with MNP. For this, the total amount of MNP in cases previously diagnosed with TCMR was compared with that in control cases previously diagnosed with medullary ray injury (MRI), based

Table 2. Histological characteristics of the patients

	Total (n = 58)	Group A (n = 24)	Group B (n = 34)	p value
Total amount of MNP	5.4±9.9	10.6±14.0	1.7±1.1	<0.001
Length of renal tissue, mm	20.3±7.8	21.0±8.1	19.8±7.7	0.555
ah-score (1-h biopsy)	0.36±0.61	0.50±0.72	0.26±0.51	0.201
aah-score (1-h biopsy)	0.03±0.18	0.04±0.20	0.03±0.17	0.803
t-score (<1-year biopsy, maximum)	0.39±0.73	0.61±0.84	0.24±0.61	0.031
TCMR (<1-year biopsy), n (%)	1 (1.7)	0 (0)	1 (2.9)	1.000
ABMR (<1-year biopsy), n (%)	9 (15.5)	5 (20.8)	4 (11.8)	0.467
BKV nephropathy (<1-year biopsy), n (%)	1 (1.7)	0 (0)	1 (2.9)	1.000
t-score (1-year biopsy)	0.26±0.61	0.33±0.76	0.21±0.48	0.694
ah-score (1-year biopsy)	0.57±0.68	0.75±0.68	0.44±0.66	0.060
aah-score (1-year biopsy)	0.31±0.65	0.33±0.64	0.29±0.68	0.696
ct-score (1-year biopsy)	0.82±0.50	0.75±0.53	0.88±0.49	0.333
ci-score (1-year biopsy)	0.51±0.60	0.46±0.59	0.55±0.62	0.594
TCMR (1-year biopsy), n (%)	2 (3.4)	2 (8.3)	0 (0)	0.167
ABMR (1-year biopsy), n (%)	10 (17.2)	6 (25.0)	4 (11.8)	0.291
BKV nephropathy (1-year biopsy), n (%)	0 (0)	0 (0)	0 (0)	

Data are shown as mean ± standard deviation unless otherwise indicated. TCMR, T-cell-mediated rejection; ABMR, antibody-mediated rejection; BKV, BK virus.

Table 3. Risk factors for MNP in the multivariate analysis

	Odds ratio (95% CI)	p value
t-score (<1-year biopsy, maximum)	2.43 (1.02–5.79)	0.045
ATN grade	0.55 (0.27–1.13)	0.105
CMV C7HRP (<1-year biopsy, maximum)	1.00 (1.00–1.01)	0.568

ATN, acute tubular necrosis; CMV, cytomegalovirus.

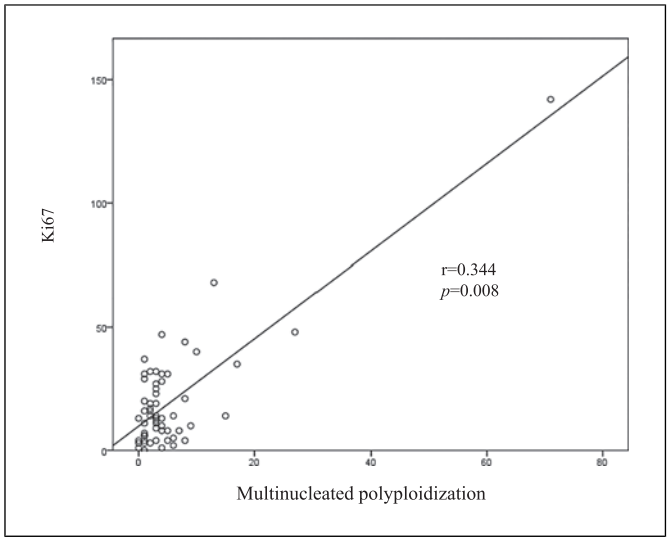

Fig. 3. Amount of Ki67-positive epithelial cells is significantly correlated with amount of MNP.

on a previous study [13]. MRI was defined as interstitial fibrosis and inflammation confined to the medullary ray and caused by nonimmune injury in the previous study. Additionally, in this study, MRI definition also required a score of 0 in both the t- and v-scores. MRI cases were selected as controls because they probably reflect ischemic changes without rejection [13]. We evaluated the next biopsies (index biopsies) just after the diagnosis of TCMR or MRI from January 2011 to October 2021. We excluded cases with borderline changes, TCMR, antibody-mediated rejection, or BK virus nephropathy in the index biopsy. Finally, we included 16 precedent TCMR cases and 13 precedent MRI cases. The total amount of MNP was significantly greater in the TCMR cases compared with the MRI cases (p = 0.037), as shown in Table 4. The TCMR cases had higher ct- and ci-scores than the MRI cases in the precedent biopsies. In the index biopsies, the ct-score was higher in the MRI cases compared with the TCMR cases, while the ci-score showed no difference.

Table 4. Comparison of MNP between precedent TCMR cases and precedent MRI cases

	Precedent TCMR (n = 16)	Precedent MRI (n = 13)	p value
Total amount of MNP	14.8±13.2	7.7±8.3	0.037
Time between biopsies, months	19.0±10.8	17.4±8.2	0.492
t-score (precedent biopsy*)	2.19±0.40	0±0	<0.001
i-score (precedent biopsy)	2.13±0.34	0.08±0.28	<0.001
ct-score (precedent biopsy)	1.64±0.75	0.92±0.28	0.003
ci-score (precedent biopsy)	1.14±0.66	0.46±0.52	0.010
t-score (index biopsy**)	0±0	0±0	
i-score (index biopsy)	0.38±0.62	0±0	0.030
ct-score (index biopsy)	0.75±0.58	1.15±0.38	0.039
ci-score (index biopsy)	0.44±0.51	0.85±0.69	0.100

Data are shown as mean ± standard deviation. TCMR, T-cell-mediated rejection; MRI, medullary ray injury. *Precedent biopsy represents the biopsy that showed TCMR or MRI in each group. **Index biopsy represents the next biopsy just after the precedent biopsy.

The diagnostic efficacy of MNP was further evaluated by receiver operating characteristic (ROC) curve analysis, as shown in Figure 4. The area under the curve in the ROC curve analysis was 0.728 (95% CI: 0.532–0.924, p = 0.037) and the cut-off value of MNP to predict precedent TCMR was 8.5 (sensitivity: 0.750, specificity: 0.769) according to the Youden index.

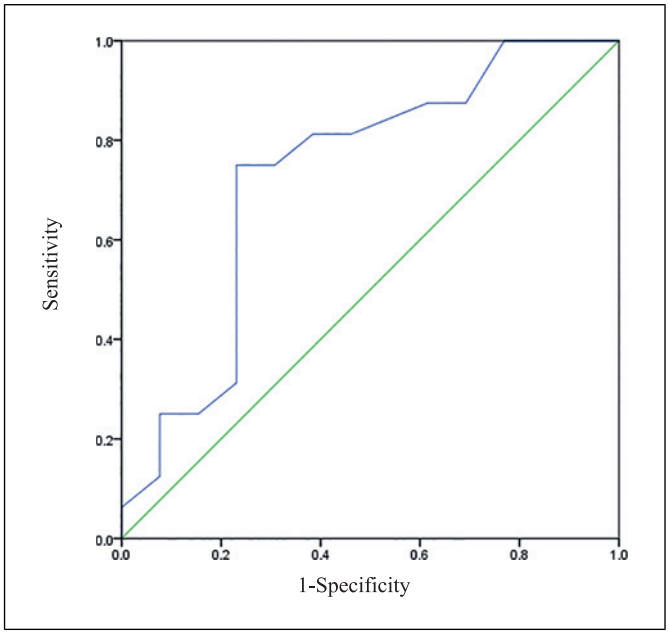

Fig. 4. ROC curve analysis for total amount of MNP to predict precedent TCMR.

Discussion

In the present study, MNP was found in 54 of 58 (93%) kidney allograft biopsies at 1 year after transplantation. According to the two groups divided by the median total amount of 3 for MNP, maximum t-score before the 1-year biopsy was significantly higher in group A than in group B. MNP in tubular epithelial cells was independently associated with previous maximum t-score. Amount of Ki-67-positive cells was significantly correlated with total amount of MNP. The additional study revealed that MNP was more prevalent in precedent TCMR cases than in precedent MRI cases. In the ROC curve analysis for total amount of MNP, the area under the curve was 0.728 and the cut-off value of MNP to predict precedent TCMR was 8.5.

Previous maximum t-score was significantly related to MNP in the 1-year biopsy (Table 3), while t-score at the 1-year biopsy did not differ between the two groups (Table 2). These findings indicate that preceding, but not ongoing, tubular inflammation is associated with MNP formation. To explore which kind of tubular injury was correlated with induction of MNP, we compared precedent TCMR cases with precedent MRI cases in an additional cohort. MRI was shown to be related to ischemic injuries [13], and we therefore selected precedent MRI cases as controls. In the additional study, the precedent TCMR cases had a higher amount of MNP than the precedent MRI cases (Table 4). However, the precedent MRI cases also had a moderately high amount of MNP. These findings suggest that MNP is not a specific change for precedent TCMR and may depend on the intensity or quality of tubular injuries. The results of the

ROC curve analysis indicated that a cut-off value for MNP of 8.5 could indicate precedent TCMR with high probability. Precedent TCMR was shown to be associated with an increased risk for de novo production of donor-specific antibodies [14]. Therefore, MNP may be a potentially useful marker to predict de novo production of donor-specific antibodies.

Amount of Ki67-positive cells was positively correlated with total amount of MNP. Ki67 is a cell cycle marker expressed from G1 phase to mitosis phase [15], suggesting that MNP may reflect cell cycle upregulation. A previous study demonstrated that there were two types of tubular epithelial cells: one that had the capacity to complete mitosis and regenerate and another that lacked the capacity for mitosis and instead entered the alternative cell cycle of endoreplication in mice and humans [7]. Endoreplication is a cell cycle that differs from mitosis and causes cell polyploidy [2]. Endoreplication was previously observed in kidney tubular injury [7]. Endoreplication comprises endomitosis and endocycle: the former causes mononucleated or binucleated polyploidy and the latter causes mononucleated polyploidy, and both occur in the cell cycle with Ki67 expression [2]. Therefore, it is impossible to distinguish endoreplication from typical mitosis using Ki67 staining [2]. Taken together, we assumed that Ki67-positive staining in allografts represents cell cycle acceleration and that this may be partially due to endoreplication because endoreplication can cause multinucleated polyploidy.

It is important to clarify whether MNP is clinically or histologically related to the normal healing process. At the time of the 1-year biopsy, there were no differences in the ct- and ci-scores and graft function. In addition, the ct- and ci-scores showed improvement over time in the comparisons between the precedent TCMR cases and precedent MRI cases. These findings may indicate that MNP can reflect the normal healing process of tubular inflammation. However, a longer observation period is needed to explore the future prognosis in cases with MNP.

We should consider the limitations of the present study. First, unknown covariates for MNP may not have been entirely excluded. Second, the population was limited to patients with an available 1-year biopsy specimen. Third, it remains unclear whether MNP is specific for tubular injury in kidney allografts. Most biopsies for tubular injury in the native kidney are typically performed only once. Therefore, we were unable to investigate MNP using precedent tubular injury in native kidney cases under the same conditions as the kidney allograft biopsies.

In conclusion, MNP in kidney allograft biopsies was significantly associated with maximum t-score before the 1-year biopsy, indicating prior tubular inflammation. Higher amount of MNP was found in precedent TCMR cases than in precedent MRI cases. The cut-off value for MNP to predict precedent TCMR was 8.5. MNP was correlated with Ki67 expression in tubular epithelial cells, implying that it is caused by endoreplication during recovery from tubular injuries.

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Statement of Ethics

This study was approved by the Ethics Committee of Toho University Omori Medical Center (approval numbers: M21291 21129). Patients were provided the opportunity to refuse participating the present study by opt-out.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Noriyuki Kounoue designed the study, collected data, performed analyses, and wrote the paper. Hideyo Oguchi designed the study, contributed to the pathological evaluation, and revised the paper. Naobumi Tochigi contributed to the pathological methodology and data interpretation. Tetuo Mikami contributed to the pathological evaluation and data interpretation. Yutaka Yamaguchi contributed to the study conception and pathological evaluation. Kazuho Honda, Masaki Muramatsu, Yoshihiro Itabashi, and Ken Sakai contributed to the data interpretation and revised the paper. Takashi Yonekura contributed to the methodology and data interpretation.

Data Availability Statement

All data can be available upon reasonable request to the corresponding author.

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