OPEN

Rejection-associated Phenotype of De Novo Thrombotic Microangiopathy Represents a Risk for Premature Graft Loss

Vojtech Petr, MD,[1] Petra Hruba, PhD,[2] Marek Kollar, MD,[3] Karel Krejci, MD, PhD,[4] Roman Safranek, MD, PhD,[5] Sona Stepankova, MD,[6] Jarmila Dedochova, MD,[7] Jana Machova, MD,[8,9] Jakub Zieg, MD, PhD,[10] Janka Slatinska, MD,[1] Eva Pokorna, MD, PhD,[11] and Ondrej Viklicky, MD, PhD[1,2,11]

Background. Thrombotic microangiopathy (TMA) significantly affects kidney graft survival, but its pathophysiology remains poorly understood. **Methods.** In this multicenter, retrospective, case–control paired study designed to control for donor-associated risks, we assessed the recipients' risk factors for de novo TMA development and its effects on graft survival. The study group consists of patients with TMA found in case biopsies from 2000 to 2019 (n = 93), and the control group consists of recipients of paired kidney grafts (n = 93). Graft follow-up was initiated at the time of TMA diagnosis and at the same time in the corresponding paired kidney graft. **Results.** The TMA group displayed higher peak panel-reactive antibodies, more frequent retransplantation status, and longer cold ischemia time in univariable analysis. In the multivariable regression model, longer cold ischemia times (odds ratio, 1.18; 95% confidence interval [CI], 1.01-1.39; $P = 0.043$) and higher peak pretransplant panel-reactive antibodies (odds ratio, 1.03; 95% CI, 1.01-1.06; $P = 0.005$) were found to be associated with increased risk of de novo TMA. The risk of graft failure was higher in the TMA group at 5 y (hazard ratio [HR], 3.99; 95% CI, 2.04-7.84; $P < 0.0001$). Concomitant rejection significantly affected graft prognosis at 5 y (HR, 6.36; 95% CI, 2.92-13.87; $P < 0.001$). De novo TMA associated with the active antibody-mediated rejection was associated with higher risk of graft failure at 5 y (HR, 3.43; 95% CI, 1.69-6.98; $P < 0.001$) compared with other TMA. **Conclusions.** Longer cold ischemia and allosensitization play a role in de novo TMA development, whereas TMA as a part of active antibody-mediated rejection was associated with the highest risk for premature graft loss.

(*Transplantation Direct* 2021;7: e779; doi: 10.1097/TXD.0000000000001239. Published online 22 October, 2021.)

INTRODUCTION

Thrombotic microangiopathy (TMA) is a well-recognized complication affecting the long-term outcome in kidney transplantation.[1] Although recurrent TMA in patients with atypical hemolytic–uremic syndrome is a rare event driven mainly by the recipient's gene mutation of complement factors and regulatory proteins, de novo TMA is a much more frequent posttransplant pathology that negatively

Received 30 June 2021. Revision received 19 August 2021.

Accepted 8 September 2021.

[1] Department of Nephrology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

[2] Transplant Laboratory, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

[3] Department of Clinical and Transplant Pathology, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

[4] 3rd Department of Internal Medicine—Nephrology, Rheumatology and Endocrinology, Teaching Hospital Olomouc, Czech Republic.

[5] Hemodialysis Centre, Teaching Hospital Hradec Kralove, Czech Republic.

[6] Centre for Cardiovascular and Transplantation Surgery, Brno, Czech Republic.

[7] Department of Medicine, Teaching Hospital Ostrava, Czech Republic.

[8] Department of Internal Medicine I, Faculty of Medicine in Pilsen, Charles University, Teaching Hospital Pilsen, Czech Republic.

[9] Biomedical Centre, Faculty of Medicine in Pilsen, Charles University, Pilsen, Czech Republic.

[10] Department of Paediatrics, Second Faculty of Medicine, Charles University in Prague and Motol University Hospital, Prague, Czech Republic.

[11] Transplant Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic.

The authors declare no conflicts of interest.

This work was supported by the grants from Ministry of Health of the Czech Republic NV19-06-00031 and NU21-06-00021, by its conceptual development of research organizations (Institute for Clinical and Experimental Medicine-IKEM, IN 00023001), and, in part, by the Charles University Research Fund Progres Q39.

K.K., R.S., S.S., J.D., J.M., J.K., J.S., and E.P. participated in the acquisition of the data. V.P. participated in research design, writing the article, performance of the research, acquisition of the data, and data analysis. P.H. participated in research design, writing the article, performance of the research, and data analysis. M.K. participated in data analysis. O.V. participated in research design, writing the article, performance of the research, and acquisition of the data.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

Correspondence: Ondrej Viklicky, MD, PhD, Department of Nephrology, Transplant Center, Institute for Clinical and Experimental Medicine, Videnska 1958/9, 140 21, Prague, Czech Republic. (ondrej.viklicky@ikem.cz).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001239

affects graft survival.[1-6] The pathogenesis of de novo TMA remains poorly understood. Multiple triggers have been implicated, such as ischemia-reperfusion injury,[7] immunosuppression with calcineurin inhibitors (CNIs) or the mechanistic target of rapamycin inhibitors,[3, 4] severe antibody-mediated rejection (AMR),[5, 8] and viral infections.[7] The common pathophysiology of de novo TMA links severe endothelial injury and complement dysregulation.[9] The aberrant complement regulation as a response to endothelial injury varies among individuals, which suggests hereditary susceptibility.[10] Availability of an assessment of polymorphisms or mutations of genes associated with complement system is not realistic at the time of transplantation. Therefore, better understanding of TMA clinical phenotypes may be of aid to clinicians in therapy modification. Previous clinical descriptions of de novo TMA suffered from small sample sizes and ill-defined control groups. In this large, national, retrospective, multicenter, case–control study with paired kidney grafts, we evaluated the risk factors for de novo TMA and the outcomes of its clinical phenotypes.

MATERIALS AND METHODS

Study Cohorts

We retrospectively evaluated histological reports of 4487 patients who had undergone kidney transplantation from 2000 to 2019, and we identified 122 biopsies with histologically proven posttransplant TMA (2.7%). To eliminate the effects of the donor-associated risk factors, a control group of paired kidney graft recipients was established. These kidney grafts had been transplanted in transplant centers all over the country.

As we focused on donor-controlled recipient risks for de novo TMA, cases with recurrent TMA, donor-derived TMA, living donor transplants, and/or an incomplete data set from both kidney grafts were not included (Figure 1).

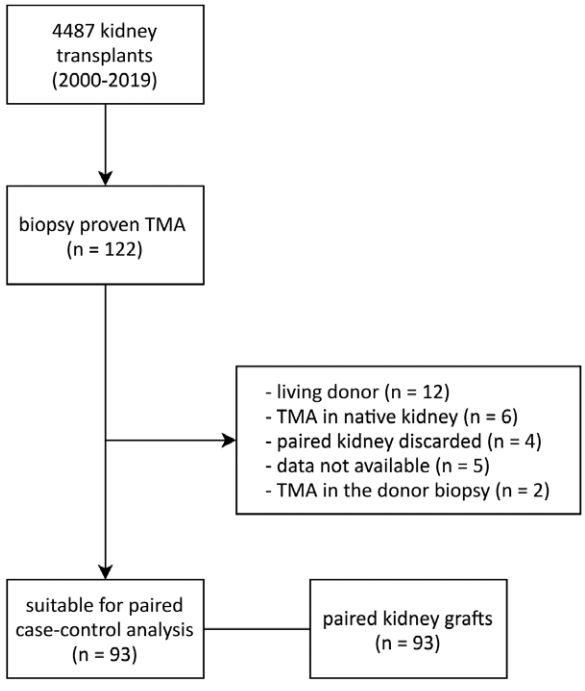

FIGURE 1. Study flow diagram. TMA, thrombotic microangiopathy.

Clinical data were collected from patients' medical records, and survival data were collected from the transplant registry. Patient demographics are presented in Table 1.

Kidney transplant recipients were HLA-typed for HLA-A, -B, and -DR loci (polymerase chain reaction sequence specific oligonucleotide probe technique, One Lambda, Inc), and deceased organ donors were HLA-typed for HLA-A, -B, -DR, and -DQ loci (polymerase chain reaction sequence specific primer (SSP) low-resolution kits, Olerup SSP, and Histo Type SSP, BAG). Pretransplant detection of antibodies specific to HLA-A, -B, -DR, and -DQ antigens was performed in 42% and 33% of recipients in the TMA and the control group, respectively (Table 1). Fifty-three percent of patients at the TMA diagnosis had performed anti-HLA antibodies detection (Table S1, SDC, http://links.lww.com/TXD/A376). Serum samples were analyzed using the LabScreen Mixed technique and, in the case of positivity, using the LabScreen Single Antigen Luminex technique (One Lambda, Inc). In indicated cases also, antibodies specific to HLA-DP and Cw antigens were determined.

TABLE 1.

Demographics and outcomes

	TMA group (n = 93)	Control group (n = 93)	*P*
Biopsy-proven TMA, n (%)	93 (100)	2 (2.1)	NA
Days to TMA occurrence, median (IQR)	9 (6–25.5)		NA
Male sex, n (%)	60 (65)	58 (62)	0.480
Recipient age, y (IQR)	53 (42–59)	54 (44–62)	0.690
Donor age, y (IQR)	55 (42–60)	55 (42–60)	NA
Extended criteria donor, n (%)	49 (53)	49 (53)	NA
Dialysis vintage duration, mo (IQR)	27 (13–44)	21 (14–35)	0.083
HLA mismatch (IQR)	3 (3–4)	3 (2–4)	0.502
PRA peak (IQR)	10 (2–40)	2 (0–10)	**0.004**
DSA at time of transplantation (positive/negative/not available)	10/29/54 (10/31/58%)	6/24/63 (6/25/67%)	0.375
FACS crossmatch at time of transplantation (positive/negative/not available)	5/29/59 (5/31/63%)	1/5/87 (1/5/93%)	**<0.001**
Retransplantation, n (%)	18 (19)	8 (9)	**0.004**
Mean cold ischemia time (h) (SD)	17.2 (±4.01)	15.8 (±3.63)	**0.006**
CNI-based maintenance regimen, n (%)	93 (100)	93 (100)	NA
Recipient CMV IgG positivity at transplantation, n (%)	78 (84)	74 (79)	0.458
CMV mismatch (D^+/R^-), n (%)	13 (14)	14 (15)	0.835
T cell–depletive induction, n (%)	44 (47)	22 (24)	**<0.001**
Delayed graft function, n (%)	60 (68)	28 (32)	**<0.001**
Rejection at time of TMA diagnosis, n (%)	28 (30)	N/A	N/A
Acute rejection in first year posttransplant	39 (42)	18 (19)	**<0.01**

Categorical variables are shown as the frequency and percentage within parentheses. Continuous variables are shown as the median and interquartile range within parentheses. Values printed in bold indicate statistical significance.

CMV, cytomegalovirus; CNI, calcineurin inhibitor; D^+/R^-, donor positive, recipient negative; DSA, donor-specific antibodies; FACS, fluorescence-activated cell sorting; IQR, interquartile range; NA, not applicable; PRA, panel-reactive antibodies; TMA, thrombotic microangiopathy.

Thrombocytopenia was noted in 36 patients (39%), and anemia was present in 69 patients (74%) at the time of TMA histological diagnosis (Table S1, SDC, http://links.lww.com/TXD/A376). The systemic manifestation of TMA was not further evaluated because of potential bias, as TMA was found in most cases shortly after transplantation. During this period, anemia and thrombocytopenia were generally frequently occurring consequences of surgery, bleeding, or depletive induction therapy.

Pathological Definitions

TMA diagnosis was based on the presence of 1 or more fibrin thrombi in glomeruli or small arteries and arterioles; endothelial swelling with luminal compromise of the glomerular capillaries with or without fragmented erythrocytes; and vascular fibrinoid necrosis or mucoid thickening of intima of small arteries/arterioles. The biopsies with TMA were reassessed by a pathologist (M.K.) for the purpose of this study. All biopsies were reassessed according to the most recent Banff classification 2019.[8] As many TMAs occurred as a part of acute rejection, 2 clinical phenotypes of de novo TMA were identified: TMA with rejection (TMA R^+) and TMA without rejection (TMA R^-). TMA R^+ was defined when both TMA and rejection were detected in the same biopsy. Because this is a retrospective study spanning 20 y, donor-specific antibodies analysis was available in 46 patients (49%) (Table S1, SDC, http://links.lww.com/TXD/A376).

Statistics

The statistics were calculated using R-Studio software, version 1.2.5019 (Development for RStudio, Inc, Boston, MA). Continuous variables were reported as medians and interquartile ranges (IQRs) or means with SD, and the paired *t* test or Mann-Whitney U test was used for a simple comparison of groups in univariable analysis. Categorical variables were reported as proportions, and the McNemar test was used to compare the groups in univariable analysis. Univariable conditional logistic regression was performed, and variables with $P < 0.01$ from univariable analysis were entered into the multivariable conditional logistic regression analysis. The results of conditional logistic regression analyses were expressed as odds ratios (ORs) and 95% confidence intervals (CIs). The Kaplan-Meier method and log-rank test were used to analyze time to graft loss. Graft survival was analyzed from the time of TMA diagnosis, and follow-up of the corresponding paired kidney graft was initiated at the same time. Censored patients were defined as death with a functional graft. Cox proportional-hazards models were used to estimate the hazard ratios (HRs) and 95% CIs for kidney allograft loss. An overall *P* value <0.05 was considered statistically significant.

The study was approved by the institutional review board of the Institute for Clinical and Experimental Medicine, Prague, Czech Republic. The study is in accordance with the 1964 Helsinki Declaration and its later amendments. The clinical and research activities being reported are consistent with the Principles of the Declaration of Istanbul as outlined in the "Declaration of Istanbul on Organ Trafficking and Transplant Tourism."

RESULTS

De Novo TMA Incidence and Manifestation

The incidence of de novo TMA was 2.1% in the study cohort (n = 93 out of 4487 patients). The TMA was diagnosed at a median of 9 postoperative days (PODs; range, 4–1584 d) (Figure S1, SDC, http://links.lww.com/TXD/A376). All TMA cases were found in indication biopsies. Most TMA cases developed soon after transplantation; 72 cases were diagnosed within the first 30 d (77%), 2 cases between 31 and 90 d (2%), and 12 cases between 91 d and 1 y (13%). Beyond 1 y, TMA occurred in just 7 cases. Treatment of TMA cases is outlined in detail in Tables S1 and S2 (SDC, http://links.lww.com/TXD/A376).

Interestingly, TMA was also found in the control group in 2 cases (Table 1).

In 1 case, TMA was found as a part of early AMR (C4d3, g1, ptc3) at POD 6 in the control group, whereas in the corresponding paired graft from study group, TMA was found at POD 50 without any signs of rejection.

In the second case, TMA was diagnosed at POD 210 in the control group with some degree of inflammation (i1, t1), whereas in corresponding paired graft from the study group, TMA was found as early as at POD 5, and no rejection was discovered.

Risk Factors of De Novo TMA Development

Demographics of both the TMA group and the control groups are presented in Table 1. Comparisons revealed that patients in the TMA group experienced longer cold ischemia time (CIT), more frequent retransplantation status, and higher peak pretransplant panel-reactive antibodies (PRAs). As a consequence of higher immunological risk in the TMA group, T cell–depletive induction was more frequently used. Patients in the TMA group experienced delayed graft function more frequently than the control group (68% versus 32%; $P < 0.001$).

There were no differences in the chronic kidney disease diagnosis between the 2 groups, as indicated in Table S3 (SDC, http://links.lww.com/TXD/A376).

Univariable conditional logistic regression was used to assess pretransplant risk factors for de novo TMA development, the result of which is presented in Table 2. Longer dialysis vintage, higher peak PRA, longer CIT, and retransplantation status were associated with higher risk of de novo TMA development in univariable analysis. In multivariable analysis, longer CIT (OR, 1.18; 95% CI, 1.01-1.39; $P = 0.043$) and higher peak pretransplant PRA (OR, 1.03; 95% CI, 1.01-1.06; $P = 0.005$) were both associated with increased risk of de novo TMA development (Table 2).

De Novo TMA Is Associated With Acute Rejection

TMA was associated with both antibody- and T cell–mediated rejection (TCMR) in a significant proportion of cases. Twenty-eight out of 93 patients (30%) in the TMA group (Table 3) experienced rejection. TMA was mostly associated with AMR in 17 out of 28 (61%) cases, whereas acute TCMR occurred in 11 out of 28 (39%) cases, respectively. Interestingly, in all cases of acute TCMR, endarteritis ($v > 0$) was present. Banff scores of the 93 biopsies with TMA are provided in Table S4 (SDC, http://links.lww.com/TXD/A376). Additional data on timing of rejections before and within

TABLE 2.

Pretransplant factors associated with de novo TMA development in univariable and multivariable conditional logistic regression analyses

	Univariable analysis			Multivariable analysis		
Pretransplant factors	**OR**	**95% CI**	***P***	**OR**	**95% CI**	***P***
Male sex	0.810	0.329-1.990	0.647			
Recipient age (y)	0.991	0.960-1.020	0.595			
Dialysis vintage (mo)	1.030	1.010-1.050	**0.005**	1.020	0.994-1.040	0.169
Retransplantation	5.060	1.560-16.40	**0.007**	1.140	0.264-4.890	0.864
HLA mismatch	1.190	0.808-1.760	0.377			
PRA peak	1.040	1.020-1.060	**<0.001**	1.030	1.010-1.060	**0.005**
Cold ischemia time (h)	1.330	1.140-1.550	**<0.001**	1.180	1.010-1.390	**0.043**

Values printed in bold indicate statistical significance.
CI, confidence interval; OR, odds ratio; PRA, panel-reactive antibodies; TMA, thrombotic microangiopathy.

TABLE 3.

Rejection in TMA biopsy

Rejection	TMA group (n = 93)
No rejection, n (%)	65 (69)
Active AMR, n (%)	17 (18)
Acute TCMR, n (%)[a]	11 (12)

[a]All patients experienced acute TCMR with $v > 0$; in addition, 1 patient in the TMA group also fulfilled criteria for grade I acute TCMR.
AMR, antibody-mediated rejection; TCMR, T cell–mediated rejection; TMA, thrombotic microangiopathy.

the first year after TMA diagnosis are available in Tables S1, S5, and S6 (SDC, http://links.lww.com/TXD/A376).

De Novo TMA Is Associated With Inferior Graft Outcome

It has been accepted that TMA after kidney transplantation represents a risk for graft failure. Similarly, in this study, 5- and 10-y graft survivals were inferior in the TMA cohort.

The death-censored graft survival was 56% and 56% in the TMA group and 88% and 87% in the control group at 5 and 10 y, respectively (Figure 2). The total follow-up was 385 person-years in the TMA group (median 3.22, maximum 17.8 y) and 526 person-years in the control group (median, 5.04; maximum, 17.8 y); no patient was lost to follow-up.

The risk of graft failure was nearly 4 times higher (HR, 3.99; 95% CI, 2.04-7.84; $P < 0.001$) at 5 y and 4 times higher at 10 y (HR, 4.13; 95% CI, 2.11-8.11; $P < 0.001$) in the TMA cohort compared with the control group.

Rejection Phenotype of De Novo TMA Is Associated With Worst Graft Outcome

The inferior graft survival in the TMA group prompted questions about what drives the poor survival. Concomitant rejection was the main driver leading to premature kidney graft failure. Patients who experienced acute rejection and TMA at biopsy (TMA R^+) had an inferior graft outcome compared with patients with rejection-free TMA (TMA R^-) phenotype

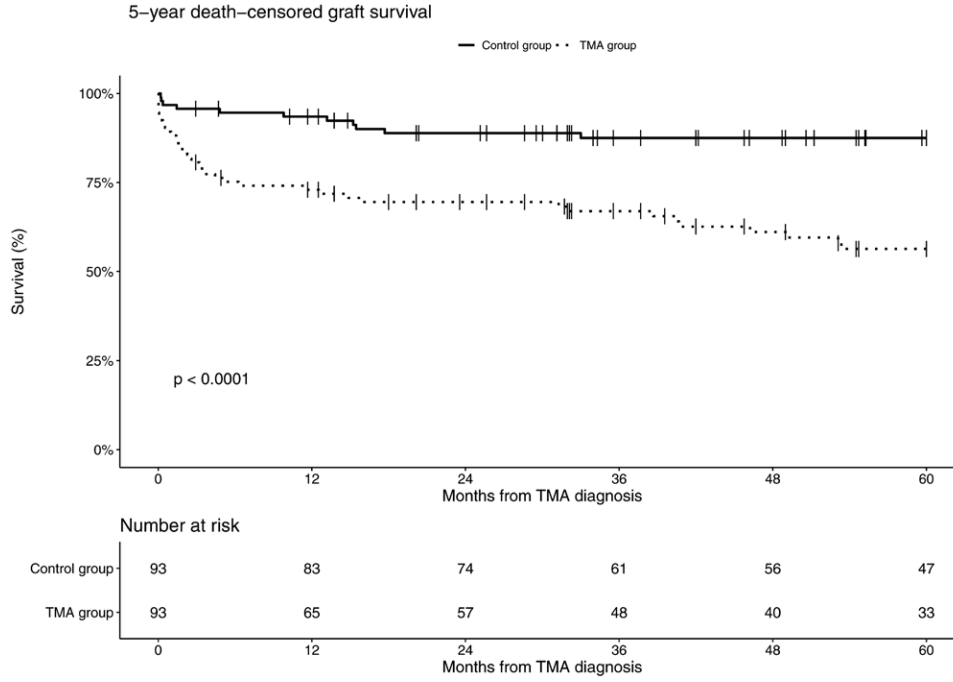

FIGURE 2. Five-y death-censored graft survival in the TMA group and the control group. TMA, thrombotic microangiopathy.

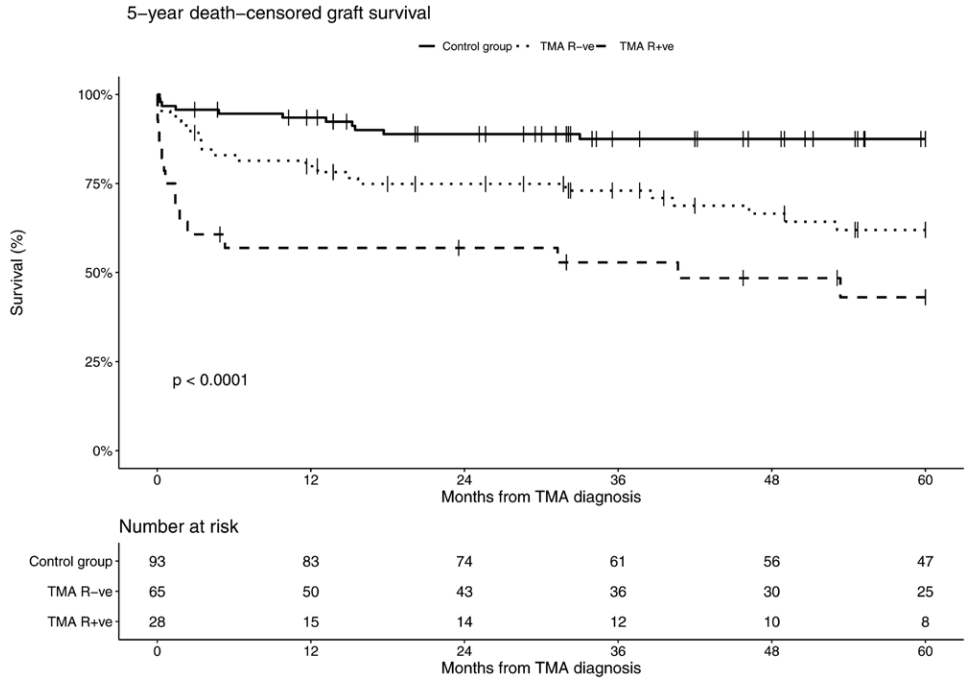

FIGURE 3. Five-y death-censored graft survival in patients with TMA coupled with rejection (TMA R^+), TMA without rejection (TMA R^-), and controls. TMA, thrombotic microangiopathy.

(Figure 3). The presence of rejection increased the risk of graft failure at 5 and 10 y, respectively. Compared with controls, the risk of graft failure at 5 and 10 y was >6 times higher in TMA R^+ subgroup (HR, 6.36; 95% CI, 2.92-13.87; $P<0.0001$ at 5 y and HR, 6.58; 95% CI, 3.02-14.36; $P<0.001$ at 10 y).

Graft survival at 5 y was worse in the TMA R^+ subgroup in comparison with the TMA R^- subgroup (HR, 1.99; 95% CI, 1.03-3.84; $P=0.04$).

We have also analyzed the effect of either active AMR or vascular rejection on graft survival at 5 y (Figure 4). Active AMR was associated with the worst graft survival (HR, 3.43; 95% CI, 1.69-6.98; $P<0.001$), whereas vascular rejection showed a similar risk of graft failure as the TMA R^- group (HR, 0.75; 95% CI, 0.22-2.49; $P=0.64$).

DISCUSSION

In this study, we demonstrate CIT and PRA as risk factors for de novo TMA development in kidney transplantation and show a detrimental effect of de novo TMA on graft survival. Moreover, we showed that de novo TMA in association with rejection, particularly with active AMR, represents the phenotype with the highest risk for premature graft loss in comparison with de novo TMA without rejection.

TMA is characterized by microvascular thrombosis that results from activation of glomerular endothelial cells. Glomerular endothelium exhibits distinct characteristics that increase its susceptibility to oxidative stress[11]; glomerular endothelial cells produce fibrinolytic factors[12] and glycocalyx avidly binding complement factor H.[13] In response to HLA-antibody binding[14] or ischemia-reperfusion injury,[15] these functions are lost, and local inflammation is enhanced to a higher extent than other endothelial cells exposed to similar conditions. Glomerular endothelium injury is therefore associated with poorly controlled alternative complement pathway activation. Loss of fibrinolytic phenotype enhances microvascular thrombosis, and ischemic insult further worsens dysfunction of glomerular endothelium[16, 17]; thus, TMA develops.

In the present study, besides CIT, the PRAs were found to be an independent risk factor for de novo TMA development. Therefore, enhanced alloimmune response in sensitized patients, as well as well-known ischemic injury, is likely to be involved in de novo TMA pathogenesis. The presence of de novo TMA decreases graft survival regardless of cause. In the present study, we found that 5-y death-censored graft survival was 56% in the TMA group, whereas it was 88% in control groups. Others have reported similarly inferior graft survival.[2-4, 6]

One-third of our TMA cases were associated with rejection, either active AMR, or vascular rejection. We found that the rejection phenotype of de novo TMA is associated with worse graft survival. Interestingly, decreased graft survival in the TMA R^+ group was affected only by active AMR cases. Endothelial injury caused by donor-specific antibodies is a cornerstone of AMR pathogenesis, as evidenced by transcriptomic analyses,[18, 19] and TMA is thus a consequence of severe antibody-mediated endothelial injury,[20] as reflected by Banff classification.[8]

In the present study, intimal arteritis (v-lesion) along with de novo TMA did not represent additional risk as compared with de novo TMA alone. Intimal arteritis has been widely studied; however, its clinical impact remains to be fully elucidated. Salazar et al[21] showed that v-lesion does not influence graft survival, especially in cases of early v-lesion. Previously, our group has reported that early isolated v-lesions represent rather a nonalloimmune injury pattern.[22] In the present study, we found 9 out of 11 cases of intimal arteritis very early—within the first 7 d after transplantation. It is therefore likely that intimal arteritis with TMA was associated with severe peritransplant rather than alloimmune injury and thus cannot affect the graft survival more than de novo TMA itself.

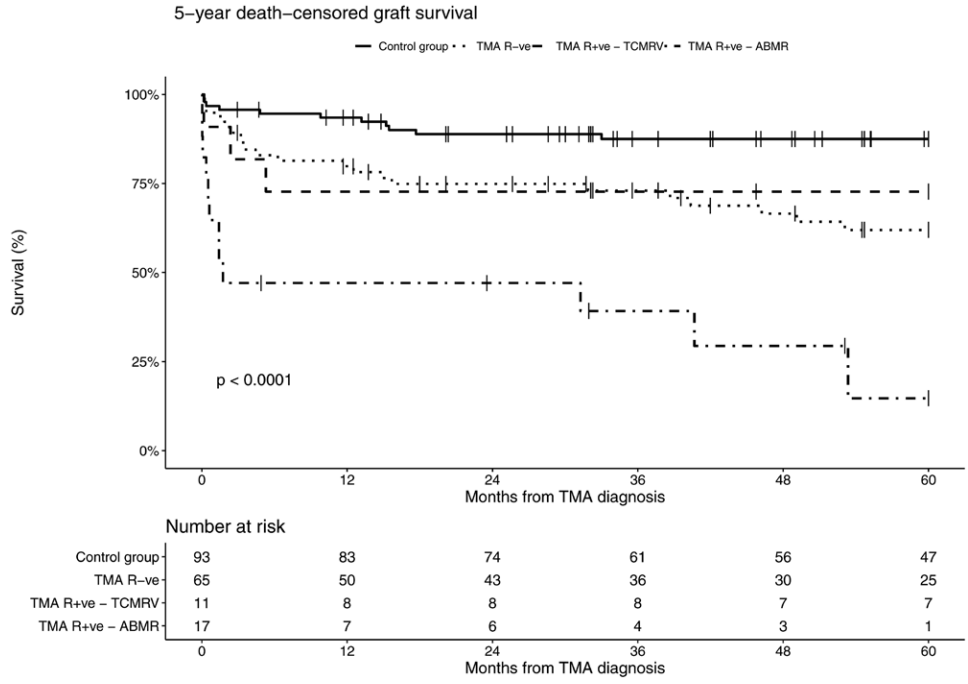

FIGURE 4. Five-y death-censored graft survival in TMA with active antibody-mediated rejection (TMA R^+—AMR), TMA with vascular rejection (TMA R^+—TCMRV), TMA without rejection (TMA R^-), and controls. TMA, thrombotic microangiopathy.

There are only 3 studies that reported phenotypes of TMA associated with rejection. Satoskar et al[5] reported 33 cases of C4d-positive TMA that were defined as AMR, Wu et al[23] reported 18 cases of TMA associated with active AMR, and Teixeira et al[6] reported 11 cases of TMA associated with AMR.

Wu et al[23] defined the rejection background of de novo TMA using Banff criteria; their results are therefore best comparable with ours. Authors compared TMA with and without concomitant rejection. In that study, patients with TMA R^+ experienced higher PRA, more frequent retransplantation, and a higher proportion of T-depletive induction; dialysis vintage duration was not studied. They reported inferior graft survival in active AMR cases (48% versus 70%; P=NS), which is in accordance with our results. Contrary to our results, they reported that TMA with intimal arteritis was associated with strikingly worse graft survival (74% versus 28% at 5 y); however, 3 out of 14 cases of intimal arteritis were assigned to the TMA group without rejection. Moreover, in that study, most TMAs in the rejection group occurred late after transplantation, and intimal arteritis may thus represent true rejection phenotype.[23]

Satoskar et al[5] assigned active AMR background only to C4d-positive cases; the 2-y graft survival was 42% and 40% in C4d-positive and C4d-negative groups, respectively, and v-scores were not reported. Teixeira et al[6] reported inferior 1-y graft survival in the AMR group (41% versus 70%); however, the authors did not provide a definition of the AMR group.

In our study, the active AMR defined by Banff 2019 criteria[8] with TMA features was associated with the worst graft survival; at 5 y, most grafts had failed in comparison with other TMA phenotypes. The clinical outcome of active AMR, along with de novo TMA, remains unclear and was not systematically studied in larger cohorts. Inferior short-term graft survival was reported in smaller reports.[5, 6, 23] Former studies lack AMR definitions, and, therefore, CNI-driven de novo TMA pathogenesis might be overestimated.[4, 24] In other reports, rejections were excluded from the analyses.[2, 25]

Our study was designed to eliminate the effect of the donor risk factors. Interestingly, previously published paired kidney analyses showed that both grafts from the same donor have a comparable survival up to 3 y, and recipient factors affected outcomes later in US[26] and European[27] cohorts. In this study, we thus showed that the occurrence of de novo TMA dramatically affects the fate of kidney allografts, which might be otherwise functioning much longer if the CIT was shorter, and recipients were not at risk of AMR.

CIT was frequently suggested to be the risk factor for de novo TMA development.[1] In our study, CIT was longer in de novo TMA as well. Clearly, CIT itself cannot play such an important role in de novo TMA, whose pathogenesis is rather complex.

Hereditary abnormalities in complement gene proteins and regulatory factors in de novo TMA pathogenesis have been proposed in several studies.[10, 28, 29] Donor risk factors may affect de novo TMA development, such as preexisting pathologies, brain-death–associated circulatory events, or hereditary susceptibility. Therefore, in our study, we used the paired kidney analysis to control for the donor factors as described previously in other studies on different topics.[30-33] To the best of our knowledge, our study is the first paired kidney analysis to study de novo TMA in kidney transplantation.

Treatment of de novo TMA is not clearly defined. Although TMA as a part of active AMR is usually treated according to center-specific protocols that include plasmapheresis, intravenous immunoglobulins, and rituximab along with steroids, de novo TMA without active AMR is a complement-mediated disease; thus, plasma exchanges, CNI discontinuation, and supportive care are more frequently used.[1, 34]

In conclusion, this multicenter, national, retrospective, case–control study showed that de novo TMA is a rather rare complication after kidney transplantation, yet it has

a detrimental effect on graft survival. We have shown that its pathogenesis is complex and dominantly driven by recipients' risk factors. Aberrant humoral alloimmune response in sensitized kidney transplant recipients is the most important one, and, therefore, patients with de novo TMA and AMR should be considered to be at the highest risk and to likely require more aggressive antirejection treatment and monitoring. In the cases of those not associated with rejection, the elimination of complement aggravating factors and CNI withdrawal or minimization may be considered.

ACKNOWLEDGMENTS

The authors are grateful to Sylvie Dusilova-Sulkova and Tomas Reischig for their assistance in the control cohort definition.

REFERENCES

1. Garg N, Rennke HG, Pavlakis M, et al. De novo thrombotic microangiopathy after kidney transplantation. *Transplant Rev (Orlando).* 2018;32:58–68.
2. Caires RA, Marques ID, Repizo LP, et al. De novo thrombotic microangiopathy after kidney transplantation: clinical features, treatment, and long-term patient and graft survival. *Transplant Proc.* 2012;44:2388–2390.
3. Reynolds JC, Agodoa LY, Yuan CM, et al. Thrombotic microangiopathy after renal transplantation in the United States. *Am J Kidney Dis.* 2003;42:1058–1068.
4. Zarifian A, Meleg-Smith S, O'donovan R, et al. Cyclosporine-associated thrombotic microangiopathy in renal allografts. *Kidney Int.* 1999;55:2457–2466.
5. Satoskar AA, Pelletier R, Adams P, et al. De novo thrombotic microangiopathy in renal allograft biopsies-role of antibody-mediated rejection. *Am J Transplant.* 2010;10:1804–1811.
6. Teixeira CM, Tedesco Silva Junior H, Moura LAR, et al. Clinical and pathological features of thrombotic microangiopathy influencing long-term kidney transplant outcomes. *PLoS One.* 2020;15:e0227445.
7. Ponticelli C, Banfi G. Thrombotic microangiopathy after kidney transplantation. *Transpl Int.* 2006;19:789–794.
8. Loupy A, Haas M, Roufosse C, et al. The Banff 2019 Kidney Meeting Report (I): updates on and clarification of criteria for T cell- and antibody-mediated rejection. *Am J Transplant*. 2020;20:2318–2331.
9. Cernoch M, Viklicky O. Complement in kidney transplantation. *Front Med (Lausanne).* 2017;4:66.
10. Jodele S, Zhang K, Zou F, et al. The genetic fingerprint of susceptibility for transplant-associated thrombotic microangiopathy. *Blood.* 2016;127:989–996.
11. Gomez SA, Abrey-Recalde MJ, Panek CA, et al. The oxidative stress induced in vivo by Shiga toxin-2 contributes to the pathogenicity of haemolytic uraemic syndrome. *Clin Exp Immunol.* 2013;173:463–472.
12. Louise CB, Obrig TG. Human renal microvascular endothelial cells as a potential target in the development of the hemolytic uremic syndrome as related to fibrinolysis factor expression, in vitro. *Microvasc Res.* 1994;47:377–387.
13. Meri S, Pangburn MK. Discrimination between activators and nonactivators of the alternative pathway of complement: regulation via a sialic acid/polyanion binding site on factor H. *Proc Natl Acad Sci U S A.* 1990;87:3982–3986.
14. Reyes-Vargas E, Pavlov IY, Martins TB, et al. Binding of anti-HLA class I antibody to endothelial cells produce an inflammatory cytokine secretory pattern. *J Clin Lab Anal.* 2009;23:157–160.
15. Azuma H, Nadeau K, Takada M, et al. Cellular and molecular predictors of chronic renal dysfunction after initial ischemia/reperfusion injury of a single kidney. *Transplantation.* 1997;64:190–197.
16. Ponticelli C. Ischaemia-reperfusion injury: a major protagonist in kidney transplantation. *Nephrol Dial Transplant.* 2014;29:1134–1140.
17. Agustian PA, Bockmeyer CL, Modde F, et al. Glomerular mRNA expression of prothrombotic and antithrombotic factors in renal transplants with thrombotic microangiopathy. *Transplantation.* 2013;95:1242–1248.
18. Sis B, Jhangri GS, Bunnag S, et al. Endothelial gene expression in kidney transplants with alloantibody indicates antibody-mediated damage despite lack of C4d staining. *Am J Transplant.* 2009;9:2312–2323.
19. 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;10:1812–1822.
20. Drachenberg CB, Papadimitriou JC. Endothelial injury in renal antibody-mediated allograft rejection: a schematic view based on pathogenesis. *Transplantation.* 2013;95:1073–1083.
21. Salazar ID, Merino López M, Chang J, et al. Reassessing the significance of intimal arteritis in kidney transplant biopsy specimens. *J Am Soc Nephrol.* 2015;26:3190–3198.
22. Wohlfahrtova M, Hruba P, Klema J, et al. Early isolated V-lesion may not truly represent rejection of the kidney allograft. *Clin Sci (Lond).* 2018;132:2269–2284.
23. Wu K, Budde K, Schmidt D, et al. The inferior impact of antibody-mediated rejection on the clinical outcome of kidney allografts that develop de novo thrombotic microangiopathy. *Clin Transplant.* 2016;30:105–117.
24. Young BA, Marsh CL, Alpers CE, et al. Cyclosporine-associated thrombotic microangiopathy/hemolytic uremic syndrome following kidney and kidney-pancreas transplantation. *Am J Kidney Dis.* 1996;28:561–571.
25. Schwimmer J, Nadasdy TA, Spitalnik PF, et al. De novo thrombotic microangiopathy in renal transplant recipients: a comparison of hemolytic uremic syndrome with localized renal thrombotic microangiopathy. *Am J Kidney Dis.* 2003;41:471–479.
26. Louvar DW, Li N, Snyder J, et al. "Nature versus nurture" study of deceased-donor pairs in kidney transplantation. *J Am Soc Nephrol.* 2009;20:1351–1358.
27. Traynor C, O'Kelly P, Denton M, et al. Concordance of outcomes of pairs of kidneys transplanted into different recipients. *Transpl Int.* 2012;25:918–924.
28. Westra D, Volokhina EB, van der Molen RG, et al. Serological and genetic complement alterations in infection-induced and complement-mediated hemolytic uremic syndrome. *Pediatr Nephrol.* 2017;32:297–309.
29. Le Quintrec M, Lionet A, Kamar N, et al. Complement mutation-associated de novo thrombotic microangiopathy following kidney transplantation. *Am J Transplant.* 2008;8:1694–1701.
30. Michielsen LA, Wisse BW, Kamburova EG, et al. A paired kidney analysis on the impact of pre-transplant anti-HLA antibodies on graft survival. *Nephrol Dial Transplant*. 2019;34:1056–1063.
31. Kayler LK, Magliocca J, Zendejas I, et al. Impact of cold ischemia time on graft survival among ECD transplant recipients: a paired kidney analysis. *Am J Transplant.* 2011;11:2647–2656.
32. Kaplan B, Schold JD, Meier-Kriesche HU. Long-term graft survival with neoral and tacrolimus: a paired kidney analysis. *J Am Soc Nephrol.* 2003;14:2980–2984.
33. Doshi MD, Garg N, Reese PP, et al. Recipient risk factors associated with delayed graft function: a paired kidney analysis. *Transplantation.* 2011;91:666–671.
34. Schinstock CA, Mannon RB, Budde K, et al. Recommended treatment for antibody-mediated rejection after kidney transplantation: the 2019 expert consensus from the Transplantion Society Working Group. *Transplantation*. 2020;104:911–922.