Everolimus, Cyclosporine, and Thrombotic Microangiopathy: Clinical Role and Preventive Tools in Renal Transplantation

F. Nava*, G. Cappelli, G. Mori, M. Granito, G. Magnoni, C. Botta, A. Solazzo, F. Fontana, A. Baisi, and D. Bonucchi

Nephrology Dialysis and Kidney Transplantation, AOU Policlinico, Modena, Italy

ABSTRACT

Introduction. Thrombotic microangiopathy (TMA) is characterized by endothelial cell injury and formation of fibrin thrombi within capillary and arterioles. In renal allograft recipients, TMA mainly presents as hemolytic uremic syndrome. Its occurrence is rare, and diagnosis requires a high degree of suspicion. Drug toxicity, in particular from calcineurin inhibitors (CNIs) and mTOR inhibitors (mTORi), is the most common cause posttransplant and has recently been emphasized in the setting of lung transplantation.

Objective. The goal of this study was to investigate the role of mTORi as an added risk factor in the development of TMA to propose strategies for modulation of immunosuppressive (IS) therapy.

Patients and Methods. From a database of 496 renal graft recipients, we analyzed 350 renal graft biopsy specimens gathered at our center from 1998 to 2012. In patients undergoing combined therapy with mTORi and CNI, we compared drugs levels in TMA-affected and TMA-free groups, using mTORi and CNI TLC and the summation of [everolimus TLC + (cyclosporine C2/100)] (Σ) as a surrogate marker of combined exposition to 2 drugs. Receiver-operating characteristic analysis of association of EVL TLC + (C2/100) was performed for patients exposed to mTORi.

Results. Histologic features of TMA were found in 36 patients (prevalence of 7.3%). The caseload was divided into 2 groups: not drug-related TMA (n = 19) and drug-related TMA (n = 17). Despite the prevalence of TMA in patients exposed to mTORi being greater (8 of 153; prevalence, 5.3%) compared with therapies without mTORi (9 of 324; prevalence, 2.8%), statistical difference was not reached. Patients treated with mTORi who developed de novo drug-related TMA had higher blood levels of IS drugs compared with those who did not develop TMA. Receiver-operating characteristic analysis found a significant threshold of 12.5 ng/mL (area under the curve, 0.803; $P = .006$).

Conclusions. Results confirm the pivotal role of IS drugs in the onset of de novo TMA. On the basis of literature, we could speculate a sequence of endothelial damage by CNI, on which everolimus fits hindering the repair of endothelial injury. Therefore, high blood levels of CNI and mTORi seem to predispose patients to posttransplant TMA. Combined monitoring of these 2 drugs might be used to prevent the complication. Σ [everolimus TLC + (cyclosporine C2/100)] >12.5 ng/mL should be avoided as a surrogate risk factor for adverse effects.

THROMBOTIC MICROANGIOPATHY (TMA) is a pathologic condition characterized by the formation of fibrin thrombi and platelet aggregations within capillary and arterioles leading to endothelial cell injury and small vessel occlusion. This causes thrombocytopenia, hemolytic anemia,

*Address correspondence to Dr. Fabio Nava (MD, resident) Nephrology Dialysis and Kidney Transplantation, AOU Policlinico, Modena, Italy. E-mail: fabio.nava24k@gmail.com

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360 Park Avenue South, New York, NY 10010-1710

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purpura, and renal failure. TMA mainly presents as hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura; although HUS is distinguished by the presence of more severe and refractory renal involvement, thrombotic thrombocytopenic purpura has a typical neurologic involvement and depends on ADAMTS13 dysfunction.

In renal allograft recipients, HUS is rare, with an incidence ranging between 0.8% and 14% [1]. When unrecognized and not treated early, it can lead to graft loss and, in severe cases, death. Toxicity by calcineurin inhibitors (CNIs) and mTOR inhibitors (mTORi), viral infection, ischemia-reperfusion injury, antibody-mediated rejection (AMR), and presence of antiphospholipid antibodies [2] are the most frequent etiologies. Drug toxicity, in particular from CNIs, is the most common cause of posttransplant HUS; TMA has been a well-known adverse effect of cyclosporine (CsA) since its first use in transplantation [3]. Systemic signs of CsA-related TMA are uncommon, underscoring the importance of a graft biopsy for diagnosis. Conversely, mTORi sirolimus (SIR) and everolimus (EVE) inhibit glomerular endothelial cell proliferation and vascular endothelial growth factor activity in experimental TMA [4,5]. This could delay the repair of endothelial injury caused by CNI cytotoxicity as a first step in posttransplant TMA pathogenesis. A recent study on lung transplantation found that EVE and CsA association induced a high risk of TMA/HUS (related to the drugs' plasma levels) [6].

We retrospectively analyzed our transplant caseload with 3 main objectives: (1) to estimate the prevalence of biopsy-proven TMA in our renal transplant patients; (2) to evaluate the role of immunosuppressive (IS) combinations and their levels in the onset of TMA, with particular focus on mTORi; and (3) to determine a surrogate indicator of toxicity by IS combinations (CsA minimization and EVE).

MATERIALS AND METHODS

We analyzed 350 renal graft biopsy specimens gathered from 1998 to 2012 in 496 renal graft recipients (excluding kidney/pancreas, kidney/liver allografts, and primary nonfunction). Patients provided written informed consent before graft biopsy; the biopsy was indicated on clinical grounds (worsening renal function and/or proteinuria, signs of hemolitic anemia such as lactate dehydrogenase elevation, without other explanation). No protocol biopsies were performed.

In each patient with biopsy-proven TMA, we evaluated therapy at the time of biopsy and time from transplantation at the onset, interventions, and outcome expressed as graft loss. The cases were divided into 2 groups: not drug-related TMA and drug-related TMA. In the first group, TMA was related to various clinical pathologic conditions; for example, AMR, viral infections, systemic lupus erythematosus, and recurrence of atypical HUS. In the second group, TMA was linked exclusively to IS drugs.

Mean IS drug levels were measured in patients exposed to mTORi comparing TMA and TMA-free groups. To ensure the evaluation of a stabilized IS therapy, for the first group we considered the 3 months before biopsy, taking into account peak values before the procedure.

For the latter (TMA-free as control subjects), we analyzed TLCs for 6 months, after a minimum of 6 months of stabilized mTORi therapy. For statistical comparisons between patients with TMA who were exposed or not exposed to mTORi, and between drug-related and not drug-related TMA groups, a χ^2 analysis with Yates' correction was used. Drug blood levels were compared by using the Student unpaired t test or nonparametric Mann-Whitney U test where appropriate. SPSS (IBM SPSS Statistics 21.0, IBM Corporation, Armonk, NY, United States) was used for statistical analysis, and $P < .05$ was considered statistically significant. Data are presented as medians with ranges. Receiver-operating characteristic analysis of the association of EVL TLC + (C2/100) was performed for patients exposed to mTORi.

RESULTS

Thirty-six cases of TMA were found in 350 renal biopsy specimens in a population of 496 patients receiving a renal allograft. Prevalence was 7.3% (36 of 496). TMA that was not drug related (n = 19) included AMR (n = 11), viral infection (cytomegalovirus, human immunodeficiency virus) (n = 5), vasculitis (n = 1), systemic lupus erythematosus (n = 1), and recurrent atypical HUS (n = 1); drug-related TMA was found in 3.4% of patients (n = 17). Statistical analysis found no significant differences between the 2 groups (drug related and not drug related TMA), except for distribution of histopathologic features of rejection, resulting prevalent in not drug related group (Tables 1 and 2).

The present study focused on the 17 patients of the drug-related TMA group to investigate the specific role of IS drugs. Drug-related TMA group prevalence was 3.4% (17 of 496). Patients with drug-related TMA did not differ significantly with respect to age (52 ± 12 years) and sex (9 male subjects, 8 female subjects). The allografts were single grafts from a cadaveric donor in 14 of 17 cases. In 2 cases, it was a double renal transplant; only 1 was from a living related donor. The mean time between transplantation and onset of de novo TMA was 276 days (range, 9 days to 4 years).

Eight cases of TMA were found in the mTORi-treated group (n = 153; prevalence, 5.3%); 6 were associated with CsA-EVE and 2 with FK-EVE. Nine cases of TMA were observed in patients not receiving mTORi (n = 324), with a prevalence of 2.8%; of these, 8 were treated with FK506 and 1 with CsA. A χ^2 analysis with Yates' correction yielded no significant difference in the prevalence of TMA between the 2 groups ($P = .28$). There were no cases of TMA in our patients treated with SIR.

Patients treated with mTORi who developed de novo drug-related TMA had higher blood levels of IS drugs compared with those who did not develop TMA. In particular, serum CsA TLC (C0) was 134.8 ± 108.1 ng/mL in patients with TMA (6 cases) and 53.6 ± 23.6 ng/mL in patients without TMA treated with CsA ($P < .05$). C2 levels were statistically nonsignificant, despite higher values in cases with TMA (588.8 ± 433.2 ng/mL vs 317.0 ± 95.5 ng/mL; $P = .095$). EVE TLC mean values, when used in association with CsA, were 9.9 ± 3.9 ng/mL in patients with TMA and 7.5 ± 2.2 ng/mL in patients without TMA ($P = .0381$). Noteworthy was the difference ($P = .034$) between the summation of concentrations of the 2 drugs: Σ [EVE TLC + (C2/100)]: 15.2 ± 6.3 ng/mL in patients with

Table 1. Renal Graft Recipients With Drug-Related TMA

Patient	Sex	Age (y)	Native Renal Disease	Allograft	TMA Onset Posttransplant (d)	Prebiopsy Therapy	CMV	HIV	HR	DSA	C4d	LDH	Blood Smear
1	M	35	Alport	Living	128	FK + EVE	Neg	Neg	Neg	–	–	=	–
2	F	45	Pyeloneph	Single	253	CsA + EVE	Neg	Neg	Neg	Neg	Neg	↑	Neg
3	M	39	VUR	Single	1494	CsA + EVE	Neg	Neg	Neg	Neg	Neg	=	Neg
4	M	44	IgAN	Single	138	CsA + EVE	Neg	Neg	Neg	Neg	Pos	↑	Neg
5	F	66	Lithiasis	Double	343	CsA + EVE	Neg	Neg	Neg	I	Pos	↑	Neg
6	F	60	Interstitial	Single	109	FK + EVE	Neg	Neg	Neg	Neg	Neg	↑	Neg
7	F	61	DN	Single	33	CsA + EVE	Neg	Neg	Neg	–	Neg	↑	Hemolysis
8	M	64	DN	Single	254	CsA + EVE	Neg	Neg	Neg	II	–	↑	–
9	M	54	Extracapillary GN	Single	12	FK	Neg	Neg	Neg	I	Neg	=	Hemolysis
10	M	58	DN	Single	558	FK	Neg	Neg	Neg	Neg	Neg	↑	–
11	M	52	Mesangial GN	Single	85	CsA	Neg	Neg	Neg	Neg	Neg	↑	–
12	M	36	Focal GN	Single	na	FK	Neg	Neg	Neg	–	–	–	–
13	F	40	Focal GN	Single	17	FK	Neg	Neg	Neg	–	–	–	Hemolysis
14	F	40	ESRD	Single	9	FK	Neg	Neg	Neg	–	–	↑	Neg
15	M	71	Hypertensive	Single	27	FK	Neg	Neg	Neg	Neg	Neg	↑	Hemolysis
16	F	62	MPGN	Double	336	FK	Neg	Neg	Neg	Neg	Neg	↑	Hemolysis
17	F	43	GIN	Single	626	FK	Neg	Neg	Neg	II	Neg	=	–
TOT	Male	Mean ± SD		S/D	Mean ± SD	mTORi	Pos	Pos	Pos	DSAII	Pos	↑LDH	Hemolysis
17	9	51 ± 12		15/2	276 ± 377	8	0	0	0*	2	2	11	5

Abbreviations: CMV, cytomegalovirus; CsA, cyclosporine; DSA, donor-specific antibody; ESRD, end-stage renal disease; EVE, everolimus; F, female; GIN, granulomatous interstitial nephritis; HIV, human immunodeficiency virus; HR, histology compatible with rejection; LDH, lactate dehydrogenase; M, male; MPGN, membranoproliferative glomerulonephritis; Neg, negative; Pos, positive; SLE, systemic lupus erythematosus; TMA, thrombotic microangiopathy; VUR, vesicoureteral reflux.

*Considered elevated if >400 UI/L.

$P < .05$ between drug-related and not drug-related groups.

Table 2. Renal Graft Recipients With TMA That Is Not Drug Related

Patient	Sex	Age (y)	Native Renal Disease	Allograft	TMA Onset Posttransplant (d)	Prebiopsy Therapy	CMV	HIV	HR	DSA	C4d	LDH	Blood Smear
1	F	59	ESRD	Single	19	CsA	Neg	Neg	Pos	–	–	–	–
2	M	39	ESRD	Single	187	CsA	Neg	Neg	Pos	–	–	–	Neg
3	M	51	HIVAN	Single	23	CsA	Neg	Pos	Neg	Neg	Neg	–	Hemolysis
4	F	56	ESRD	Single	10	FK	Neg	Neg	Pos	I + II	Pos	↑	Hemolysis
5	M	52	ESRD	Single	969	CsA	Neg	Neg	Pos	–	–	–	Hemolysis
6	M	34	ESRD in HCV+	Single	8	CsA	Neg	Neg	Pos	I	Pos	–	Hemolysis
7	M	62	GN	Single	2569	CsA	Neg	Neg	Pos	I	Pos	=	–
8	M	32	VUR	Single	115	FK	Neg	Neg	Pos	Neg	–	–	–
9	M	77	Hypertensive	Single	2768	CsA	Neg	Neg	Pos	–	–	=	–
10	M	40	IgAN	Single	2962	CsA	Neg	Neg	Neg	I + II	Pos	=	Hemolysis
11	M	54	Extracapillary GN	Living	2463	FK	Neg	Neg	Pos	Neg	Neg	=	–
12	M	60	V-U reflux	Single	11	Thymogl.	Neg	Neg	Pos	–	–	–	–
13	M	48	HIVAN	Single	3	EVE	Neg	Pos	Neg	Neg	Neg	=	Hemolysis
14	M	71	ESRD	Double	15	EVE	Pos	Neg	Neg	Neg	Neg	=	Hemolysis
15	F	52	Atypical HUS	Single	144	Sir	Neg	Neg	Neg	–	Neg	–	–
16	M	50	Focal GN	Single	199	FK	Pos	Neg	Neg	–	–	–	Neg
17	M	37	SLE	Single	3178	CsA	Neg	Neg	Neg	I	Pos	=	–
18	M	50	DN, HIVAN	Single	653	CsA + EVE	Neg	Pos	Neg	Neg	Neg	–	–
19	M	65	Cryo GN	Single	10	MMF	Neg	Neg	Pos	Neg	Neg	↑	Neg
Total	Male	Mean ± SD		S/D	Mean ± SD	mTORi (EVE/Sir)	Pos	Pos	Pos	DSAII	Pos	↑LDH*	Hemolysis
19	16	52 ± 12		18/1	858 ± 1217	4 (3/1)	2	3	11†	2	5	2	7

Abbreviations: CMV, cytomegalovirus; CsA, cyclosporine; DSA, donor-specific antibody; ESRD, end-stage renal disease; EVE, everolimus; F, female; GIN, granulomatous interstitial nephritis; HIV, human immunodeficiency virus; HR, histology compatible with rejection; LDH, lactate dehydrogenase; M, male; MPGN, membranoproliferative glomerulonephritis; Neg, negative; Pos, positive; SLE, systemic lupus erythematosus; TMA, thrombotic microangiopathy; VUR, vesicoureteral reflux.

*Considered elevated if >400 UI/L.

†$P < .05$ between drug-related and not drug-related groups.

TMA and 10.7 ± 2.1 ng/mL in patients without TMA (Fig 1). Receiver-operating characteristic analysis found a significant threshold of 12.5 ng/mL (area under the curve, 0.803; $P = .006$) (Fig 2). Tacrolimus (FK) TLC mean blood levels were 7.2 ± 1.3 ng/mL in patients with TMA and 4.7 ± 0.8 ng/mL in patients without TMA ($P = .0075$). Similarly, EVE TLC in these patients was 15.3 ± 8.1 ng/mL in patients with TMA and 4.9 ± 0.9 ng/mL in patients without TMA ($P = .0011$). EVE TLC + FK TLC was 21.1 ± 11.3 ng/mL in patients with TMA and 9.6 ± 1.3 ng/mL in patients without TMA.

DISCUSSION

The goal of this study was to investigate the role of IS drugs in posttransplant TMA. We selected a TMA population not influenced by classical risk factors (eg, AMR, viral infections, vasculitides, systemic lupus erythematosus, recurrence of atypical HUS), in whom the role of the antirejection medication appeared predominant. However, we could not exclude the possible presence of underlying causes unknown at the time of onset.

According to the literature, drug-induced HUS represents 13% of all HUS cases [7]. The real incidence of drug-related TMA in renal graft recipients is not known; renal biopsy is the only available method for diagnosis of TMA, and indications for renal biopsy vary among medical centers. The overall prevalence of TMA observed in our center since 1998 is 7.3%, which is in line with the reported series in several studies [7–9].

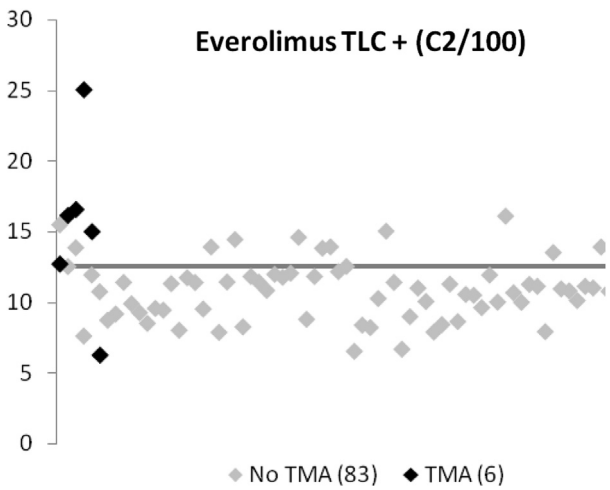

Fig 1. Summation of blood levels of everolimus TLC + (C2/100) in patients with drug-related thrombotic microangiopathy (TMA) treated with the combination of cyclosporine (CsA) and everolimus. Black diamonds represent Σ blood levels in patients with TMA; grey diamonds represent Σ blood levels in graft recipients without TMA. Vertical axis indicates blood concentration of drug expressed in nanograms per milliliter. The number of patients examined for each group is indicted in brackets. The line represents the Σ safety threshold of 12.5 ng/mL [everolimus TLC + (C2/100)] suggested by the receiver-operating characteristic analysis.

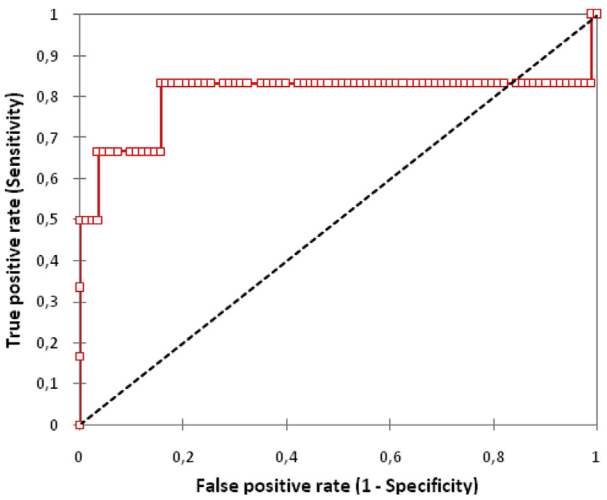

Fig 2. Receiver-operating characteristic curve of association of everolimus TLC + (C2/100) (∑) in patients exposed to mTOR inhibitors. Area under the curve, 0.803; $P = .006$.

The association between TMA and CsA is well established [10]. Although the prevalence of TMA in patients exposed to mTORi in our study was greater (5.3% vs 2.8% in patients exposed to CNI only), these differences were not statistically significant. The data regarding exposure to IS could provide an explanation of this difference. Analyzing blood levels of drugs in relation to the presence of TMA, we found significantly higher drug blood levels in patients with TMA. Apart from the well-known relationship with CsA basal levels, the values of the Σ are noteworthy; they reached an average of 15.2 ng/mL in patients with TMA, and in patients without TMA, the average summation was 10.7 ng/mL ($P < .05$).

Combined monitoring of IS drugs has recently proved to be a useful tool for reducing the risk of rejection within the first months after transplant [11]. The results of the present study support the use of combined monitoring to also predict and prevent the onset of de novo HUS. In fact, TMA in renal allograft recipients treated with mTORi is very infrequent below specific values 12.5 ng/mL of Σ (Fig 1). Toxic effects of CNI and mTORi interact in a synergistic way. We hypothesize that the endothelial injury is due to the action of a constant high level of CsA amplified by EVE-mediated inhibition of endothelial cell regeneration. In other words, endothelial damage caused by CsA does not heal under the effect of mTORi. A similar interaction should also be assumed for the association of EVE-FK, but the low number of such cases in our study does not allow us to draw conclusions.

EVE-based IS therapy is an important prevention strategy of chronic drug-induced renal toxicity, allowing minimization of CNI doses. However, as well as SIR, EVE therapy seems to play a predisposition role in the onset of de novo TMA. A specific nephrotoxicity of mTORi has not been documented to be present on undamaged

endothelium. As reported in the literature for SIR, EVE may be a causal factor in kidneys suffering from ischemic damage, such as double transplant, or marginal donors [5]. In this study, 2 cases fit this category: 1 double transplant and 1 extended-criteria donor.

Our study is limited by its retrospective design and the low number of cases. However, our results are strengthened by the use of a novel cognitive tool, represented by the combined monitoring of 2 drugs.

In conclusion, according to our data, TMA is a rare condition in the setting of renal transplant compared with the field of lung transplantation. Maintaining lower drug exposure could be the explanation for the different outcome. In fact, CsA TLC as reported by the German group (200–250 ng/mL) corresponds to 1000 ng/mL of C $(\text{concentration})_{\max}$ and therefore means no minimization of CNI. On the contrary, in our minimization protocol, CsA $C_{\max}$ (C2) is maintained at ~300 to 350 ng/mL. We also postulate a sequence of endothelial damage by CNI, on which EVE fits hindering the repair of endothelial injury.

High blood levels of CNI and mTORi seem to predispose patients to posttransplant TMA. Combined monitoring of these 2 drugs might be useful for preventing this complication. Summated blood levels of [everolimus TLC + (C2/100)] greater than 12.5 ng/mL should be avoided.

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