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ORIGINAL ARTICLE

Risk factors for thrombotic microangiopathy in allogeneic hematopoietic stem cell recipients receiving GVHD prophylaxis with tacrolimus plus MTX or sirolimus

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Transplantation-associated thrombotic microangiopathy (TA-TMA) is a feared complication of allogeneic hematopoietic SCT (HSCT) owing to its high mortality rate. The use of calcineurin inhibitors or sirolimus (SIR) for GVHD prophylaxis has been suggested as a potential risk factor. However, the impact of tacrolimus (TAC) and SIR combinations on the increased risk of TA-TMA is currently not well defined. We retrospectively analyzed the incidence of TA-TMA in 102 allogeneic HSCT recipients who consecutively received TAC plus SIR (TAC/SIR) ($n=68$) or plus MTX (TAC/MTX) ± ATG ($n=34$) for GVHD prophylaxis. No significant differences were observed in the incidence of TA-TMA between patients receiving TAC/SIR vs TAC/MTX ± ATG (7.4% vs 8.8%, $P=0.8$). Only grade III–IV acute GVHD, previous HSCT and serum levels of TAC >25 ng/mL were associated with a greater risk of TA-TMA. Patients developing TA-TMA have significantly poorer survival ($P<0.001$); however, TA-TMA ceased to be an independent prognostic factor when it was included in a multivariate model. In conclusion, the combination of TAC/SIR does not appear to pose a higher risk of TA-TMA. By contrast, we identified three different risk groups for developing TA-TMA.

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INTRODUCTION

Transplantation-associated thrombotic microangiopathy (TA-TMA) is a well-documented complication after allogeneic hematopoietic SCT (HSCT).[1] The reported incidence of TA-TMA varies greatly from 0.5–63.6%, which is at least partly a consequence of the marked discrepancy in the definitions used and the lack of uniform criteria for diagnosis.[2] A generalized endothelial dysfunction, independent of ADAMTS-13 activity, appears to be the key event that represents the final common pathway of the disease, resulting in thrombosis and fibrin deposition in the microcirculation.[3–6] However, the exact pathophysiology of TA-TMA remains unclear. A variety of potential risk factors has been proposed, such as different conditioning regimens,[7–11] the development of acute GVHD,[1,3,8,10–14] viral or fungal infections,[1,2] the use of unrelated donors,[11] HLA mismatch,[1] ABO incompatibility[10] and the use of calcineurin inhibitors (CYA and tacrolimus (TAC))[15] or sirolimus (SIR) for GVHD prophylaxis.[16] The combination of TAC and SIR (TAC/SIR) in both solid organ transplantation and HSCT is associated with an increased risk of TA-TMA in some studies.[17–22] Moreover, few comparisons of TAC/SIR with other TAC-based regimens have been reported. In fact, two recent studies suggest that TA-TMA incidence does not differ significantly between patients who received TAC/SIR and those who received TAC/MTX for GVHD prophylaxis.[23,24] However, these two studies were not focused on TA-TMA and only described the incidence of TA-TMA without analyzing risk factors for TA-TMA, management or clinical outcome.

In the current study we report the results of a retrospective analysis of 102 allogeneic HSCT recipients to determine the incidence of TA-TMA with a combination of TAC plus SIR vs other TAC-based regimens. In addition, we aimed to identify risk factors and the clinical outcome of TA-TMA after allogeneic HSCT.

PATIENTS AND METHODS

Patients

In 2007 TAC was introduced into our practice as GVHD prophylaxis for unrelated allogeneic HSCT. TAC was associated with MTX (TAC/MTX) in myeloablative allo-HSCT. By contrast, in the reduced-intensity conditioning regimen setting, TAC/MTX was also used until October 2008; subsequently, SIR plus TAC was used in the context of a phase II prospective multicenter trial (2007-006416-32 trial by GEL-TAMO/GETH) until October 2010, and as a standard procedure for patients receiving reduced-intensity conditioning allo-HSCT thereafter.[25]

In the current study, we analyzed retrospectively all consecutive allogeneic HSCT recipients (aged over 18 years) who received a TAC-based regimen for GVHD prophylaxis between April 2007 and July 2012 in our unit ($n=102$). Thirty-four received TAC/MTX and 68 TAC/SIR combinations. Demographic data, clinical course, occurrence of GVHD and immunosuppressive levels were recorded. Twenty-eight out of 68 patients in the TAC/SIR group have been included in a phase II trial, and the results have already been published.[25]

Supportive care

The day of stem cell infusion was designated as day 0. Antibacterial, antiviral and antifungal prophylaxis was performed according to our

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institutional guidelines with no differences between the two groups except for the use of azoles, which were not allowed for patients receiving TAC/SIR per protocol (with the exception of fluconazole). Ursodeoxycholic acid (600–900 mg once daily p.o.) was used to prevent veno-occlusive disease from the beginning of conditioning.

Acute GVHD prophylaxis

GVHD prophylaxis consisted of TAC/SIR ($n=68$), TAC/MTX ($n=16$) and TAC/MTX with antithymocyte globulin (TAC/MTX + ATG) ($n=18$), the latter being used for those patients receiving mismatched unrelated donor transplants and myeloablative conditionings. For patients receiving TAC/MTX ± ATG, TAC was administered daily at a dose of 0.01 mg/kg i.v., starting on day −7, followed by 0.03 mg/kg i.v. Doses were adjusted as necessary to maintain serum levels between 5–15 ng/mL. Among patients receiving TAC/SIR, TAC was started on day −3 at a dose of 0.02 mg/kg/day as a continuous i.v. infusion and doses were adjusted for target blood levels of 5–10 ng/mL. TAC was switched to an equivalent oral dose when oral intake was sufficient to maintain the target serum levels. SIR was administered at a dose of 6 mg p.o. on day −6 (loading dose), followed by 4 mg once daily p.o. Doses were adjusted to maintain serum levels between 6 and 10 ng/mL. Both drug levels were assessed using immunoassays.

Post-HSCT complications

Post-HSCT complications, such as acute GVHD, TA-TMA, veno-occlusive disease, fungal or viral infection and relapse or progression after HSCT, were recorded. Acute GVHD were assessed and graded according to established criteria.[26]

Screening of TA-TMA: regular blood cell count and assay of lactate dehydrogenase were performed in all patients. Peripheral blood smear, Coombs test and assay of haptoglobin were performed in those with reduced Hb level and/or thrombocytopenia $<50\times10^9$/L or a decrease in platelet count of $\geqslant50\%$.

The diagnosis of TA-TMA was made according to the probable TMA criteria:[1] $\geqslant2$ schistocytes per high-power field in peripheral blood, concurrent increased serum lactate dehydrogenase above the institutional baseline, thrombocytopenia $<50\times10^9$/L or a decrease in platelet count of $\geqslant50\%$, reduced Hb level, negative Coombs test results, reduced haptoglobin level and an absence of coagulopathy.

Statistical analysis

Differences between groups were evaluated by Student's *t*-test, for quantitative variables, and the χ^2-test, for categorical variables, using IBM SPSS Statistics 20 (SPSS, Chicago, IL, USA). The incidence of TA-TMA and the overall survival were calculated using the Kaplan–Meier method. The two-sided log-rank test was used to identify risk factors for developing TA-TMA and to compare survival curves. All the parameters that were significant in univariate analyses were included in a multivariate analysis using Cox proportional hazard models. Statistical significance of all tests was accepted for values of $P<0.05$.

RESULTS

Baseline characteristics of patients

We analyzed retrospectively 102 consecutive allogeneic HSCT recipients who received a TAC-based regimen for GVHD prophylaxis. Thirty-four received TAC/MTX and 68 TAC/SIR combinations. Clinical and laboratory characteristics are presented in Table 1. The mean age of recipients was significantly higher in

Table 1. Baseline characteristics of patients

Variable	*Total* (n = *102*)	*TAC/SIR* (n = *68*)	*TAC/MTX* (n = *34*)	P-*value*
Age (median, range)	51 (20–68)	53 (30–68)	43.5 (20–60)	<0.001
Sex (male/female)	61/41 (59.8/40.2)	40/28 (58.8/41.2)	21/13 (61.8/38.2)	NS
Stage of the disease[a]				*NS*
Low risk	40 (39.2)	24 (35.3)	16 (47.1)	
Intermediate risk	24 (23.5)	15 (22.1)	9 (26.5)	
Advanced risk	37 (36.3)	29 (42.6)	8 (23.5)	
Prior allogeneic HSCT	6 (5.9)	5 (7.4)	1 (2.9)	NS
Donor				*NS*
Related allogeneic	34 (33.3)	23 (33.8)	11 (32.4)	
Unrelated allogeneic	68 (66.7)	45 (66.2)	23 (67.6)	
HLA				*NS*
Identical	76 (74.5)	48 (70.6)	28 (82.4)	
9/10 vs 7/8 vs 8/10 match	11/8/7 (10.8/7.8/6.9)	7/8/5 (10.3/12.5/7.3)	1/3/2 (2.9/8.8/5.9)	
ABO compatibility				*NS*
Identical	60 (58.8)	43 (63.2)	17 (50)	
ABO mismatch	42 (41.2)	25 (36.8)	17 (50)	
Source of stem cell				*NS*
Peripheral blood/BM	87 (85.3)/15 (14.7)	59 (86.8)/9 (13.2)	28 (82.4)/6 (17.6)	
ATG	18 (17.6)	0 (0)	18 (52.9)	<0.001
Conditioning regimen				*<0.001*
RIC[b]	77 (76.2)	66 (97.1)	11 (32.4)	
Myeloablative[c]	25 (24.5)	2 (2.9)	23 (67.6)	
Grade III–IV acute GVHD	11 (10.8)	8 (11.8)	3 (8.8)	NS

Abbreviations: ATG = antithymocyte globulin; HSCT = hematopoietic SCT; RIC = reduced-intensity conditioning; SIR = sirolimus; TAC = tacrolimus. [a]Disease stage was reported according to previously described criteria.[36] Early stage (acute leukemia transplanted in first CR, myelodysplastic syndrome transplanted, either untreated or in first CR, CML in first chronic phase and non-Hodgkin lymphoma and multiple myeloma, transplanted untreated or in first CR); intermediate stage (acute leukemia in second CR, CML in all other stages except chronic phase or blast crisis, myelodysplastic syndrome in second CR or in PR and non-Hodgkin lymphoma and multiple myeloma in second complete or PR or stable disease) and advanced stage (acute leukemia in all other disease stages, CML in blast crisis, myelodysplastic syndromes in all other disease stages and multiple myeloma and lymphoma in all disease stages except those defined as early or intermediate). Stage was not applicable for patients with aplastic anemia. [b]RIC: Fudarabine (FLU) 150 mg/m^2 + BU 8–10 mg/kg p.o. or 9.6 mg/kg i.v., FLU (150 mg/m^2) + melpahalan (MEL) 140 mg/m^2, FLU (150 mg/m^2) + MEL (140 mg/m^2) + thiotepa (THIO) 10 mg/kg, FLU (90 mg/m^2) + MEL (140 mg/m^2) + Bortezomib (1.3 mg/m^2 i.v. on days −9 and −2) ($n=2$), Yttrium-90 ibritumomab tiuxetan (0.4 mCi/kg) + FLU (150 mg/m^2) + MEL (140 mg/m^2) + THIO (10 mg/kg) ($n=1$); clofarabine (200 mg/m^2) + MEL (100 mg/m^2) ($n=1$); etoposide (40 mg/kg) + Ara-C (18 g/m^2) ($n=1$); CY 1200 mg/m^2 + FLU (120 mg/m^2) + ATG (7.5 mg/kg) + TBI 200 cGy ($n=1$), FLU (90 m/m^2) + once daily i.v. BU (6.4 mg/kg) + THIO (10 mg/kg) ($n=1$). [c]Myeloablative conditioning: FLU (160 mg/m^2) + once daily i.v. BU 12.8 mg/kg ($n=10$); CY 120 mg/kg + TBI 12 Gy × 6 fractions ($n=5$); BU 12.8 mg/kg i.v. or 16 mg orally + CY 120 mg/kg ($n=5$); and others ($n=5$), consisting of BU (8 mg/kg i.v.) + CY (120 mg/kg) + THIO (750 mg/m^2) 3, CY (120 mg/kg) + TBI (13.2 Gy × 11 fractions) 1, CY (120 mg/kg) + TBI (12 Gy × 6 fractions) + THIO (400 mg/m^2) 1.

Table 2. Clinical, laboratory findings, management and outcome of patients diagnosed with TA-TMA

Age[a]/ sex	*Group*	*Schist. per field*	*EBL/ 100 WBC*	*LDH (IU/L)*	*Hb (g/dL)*	*Platelet count ($\times 10^9$/L)*	*First-line treatment*	*Second-line treatment*	*Third-line treatment*	*Evolution of EBL*	*Cause of death*
38/M	TAC/MTX	8	2	1346	8.1	15	TAC reduction	Vincristine[b]	—	ND	TA-TMA, GI bleeding and pulmonary mucormycosis
59/F	TAC/SIR	1	4	481	9	38	TAC withdrawal	—	—	↓	Relapse
28/M	TAC/MTX	3	3	393	6.6	14	TAC withdrawal, adding CsA	Vincristine[b]	CsA withdrawal, adding MMF	↓	Alive
61/M	TAC/SIR	12	ND	5474	7.3	17	TAC withdrawal	Vincristine[b]	—	ND	TA-TMA, pulmonary aspergillosis, GI bleeding and GVHD
43/F	TAC/MTX	6	22	1350	8.5	28	TAC withdrawal + rituximab[c]	MMF + CsA	CsA withdrawal	↑	TA-TMA, respiratory insufficiency, GI bleeding and GVHD
62/F	TAC/SIR	2	7	818	8.9	36	TAC withdrawal	—	—	↓	Alive
48/M	TAC/SIR	7	2	881	9.6	26	TAC withdrawal + rituximab[c]	SIR withdrawal, adding MMF	—	↑	TA-TMA, pulmonary aspergillosis and GVHD
56/F	TAC/SIR	ND	ND	179	10.7	11	TAC withdrawal + rituximab[c]	Vincristine[b]	—	ND	TA-TMA, disseminated aspergillosis, CMV infection and GVHD

Abbreviations: EBL = erythroblasts; F = female; GI = gastrointestinal; LDH = lactate dehydrogenase; M = male; MMF = mycophenolate mofetil; ND = not determined; Schist = schistocytes; SIR = sirolimus; TA-TMA = transplantation-associated thrombotic microangiopathy; TAC = tacrolimus. [a]Age on the day of HSCT. [b]Vincristine was administered at a dose of 1 mg i.v. on days +1, +4, +8 and +11. [c]Rituximab was administered at a dose of 375 mg/m^2 i.v. weekly × 4 doses.

the TAC/SIR group (53.3 ± 7.9 vs 41.3 ± 11.9, $P < 0.001$), and a higher proportion of patients received the reduced-intensity conditioning regimen in the TAC/SIR group (97.1% vs 32.4%). Other characteristics are described in Table 1.

Incidence of TA-TMA

With a median follow-up of 451 days (range, 28–1946 days), eight out of 102 allogeneic HSCTs developed TA-TMA (7.8%): three out of 34 patients in the TAC/MTX ± ATG group (8.8%) and five out of 68 in the TAC/SIR group (7.4%) ($P = 0.8$). The median time from the day of stem cell infusion until diagnosis of TA-TMA was 81 days (range, 39–405 days). Six out of eight patients were diagnosed before the +110 post HSCT.

Clinical and laboratory findings from patients diagnosed with TMA are shown in Table 2. The median age of the patients was 52 years (range, 24–63 years), and there were equal numbers of males and females. Two patients developed TA-TMA after second allogeneic HSCT. Six out of eight patients diagnosed with TA-TMA fulfilled all the TMA criteria; one of the remaining two patients had only one schistocyte per field in peripheral blood, but fulfilled the other criteria, and the second patient did not fulfill all the criteria because neither a peripheral blood smear nor haptoglobin serum levels were available, although there was histological evidence of TA-TMA in a biopsy specimen after colonoscopy for severe diarrhea. Interestingly, in 6/8 patients with an available peripheral blood smear we could detect the presence of erythroblasts both at diagnosis and during TA-TMA evolution. Concurrent renal and/or neurologic dysfunctions were observed in only 3/8 patients diagnosed with TA-TMA.

Serum levels of immunosuppressive drugs

TAC levels above the upper limit were observed at least once in 87.5% ($n = 7/8$) of patients before a TA-TMA diagnosis was made and in 88.3% of patients without TA-TMA ($n = 83/94$), $P = 0.724$. TAC levels >25 ng/mL were observed in 4/8 (50%) patients who developed TA-TMA vs 10/94 (10.6%) patients who did not ($P = 0.004$). The mean number of days with toxic levels of TAC was also significantly greater in patients who developed TA-TMA (9 ± 7 days) compared with those without TA-TMA (4.6 ± 4.1 days) ($P = 0.008$).

Within the TAC/SIR group, 41 patients (60%) had levels of SIR >12 ng/mL at least once, but no association with an increase incidence of TA-TMA was found (7.3% and 7.4% TA-TMA for patients with or without SIR levels above the upper limit at least once, respectively; $P = 0.99$). There were also no differences in terms of TA-TMA incidence with respect to the mean number of days with toxic levels of SIR between patients who developed TA-TMA (2.2 ± 2.3 days) compared with those who did not (2.05 ± 2.7) ($P = 0.900$).

Management and outcome of TA-TMA

The results concerning the management and outcome of TA-TMA are presented in Table 2. The initial treatment strategy for patients who experienced TA-TMA ($n = 8$) was complete withdrawal from ($n = 7$), or dose reduction ($n = 1$) of, TAC. In addition, CYA ($n = 1$) or rituximab ($n = 3$) were added after TAC had been discontinued.

Four out of eight patients achieved CR of TA-TMA. This occurred in two patients after TAC withdrawal alone (both in the TAC/SIR group), in one patient from the TAC/SIR group after discontinuation of TAC and SIR (mycophenolate mofetil was added as GVHD prophylaxis); however, in this patient TA-TMA was subsequently identified in necropsy. In the other patient (from the TAC/MTX group), TAC was substituted by CsA, without response, so vincristine was subsequently administered, also without response. The patient finally responded after substitution of CsA by mycophenolate mofetil.

Of the four patients who did not respond, after TAC withdrawal, three had received vincristine as second-line therapy. The fourth

patient received rituximab as first-line therapy and TAC was substituted by mycophenolate mofetil plus CYA (CsA was subsequently stopped, with no response).

Six out of eight patients who developed TA-TMA died. TA-TMA was a contributing cause of death in the four non-responding patients. In addition to the TA-TMA, other contributing causes of death in the non-responding patients were: GVHD ($n = 3/4$), invasive fungal infection ($n = 3/4$), CMV infection ($n = 1/4$) and hemorrhage ($n = 3/4$). Of the responder patients, two died after resolution of TA-TMA (one due to pulmonary aspergillosis and the other due to relapse of her disease); however, in one responder patient TA-TMA was identified in necropsy indicating a misdiagnosed relapse of TA-TMA.

Risk factors for TA-TMA

Table 3 shows the results of the univariate analyses carried out to identify variables predicting TA-TMA in allogeneic HSCT recipients and who received TAC-based regimens for GVHD prophylaxis. Lymphoid malignancies, prior HSCT (autologous or allogeneic), conditioning regimens other than FLU + BU at any dose, use of thiotepa, grade III–IV acute GVHD, serum levels of TAC >25 ng/mL, TAC levels at the upper limit for more than 7 days and development of an invasive fungal infection were significantly associated with TA-TMA in the univariate analysis. In multivariate analyses, only grade III–IV acute GVHD, previous HSCT and serum levels of TAC >25 ng/mL were identified as risk factors for developing TA-TMA (Table 4).

We also observed that patients without any of these three risk factors, or with any single factor but without grade III–IV acute GVHD, had a low risk of TA-TMA ($n = 1/86$, 1.2%). Patients who developed grade III–IV acute GVHD or who had a previous HSCT + serum levels of TAC >25 ng/mL had an intermediate risk of TA-TMA ($n = 3/11$, 27.3%). Those patients who developed grade III–IV acute GVHD plus previous HSCT and/or serum levels of TAC >25 ng/mL had a very high risk of TA-TMA ($n = 4/5$, 80%) (Figure 1).

Prognostic impact of TA-TMA

The presence of TA-TMA after HSCT was associated with an adverse outcome. In this regard, patients developing TA-TMA had a significantly poorer survival at 6 months and at 15 months than those without TA-TMA (Kaplan–Meier estimate: 37.5% vs 91.4% and 18.8% vs 80.1%, respectively; log-rank test: $P < 0.001$) (Figure 2). However, when TA-TMA was included in the multivariate model, it ceased to be an independent prognostic factor ($P = 0.595$) probably because of the development of grade III–IV acute GVHD, which was the strongest determinant of TA-TMA development (Table 4), and was also the most powerful factor determining prognosis in our series(HR 12.52: 95% confidence interval: 4.54–34.54, $P < 0.001$).

Table 3. Univariate analyses of factors influencing TA-TMA

Variable	N	*No. (%) patients with TA-TMA*	P-*value*
Age >45 years	77	5 (6.5)	0.36
Sex (female)	41	4 (9.7)	0.574
Lymphoid malignancy[a]	38	6 (15.8)	0.021
Advanced disease	37	5 (13.5)	0.092
Prior allogeneic HSCT[b]	6	2 (33.3)	0.010
Prior HSCT (autologous or allogeneic)	29	5 (17.2)	0.018
Donor			*0.117*
Related/unrelated allogeneic	34/68	4 (11.7)/4 (5.9)	
HLA			*0.962*
HLA—identical/ mismatched	76/26	6 (7.9)/2 (7.7)	
ABO compatibility			*0.497*
Identical/ABO-mismatched	60/42	4 (6.6)/4 (9.5)	
Source of stem cell			*0.762*
Peripheral blood/BM	87/15	7 (8)/1 (6.6)	
Myeloablative conditioning	25	2 (8)	0.876
Regimens other than FLU + BU[c]	34	7 (20.6)	0.046
Thiotepa	11	3 (27.3)	0.008
TBI	8	1 (12.5)	0.578
ATG	18	1 (5.5)	0.592
Prophylaxis of GVHD			*0.798*
TAC/MTX ± ATG	34	3 (8.8)	
TAC/SIR	68	5 (7.4)	
TAC levels above the upper limit	90	7 (7.7)	0.724
Serum levels of TAC >25 ng/mL	14	4 (28.6)	0.004
Toxic levels of TAC for >7 days	24	5 (20.4)	0.016
Acute GVHD			*0.000*
Grade 0–I	40	0 (0)	
Grade II	51	3 (5.9)	
Grade III–IV	11	5 (45.4)	
CMV reactivation/infection	38	3 (7.9)	0.992
Invasive fungal infection	9	2 (22.2)	0.051

Abbreviations: ATG = antithymocyte globulin; FLU = fludarabine; HSCT = hematopoietic SCT; SIR = sirolimus; TA-TMA = transplantation-associated thrombotic microangiopathy; TAC = tacrolimus. FLU + BU: conditioning regimens containing FLU and BU at any dose. [a]Compared with myeloid malignancies. [b]Compared with first allogeneic HSCT. [c]Compared with FLU + BU conditioning regimens.

DISCUSSION

TA-TMA is an uncommon but feared complication of allogeneic HSCT owing to its high mortality rate (>60%).[2] The exact pathophysiology of TA-TMA remains unclear, but a variety of potential risk factors have been suggested.[1,3,5,8,10–14] The use of TAC plus SIR as GVHD prophylaxis has been associated with an increased incidence of TA-TMA, which ranges from 10.8–55%, the latter in patients who received BU plus CY as part of their conditioning regimen.[17–19,21,22] In a recent phase II multicenter prospective trial conducted by our group, including some of the patients in this study, no differences were observed in the incidence of TA-TMA when TAC/SIR was compared with patients included in a prior prospective trial using CYA–mycophenolate (the overall incidences of TA-TMA were 10% and 6%, respectively).[25] In addition, very few studies[23,24] have evaluated the risk of TA-TMA among patients receiving the TAC/SIR combination in comparison with other TAC-based regimens. To shed further light on this matter, we performed a retrospective analysis in 102 allogeneic HSCT recipients who consecutively received TAC/SIR ($n = 68$) or TAC/MTX ± ATG ($n = 34$) for GVHD prophylaxis.

Unlike previously published evidence,[17,18,21,22] the combination of TAC and SIR did not appear to pose a higher risk of TA-TMA than with TAC/MTX ± ATG. These results are in agreement with a retrospective study in which the incidence of TA-TMA in patients who were given TAC/SIR ± ATG was not significantly different from that in patients who received MTX with TAC or CYA (10.2% vs 4.3%),[23] or with those of a recently randomized phase II trial comparing TAC/SIR with TAC/MTX,[24] although the high incidence of TA-TMA reported in this latter trial (24.3% with TAC/SIR and 18.9% with TAC/MTX) should be noted.[24]

Endothelial cells can be activated and damaged by several factors after HSCT.[5] As this endothelial cell injury is critical for the development of TA-TMA,[3,5,12,27] it is not surprising that grade III–IV

Table 4. Multivariate analysis of factors influencing TA-TMA

Variable	P-*value*	*HR*	*95% CI*
Lymphoid malignancy	0.298	—	—
Advanced disease	0.556	—	—
Prior allogeneic HSCT	0.288	—	—
Prior HSCT (autologous or allogeneic)	0.006	12.2	(2.07–71.95)
Use of regimens other than FLU + BU	0.106	—	—
Use of thiotepa	0.277	—	—
Grade III–IV acute GVHD	<0.001	70.48	(7.24–685.6)
Serum levels of TAC >25 ng/mL	0.015	7.34	(1.48–36.3)
Toxic levels of TAC for >7 days	0.257	—	—
Invasive fungal infection	0.051	6.56	(0.99–43.28)

Abbreviations: FLU = fludarabine; HSCT = hematopoietic SCT; TA-TMA = transplantation-associated thrombotic microangiopathy. FLU + BU: conditioning regimens containing FLU and BU at any dose.

acute GVHD was the most important risk factor for TA-TMA in our series. These results are concordant with other reports of close associations between TA-TMA and GVHD.[3,5,8,10–12,14] This may be because, during engraftment, donor T lymphocytes first encounter host endothelial cells.[4] Moreover, increased levels of coagulation factors (Von Willebrand factor, soluble thrombomodulin), inflammatory cytokines (TNF-α, IL-1, IFN-γ, IL-8) associated with cell injury or adhesion molecules (sVCAM-1) have been reported in the setting of acute GVHD, as well as circulating endothelial cells and microparticles to favor platelet aggregation and microthrombosis expressing markers of endothelial activation (CD62, anexin V).[3,5] Interestingly, in a pilot study of TAC/SIR carried out in patients who had not received chemotherapy before the conditioning regimen, none of the patients developed grade III–IV acute GVHD or TA-TMA, which is in favor of TA-TMA caused by GVHD and not by the calcineurin inhibitor.[28] Therefore, aggressive treatment of GVHD is essential. In fact, GVHD achieved a CR in 3/4 responder patients. By contrast, GVHD did not achieved a CR in any of the non-TA-TMA responders, and was a contributing cause of death in the initially responder patient with misdiagnosed TA-TMA relapse.

On the other hand, although the incidence of TA-TMA in our institution (7.8%) was similar to that reported in other studies, it is difficult to compare these results owing to the marked differences among the definitions used.[2] In an attempt to standardize the diagnosis, the Blood and Marrow Transplant Clinical Trials Network and the International Working Group proposed their own guidelines.[29,30] Subsequently, a retrospective study was performed in order to validate these proposed criteria.[31] This study noted limitations in the guidelines and introduced the concept of probable TMA, which does not rely on renal or neurological findings.[1] We used probable TMA criteria to reduce the risk of underreporting cases of TA-TMA in our series. In fact, two patients in our study did not fulfill the Clinical Trials Network or International Working Group criteria, another patient was diagnosed by histological findings without fulfilling the clinical criteria and one patient suffered a misdiagnosed relapse (diagnosed by necropsy). Remarkably, both patients who only fulfilled the probable TMA criteria responded to TAC withdrawal alone, whereas none of the other six patients responded to TAC discontinuation ($P = 0.005$), which supports the role of GVHD on TA-TMA development and stresses the proper treatment for GVHD. Moreover, this finding is consistent with the fact that an early diagnosis of TA-TMA is crucial in obtaining a faster response and a better clinical evolution of these patients, before the development of an acute kidney injury, as TA-TMA is one of the most common causes of chronic kidney disease after HSCT, which

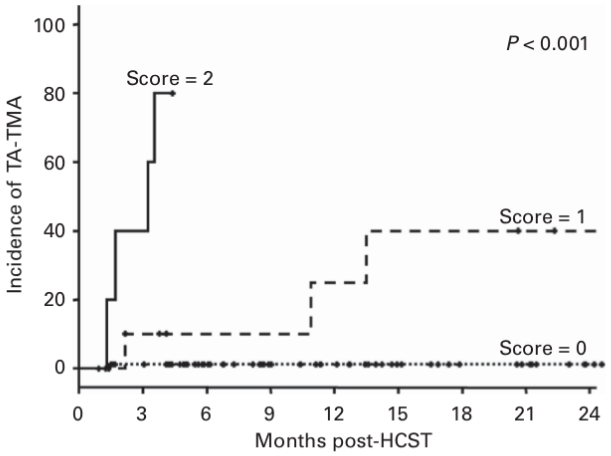

Figure 1. Score for TA-TMA risk assessment. Risk factors for TA-TMA development: grade III–IV acute GVHD, previous hematopoietic SCT and serum levels of tacrolimus >25 ng/mL. Score 0 = no risk factors or previous HSCT or serum levels of tacrolimus >25 ng/mL. Score 1 = Grade III–IV acute GVHD or previous HSCT + serum levels of tacrolimus >25 ng/mL. Score 2 = Grade III–IV acute GVHD + (previous HSCT and/or serum levels of tacrolimus >25 ng/mL).

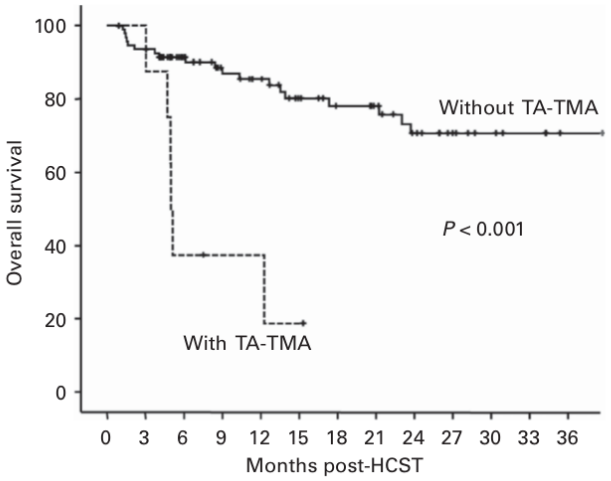

Figure 2. Overall survival of patients with or without TA-TMA.

is supposed to be a worse survival in these patients.[32] In this regard, we have described for the first time the presence of circulating erythroblasts in all patients developing probable TMA. As previously reported by Oberic *et al.*,[33] patients with cancer-associated TMAs had a typical presentation on diagnosis that was clearly distinct from that of idiopathic TMA. In particular, patients usually displayed moderate/massive erythroblastosis in peripheral blood a typical feature at presentation in comparison with patients with an idiopathic TMA. Our results are in agreement with this observation, as the great majority of our patients who developed TMA presented this feature. This fact could help not only for diagnosis, sometimes difficult, but also, and more importantly, adds prognostic information, as all patients achieving a good response show a decrease in the number of circulating erythroblasts. Although our study is limited by the relatively small number of patients, our findings are encouraging enough so as to validate them in further series. Prospective studies analyzing this data should be performed in order to confirm this original observation.

The effect of serum levels of immunosuppressive drugs on the development of TA-TMA has not so far been evaluated effectively.[17,20,34] Our results show that, although levels above the upper normal limit of TAC (>10 ng/mL in the TAC/SIR group or >15 ng/mL in the TAC/MTX group) are not associated with TA-TMA, very high levels (>25 ng/mL) are an independent risk factor for TA-TMA development. Accordingly, correct drug management is crucial to prevent this complication,[17,20,34,35] especially among patients who suffer from compromised organ function, either due to acute GVHD or from transplantation-related morbidity. This close monitoring is especially critical in the first 4 months after transplantation, as 75% of patients with TA-TMA were diagnosed before day +110 post HSCT, mainly when patients have developed severe grade III–IV acute GVHD.

Other studies have reported a trend toward a higher risk of TA-TMA in patients receiving prior myeloablative conditioning.[10,14] In our study, patients who received a prior autologous or allogeneic HSCT were at higher risk of developing TA-TMA. However, in the current study, we found no association between the risk of TA-TMA and the type of conditioning regimen.

The treatment strategies in the eight affected patients varied considerably in our series, which could be explained owing to the lack of consensus about the most appropriate treatment for patients with TA-TMA. The results of the current study, although from a small number of patients, indicate that the initial treatment strategy should include withdrawal of TAC if serum levels are >25 ng/mL. But, as aggressive treatment of the GVHD should be employed, levels >25 ng/mL for TAC are a time for holding doses and getting the level into a more appropriate range but perhaps, discontinuation all together is not the correct therapy. The choice of second-line treatment remains unresolved, however.

In conclusion, our findings suggest that the use of TAC/SIR for GVHD prophylaxis does not increase the risk of TA-TMA compared with the TAC/MTX ± ATG regimen. Severe acute GVHD is the major important risk factor for TA-TMA and transplant-related mortality; therefore, efforts should be made to improve GVHD prophylaxis and treatment. This should be counterbalanced by the close monitoring of immunosuppressive drug serum levels so that drug toxicity can be minimized. However, owing to the limitations of this retrospective study with a low number of cases, these results should be confirmed in a prospective large cohort study specifically focused on TA-TMA.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

DC and JL conceived the study; JL performed the statistical analysis; JL, LL-C and DC wrote the paper; JL, OL-G and RP-L collected the data and critically reviewed the paper; LL-C, LV, MC-C, MD-C, FS-G, EP-L, CG, IA, JAP-S and DC provided the patients and critically reviewed the paper; all authors approved the final version of the paper.

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