TITLE PAGE

Title:

Risk factors for thrombotic microangiopathy in allogeneic hematopoietic stem cell recipients receiving graft versus host disease prophylaxis with tacrolimus plus methotrexate or sirolimus.

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Running head: TA–TMA in allogeneic-HSCT treated with tacrolimus

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MDC and JL conceived the study; JL performed the statistical analysis; JL, LLC and MDC wrote the paper; JL, OLG and RPL collected the data and performed a critical revision of the paper; LLC, LV, MCC, EPL, CG, FMSG, JAPS, IA and MDC included the patients and performed a critical revision of the paper; and JL, MDC, JAPS, JRGP and JFS approved the final version of the paper.

JFSM (uniformidad) has served on speakers' bureaus and advisory boards for Millennium, Janssen, Onyx, Novartis and Celgene. The remaining authors declare no competing financial interests.

ABSTRACT:

Background

Transplantation-associated thrombotic microangiopathy (TA-TMA) is an uncommon but feared complication of allogeneic hematopoietic stem cell transplant (HSCT) due to its high mortality rate. The use of calcineurin inhibitor or sirolimus for graft versus host disease (GVHD) prophylaxis has been suggested as potential risk factors. However, the impact of tacrolimus and sirolimus combinations in the increased risk of TA-TMA is currently not well defined.

Design and Methods

We retrospectively analyzed data from 102 allogeneic HSCT recipients who consecutively received tacrolimus plus sirolimus (TAC/SIR) (n = 68) or plus methotrexate (TAC/MTX) ± ATG (n = 34) for GVHD prophylaxis to identify the incidence of TA-TMA. Other objectives were to determine the risk factors, including the role of immunosuppressive toxic levels in the development of TA-TMA.

Results

No significant differences were observed in the incidence of TA-TMA when TAC/SIR was compared with TAC/MTX ± ATG (7.4% vs 8.8%, p = 0.8). Only grade III – IV acute GVHD, previous HSCT and serum levels of tacrolimus > 25 ng/mL were associated with an increased risk of TA-TMA. Patients developing TA-TMA have a significantly poorer survival when compared with those without TA-TMA (p < 0.001). However, when TA-TMA was included in the multivariate model, it did not remain an independent prognostic factor (p = 0.595).

Conclusions

The combination of TAC/SIR does not appear to pose a higher risk of TA-TMA compared with TAC/MTX ± ATG for GVHD prophylaxis. Grade III – IV acute GVHD and very high tacrolimus levels (>25 ng/mL) were the strongest determinant of TA-TMA and mortality.

INTRODUCTION:

Transplant-associated thrombotic microangiopathy (TA-TMA) is a well documented complication of allogeneic hematopoietic stem cell transplantation (HSCT) usually diagnosed within 150 days after allo-HSCT (1). The exact incidence rate of TA-TMA is difficult to determine due to the marked heterogeneity in the definitions used and the lack of uniform criteria. Thus, the reported incidence of TA-TMA varies enormously from 0.5% to 63.6% (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, 4). However, the exact pathophysiology of TA-TMA remains unclear (3, 4), which could explain that this disorder responds poorly to conventional treatments for thrombotic thrombocytopenic purpura (3, 4). In the absence of randomized clinical trials, there is no consensus regarding the best approach to treat TA-TMA. A reasonable measure is to reduce or stop the use of calcineurin inhibitors (Otra forma: A reasonable measure is the replacement of cacineruin inhibitors by other agents), adding another agent for graft versus host disease (GVHD) prophylaxis or treatment (such as corticosteroids or mycophenolate mofetil) (3, 5). Other frequently reported treatment option includes plasma exchange, but its effectiveness is uncertain (response rate of 27 – 85%, and high rate of serious complications) (4, 6). Successful results have been reported with rituximab or defibrotide (4). Although mortality rate is difficult to discern due to the variations in the definitions used, it exceeds up to 60% and TA-TMA continues to be a feared complication of HSCT (2).

A variety of potential risk factors have been proposed such as different conditioning regimens (7-11), the development of acute GVHD (1, 3, 8, 10-14), virus or fungal infections (1, 2), unrelated donor (11), HLA mismatch (1), ABO incompatibility (10) and the use of calcineurin inhibitors (cyclosporine, tacrolimus) (15) or sirolimus GVHD prophylaxis (16). With the recent combination of tacrolimus and sirolimus in both, solid organ transplantation and HSCT, an increased risk for TA-TMA has been reported (17-21). Although most of these studies have evaluated the risk of TA-TMA due to tacrolimus/sirolimus combination (TAC/SIR) for GVHD prophylaxis, very few comparisons with other tacrolimus-based regimens have been reported. Two recent studies suggest that TA-TMA incidence does not significantly differ between

patients who received TAC/SIR and patients treated with tacrolimus/methotrexate (TAC/MTX) for GVHD prophylaxis (22, 23).

We report on the results of a retrospective analysis of 102 allogeneic HSCT to determine the incidence of TA-TMA with combination of tacrolimus plus sirolimus vs. other tacrolimus-based regimens. In addition, we have analyzed the influence of serum levels monitoring, as well as risk factors and clinical outcome of TA-TMA in allogeneic-HSCT.

PATIENTS AND METHODS:

Patients:

In 2007 tacrolimus was introduced in our Unit as GVHD prophylaxis in unrelated transplant: associated to MTX in Myeloablative transplants (esto no me suena bien). In the setting of reduce intensity conditioning regimen (RIC), we performed a phase II prospective multicenter trial (2007-006416-32 trial by GEL-TAMO/GETH) in which patients received TAC/SIC. Since then, all our patients receiving a RIC allo-HSCT have been given this GVHD prophylaxis (24).
Now we analyzed retrospectively 102 consecutives allogeneic-HSCT (aged over 18 years) who received a tacrolimus-based regimen for GVHD prophylaxis between April 2007 and July 2012 in our Unit. From them, 34 received TAC/MTX and 68 TAC/SIR combinations. Demographic data, clinical course, occurrence of GVHD and immunosuppressive levels were recorded. Clinical and laboratory characteristics are presented in Table 1. The mean age of recipients was significantly increased in the TAC/SIR group (53.3 ± 7.9 vs. 41.3 ± 11.9, $p < 0.001$); also, a higher proportion of patients received reduce intensity conditioning regimen in the TAC/SIR group (97.1% vs. 32.4%); both differences were observed because TAC/MTX GVHD prophylaxis was administered to younger patients, in the context of a myeloablative regimen. Otra posibilidad: Both differences were observed due to the administration of TAC/MTX GVHD prophylaxis to younger patients….Other differences found between the two subgroups are described in table 1. Twenty-eight out of 68 patients in the TAC/SIR group have been included in the phase II trial, and results have been already published (24).

Supportive care

The day of stem cell infusion was designated as day 0. Prophylactic platelet transfusion was given when the platelet count fell below 20 $x10^9$/L. Antibacterial, antiviral and antifungal

prophylaxis was performed according to our institutional guidelines, without differences between both 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/day orally) was used to prevent veno-occlusive disease from the beginning of conditioning.

Acute GVHD Prophylaxis

GVHD prophylaxis consisted on tacrolimus and sirolimus (TAC/SIR) (n = 68); tacrolimus and methotrexate (TAC/MTX) (n = 16); and tacrolimus plus MTX with antithymocyte globulin (TAC/MTX + ATG) (n = 18), mainly for those receiving HLA non-identical donor transplants (esto de “mainly”… se refiere solo al último grupo o a los 3?). For patients receiving TAC/MTX ± ATG, tacrolimus was administered intravenously daily at a dose of 0.01 mg/kg starting on day -7, followed by 0.03 mg/kg intravenously daily on day -1, levels were monitored on day +1 and doses were adjusted as necessary to maintain serum levels between 5 to 15 ng/mL. In the case of patients receiving TAC/SIR, tacrolimus was started on day -3 at a dose of 0.02 mg/kg/day as a continuous i.v. infusion, levels were monitored from day -1 and doses were adjusted for target blood levels of 5-10 ng/mL. Tacrolimus was switched to an equivalent oral dose when oral intake was adequate to maintain the target serum levels. Sirolimus was administered at a dose of 6 mg by mouth on day -6 (loading dose), followed by 4 mg qd p.o. Levels were monitored on day -1 and doses were adjusted to maintain serum levels between 6 and 10 ng/mL. Tacrolimus and sirolimus levels were measured twice a week for income, and then at least weekly, and the dose was adjusted for the target levels and for clinical toxicity. Both drug levels were assessed using immunoassays. The dose of tacrolimus was planned to be tapered at 5% weekly, starting on day +56 and stopped on day +180 in the TAC/SIR regimen. Tapering of tacrolimus in the TAC/MTX regimen was planned to start on day +56 (in the case of bone marrow HSCT from related donor or use of ATG), on day +100 (in the case of bone marrow HSCT from unrelated donor or peripheral blood HSCT without GVHD risk factors at day +100), or on day +240 (in presence of GVHD risk factors at day +100). Es una frase larguísima, quizá con los parentesis queda mas claro.The dose of sirolimus was planned to be tapered on day +180 and stopped on day +240. This tapering management for both drugs was for patients who did not developed GVHD, with negative minimal residual disease and complete chimerism. Intravenous administration of MTX was performed at 15 mg/m2 on day + 1 and 10 mg/m2 on days + 3, + 6

and + 11, followed by folinic acid rescue. Antithymocyte globulin (ATG) was administered intravenously at a dose of 2.5 mg/Kg on days - 4 to – 2.

Post-HSCT complications

Post-HSCT complications such as acute GVHD, TA-TMA, fungal or virus infection and relapse or progression after HSCT, were also recorded. Acute GVHD were assessed and graded according to published criteria (25).

The diagnosis of TA-TMA was considered according to probable TMA criteria as defined by validation study by Cho et al (1): ≥ 2 schistocytes per high-power field on peripheral blood, concurrent increased serum LDH above institutional baseline, thrombocytopenia < 50 x 10^9/L or a ≥ 50% decrease in platelet count, decreased hemoglobin, negative Coombs test results, decreased haptoglobin and absence of coagulopathy.

Statistical analysis

Data were initially included in an Excel (Microsoft) spreadsheet and a descriptive statistical analysis performed. Results are expressed as percentages for categorical variables and as medians (and standard deviations) for continuous variables. Differences between groups were evaluated with IBM SPSS Statistics 20 (SPSS, Chicago, IL, USA), using Student's t-Test for independent samples to compared quantitative variables, and Chi-squared test to compared categorical variables. The incidence of TMA was calculated and plotted by using Kaplan-Meier analysis. The log-rank test was used to identify risk factors for the development of TA-TMA. Variables associated with TA-TMA in the univariate analyses were included in a Cox proportional hazard model using the forward conditional variable selection method. Overall survival was defined as the time elapsed between HSCT day 0 and death or last follow-up and calculated by the Kaplan–Meier method. The log-rank test was used to assess differences between groups of patients with or without TA-TMA. All the parameters that were significant in the univariate analyses were included in the multivariate analysis. Multivariate survival analysis involved developing Cox proportional hazards models with stepwise variable selection. Statistical significance of all tests was concluded for values of $p < 0.05$.

RESULTS:

Between April 2007 and July 2012, 102 out of 238 allogeneic-HSCTs performed at our unit (aged over 18 years), were given tacrolimus regimen for prevention GVHD and were included in this retrospective study. The primary end-point was to analyze the incidence of TA-TMA in patients who received TAC/SIR GVHD prophylaxis vs. other tacrolimus-based regimens. Other objectives were to determine the risk factors, including the role of immunosuppressive toxic levels in the development of TA-TMA.

Incidence of transplant-associated thrombotic microangiopathy

With a median follow-up of 451 days (range, 28 – 1946 days), eight out of 102 allogeneic HSCTs developed TA-TMA (7.8%): 3/34 patients in the TAC/MTX ± ATG regimen group (8.8%) and 5/68 in the TAC/SIR regimen 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 8 patients were diagnosed before the day +110 post-HSCT. None of them developed chronic GVHD previously to the diagnosis of TA-TMA.

Clinical and laboratory findings leading to the diagnosis of TMA are presented in table 2. Median age of the patients was 52 years (range, 24 - 63), and 50% were male. Two patients developed TA-TMA during their second allogeneic-HSCT. Six out of 8 patients diagnosed of TA-TMA fulfilled all TMA criteria: one of the other 2 patients had only 1 schistocyte per field in peripheral blood, but fulfilled the rest of required criteria and serum levels of sirolimus were toxic (16.7 ng/mL), so he was finally diagnosed of TA-TMA; the second patient did not fulfilled all criteria because, neither peripheral blood smear, nor haptoglobin serum levels were performed, but had histologic evidence of TA-TMA in a biopsy specimen after underwent colonoscopy for severe diarrhea. Concurrent renal and/or neurologic dysfunction were observed only in 3/8 patients diagnosed of TA-TMA.

Serum levels of immunosuppressive drugs

Previous toxic levels of tacrolimus were observed in 87.5% of patients with TA-TMA (n = 7/8) and in 88.3% of patients without TA-TMA (n = 83/94), p = NS. Patient with TA-TMA and without toxic tacrolimus levels belonged to the TAC/SIR group and presented toxic levels of sirolimus. Moreover, tacrolimus levels > 25 ng/mL were observed in 4/8 (50%) of the TA-TMA patients vs

10/94 (10.6%) of patients without TA-TMA (p = 0.004) and the mean number of days with toxic levels of tacrolimus also was significantly higher in patients who developed TA-TMA (9 ± 7 days) compared to patients without TA-TMA (4.6 ± 4.1 days), p = 0.008.

Forty one patients (60%) have toxic levels of sirolimus, but no association with an increased incidence of TA-TMA was observed (7.3 and 7.4%, respectively), p = 0.99. There were also no differences respect to the mean number of days with toxic levels of sirolimus between patients who developed TA-TMA (2.2 ± 2.3 days) compared to patients without TA-TMA (2.05 ± 2.7), p = 0.90.

Management and outcome of TA-TMA (table 3)

The initial treatment strategy for patients who experienced TA-TMA (n = 8) was complete withdrawal (n = 7) or dose reduction (n = 1) of tacrolimus. Additionally, cyclosporine (n = 1) or Rituximab (n = 3) were added to tacrolimus discontinuation. Subsequent lines of treatment included vincristine (n=4) and mycophenolate mofetil (MMF) (n=3).

Four out of 8 patients achieved a resolution of TA-TMA: 2 after tacrolimus withdrawal alone (both in the TAC/SIR group); 1 patient in the TAC/SIR group responded after second line treatment with sirolimus discontinuation and adding mycophenolate mofetil; and the last patient, in the TAC/MTX group, responded after third line treatment with MMF and cyclosporine withdrawal (which was added as first line treatment) after prior failure with vincristine.

Regarding the 4 patients who did not respond, 3 had received vincristine as second line therapy, and a fourth patient received MMF plus cyclosporine (which was stopped later) as salvage therapy.

Six out of 8 patients with TA-TMA died. TMA was a contributing cause of death in the 4 non-responded patients. Two patients died of other causes after resolution of TMA (1 patient due to pulmonary aspergillosis in GVHD context and the other patient due to relapse of her disease). Other TMA-associated causes of death were GVHD (n = 3), invasive fungal infection (n = 3) and CMV infection (n = 1) in GVHD context and hemorrhage (n = 3).

Risk factors for transplant-associated thrombotic microangiopathy

Table 4 shows results of the univariate analyses carried out to identify the variables capable of predicting TA-TMA in allogeneic-HSCT recipients who received tacrolimus-based regimen for GVHD prophylaxis. Lymphoid malignancies, prior HSCT (autologous or allogeneic), conditioning regimens different from FLU + BU at any dose, use of thiotepa, grade III – IV acute GVHD, serum levels of tacrolimus > 25 ng/mL, toxic levels of tacrolimus 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 tacrolimus > 25 ng/mL retained its association with TA-TMA (Table 5).

Moreover, we observed that patients without any of these 3 risk factors or without previous HSCT or serum levels of tacrolimus > 25 ng/mL 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 tacrolimus > 25 ng/mL had a high risk of TA-TMA (n = 3/11, 27.3%); and patients who developed grade III – IV acute GVHD and had previous HSCT and/or serum levels of tacrolimus > 25 ng/mL had a very high risk of TA-TMA (n = 4/5, 80%) (Figure 2). No tiene sentido que la figura 2 se nombre antes que la 1.

Prognostic impact of TA-TMA

The presence of TA-TMA after HSCT was associated with an adverse outcome when compared with patients without TA-TMA. Patients developing TA-TMA have a significantly poorer survival at 6 months and at 15 months as compared with those without TA-TMA (37.5% and 18.8% and 91.4% and 80.1%, respectively, Kaplan–Meier estimate, $p < 0.001$; log-rank test) (Figure 1). However, when TA-TMA was included in the multivariate model (Table 6), it did not remain an independent prognostic factor as it was superseded by negative prognostic effect of the grade III – IV acute GVHD, which was the strongest determinant of TA-TMA in our series (Table 5).

Discussion

TA-TMA is an uncommon but feared complication of allogeneic HSCT due 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, 8, 10-14). The increasing use of tacrolimus plus sirolimus as GVHD prophylaxis suggests an increase incidence of TA-TMA, which ranges

from 10.8% to 55% in patients who also received busulfan and cyclophosphamide in combination (17-19, 21). However, in a recent phase II multicenter prospective trial conducted by our group, including some of the patients of 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 with Cyclosporine-Mycophenolate (the overall incidence of TA-TMA was 10% and 6%, respectively) (24); and very few studies have evaluated the increased risk of TA-TMA due to TAC/SIR combination in comparison with other tacrolimus-based regimens. To shed further light on this matter we report a retrospective analysis of 102 allogeneic HSCT who consecutively received TAC/SIR (n = 68) or TAC/MTX ± ATG (n = 34) for GVHD prophylaxis.

In contrast to previously published evidence (17, 18, 21), the combination of tacrolimus and sirolimus does not appear to pose a higher risk of TMA compared with TAC/MTX ± ATG. These results are in agreement with a recently randomized phase II trial comparing TAC/SIR to TAC/MTX (23) and with a retrospective study in which the incidence of TA-TMA in patients given TAC/SIR ± ATG was not significantly different from that in patients who received MTX with TAC or cyclosporine (10.2% vs 4.3%) (22) and . However, it is important to note the high incidence of TA-TMA reported in this trial (24.3% with TAC/SIR and 18.9% with TAC/MTX) (23).

On the other hand, although the incidence of TA-TMA in our institution (7.8%) is similar to that in other studies, it is very difficult to compare these results due to the marked heterogeneity in the definitions used and the lack of uniform criteria, what gives rise to enormous variations in the incidence (2). In an attempt to standardize the diagnosis, the Blood and Marrow Transplants Clinical Trials Network (CTN) and the International Working Group (IWG) proposed separate guidelines (26, 27). Subsequently, a retrospective study was performed in order to validate these proposed criteria (28). This study noted limitations in the guidelines and introduced the concept of “probable-TMA,” which does not require renal or neurologic findings (1). We used “probable-TMA” criteria in order to decrease TA-TMA underreported. In fact, 2 patients in our study did not fulfilled CTN neither IWG criteria, and another patient was diagnosed by histology without fulfilled clinical criteria, what stress the limitations of clinical diagnostic criteria. It is intriguing that both patients who only fulfilled probable-TMA criteria responded to tacrolimus withdrawal alone, while only a few patients with other criteria of TA-TMA (n = 6) responded to tacrolimus discontinuation (p = 0.005). This finding is consistent with the fact that an early

diagnosis of TA-TMA is crucial to obtain a faster response and a better clinical evolution of these patients.

Moreover, the impact of serum immunosuppressive levels on the influence of the development of TA-TMA has been poorly evaluated (17, 20, 29). Our results showed that, although high levels of TAC do not associate with TA-TMA, very high levels (> 25 ng/mL) are an independent risk factor for TA-TMA development, which makes crucial a correct drug management. Therefore, an important issue is the correct monitoring of serum levels of tacrolimus and sirolimus (17, 20, 29, 30), especially in patients who suffered from organ function compromise, either due to acute GVHD or from transplant-related morbidity, avoiding toxic levels and adjusting doses to maintain toxic levels the fewest number of days as possible. This close monitoring is especially critical in the first 4 months after transplant, since 75% of the patients with TA-TMA were diagnosed before the day +110 post-HSCT, mainly when patients have developed severe grade III-IV acute GVHD.

Otherwise, since endothelial cell injury is critical for the development of TA-TMA (3, 12, 31), it is not surprising that grade III-IV acute GVHD and prior HSCT (autologous, allogeneic) were the most important risk factor of TA-TMA in our series. These results are according to other reports that also showed a close association between TMA and GVHD (3, 8, 10-12, 14). On the other hand, in a pilot study of TAC/SIR in patients who received allogeneic-HSCT, mostly for non-malignant disorders and untreated with chemotherapy prior to conditioning for HSCT, none of the patients developed TA-TMA nor grade III-IV acute GVHD (32).

Other studies have shown that patients with previous autologous- HSCT, or a prior ablative HSCT within 6 months, exhibit a trend to developed TA-TMA (10, 14). However, we advertised an association of prior HSCT with TA-TMA, independent of conditioning regimen or the time from the first transplant. Therefore, we must pay attention to patients suffering from organ function compromise, either due to GVHD or from other causes, since they are at the highest risk to develop TA-TMA.

There is no consensus on the most appropriate treatment for patients with TA-TMA. The results described in the current study, although in a low number of patients, show that the initial treatment strategy should include dose reduction or tacrolimus withdrawal. Second line

treatment is full of controversy. It must be pointed out the unfavorable results obtained with vincristine, which is contrary to previous report by our group (33).

Finally, our results illustrates that although TA-TMA is a significant problem in patients with allogeneic HSCT, it is not associated with increased mortality in the multivariate analysis. This can be explained because it was superseded by negative prognostic effect of the grade III – IV acute GVHD, which was the strongest determinant of TA-TMA and mortality.

In conclusion, our data support that the use of TAC/SIR GVHD prophylaxis does not increase the risk of TA-TMA as compared to TAC/MTX ± ATG regimen. On the other hand, although immunosuppressive drugs are necessary to control development of acute GVHD, it is very important a correct monitoring of serum levels to avoid both, severe acute GVHD and drug toxicity.

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Legend to tables:

Table 1.-

Header. Baseline characteristics of patients (n = 102)

Foot. NS: non significant. TAC: Tacrolimus; SIR: sirolimus; MTX: methotrexate; HSCT: hematopoietic stem cell transplantation. GVHD: graft versus host disease; MTX: methotrexate; ATG: antithymocyte globulin.

[a] Stage disease was reported according criteria previously described (34). Early stage (acute leukemia transplanted in first complete remission, myelodysplastic syndrome transplanted either untreated or in first complete remission, chronic myeloid leukemia in first chronic phase, and non-Hodgkin lymphoma and multiple myeloma transplanted untreated or in first complete remission), intermediate stage (acute leukemia in second complete remission; chronic myeloid leukemia in all other stages than chronic phase or blast crisis; myelodysplastic syndrome in second complete remission or in partial remission; and non-Hodgkin lymphoma and multiple myeloma in second complete remission, in partial remission, or stable disease) and advance stage (acute leukemia in all other disease stages, chronic myeloid leukemia in blast crisis, myelodysplastic syndromes in all other disease stages, and multiple myeloma and lymphoma in all other disease stages than those defined as early or intermediate). Stage was not applicable for patients with aplastic anemia.

[b] RIC: Reduced intensity conditioning: Fudarabine (FLU) 150 mg/m2 + Busufan (BU) 8 to 10 mg/kg orally or 9.6 mg/Kg iv, FLU (150 mg/m2) + melpahalan (MEL) 140 mg/m2, FLU (150 mg/m2) + MEL (140 mg/m2) + thiotepa (THIO) 10 mg/Kg, FLU (90 mg/m2) + MEL (140 mg/m2) + Bortezomib (1.3 mg/m2 on days -9 and -2) (n = 2), Yttrium-90 ibritumomab tiuxetan (0.4 mCi/Kg) + FLU (150 mg/m2) + MEL (140 mg/m2) + THIO (10 mg/Kg) (n = 1); clofarabine (200 mg/m2) + MEL (100 mg/m2) (n = 1); etoposide (40 mg/Kg) + Ara-C (18 g/m2) (n = 1); CY (1200 mg/m2) + FLU

(120 mg/m2) + ATG (7.5 mg/Kg) + TBI (200 cGy) (n = 1), FLU (90 m/m2) + once-daily intravenous BU (6.4 mg/Kg) + THIO (10 mg/Kg) (n = 1).

[c] Myeloablative conditioning: FLU (160 mg/m2) + once-daily intravenous (iv) busulfan (BU) 12.8 mg/Kg (n = 10); cyclophosphamide (CY) 120 mg/Kg + total body irradiation (TBI) 12 Gy x 6 fraction (n = 5); BU 12.8 mg/Kg iv or 16 mg orally + CY 120 mg/Kg (n = 5); and others (n = 5), consisting in BU (8 mg/Kg iv) + CY (120 mg/Kg) + thiotepa (THIO) (750 mg/m2) 3, CY (120 mg/Kg) + TBI (13.2 Gy x 11 fraction) 1, CY (120 mg/Kg) + TBI (12 Gy x 6 fraction) + THIO (400 mg/m2) 1.

Table 2.-

Header. Clinical and laboratory findings leading to the diagnosis of TA-TMA

Foot. TAC: tacrolimus. SIR: sirolimus. MTX: methotrexate. ND: Not determined * Age at day of HSCT. ** Patient under treatment with continuous venovenous hemodialysis.

Table 3.-

Header. Management and outcome of TA-TMA

Foot. TA-TMA: Transplant-associated thrombotic microangiopathy. TAC: tacrolimus. SIR: sirolimus. MTX: methotrexate. CsA: cyclosporine. MMF: mycophenolate mofetil. GI: gastrointestinal. IFI: invasive fungal infection. GVHD: graft versus host disease. * Age at day of HSCT. ** Vincristine was administered at a dose of 1mg iv on days +1, +4, +8 and +11. *** Rituximab was administered at a dose of 375 mg/m2 iv weekly x 4 doses.

Table 4.-

Header. Univariate analysis of factors influencing TA-TMA

Foot. TA-TMA: Transplant-associated thrombotic microangiopathy; HSCT: hematopoietic stem cell transplantation; FLU: fludarabine; BU: busulfan; FLU

+ BU: conditioning regimens containing FLU and BU at any dose; TBI: total body irradiation; ATG: antithymocyte globulin; GVHD: graft versus host disease; CMV: cytomegalovirus. * Compared with myeloid malignancies. ** Compared with first allogeneic-HSCT.

Table 5.-

Header. Multivariate analysis of factors influencing TA-TMA

Foot. TA-TMA: Transplant-associated thrombotic microangiopathy; HSCT: hematopoietic stem cell transplantation; FLU: fludarabine; BU: busulfan; FLU + BU: conditioning regimens containing FLU and BU at any dose; TBI: total body irradiation; ATG: antithymocyte globulin; GVHD: graft versus host disease; CMV: cytomegalovirus

Table 6.-

Header. Univariate and Multivariate analysis of factors influencing overall survival

Foot. HSCT: hematopoietic stem cell transplantation; GVHD: graft versus host disease; CMV: cytomegalovirus.

Table 1: Baseline characteristics of patients (n = 102)

Variable	Total	TAC/SIR	TAC/MTX	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)	*p* = NS
Diagnosis				0.021
Acute myeloid leukemia	35 (34.3)	20 (29.4)	15 (44.1)	
Myelodysplastic syndrome	20 (19.6)	17 (25)	3 (8.8)	
Non-Hodgkin lymphoma	14 (13.7)	11 (16.2)	3 (8.8)	
Acute lymphocytic leukemia	8 (7.8)	2 (2.9)	6 (17.6)	
Chronic lymphocytic leukemia	8 (7.8)	7 (10.3)	1 (2.9)	
Chronic myeloid leukemia	6 (5.9)	2 (2.9)	4 (11.8)	
Others	11 (10.9)	9 (13.2)	2 (6)	
Stage of the disease [a]				*p* = 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)	*p* = NS
Donor				*p* = NS
Related allogeneic	34 (33.3)	23 (33.8)	11 (32.4)	
Unrelated allogeneic	68 (66.7)	45 (66.2)	23 (67.6)	
HLA				*p* = NS
Identical	76 (74.5)	48 (70.6)	28 (82.4)	
9/10 *vs.* 7/8 *vs.* 8/10 match	11 / 8 / 7	7 / 8 / 5	1 / 3 / 2	
	(10.8 / 7.8 / 6.9)	(10.3 / 12.5 / 7.3)	(2.9 / 8.8 / 5.9)	
ABO compatibility				*p* = NS
Identical	60 (58.8)	43 (63.2)	17 (50)	
Minor mismatch	14 (13.7)	7 (10.3)	7 (20.6)	
Major mismatch	21 (20.6)	13 (19.1)	8 (23.5)	
Bidirectional incompatible	7 (6.9)	5 (7.4)	2 (5.9)	
Source of stem cell				*p* = NS
Peripheral blood	87 (85.3)	59 (86.8)	28 (82.4)	
Bone marrow	15 (14.7)	9 (13.2)	6 (17.6)	
Conditioning regimen				< 0.001
RIC [b]	77 (76.2)	66 (97.1)	11 (32.4)	
FLUBU	37	32	5	
FLUMEL	28	26	2	
FLU + MEL + THIO	5	2	3	
Other	7	6	1	
Myeloablative [c]	25 (24.5)	2 (2.9)	23 (67.6)	
FLUBU (once – daily intravenous)	10	2	8	
CY – TBI	5	-	5	
BUCY	5	-	5	
Other	5	-	5	
Complications after HSCT				
Grade III – IV acute GVHD	11 (10.8)	8 (11.8)	3 (8.8)	*p* = NS
Thrombotic microangiopathy	8 (7.8)	5 (7.4)	3 (8.8)	*p* = NS

Table 2.- Clinical and laboratory findings leading to the diagnosis of TMA

Age*/ Sex	Group	Days post-HSCT	Schistocytes per field	LDH (IU/L)	Hemoglobin (g/dL)	Platelet count (x109/L)	Haptoglobin (mg / dL)	Direct Coombs test	Coagulopathy	Creatinine (mg/dL)	Neurologic dysfunction
38 M	TAC/MTX	97	8	1346	8.1	15	5.2	Negative	Absent	0,79	No
59 F	TAC/SIR	51	1	481	9	38	16.5	Negative	Absent	1.30	Yes
28 M	TAC/MTX	39	3	393	6.6	14	< 6.63	Negative	Absent	1.35	No
61 M	TAC/SIR	65	12	5474	7.3	17	< 7.38	Negative	Absent	0,75**	No
43 F	TAC/MTX	327	6	1350	8.5	28	51	Negative	Absent	0.80	No
62 F	TAC/SIR	405	2	818	8.9	36	44	Negative	Absent	0.60	No
48 M	TAC/SIR	39	7	881	9.6	26	< 6.18	Negative	Absent	0.88	No
56 F	TAC/SIR	106	ND	179	10,7	11	ND	Negative	Absent	0,7	No

Table 3.- Management and outcome of TA-TMA

Age* / Sex	Group	First line treatment	Response	Second line treatment	Response	Third line treatment	Response	Situation	Cause of dead
38 M	TAC/MTX	TAC reduction	No response	Vincristine**	No response	-	-	Dead	TA-TMA. GI bleeding. IFI
59 F	TAC/SIR	TAC withdrawal	Good response	-	-	-	-	Dead	Relapse
28 M	TAC/MTX	TAC withdrawal, adding CsA	No response	Vincristine**	No response	CsA withdrawal, adding MMF	Good response	Alive	-
61 M	TAC/SIR	TAC withdrawal	No response	Vincristine**	No response	-	-	Dead	TA-TMA. IFI. GI bleeding. GVHD
43 F	TAC/MTX	TAC withdrawal + Rituximab ***	No response	MMF + CsA cyclosporine	No response	CsA withdrawal	No response	Dead	TA-TMA. Respiratory insufficiency. GI bleeding. GVHD
62 F	TAC/SIR	TAC withdrawal	Good response	-	-	-	-	Alive	-
48 M	TAC/SIR	TAC withdrawal + Rituximab ***	No response	SIR withdrawal, adding MMF	Good response	-	-	Dead	Pulmonary aspergillosis. GVHD
56 F	TAC/SIR	TAC withdrawal + Rituximab ***	No response	Vincristine**	No response	-	-	Dead	TA-TMA. IFI. CMV infection. GVHD

Table 4. Univariate analysis of factors influencing TA-TMA

Variable	N	No. (%) patients with TA-TMA	P-value
Infection/inflammation	9	2 (22.2)	0.051
Age > 45 years	77	5 (6.5)	0.36
Sex (female)	41	4 (9.7)	0.574
Diagnosis			0.126
Acute myeloid leukemia	35	2 (5.7)	
Myelodysplastic syndrome	20	0 (0)	
Non-Hodgkin lymphoma	14	2 (14.3)	
Acute lymphocytic leukemia	8	2 (25)	
Multiple Myeloma	5	0 (0)	
Chronic myeloid leukemia	6	0 (0)	
Others	14	2 (14.3)	
Lymphoid malignancy *	38	6	0.021
Advanced disease	37	5 (13.5)	0.092
Prior allogeneic – HSCT **	6	2 (33.3)	0.010
Prior HSCT (Auto or allo)	29	5 (17.2)	0.018
Donor			0.117
Related allogeneic	34	4 (11.7)	
Unrelated allogeneic	68	4 (5.9)	
HLA			0.962
HLA – identical	76	6 (7.9)	
HLA – mismatched	26	2 (7.7)	
ABO compatibility			0.736
Identical	60	4 (6.6)	
Minor mismatch	14	2 (14.3)	
Major mismatch	21	2 (9.5)	
Bidirectional incompatible	7	0 (0)	
ABO-mismatched	42	4 (9.5)	0.497
Source of stem cell			0.762
Peripheral blood	87	7 (8)	
Bone marrow	15	1 (6.6)	
Myeloablative conditioning	25	2 (8)	0.876
FLU + BU conditioning regimens	48	1 (2.1)	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
Tacro/MTX +/- ATG	34	3 (8.8)	
Tacro/SIR	68	5 (7.4)	
Toxic levels of tacrolimus	90	7 (7.7)	0.724
Serum levels of tacrolimus > 25ng/mL	14	4 (28.6)	0.004
Toxic levels of tacrolimus 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

Table 5.- Multivariate analysis of factors influencing TA-TMA

Variable	P	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 Flu + BU conditioning	0.106	-	-
Use of thiotepa	0.277	-	-
Grade III – IV acute GVHD	0.000	70.48	(7.24 – 685.6)
Serum levels of tacrolimus > 25 ng/mL	0.015	7.34	(1.48 – 36.3)
Toxic levels of tacrolimus for > 7 days	0.257	-	-
Invasive fungal infection	0.051	6.56	(0.99 – 43.28)

Table 6.- Univariate and Multivariate analysis of factors influencing mortality

Variable	P – Univariate	Hazard ratio	95% CI	P – Multivariate
Age, <45 years	0.599	-	-	-
Male Sex	0.457	-	-	-
Advanced disease	0.15	-	-	0.197
Prior HSCT	0.004	2.77	(1.19 – 6.48)	0.018
Myeloablative conditioning	0.682	-	-	-
Grade III-IV acute GVHD	0.000	12.52	(4.54 – 34.54)	0.000
Thrombotic microangiopathy	0.000	-	-	0.595
CMV reactivation/infection	0.967	-	-	-
Invasive fungal infection	0.036	6.29	(1.80 – 21.94)	0.004
Progression or relapse after HSCT	0.000	5.97	(2.3 – 15.54)	0.000

Legends to figures and figures:

Figure 1.- Overall survival of patients with and without TA-TMA

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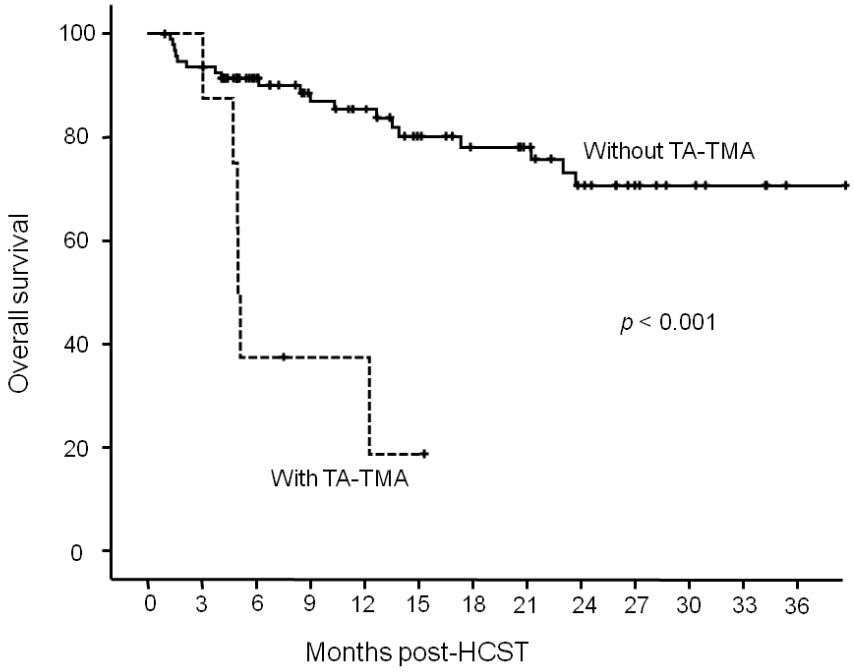

Figure 2.-

Header. Score for TA-TMA risk assessment

Foot. Risk factors for transplant associated – thrombotic microangiopathy (TA-TMA) development: grade III – IV acute graft versus host disease (GVHD), previous hematopoietic stem cell transplant (HSCT) 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)