Minnesota acute graft-*versus*-host disease risk score predicts survival at onset of graft-*versus*-host disease after post-transplant cyclophosphamide prophylaxis

Prevention and treatment of graft-*versus*-host disease (GvHD) still represent a major unmet need of allogeneic stem cell transplantation (HSCT). Roughly half of the patients receiving HSCT will develop acute GvHD (aGvHD) and half will obtain complete resolution of GvHD through corticosteroids in combination with immunosuppressive therapy; steroid refractory aGvHD will lead to death of around 20% of patients and the remaining will experience an aberrant immune activation possibly leading to chronic GvHD (cGvHD).[1-2]

Early identification of patients at higher risk of developing steroid-refractory aGvHD and GvHD-related mortality is a paramount.

Refined immunologic profiles and machine learning algorithms have been recently investigated,[3] aiming at the estimation of clinical outcomes after post-transplant cyclophosphamide (PTCy)–based GvHD prophylaxis. Unfortunately, clinical scoring systems, easier to apply in clinical practice, have intrinsic limitations: if the maximum grade of aGvHD is strongly correlated with mortality, this can be determined only retrospectively, while the grade at onset is dramatically inconsistent due to subsequent evolutions. The Minnesota group has provided a risk score model for aGvHD classifying patients into high risk (HR) or standard risk (SR) at aGvHD onset. This score was firstly validated in a CIBMTR cohort of 1,723 patients and recently confirmed in a multicenter cohort of 355 patients.[1,4-6] The original study of the Nineties involved over 800 patients. The Minnesota aGvHD risk score can be used in real time at the bedside and proved to offer a reliable stratification of patients with reference to both probability of aGvHD overall response and transplant related mortality (TRM).

The aim of our study was to assess the efficacy of the Minnesota risk score as a tool to identify patients at higher risk of mortality at onset of aGvHD in the setting of PTCy, increasingly adopted by the worldwide community in all types of donor type and matching. Of note, GvHD prophylaxis was based on PTCy with sirolimus alone for matched related donor (MRD) HSCT, or in combination with mycophenolate mofetil in case of a mismatched related (MMRD) or matched unrelated donor (MUD), in accordance with local guidelines.[7]

Categorical variables were described as frequencies and continuous variables as median value and interquartile range. Acute GvHD and cGvHD were graded according to MAGIC criteria[8] and NIH 2014 criteria.[9] TRM was defined as death from any cause while in continuous remission of the primary disease. Overall survival (OS) was defined as the interval from allogeneic HSCT to death whatever the cause, and patients were censored at the date of last contact if alive. Progression-free survival (PFS) was defined as the interval from HSCT to either relapse or progression or death in remission (whichever came first). The probabilities of OS and PFS were estimated using the Kaplan-Meyer estimator.[10] Cumulative incidences were estimated for engraftment, GvHD, relapse and TRM to accommodate competing risks. Relapse or progression was a competing risk for TRM. Relapse/progression and death from any causes were competing risks for GvHD.[11] Log-rank test was used for univariate comparisons of survival curves,[12] while the Gray's test was conducted to compare cumulative incidences of competing-risks endpoints.[13] Factors predicting aGvHD were studied using multivariable Cox regression analysis.[14] The proportional hazard assumption was met for all variables. All tests were 2-sided, and a α-1 error of 0.05 was considered significant for the determination of factors associated with time to event. Statistical analyses were performed with R 4.0.4 (R Development Core Team, Vienna, Austria) software. The Minnesota risk score was calculated based on the number of involved organs and organ stage, thus determining the severity of GvHD at onset as previously detailed.[4-5]

Our analysis consisted in a prospective single-center study, involving all consecutive allogeneic transplants performed in 315 patients at our center for any disease, from any donor type and under PTCy-based GvHD prophylaxis,7 between January 2016 and June 2020. Patients' median age was 52.7 years (range, 15.3-75.6). The median follow-up was 2.4 years (range, 1.4-3.5). Patients and transplant features are described in Table 1.

The 2-years probability of OS was 66.2% (95% confidence interval [CI]: 60.4-71.4), the 2-years probability of PFS was 62.5% (95% CI: 56.6-67.8). The 2-years cumulative incidence of relapse was 20% (95% CI: 15.6-24.8) while the 2-years cumulative incidence of TRM was 17.5% (95% CI: 1.4-22.1) (*Online Supplementary Figure S1*).

Acute GvHD was diagnosed in 139 patients and the median time from transplant to GvHD onset was 30 days (range, 1-250). Day-100 cumulative incidence of aGvHD grade II-IV and III-IV was 24.8% (95% CI: 26.5–37.4) and 14.9% (95% CI: 11.2–19.1) respectively. First-line systemic treatment at diagnosis of aGvHD grade II-IV – irrespective of single-organ

manifestation or multi-organ manifestation - consisted of high dose steroids (methylprednisolone 2 mg/kg/day) according to the EBMT recommendation[15] and agents beyond the first line included extracorporeal photopheresis, ruxolitinib and infliximab. Multivariate analysis outlined donor type (MRD *vs*. MUD *vs*. MMRD) and donor age independently associate with aGvHD (*Online Supplementary Table S1)*.

The 2-years cumulative incidence of cGvHD was 31.9% (95% CI: 26.5-37.4) overall, while moderate/severe cGvHD was 24.5% (95% CI: 19.6-29.6) (*Online Supplementary Figure S2*). First-line systemic treatment consisted of steroids (prednisone 1 mg/kg/day) and agents beyond the first line included extracorporeal photopheresis, ruxolitinib, ibrutinib and methotrexate.

Among patients diagnosed with aGvHD, initial GvHD organ involvement was skin only (54%), upper and/or lower gastro-intestinal tract only (10%), liver only (1.4%) or multi-organ (31.7%).

Of the 139 patients, 46 (33.1%) were categorized as Minnesota HR aGvHD and 93 (66.9%) as Minnesota SR aGHHD. GvHD treatment was initiated at GvHD declaration in both SR and HR. At onset of steroid therapy, 36% of patients had grade I GvHD, 25.9% grade II GvHD, 24.5% had grade III GvHD and 12.2% had grade IV GvHD. Overall, three patients did not receive any steroid systemic or topical treatment; nine patients with topical therapy only were in complete response at day 28, one in partial response.

Day-28 overall response (complete remission [CR], partial remission [PR]) was higher in Minnesota SR (96% - 78 patients CR, 9 patients PR) *versus* Minnesota HR aGvHD (63% - 27 patients CR, 2 patients PR) *P*<0.0001. Overall, in the SR aGvHD cohort two patients were not evaluable due to disease progression and related treatment, while in the HR aGvHD cohort 17 patients were considered non-responders and all but one died due to aGvHD (n=12), disease progression (n=2), infections (n=2).

The 1-year cumulative incidence of cGvHD was not significantly different between SR and HR aGvHD patients: 9% (95% CI: 1-36) for Minnesota SR and 26% (95% CI: 14-49) for Minnesota HR, *P*=0.065.

In multiple regression analysis, adjusting for clinically significant variables, the 2-year OS were lower in HR *versus* SR GvHD patients: 57% (95% CI, 37.8-72.4) for Minnesota SR and 30.7% (95% CI: 17.4-45) for Minnesota HR, *P*=0.00389; conversely the 2-year TRM were higher in HR *versus* SR GvHD patients: 20.6% (95% CI: 9.5-34.7) for Minnesota SR and 52.7% (95% CI, 36.3-66.7) for Minnesota HR, *P*=0.00156 (Figure 1A and B).

Of note, the 2-year OS according to day-28 response was 68% (95% CI: 57-76) for patients in CR, 55% (95% CI: 23-78) for patients in PR and 10% (95% CI: 2-27) for non-responders (*P*<0.0001). Similarly, the 2-year TRM according to day-28 response was 13% (95% CI: 7-21) for patients in CR,

Table 1. Patients' characteristics.

	All patients N=315	Acute GvHD patients N=139
Patient sex, N (%)		
F	117 (37.1)	52 (37.4)
M	198 (62.9)	87 (62.6)
Diagnosis, N (%)		
AML	176 (55.9)	70 (50.4)
ALL	37 (11.7)	17 (12.2)
NHL/HL	41 (13.0)	15 (10.8)
MDS or MPN	57 (18.1)	33 (23.7)
Other	4 (1.3)	4 (2.9)
Disease status, N (%)		
AD	135 (42.9)	66 (47.5)
CR>1	56 (17.8)	23 (16.5)
CR1	122 (38.7)	50 (36)
DRI, N (%)		
Low-int	174 (55.2)	77 (55.4)
High	94 (29.8)	40 (28.8)
Very high	22 (7.0)	8 (5.8)
Donor, N (%)		
MMRD	126 (40.0)	67 (48.2)
MRD	62 (19.7)	18 (12.9)
MUD	127 (40.3)	54 (38.9)
Donor sex, N (%)		
F	121 (38.4)	52 (37.4)
M	192 (61.0)	86 (61.9)
Female donor to male host, N (%)		
No	247 (78.4)	109 (78.4)
Yes	66 (21.0)	29 (20.9)
CMV matching, N (%)		
Neg/neg	22 (7.0)	12 (8.6)
Neg/pos	9 (2.9)	4 (2.9)
Pos/neg	81 (25.7)	33 (23.7)
Pos/pos	202 (64.1)	89 (64)
Conditioning, N (%)		
MAC	212 (67.3)	98 (70.5)
RIC	103 (32.7)	40 (28.8)
Graft source, N (%)		
BM	13 (4.1)	8 (5.8)
PBSC	302 (95.9)	131 (94.2)
Minnesota risk, N (%)		
HR	46 (14.6)	46 (33.1)
SR	93 (29.5)	93 (66.9)

GvHD: graft-*versus*-host disase; F: female; M: male; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; NHL: non-Hodgkin lymphoma; HL: Hodgkin lymphoma; MDS: myelodysplastic syndromes; MPN myeloproliferative syndromes; CR: complete remission; AD: active disease; DRI: Disease Risk Index; MMRD: mismatched related donor; MRD: matched related donor; MUD: matched unrelated donor; CMV: cytomegalovirus matching; MAC: myeloablative conditioning, RIC: reduced intensity conditioning; PBSC: peripheral blood stem cells; BM: bone marrow; CBU: cord blood unit; HR: high risk; SR: standard risk.

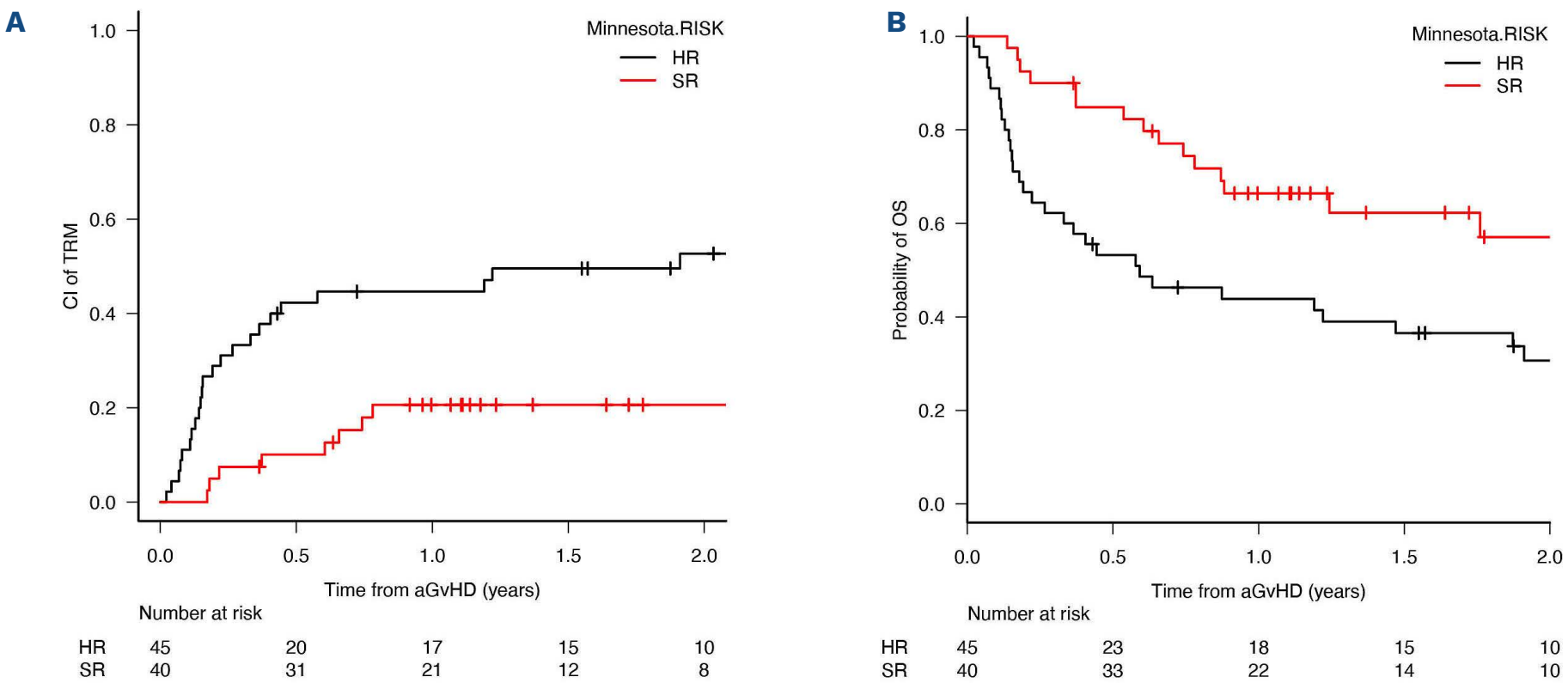

Figure 1. Overall survival and transplant-related mortality analysis. (A) Cumulative incidence of transplant-related mortality and (B) probability of overall survival (OS) according to Minnesota acute graft-*versus*-host disease (aGvHD) risk score stratification (red line standard risk – black line high risk). HR: high risk; SR: standard risk.

18% (95% CI: 3-46) for patients in PR and 75% (95% CI: 48-89) for non-responders (P<0.0001).

Identification of patients at higher risk of steroid refractory aGvHD and consequently of TRM is crucial to optimize treatment and counseling. Minnesota risk score proved to be a reliable and easy-to-use tool for stratification of patients at onset of aGvHD.

In the setting of PTCy GvHD prophylaxis, the Minnesota risk score clearly identifies patients with lower possibility of GvHD overall response by day-28, higher risk of TRM and lower probability of survival. Of note, in our cohort a higher percentage of patients was classified as high risk in comparison with the original Minnesota reports. A possible speculation may be related to the different proportion in stem cell donor source: while in the original reports a consistent proportion of patients received cord blood stem cells and bone marrow, in our experience the majority (94.2%) received peripheral blood stem cells. Not only, in our experience the conditioning was full myeloablative in most patients (70.5% *vs*. 50% new Minnesota cohort and 47% old Minnesota cohort) and age was slightly higher (52.7 years *vs*. 49 years in the new Minnesota cohort and 40 years in the old Minnesota cohort).

Among the risk factors, graft cell composition, patients' age and conditioning regimens are well-recognized key players for aGvHD development.

Major outcomes – OS and TRM – significantly differ according to the day-28 response, pointing out for a dismal prognosis for patients that fail to obtain a complete or partial response. Notably, day-28 overall response rate - with current available treatment - is significantly better in SR aGvHD patients than in HR patients, strengthening the indication to candidate HR patients to clinical trials where possible. Conversely, the Minnesota risk score does not predict the development of cGvHD.

It is interesting to observe that, in our cohort the response rate to first line steroid therapy was higher than in the original Minnesota report. We can only speculate on the possible effect exerted by PTCy in reshaping the immune system through a more tolerogenic cytokines and lymphocytes milieux able to promote a better response to treatment. Of course, further evaluations are warranted to clarify this point.

Today considerable efforts are aimed at identifying biomarkers capable of refining diagnosis, prognosis and predictivity of response to treatment in both aGvHD and cGvHD.[16] Biomarkers certainly can increase both prediction and prognostication on aGvHD. Unfortunately, so far, the use of biomarkers is not available in clinical practice on a large scale and in all centers, thus constituting a limit in the applicability of more refined algorithms. Coupling the Minnesota risk score with the MAGIC algorithm probability (MAP) will provide additional insight in the comprehension of risk signature of GvHD patients, fostering the identification not only of high-risk patients but also of low-risk patients, who will be ideally candidates to de-escalating approaches in the GvHD treatment.

Waiting for a systematic implementation of biomarkers applicability, the Minnesota risk score calculated at the onset of aGvHD is a reliable prognostic score irrespective of the donor source within the frame of PTCy GvHD prophylaxis.

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Contributions

MTLS, FA, RG, FL designed the study and interpreted the data. MTLS, FA and AB collected and assembled the data. FL performed the statistical analysis. MTLS, FA and FL prepared the first draft of the manuscript; and all authors contributed to data interpretation, helped revise the manuscript, and gave final approval of the manuscript.

Data-sharing statement

The datasets generated for this study are available on request to the corresponding author.

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