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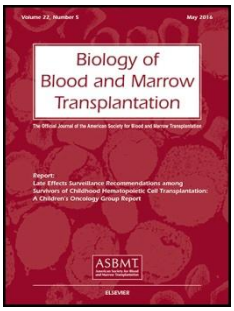

Title: A New Clinico-Biological Scoring System for the Prediction of Infection-Related Mortality and Survival after Allogeneic Hematopoietic Stem Cell Transplantation

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MANUSCRIPT TITLE:

A new clinico-biological scoring system for the prediction of infection-related mortality and survival after allogeneic hematopoietic stem cell transplantation

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Running title: A scoring system predicting IRM after allo-HSCT

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Highlights:

- Infection-related mortality (IRM) is a major challenge after allo-HSCT
- Only pre-transplant variables are challenged in the score
- Age, CMV serostatus and pre-transplant levels of IgA/IgM predict IRM
- This clinico-biological score also predicts overall survival after allo-HSCT
- Pre-transplant IgA/IgM levels can be modulated by immunoglobulins administration

Abstract

Infection-related mortality (IRM) accounts for a substantial component of non-relapse mortality (NRM) after allogeneic hematopoietic stem cell transplantation (allo-HSCT). No scores have been developed to predict IRM before transplant. Pre-transplant clinical and biochemical data were collected in a study cohort of 607 adult patients receiving allo-HSCT from January 2009 to February 2017. In a training set of 273 patients, multivariate analysis revealed that age >60 years (P=0.003), CMV host/donor serostatus different from negative/negative (P<0.001) and pre-transplant levels of IgA <1.11 g/L (P=0.004) and of IgM <0.305 g/L (P=0.028) were independent predictors of increased IRM. On the basis of these results, a 3-tiered weighted prognostic index for IRM was developed and subsequently validated in a retrospective (n=219) and in a prospective (n=115) set of patients. According to the score, patients were assigned to three different IRM risk-classes. The score significantly predicted IRM both in the training and in the retrospective and prospective validation sets (P<0.001, P=0.044 and P=0.011). In the training set, 100-day IRM for low, intermediate and high-risk groups was 5%, 11% and 16%, respectively. In the retrospective validation set it was 7%, 17% and 28% and in the prospective set 0%, 5% and 7%. This score predicted also overall survival (P<0.001, P=0.041 and P=0.023, respectively). As pre-transplant levels of IgA/IgM can be modulated by the supplementation of enriched

immunoglobulins, these results suggest the possibility of prophylactic interventional studies to improve transplant outcomes.

Keywords: prognostic score, infection-related mortality, IgM/IgA levels.

Introduction

Many advances have been made in the field of allogeneic hematopoietic stem cell transplantation (allo-HSCT) over the past 20 years. Reduced-toxicity regimens, advances in donor and graft selection, innovative cellular and pharmacological strategies able to control graft-versus-host disease (GvHD)[1-4] and to speed up immune reconstitution, are at the basis of these successes and have greatly spurred transplant activity. Today allo-HSCT can be virtually offered to every patient in need, without expecting a substantial increase in non-relapse mortality (NRM), particularly in elderly patients or in case of HLA-mismatched donor grafts. Similar outcomes have been recently reported when using HLA-haploidentical donors in comparison with matched unrelated donors (MUD) or even HLA-identical donors[5]. However, despite NRM has been considerably reduced during the last years[6, 7], infection-related mortality (IRM) still remains a major challenge, especially when alternative donors are used. Moreover, the emerging onset of multi-drug resistant pathogens has become a global threat, especially for immunocompromised patients[8,9.] Immune recovery may take months to get established after allo-HSCT, and life-threatening opportunistic infections place patients at risk of early and late IRM, clearly extending beyond 100 days after transplant[10.]

Multiple factors are believed to contribute to the risk of severe infections and to IRM. The duration of post-conditioning absolute neutropenia is considered as one of

the main risk factors. Other factors include older age, comorbidities, disease status, donor type and CMV serostatus, muco-cutaneous damage due to mucositis or to the onset of severe acute or GvHD, CMV reactivations and ensuing treatments[11-16]. Most of these factors occur during the post-transplant period and some of them can contribute simultaneously to the risk of lethal infections.

The aim of the present study was to elaborate a new scoring system based exclusively on pre-transplant clinical and biochemical factors (patient's age and levels of IgA and IgM, patient and donor CMV serostatus) capable of predicting IRM and survival after allo-HSCT. This work represents to our knowledge the first study investigating the role of pre-transplant factors, particularly of IgA and IgM levels, in the prediction of IRM. The proposed scoring system may provide a clinical tool for the infection-risk assessment evaluation in candidate patients to allo-HSCT before transplant.

Methods

Patients

Patients aged 18 years or more, receiving a first allograft for hematological disorders at IRCCS San Raffaele Scientific Hospital from 2009 to February 2017 were considered eligible for the study, while patients undergoing a second transplant during the study period were excluded. A total of 607 patients, for which also pre-transplant biological variables were available, met the inclusion criteria.

First we devised the IRM prognostic score on a training set of patients (n=273) receiving transplant from January 2012 to May 2015, then we validated the scoring system both retrospectively, in patients transplanted from January 2009 to December

2011 (n=219) and prospectively, in patients transplanted from June 2015 to February 2017 (n=115). All patients received an antimicrobial prophylaxis according to Institutional guidelines. For patients developing clinically relevant infectious complications the most appropriate antimicrobial therapy was administered according to physician's judgment and to local policy.

Prognostic factors

Clinical and transplant variables under evaluation included: age (≤ 60 vs >60 years), disease type, disease status at transplant, donor type, source of stem cell harvest, CMV serology of donor and recipient, AB0 blood major incompatibility, intensity of conditioning regimen, use of total body or total marrow irradiation, use of *in vivo* T- or B-cell depletion and the previous history of colonization or infection by multi-drug resistant gram-negative bacteria. In this context, B-cell depletion is defined as the use of an anti-$CD20^{+}$ monoclonal antibody therapy during the conditioning regimen. Pre-transplant biological variables under study included: serum levels of IgG, IgA and IgM, ferritin and free iron (all those variables were collected within 30 days before the start of conditioning chemotherapy) while CRP levels and the absolute neutrophil count (ANC) were evaluated the day before the start of conditioning chemotherapy. This data collection was part of the routine patient's pre-transplant assessment and did not require further blood sampling. Analytical procedures and reference intervals for adults were those reported in the manufacturer's instructions.

Ethical statement

Informed consent for the use of clinical data for scientific purposes was obtained from all patients receiving allo-HSCT. This was a non-interventional, retrospective and prospective, observational cohort study. Data collection and storage was performed according to current Institutional rules for ensuring privacy.

Statistical analysis and definitions

Comparison of numerical variables between groups was performed with the Mann-Whitney test, while Chi-square or Fisher's test were employed for the comparison of categorical variables, as appropriate. Overall survival (OS) and progression-free survival (PFS) were calculated from the day of transplantation to the day of death or relapse, using the Kaplan-Meier method. The log-rank test was applied for comparison among groups. We defined patients as having "early diseases" when receiving allo-HSCT upfront or in first or second complete remission (CR), including also very good partial remission (VGPR); all other patients, in remission beyond second CR (CR2) or with active disease, were considered as having "advanced diseases".

NRM was defined as time from transplant to death without relapse/recurrence. IRM was considered as the time from transplant to death caused by uncontrolled infection. Infections leading to death were diagnosed clinically, with or without a microbiological finding. For patients experiencing infection concomitant to GvHD, we considered infection as the primary cause of death only if GvHD was controlled by treatment, otherwise we considered GvHD as the primary cause of death. Cumulative incidence curves of NRM and IRM were estimated using the competing risk approach (considering as competing event: relapse/progression for NRM and relapse/progression, GvHD and other causes of death for IRM) and Gray's test was performed for comparing them among groups.

The Receiver Operating Characteristics (ROC) curve analysis was used to define the optimal cut-offs of all the biochemical variables for predicting IRM at day 100 after transplant. All biochemical variables categorized by ROC analysis, together with all clinical and transplant variables, were challenged in the multivariate Fine-Gray proportional sub-distribution hazard regression model for predicting IRM.

The final model was obtained with a backward selection procedure. On the basis of the value of the coefficients selected in the final model, a 3-tiered weighted score was developed for the prediction of IRM in the training set, and then tested on the validation sets of patients. Patients were assigned to three risk groups (low, intermediate and high risk) using the first and third quartiles. The goodness-of-fit of the prognostic score was measured with the c-index. Although not included in the analysis, we also evaluated the Disease Risk Index[17] and the HCT-CI Sorror Comorbidity Index > 2[18] to further describe the study population.

P-values less than 0.05 were considered significant. Confidence intervals were reported at level 95%. All statistical analyses were performed using R 3.2.0 (http://www.R-project.org/).

Results

Patient characteristics and outcomes

Patient characteristics of the three cohorts are summarized in **Table 1**. The median follow-up for survivors was 43 months (range, 1 to 85). Acute leukemia was the main indication to transplant, accounting for 60% (n = 356) of patients. The study population was widely heterogeneous and at high risk for severe infections due to the prevalence of patients with older age, diagnosis of acute myeloid leukemia (AML),

advanced diseases, multiple previous lines of chemotherapy. Moreover, 44% of patients received a HLA-haploidentical graft and 37% a fully HLA-matched (10/10) or single mismatch (9/10) unrelated donor (MUD) graft. Forty-seven percent (n = 277) of patients underwent transplant with advanced diseases. Conditioning regimens and GvHD prophylaxis considerably changed during the study period and among the three cohorts due to the non-overlapping transplant years (2009-2017), reflecting the advances in the field of allo-HSCT over that time. However, up to 90% of patients received a treosulfan-based conditioning. Details of conditioning regimens and GvHD prophylaxis are reported in **Table 1**.

To assess homogeneity between the training and the retrospective validation cohorts, which had both a long follow-up, we compared the OS and PFS as well as the incidence of NRM and IRM and all of them were not significantly different (P = 0.050, P= 0.440, P = 0.371 and P = 0.702, respectively). OS at 2 years was 53% (95% CI 47-60%) in the training cohort and 46% (95% CI 40-53%) in the retrospective validation cohort. PFS at 2 years was 23% (95% CI 16-23%) in the training cohort and 17% (95% CI 12-27%) in the retrospective validation cohort. NRM at 2 years was 28% (95% CI 23-34%) in the training cohort and 34% (95% CI 28-40%) in the retrospective validation cohort. In the prospective validation cohort, because of a shorter follow-up period, we were able to calculate only the cumulative incidence of NRM and IRM at day 100. These were 6% (95% CI 3-12%) and 4% (95% CI 1-8%), respectively. OS was 90% (95% CI 84-96%). NRM, IRM and OS at 100 days were 12% (95% CI 12-20%), 13% (95% CI 9-18%) and 81% (95% CI 77-86%), respectively, in the training cohort and 20% (95% CI 15-25%), 15% (95% CI 11-20%) and 77% (95% CI 71-82%) in the retrospective validation cohort.

We registered a total of 130 infection-related deaths, which are detailed in **Table 2** according to pathogen's etiology and clinical manifestation.

Development of the prognostic model

For continuous biochemical variables, we identified the optimal cut-offs predicting IRM at 100 days using ROC analysis (**Table 3**). All clinical and biochemical variables were considered in univariate analysis (**Supplementary Table 1**) and then challenged in a multivariate Fine-Gray proportional sub-distribution hazard regression analysis in order to predict IRM cumulative incidence. Four independent predictors of IRM remained from the model using a backward selection: age >60 years, CMV host/donor serostatus combination other than negative/negative and pre-transplant levels of IgA <1.11 g/L and IgM <0.305 g/L (**Table 4**).

On the basis of the coefficient of the single variables in the model, a weighted score was defined as follows: score = 0.82 (if patient's age was > 60 years) + 0.76 (if pre-transplant IgA levels were < 1.11g/L) + 0.60 (if pre-transplant IgM levels were < 0.305 g/L) + 10.16 (if CMV host/donor serostatus combination was different from negative/negative). A three-tiered prognostic index was then developed. The final score was divided using the first and the third quartiles, defining patients' risk stratification (low-risk: ≤ 10.17 points; intermediate-risk: 10.17-11.11; and high-risk: > 11.11 points).

In the training cohort, a significantly different risk of IRM was documented in the three groups by Gray's test (P = <0.001, **Figure 1A**). Low-risk patients had a 100-day and 2-year IRM of 5% (95% CI 2-10) and of 9% (95% CI 4-16), respectively; intermediate-risk patients had a 100-day and 2-year IRM of 11% (95% CI 5-18) and of

23% (95% CI 14-33), respectively; high-risk patients had a 100-day and 2-year IRM of 16% (95% CI 16-37) and of 41% (95% CI 28-53), respectively. The OS was also significantly different among the three groups (P = 0.001, **Figure 1B**). In particular, 2-year OS was 65% (95% CI 55-77), 51% (95% CI 41-64) and 41% (95% CI 30-56) in patients with low, intermediate and high risk, respectively.

Patients at high-risk according to our algorithm also showed a significantly lower CMV-reactivation free survival compared to low-risk patients: 43% (95% CI 32-59) and 74% (95% CI 66-84) (P <0.001) as shown in **Supplementary Figure 1** and a persistently impaired IgA and IgM immune recovery after transplant (**Supplementary Figure 2**). Noticeably, donor source, disease status at HSCT, conditioning intensity, use of in vivo T or B-cell depletion were not significantly associated with IRM in multivariate analysis.

Validation of the prognostic model

To assess the predictive accuracy of the scoring system, we tested it on a retrospective cohort (n = 219) and in a prospective cohort (n = 115) of patients. The prognostic index achieved a statistically significant association with the incidence of IRM by Gray's test (P = 0.044, **Figure 2A**) with a c-index of 0.608 in the retrospective validation set. Low-risk patients had a 100-day and 2-year IRM of 7% (95% CI 3-14) and of 14% (95% CI 8-22); intermediate-risk patients had a 100-day and 2-year IRM of 17% (95% CI 10-26) and of 23% (95% CI 15-33); high-risk patients had a 100-day and 2-year IRM of 28% (95% CI 15-42) and 33% (95% CI 19-47), respectively.

Overall survival in the retrospective validation cohort was also significantly different according to the three groups (P = 0.041, **Figure 2B**) with a c-index of 0.573.

Particularly, in low-risk, intermediate and high-risk groups of patients the 2-year OS was 54% (95% CI 45-65), 50% (95% CI 40-62) and 31% (95% CI 20-49), respectively. Secondary outcomes such as PFS and NRM were not significantly different among the three groups (P = 0.704 and P = 0.089).

Also in the prospective validation cohort (n=115), the three classes of risk showed a significantly different IRM (P= 0.011, **Figure 2C**) with c-index 0.787 and a significantly different OS (P= 0.023, **Figure 2D**) with c-index 0.667. 100-day IRM was of 0%, 5% (95% CI 0-15) and 7% (95% CI 1-21) for low, intermediate and high-risk classes respectively, with a 100-day OS of 95% (95% CI 80-100%), 90% (95% CI 81-100%) and 69% (95% CI 64-97%) for the three risk classes. Regarding the secondary outcomes PFS and NRM, they were both significantly different among the three risk-groups (P = 0.023 and P = 0.003). The hazard ratios (HR) for OS, NRM and PFS according to the 3-tiered prognostic model in the overall study population (n = 607) are showed in **Table 5**.

Discussion

In the current era of increasing alternative donor transplants, infections still represent a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (allo-HSCT). Impaired immune reconstitution due to the extensive immunosuppression needed to overcome HLA disparity places patients at high risk of life-threatening opportunistic infections, which account for a major part of non-relapse mortality (NRM). Patients' pre-transplant assessment is currently based on the combined evaluation of organ comorbidities and disease risk and stage[18,19].

Although this type of assessments accurately predicts overall survival, to date there are no available scoring systems able to predict infection-related mortality (IRM).

In our study, we present a prognostic scoring tool for IRM prediction in patients undergoing allo-HSCT for hematological diseases. ROC curve analysis was used to determine the optimal cut-offs of biochemical data associating with early IRM. Using multivariate analysis, we subsequently identified pre-transplant levels of IgA and IgM, age and the combination of donor and recipient CMV serostatus as independent factors predicting IRM after allo-HSCT. Our scoring system allowed the identification of three groups of patients showing significant differences in terms of IRM, independently from the type of donor or patient's disease status at transplant.

Age has been widely investigated as predictor of NRM and considered as one of the most important criteria for patient eligibility to transplant. Older age is associated with a decline in immune function, and the consequences of immune senescence include an increased risk of infections, malignancies and autoimmune disorders[20.] However, Sorror et al[21] have recently revised the impact of age in relation to comorbidities on transplant outcomes. In our study, age > 60 years was an independent factor predicting IRM, although single comorbidities or performance status were not challenged in multivariate analysis.

Several studies have shown that CMV seropositive patients[22] or CMV seronegative recipients of a seropositive graft, have a persistent mortality disadvantage, mainly due to NRM, rather than to relapse[23-28]. Indeed, CMV seropositive status contributes significantly to the risk of IRM. Also in our scoring system, the presence of any CMV seropositivity gives the highest contribution (being 10.16 its coefficient in the statistical model) to the IRM risk. In a large survey from the

EBMT, any CMV seropositivity in *de novo* AML receiving allo-HSCT associated with a significantly decreased leukemia-free survival, OS and increased NRM[26], compared to CMV seronegative patients receiving a CMV seronegative donor graft. More recently, these results have been confirmed by a CIBMTR study[27-28], showing that early CMV reactivations remain associated with increased NRM. Moreover, in the setting of alternative donor transplants, a detrimental effect on OS is observed when a CMV seropositive donor is selected for a CMV seronegative patient[29]. Our study confirms that the combination of CMV seronegative recipient with a seronegative donor is independently associated with a reduced IRM. Unfortunately, this favorable CMV-seronegative donor/recipient combination is found only in a minority of cases, especially in regions where CMV is endemic[26-27]. In the context of any CMV seropositivity, our scoring system is able to further stratify patients into low, intermediate or high-risk group for IRM, thanks to the relative contributions of the statistical coefficient of the other variables (age, IgA and IgM levels) for computing the score (**Supplementary Figure 3**). In our study population, mainly represented by alternative-donor transplants, we may argue that donor CMV seropositivity favors CMV reactivations, possibly triggering a severe GvHD. The extensive immunosuppression required for GvHD treatment subsequently places patients at risk of severe infectious complications. Although we acknowledge that GvHD may increase the risk of opportunistic infections, we did not take into account this important co-variable, as our aim was to develop a score based entirely on pre-transplant variables that could be rapidly implemented in the clinical practice.

Immunoglobulins (Ig) clearly play a role in controlling infections[30]. An important finding in this study is that only low pre-transplant levels of IgA and IgM significantly

impact on IRM. Nonetheless, the biological reason remains to be elucidated. We suggest that IgM and IgA levels may influence IRM through a direct and an indirect mechanism. The direct mechanism could rely on pathogen elimination and mucosal protection, and would provide the rationale for administration of high dose intravenous Ig during treatment of severe sepsis[31]; the indirect mechanism could rely on their correlation with GvHD, as low levels of IgA at day 100 post allo-HSCT have been recently demonstrated to be an independent risk factor for the onset of chronic GvHD[32]. In this context, we can assume that patients at higher risk for IRM according to the score have low IgA levels at the gut barrier, that in the presence of mucosal damage, favor microbial translocation and a surge in pro-inflammatory cytokines (TNF-alpha, IL-1, IL-6)[33], thus increasing the risk of bloodstream infections and potentially triggering acute GvHD.

Few studies have investigated the role of administration of IgM and IgA-enriched Ig as pre-transplant prophylaxis. Two randomized studies were conducted in the early '90s. The first study demonstrated the efficacy of prophylactic IgA and IgM-enriched Ig in reducing IRM at day 100[34], while the second study failed to demonstrate a significant reduction of IRM, but showed a clear reduction in infection rates, gut damage and endotoxemia[35]. More recently, a prospective, randomized study in pediatric allo-HSCT compared the use of prophylactic intravenous polyclonal Ig to IgM and IgA-enriched preparations, given before conditioning and until engraftment. In this study, no significant differences were reported between the two strategies[36]. According to these data and to our results, we speculate that the impact of IgM and IgA-enriched Ig prophylactic administration on infection-control might be not relevant when given without considering the endogenous levels of patients' IgM and IgA. We

believe that the beneficial effect of this prophylactic strategy could be evident if a risk-stratification, as proposed by our scoring system, is applied, reserving this option only to high-risk patients.

This single-center study was widely heterogeneous and significant differences could be noticed among conditioning intensity and regimens or type of GvHD prophylaxis due to the non-overlapping transplant years (2009-2017), reflecting the constant improvement of the transplant procedures (i.e. the use of post-transplant cyclophosphamide or use of an *in vivo* T- or B-cell depletion). However, when transplant-related variables where challenged in multivariate analysis in the training set, none of them was independently associated with increased IRM. When challenged in the retro- and prospective validation sets, the newly developed score was equally reliable across the three cohorts, suggesting the universal value of the underlying factors in shaping the risk of IRM.

These results, if validated in external cohorts, seem of particular interest since patients' pre-transplant low levels of IgA and IgM can be modulated by the exogenous administration of IgA/IgM-enriched immunoglobulins preparations in order to decrease the risk of both early and late IRM in intermediate and high-risk patients.

In summary, this new scoring system based on four independent pre-transplant variables is widely applicable, cost-effective and may provide a clinical tool for the prediction of IRM and survival after allo-HSCT. We believe that the pre-transplant assessment of IRM risk could add additional information on patient's eligibility to transplant, thus promoting post-transplant personalized strategies of intensified active surveillance and possibly pre-emptive anti-infective therapies or early vaccination. Our data suggest the possibility of an interventional study for the investigation of

prophylactic administration of IgA/IgM-enriched Ig to decrease IRM and promote survival after allo-HSCT. An Italian multicentric study performed in collaboration with the Gruppo Italiano Trapianto Midollo Osseo (GITMO) is currently on the way for the external validation of these results.

Conflict of interest: The authors declare that they have no competing interests**.**

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Author contribution

Conception and design: AF, PR, RG, MC, JP, MB, AB and FC designed the research and revised the paper. Provision of study material or patients: all authors. Collection and assembly of data: AF, VM, PR, RG, MTLS, MM. Data analysis and interpretation: all authors. Manuscript writing: all authors. Final approval of the manuscript: all authors.

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Figure Legends

Figure 1. Estimated cumulative incidence IRM and probability of OS according to the prognostic score in the Training Set (n=273). (A) 100-day and 2-yrs IRM: 5% (95% CI 2-10) and 9% (95% CI 4-16) for low-risk, 11% (95% CI 5-18) and 23% (95% CI 14-33) for intermediate-risk and 16% (95% CI 16-37) and 41% (95% CI 28-53) for high-risk patients (P=0.001). **(B)** 2-yrs OS**:** 65% (95% CI 55-77) for low-risk, 51% (95% CI 41-64) for intermediate-risk and 41% (95% CI 30-56) for high-risk patients (P= 0.001)

Figure 2: **Estimated cumulative incidence IRM and probability of OS according to the prognostic score in the Validation Sets. (A)** 100-day and 2-yrs IRM in the retrospective set (n=219): 7% (95% CI 3-14) and 14% (95% CI 8-22) for low-risk, 17% (95% CI 10-26) and 23% (95% CI 15-33) for intermediate, and 28% (95% CI 15-42) and 33% (95% CI 19-4) for high-risk patients (P= 0.044). **(B)** 2-yrs OS in the retrospective set (n=219): 54% (95% CI 45-65) for low-risk, 50% (95% CI 40-62) for intermediate-risk and 31% (95% CI 20-49) for high-risk patients (P= 0.041). **(C)** 100-day IRM in the prospective set (n=115); 0% for low-risk, 5% (95% CI 0-15) for intermediate-risk and 7% (95% CI 1-21) for high-risk patients (P= 0.011) **(D)** 100-day OS in the prospective set (n=115): 95% (95% CI 89-100) for low-risk, 90% (95% CI 81-100) for intermediate-risk and 79% (95% CI 64-97) for high-risk patients (P= 0.023)

Table 1. Characteristics of patients at allo-HSCT (n=607)

Parameters	Training set (n = 273)	Retrospective Validation set (n = 219)	P-value	Prospective Validation set (n = 115)	P-value
Age, years			0.017		0.544
Median, range	53 (18-78)	48 (18-76)		50 (19-77)	
Sex			0.041		0.911
F	111 (41%)	70 (32%)		45 (39%)	
M	162 (59%)	149 (68%)		70 (61%)	
Diagnosis			0.926		0.573
Acute leukemia	165 (60%)	134 (61%)		66 (57%)	
Others	108 (40%)	85 (39%)		49 (43%)	
Disease status at allo-HSCT					
Early	140 (51%)	112 (51%)	1.000	79 (69%)	0.001
Advanced	133 (49%)	107 (49%)		36 (31 %)	
Sorror HCT-CI at allo-HSCT			0.519		0.053
Median, range	2 (0-9)	2 (0-7)		2 (0-7)	
Disease Risk Index					
Low - Intermediate	161 (59%)	131 (60%)	0.092	59 (51%)	0.391
High	93 (34%)	62 (28%)		43 (37%)	
Very High	13 (5%)	20 (9%)		8 (7%)	
Missing	6 (2%)	6 (3%)		5 (4%)	
CMV host/donor pairs			0.737		0.534
-/-	23 (8%)	16 (7%)		7 (6%)	
Others	250 (92%)	203 (93%)		108 (94%)	
Donor type			0.336		0.080
Sibling	51 (19%)	40 (18%)		25 (22%)	
HLA-haploidentical	133 (49%)	120 (55%)		42 (37%)	
MUD and CBU	89 (32%)	59 (27%)		48 (42%)	
Conditioning intensity			<0.001		0.001
MAC	142 (52%)	46 (21%)		81 (70%)	
RIC	131 (48%)	173 (79%)		34 (30%)	
Conditioning regimen			0.001		0.023
Treosulfan-based[§]	240 (88%)	207 (95%)		111 (97%)	
Busulfan-based[*]	30 (11%)	7 (3%)		4 (3%)	
Others	3 (1%)	5 (2%)		0 (%)	
GvHD prophylaxis			0.198		<0.001
Siro-based (±PTCy)	190 (70%)	147 (67%)		103 (90%)	
CSA-based	79 (29%)	63 (29%)		10 (8%)	
Others	4 (1%)	9 (4%)		2 (2%)	

In vivo T-cell depletion	149 (55%)	171 (78%)	<0.001	10 (9%)	<0.001
In vivo B-cell depletion	110 (40%)	180 (82%)	<0.001	4 (3%)	<0.001

Abbreviations: MUD= matched unrelated donor; CBU= cord blood unit transplant; MAC= myeloablative conditioning; RIC= reduced-intensity conditioning; PTCy= post-transplant Cyclophosphamide; Siro= sirolimus; CSA= cyclosporine; §Treosulfan- Fludarabine ± Melphalan ± Thiotepa; Treosulfan-Fludarabine ± TBI 4Gy; Treosulfan-Clofarabine; *Busulfan-Fludarabine ± Thiothepa.

Table 2. Causes of infectious deaths according to pathogen and clinical presentation

Bacterial infections	
Bacteremia †	54 (41%)
Gram-negative bacteria	
Klebsiella pneumoniae	8
Other enterobacteria	1
Escherichia coli	4
Pseudomonas aeruginosa	6
Stenotrophomonas maltophilia	3
Gram-positive bacteria	
Enterococcus spp.	7
Other GP bacteria	5
No isolate	13
n.a.	7
Pneumonia ‡	35 (27%)
Gram-negative bacteria	
Escherichia coli	2
Pseudomonas aeruginosa	2
Stenotrophomonas maltophilia	2
Gram-positive bacteria	
Enterococcus spp.	2
Other GP bacteria	4
No isolate	10
n.a.	13
Invasive fungal infections	14 (11%)
Candida, non albicans	2
Invasive pulmonary aspergillosis	9
Other IFIs ¶	3
Viral infections	23 (18%)
CMV §	7
HSV6-encephalitis	3
EBV lymphoproliferative disease	1
HSV1- encephalitis	2
Respiratory viruses #	8
BK virus – hemorrhagic cystitis	2
Toxoplasmosis (CNS)	4 (3%)

Abbreviations: GP= Gram-positive; n.a.= data not available; CMV= Cytomegalovirus; HSV6 = Human herpes virus 6; EBV= Epstein-Barr virus; HSV1 = Human herpes virus 1.

† Other enterobacteria: *Citrobacter freundii* (1). Other GP bacteria: Clostridium difficile (1), *Corynebacterium jeikeium* (1), *Micrococcus luteus* (1), *Staphylococcus spp, non-MRSA* (2).

‡ Other GP bacteria: Actinomicosis, species not identified (1), *Corynebacterium jeikeium* (1), *Staphylococcus spp, non-MRSA* (2).

¶ Mucormycosis (1), *Fusarium solani* (1), P*neumocystis carinii* (1).

§ CMV-pneumomia (6), CMV colitis (1).
# Influenza A, H1N1 (4), Adenovirus (4).

Table 3. ROC Curve and Logistic Regression Analysis of Biochemical Variables for the Prediction of IRM at Day 100 in the Training Cohort.

Variable	Optimal Cut-off	Specificity	Sensibility	P-value
ANC (cell/μL)	**1050**	**0.654**	**0.556**	**0.019**
IgG (g/L)	**7.475**	**0.577**	**0.576**	**0.105**
IgA (g/L)	**1.11**	**0.631**	**0.606**	**0.012**
IgM (g/L)	**0.305**	**0.789**	**0.469**	**0.003**
Serum iron (μg/dL)	**106.5**	**0.543**	**0.690**	**0.023**
Ferritin (ng/mL)	**1473.5**	**0.613**	**0.571**	**0.044**
CRP (mg/L)	**17.5**	**0.658**	**0.528**	**0.036**

Abbreviations: ROC= Receiver Operating Characteristics; IRM= infection-related mortality; ANC= absolute neutrophil count; CRP= C-reactive protein

Table 4. Multivariable Analysis for IRM in the Training Cohort

Variable	Coef.	HR for IRM (95% CI)	P-value
Age, years			0.002
≥60 vs <60	0.82	2.28 (1.34-3.89)	
CMV host/donor serostatus			
neg/neg vs others	10.16	25800 (13919-48000)	<0.001
Pre-transplant IgA level			
< 1.11 g/L	0.76	2.14 (1.27-3.61)	0.004
Pre-transplant IgM level			
< 0.305 g/L	0.60	1.82 (1.07-3.11)	0.028

Abbreviations: HR= hazard ratio; IRM= infection-related mortality; CMV= Cytomegalovirus

Table 5. OS, NRM and PFS according to the prognostic model in the Combined Training and Validation Cohorts

		OS		**NRM**		**PFS**	
Risk Score	No.	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*	HR (95% CI)	*P-value*
Low (≤10.17)	196	1		1		1	
Intermediate (10.17-11.11)	175	1.42 (1.06-1.90)	0.018	1.46 (0.99-2.14)	0.056	1.00 (0.69-1.44)	0.994
High (>11.11)	108	2.12 (1.55-2.90)	<0.001	1.37 (1.21-1.56)	<0.001	1.53 (1.06-2.20)	0.021
Overall *P-value*			<0.001*		<0.001§		0.027*

Abbreviations: OS= overall survival; NRM= transplant-related mortality; PFS= progression-free survival; HR= hazard ratio.
*Log-rank test; §Gray's test