Tuesday, March 28th

09:00-10:30

Oral 8: GVHD (Clinical I)

**Significant association of IgG glycan structures with intensity of immunosuppression among chronic graft-versus-host disease patients: results of the NIH cohort study**

Prenc, Ema 1; Pulanic, Drazen 2; Curtis, Lauren 3; Pucic Bakovic, Maja 4; Ugrina, Ivo 4; Desnica, Lana 5; Pirsl, Filip 3; Mitchell, Sandra 6; Hakim, Fran 5; Vrhovac, Radovan 7; Nemet, Damir 7; Gress, Ronald 4; Lauc, Gordan 4; Pavletic, Steven Z. 3

1University of Zagreb School of Medicine, Zagreb, Croatia, 2University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia, and Faculty of Medicine J.J. Strossmayer University of Osijek, Osijek, Croatia, 3Experimental Transplantation and Immunology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, 4Genos Ltd., Zagreb, Croatia, 5University Hospital Center Zagreb, Zagreb, Croatia, 6Outcomes Research Branch, Division of Cancer Control and Population Sciences, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA, 7University Hospital Center Zagreb and University of Zagreb School of Medicine, Zagreb, Croatia

**Abstract**

Introduction Chronic graft-versus-host disease (cGVHD) is a systemic allo-/auto- immune disorder and a major late complication after allogeneic hematopoietic stem cell transplantation (alloHSCT). The disease is characterized by an altered homeostasis of the humoral immune response and the production of allo-/auto-antibodies. Changes in glycosylation of immunoglobulin G (IgG), the main effector molecule of the humoral immune system, are associated with a number of autoimmune and hematological diseases. Patients and Methods Plasma samples and clinical data were collected from patients enrolled in a cross-sectional natural history cGVHD study at the National Cancer Institute, National Institutes of Health, USA (NCT00092235) from 2004 to 2014. cGVHD was diagnosed according to the NIH Consensus criteria. IgG was isolated, deglycosylated and analyzed by hydrophilic interaction chromatography–ultra-performance liquid chromatography. Glycan chromatographic peaks (24) were directly measured and additional derived traits (57), representing composite traits such as total galactosylation, were calculated. Associations were tested using Wilcoxon test. Upon correction for multiple testing via false discovery rate results with p-values below 0.05 were considered significant. Results IgG glycome composition was analyzed in 242 cGVHD patients (43% female; median age 45 years [range 5-71]). A majority had received myeloablative conditioning (56%), had unrelated donors (58%), peripheral blood hematopoietic stem cells (79%), and history of acute GVHD (67%). cGVHD was characterized as de novo in 33%, progressive in 37% and quiescent in 30%, and classified as classic in 85%. Most of the patients had severe (75%) or moderate (23%) global NIH cGVHD score. cGVHD was considered to be active by clinical assessment at the time of evaluation in 50% of patients. Intensity of immunosuppression was classified as none in 19%, mild (prednisone alone <0.5 mg/kg/day) in 8%, moderate (prednisone alone ≥0.5 mg/kg/day, and/or any other single agent or modality) in 34%, and high (two or more agents or modalities (≥prednisone ≥0.5 mg/kg/day) in 39% of patients (Bone Marrow Transplant. 2010;45:762-9.). In this preliminary analysis results revealed a significant association of IgG glycans with different intensities of immunosuppression: none vs moderate: GP1 (p=0.002) and GP3 (p=0.027); and none vs high: GP1 (p=0.002), GP3 (p=0.006), GP18 (p=0.018), GP23 (p=0.004), G2 (p=0.044), IGP26 (p=0.027), IGP57 (p=0.037) and IGP69 (p=0.037). Comparisons none vs mild immunosuppression did not show significant associations with glycans. Elevated levels of agalactosylated structures (GP1 and GP3) and those with bisecting N-acetylglucosamine (GP3, IGP69) with pro-inflammatory functions were associated with increased level of immunosuppression. Sialylated (GP18, GP23, IGP26) and galactosylated (G2, IGP57) IgG glycans structures with anti-inflammatory properties (Ann NY Acad Sci.2012;1253:170-80.) showed the opposite trend; their levels reduce with increased
immunosuppression (Image 1). Conclusion There is a need for qualified biomarkers in cGVHD. Our preliminary study shows an association of IgG glycan structures with different intensities of immunosuppression in cGVHD patients. To clarify if such association is marker of disease activity, or consequence of treatment, or both, will require further continued study, preferentially in longitudinal post-transplant cohorts.

Disclosure of conflict of interest

No conflict of interest to disclose.
A Biomarker Algorithm Predicts Non-Relapse Mortality Before GVHD, at Diagnosis, and During Treatment

Levine, John 1; Ozbek, Umut 1; Renteria, Anne 1; Hartwell, Matthew 1; Major-Monfried, Hannah 1; Holler, Ernst 2; Pawarode, Attaphol 3; Aziz, Mina 1; Hogan, William 5; Ayuk, Francis 5; Efebera, Yvonne 6; Qayed, Muna 7; Hexner, Elizabeth 8; Wudhikorn, Kitsada 9; Wolf, Matthias 10; Ordemann, Rainer 11; Mielle, Stefan 10; Bunworasate, Udomsak 9; Devine, Steven 6; Kroeger, Nicolaus 5; Al-Malki, Monzr 12; Chen, Yi-Bin 13; Harris, Andrew 14; Jagasia, Madan 15; Kitko, Carrie 15; Litzow, Mark 4; Locatelli, Franco 16; Nakamura, Ryotaro 12; Reddy, Pavan 1; Reshef, Ran 17; Roesler, Wolf 19; Weber, Daniela 2; Yanik, Gregory 3; Ferrara, James 1

1Mount Sinai School of Medicine, 2University of Regensburg, 3University of Michigan, 4Mayo Clinic, 5University Medical Center, Hamburg-Eppendorf, 6Ohio State University, 7Emory University and Children’s Healthcare of Atlanta, 8University of Pennsylvania, 9Chulalongkorn University, 10University of Wurzburg, 11University Hospital TU Dresden, 12City of Hope Medical Center, 13Massachusetts General Hospital, 14University of Utah, 15Vanderbilt University, 16Ospedale Pediatrico Bambino Gesù, 17Columbia University, 18University Hospital Erlangen-Nuremberg

Abstract

BACKGROUND: The graft-versus-host disease (GVHD) reaction is often underway by day +7 after HCT although symptoms have not yet developed. We sought to identify a blood biomarker signature that could predict lethal GVHD and 6-month NRM at three separate times: day +7, at GVHD onset, and after one week of steroid treatment. PATIENTS AND METHODS: Patients (pts) from 11 centers in the Mount Sinai Acute GVHD International Consortium (MAGIC) provided samples on day 7 after HCT (n=1287), at GVHD onset (n=212), and after one week of steroid treatment for GVHD (n=378). Some pts provided samples for only one of the time points. First, we randomly selected day +7 samples from the two largest centers (n=929) to create a training set of 620 pts to develop the algorithm. We tested all 15 possible combinations of four biomarkers [ST2, REG3α, TNFR1, and IL2RA] to develop competing risk regression models to predict 6-month NRM. The best algorithm included ST2 and REG3α, which was superior to all other combinations of biomarkers. The algorithm was then validated in independent pt cohorts at day +7, at GVHD onset, and after one week of steroid treatment for GVHD. RESULTS: 6-month NRM in the training, test and validation sets were 11%, 12% and 13% respectively and lethal GVHD accounted for the majority of NRM. The algorithm identified high risk (HR) and low risk (LR) groups with 6-month NRM of 28% and 7%, respectively (p<0.001). Similar results were obtained for a test set of 309 pts from the same 2 centers and for a multicenter validation set of 358 pts from 9 centers with similar NRM rates and highly significant differences in overall survival (Fig 1A). HR pts were 3 times more likely to die from GVHD than LR pts in each cohort (p<0.001); the majority of deaths were from steroid-refractory GI GVHD. When applied to 212 samples at GVHD onset, the same algorithm separated pts into 3 distinct risk strata [Ann Arbor (AA) scores 1, 2 and 3] regarding response to treatment and NRM, similar to our previous report; 45% of pts were assigned to the most favorable risk strata (Fig 1B). We divided samples obtained from 378 pts treated for GVHD with steroids for one week into a test (n=236) and validation cohort (n=142). Unsupervised K-medoid clustering divided the test set into HR and LR groups with significantly worse 6-month NRM for the 39% of pts who were HR (50% vs 12%, p<0.0001). Approximately half of the pts in each cohort (test: 48%; validation: 44%) responded (CR+PR) to the first week of steroids, and these pts had significantly lower 6-month NRM than non-responders (NR) (test: 17% vs 36%, p=0.0002; validation: 13% vs 36%, p=0.0014). The algorithm stratified risk independently of early clinical response. In the test cohort of early responders, HR pts (28% of pts) experienced four-fold more NRM than LR pts (36% vs 8%, p<0.0001). Conversely, non-responders who were LR (almost half of the pts) had much lower NRM than non-responders who were HR (17% vs 57%, p<0.0001). These highly significant differences were also observed in the independent validation cohort (Fig 1C). IMAGE: See Figure 1. CONCLUSIONS: An algorithm that predicts NRM on day +7 post HCT was validated in two independent pt sets, at GVHD onset and after one week of steroid treatment. This algorithm can be used serially after HCT to monitor risk and may prove useful to guide risk-adapted therapy.
References

Levine *Lancet Haem* 2015

**Disclosure of conflict of interest**

Drs. Levine and Ferrara are co-inventors of a GVHD biomarker patent.
Chronic graft-versus-host-disease and B-cell reconstitution after hematopoietic stem cell transplantation in children

Verena Wiegering¹, Kirsten Haas², Marieke Frietsch¹, Matthias Wölfli¹, Paul G. Schlegel¹ and Matthias Eyrich¹

¹University Children’s Hospital, Department of stem cell transplantation, University Medical Center Wuerzburg, ²Institute for clinical epidemiology and biometry, University of Wuerzburg, Wuerzburg, Germany

Abstract

Background: Hematopoietic stem cell transplantation (HSCT) is an established therapy for many pediatric hematological diseases. Chronic GvHD (cGvHD) is the most important determinant of posttransplant morbidity and mortality. Convincing evidence suggests an important contribution of B-cells in cGvHD pathophysiology. Notably, data on B-cell reconstitution and cGvHD in children, who generally show different kinetics of immune reconstitution are lacking so far. Methods: First, in a retrospective cohort of 104 pediatric alloHSCT recipients, transplanted between 2005 and 2013, we analyzed 2151 flow cytometric immune profiles. To identify differences in lymphocyte distribution in patients with and without GvHD over time we applied hierarchial linear analysis. Second, in a prospective cohort of 74 children, we investigated more closely the distribution of B-cell subsets, serum cytokine levels including BAFF, autoantibody production and apoptosis resistance of peripheral B-cells. Results: In the first retrospective cohort, median age was 8.9±7.0 years. Incidence of cGvHD was 15%, median time to onset was 132±198days. In a univariate analysis, risk factors for cGvHD were: a history of previous aGvHD, radiation-based conditioning and a donor-host sex mismatch. As expected, relapse rate was significantly lower in cGvHD patients. Hierarchial linear analysis showed that children later experiencing cGvHD had elevated T-cell frequencies during the first 180 days (63±17 vs. 45±23% CD3+ in patients with vs. without cGvHD, resp.). In contrast, B-cell frequencies were significantly lower in cGvHD patients before the onset of the disease, but elevated during and after cessation of GvHD symptoms. Interestingly, reconstitution of naïve T-cells was delayed but not abrogated in children with cGvHD, reaching a mean of 44±17% of naïve T-cells in the CD4+ compartment after 2 years postHSCT (compared to 58±15% in children without GvHD). A more detailed analysis of B-cell subsets in the prospective cohort revealed that the lower number of B-cells in cGvHD children were due to a defect in naïve B-cell regeneration. Memory and CD24++CD38++ transitional B cells were expanded. We detected autoantibodies (mainly ANAs) in 88% of cGvHD children, compared to 13% in children without cGvHD. In approx. 50% of cGvHD children these autoantibodies became negative after 2 years postHSCT. Apoptosis resistance is a mechanism also operational in pediatric cGvHD, as B-cells from cGvHD children showed reduced rates of apoptosis after 48h in culture, a phenomenon that could be induced by exogenous BAFF in a dose dependent way. In contrast to what has been reported in adults, serum BAFF levels were not elevated continuously during cGvHD, but progressively declined over time. A detailed analysis of bone marrow B-cell precursor subsets has been performed, which is currently under biometrical evaluation. Summary: Our data represent the first large and comprehensive data set on B-cell reconstitution and cGvHD in a purely pediatric cohort. We confirm patterns of B-cell dysbalance which have been described in adult cGvHD patients before. However, other features such as the preserved thymic reconstitution despite cGvHD and the rapid decline of BAFF and autoantibodies are in contrast to previous reports. Thus, the higher regenerative capacity of children seems to have a significant impact on the disturbed B-cell homeostasis in cGvHD.

Disclosure of conflict of interest

None
Extended MHC haplotype disparity level is more relevant then the level of HLA mismatch alone for the patients survival and graft versus host disease in T cell-replate HSCT from unrelated donor

Nowak, Jacek; Nestorowicz, Klaudia \(^1\); Graczyk-Pol, Elzbieta; Mika-Witkowska, Renata \(^1\); Rogatko-Koros, Marta \(^1\); Halaburda, Kazimierz; Tomaszewska, Agnieszka \(^2\); Nasilowska-Adamska, Barbara; Szczepinski, Andrzej \(^2\); Dziopa, Joanna \(^1\); Szlendak, Urszula \(^1\); Witkowska, Agnieszka \(^1\); Gwozdowicz, Sławomir \(^1\)

\(^1\)Department of Immunogenetics, Institute of Hematology and Transfusion Medicine, Warsaw, Poland, \(^2\)Department of Hematopoietic Stem Cell Transplantation, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

Abstract

Introduction Life-threatening risks in unrelated hematopoietic stem cell transplantation (HSCT) including graft versus host disease (GvHD) and mortality are associated with HLA disparity between donor and recipient. \(^1\) The increased risks might be dependent on disparity in not-routinely-tested multiple polymorphisms in genetically dense MHC region, being organized in two extended MHC haplotypes (Ehp). For modeling we considered that in HLA mismatched donor-recipient pairs increased frequencies of SNP disparities in MHC regions adjacent to mismatched HLA loci were discovered, \(^2\) and extremely strong linkage disequilibrium across MHC is evolutionary selected. \(^3\) Patients and Methods Patients (N=889) with myeloproliferative, lymphoproliferative or non-malignant diseases received T cell-replate HSCT from 2001 to 2012. We assessed the clinical role of Ehp disparity levels in donor-recipient pairs by the in silico detection of HLA allele phase mismatch using PHASE 2.1, \(^4\) the algorithm availied for population-based prediction of donor-recipient parity and haplotype phasing. In the final study model we compared cumulative incidences of acute (a)GvHD, chronic (c)GvHD, overall survival (OS) and non-relapse mortality (NRM) in patients given HSCT from unrelated donor with 1 or 2 Ehp mismatch, in strata of unique level of HLA mismatch. In all comparisons the group with matched HLA (N=607) was set as reference. Co-ovariate adjustments were made in multivariate stepwise Cox proportional hazard analysis with backward elimination for those independent variables correlated with outcome measures in univariate models (p<0.1). Results We found highly significant increment of 100-day aGvHD (58% vs. 41%) and 5-year cGvHD (62% vs. 37%) with increasing Ehp mismatch level, even with the same level of 1 (out of 10) HLA mismatch (Figure 1A and B, blue lines). Likewise, significantly increased aGvHD (66% vs. 41%) and cGvHD (65% vs. 49%) has been found with increasing Ehp mismatch level when donor-recipient pairs were mismatched in 2-3/10 HLA (Figure 1A and B, red lines). In adjusted multivariate models Ehp disparity level remained independent prognostic factor for aGvHD (p = 0.00000018, HR=2.70, CI95% 1.75-4.14), cGvHD (p = 0.00000085, HR=3.28, CI95% 2.04-5.25), and extended cGvHD (p = 0.000024, HR=5.38, CI95% 2.77-10.44) and HLA mismatch level alone has been excluded from these models. In group restricted to single HLA mismatch and low risk phase at transplantation patients with double Ehp disparity had worse 5-year overall survival (45% vs. 56%, p = 0.014, HR=2.14, CI95% 1.13-4.05) and non-relapse mortality (40% vs. 31%, p = 0.017, HR=3.91, CI95% 1.70-8.97, Figure 1C and D), as compared to patients with single Ehp disparity.

Figure 1. Kaplan-Meyer estimates of cumulative incidence frequency in clinical outcomes of unrelated hematopoietic stem cell transplantation depending on HLA and extended MHC haplotype disparity level.

Conclusions We conclude that routinely available population based haplotype phase modeling is relevant for HSCT biology. HLA-linked factors contribute to the high morbidity and mortality in recipients given HLA-mismatched unrelated hematopoietic stem cell transplant and Ehp matching should be considered in clinical HSCT from HLA mismatched donor.

References


Disclosure of conflict of interest

The authors declare no competing financial interests.
Reg3 alpha serum levels in the course of allogeneic SCT — Synergistic impact of dysbiosis and mucosal damage

Ernst Holler1, Sakhila Ghimire1, Thomas Hehlgans2, Peter Oefner3, Katja Dettmer3, Katrin Peter1, Daniel Wolff1, Joachim Hahn1, Wolfgang Herr1, Andre Gessner4, Daniela Weber1

1Dept of Internal Medicine III, University Medical Center, 93042 Regensburg; 2Institute of Immunology, University Medical Center, 93042 Regensburg; 3Institute of functional Genomics, University of Regensburg, 93042 Regensburg; 4Institute of Microbiology and Hygiene, University of Regensburg, 93042 Regensburg

Abstract

Reg3alpha (Reg3a) serum levels have been reported as biomarkers of intestinal GvHD and correlated with Paneth cell damage and poor prognosis. To further reveal the mechanisms of early Reg3alpha release in the course of allogeneic SCT, we analyzed consecutive Reg3a serum levels at admission, d0-7,14-21 and 28-42 in 75 patients (pts) receiving allogeneic BMT. Serum levels were correlated with occurrence of severe GvHD, mucositis stage2-4, use of broad spectrum antibiotics (Abs) at the time of collection and presence of microbiota damage as indicated by urinary indoxylsulfate levels (UIS). For pretransplant Reg3a levels, only concomitant use of ABs was associated with significantly increased Reg3a levels. In posttransplant samples (n=187), presence of severe mucositis, use of Abs and occurrence of GvHD contributed stepwise to Reg3a release. Pts without Abs and without mucositis and GvHD had continuously low Reg3a levels with a mean of 36 (+/-4) pg/ml, pts with either Abs or mucositis mean levels of 84 (+/-9), pts with Abs and either Mucositis or GvHD 244 (+/- 38) (p <.001between each cohort). The highest levels were observed in pts on Abs with mucositis progressing to GvHD (429 (+/-128) pg/ml. An impact of microbiota disruption was suggested by significant upregulation of Reg3alpha in pts with UIS levels below median (196 +/- 41 pg/ml) vs 42 +/- 9 pg/ml in pts with high UIS levels ( p 0.009). Multivariate logistic regression revealed, that all factors, severity of mucositis and of GvHD as well as use of Abs independently contributed to increased Reg3a levels. Our data indicate a more complex regulation of Reg3a, which seems to be activated in relation to microbiota disruption and released as a consequence of severe mucosal and Paneth cell damage. Further studies are needed to evaluate the exact causal relation and the initiating role of microbiota disruption.

Figure 1: Mean Reg3a levels in different risk groups: 0 = no Abs, no mucositis, no severe GvHD; 1 = either ABs or mucositis, 2 = ABs with either GvHD or mucositis, 3 ABS with GvHD and mucositis. P < 0.000 between each group for 0,1,2; p 0.04 for group 2 vs 3.
Disclosure of conflict of interest

No conflicts of interest
RECIPIENT rs17281995 (CD86) AND rs2069727 (IFNg) SINGLE NUCLEOTIDE POLYMORPHISMS ARE ASSOCIATED WITH A LOWER RISK OF GRAFT VERSUS HOST DISEASE IN PATIENTS RECEIVING AN ALLOGENEIC STEM-CELL TRANSPLANT FROM A RELATED DONOR AFTER A REDUCED INTENSITY CONDITIONING REGIMEN: A MULTICENTER EXPERIENCE

Ferré, Oscar 1; García-Alvarez, María 1; López-Godino, Oriana; Rodríguez-Arboli, Eduardo 2; Esquirol, Albert 3; García-Guerrero, Estefanía 2; Castilla, Cristina 1; Martino, Rodrigo 1; Corchete, Luis 1; Marín, Luis 1; Heras, Inmaculada 2; Pérez-Simón, José Antonio 2; Pérez-López, Estefanía; Cabrero, Mónica 1; Alonso, Sara 1; Martín, Ana A. 1; Balanzategui, Ana 1; García-Sanz, Ramón 1; González, Marcos 1; López-Corral, Lucía; Caballero, M. Dolores 1; Alcoceba, Miguel 1

1Hematology Department, Hospital Universitario de Salamanca (CAUSA-IBSAL), Salamanca, 2Hematology Department, Hospital Universitario Virgen del Rocío (IBIS), Sevilla, 3Hematology Department, Hospital Santa Creu i Sant Pau, Barcelona, 4Hematology Department, Hospital General Universitario Morales Meseguer, Murcia

Abstract

INTRODUCTION: Graft versus host disease (GVHD) is the main cause of morbidity and non-relapse mortality after allogeneic hematopoietic stem cell transplantation (allo-SCT), even in HLA-identical sibling donors. Many efforts have been made trying to identify particular single nucleotide polymorphisms (SNPs) to elucidate the risk of acute GVHD (aGVHD) before transplant to personalize the procedure and improve results. However, there is little information related to allo-SCT with reduced intensity conditioning (RIC). MATERIALS AND METHODS: We included paired recipient-donor samples from 274 RIC allo-SCT patients diagnosed of AML (n=88), NHL (n=54), MDS (n=52), MM (n=28), CLL (n=25), HL (n=15), or others (n=12) in four centers in Spain. Flu-Bu conditioning was used in 56% of the transplants while Flu-Mel in 43%. Clinical characteristics were not significantly different between centers. We analyzed 52 SNP from 44 different gene loci, selected from those previously described related to GVHD in allo-SCT, known to be involved in the immune response and/or autoimmune diseases, or those with a potential role in donor/recipient interaction. SNP Genotyping was carried out by Sequenom Mass ARRAY platform (Sequenom, San Diego, CA). The analyses were carried out taking into account the receptor, donor and both profiles. Allele frequencies were estimated by direct counting and compared between groups using Fisher’s test. P-value <0.05 was considered to be statistically significant. Log-rank analysis was used to compare differences between survival curves. RESULTS: Patients’ characteristics are shown in Table 1. Cumulative incidence of aGVHD at day 200 was 56%, grades II-IV in 47% and III-IV in 18% in our series, while cGVHD at 4 years was 63%. With a median follow up for alive patients of 44 months, overall survival was 52.1%, while event free survival at 4 years was 43%. The main cause of death was disease relapse/progression (18.1%). Transplant related mortality (TRM) was 21%, and GVHD related mortality was 11.6%. In the univariate analysis, patients with genotype C/G in rs17281995 (CD86) showed a lower aGVHD (39% vs. 60%, p=0.01), and aGVHD grades II-IV (31% vs. 51%, p=0.007). Genotype T/T in rs2069727 (IFNg) in recipients was associated with lower aGVHD grade (III-IV 5% vs. 22%, p=0.008), and lower TRM (13% vs. 25%, p=0.036). Other variable influencing aGVHD grades II-IV was Flu-Mel conditioning (54% vs. 40%, 0.028). In the multivariate analysis, rs17281995 (CD86) C/G genotype (HR 0.4, 95% CI: 0.22-0.75), Flu-Mel conditioning (HR 1.7, 95% CI: 1.1-2.7), rs2069727 (IFNg) T/T genotype (HR 0.6, 95% CI: 0.35-0.99) and Tacro+Rapa GVHD prophylaxis (HR 1.7, 95% CI: 1.1-2.7), significantly associated to aGVHD grades II-IV. The rs2069727 (IFNg) T/T genotype (HR 0.31, 95% CI: 0.09-1.0), and the rs17281995 (CD86) C/G genotype (HR 0.32, 95% CI: 0.09-1.1) were the only variables showing a trend to be associated to aGVHD grades III-IV. There was no relation between rs17281995 (CD86) and rs2069727 (IFNg) polymorphisms and incidence/severity of cGVHD or OS. The rs17281995 (CD86) and rs2069727 (IFNg) donor genotypes did not influence allo-SCT outcome. CONCLUSION: The present data suggest an association between rs1781995 (CD86) and rs2069727 (IFNg) polymorphisms with incidence and severity of aGVHD. Confirmation in larger series is required. FUNDINGS: PI12/02361, RTICC (RD12/0036 grupos 0052, 0069 y 0071), BIO/SA60/13 e Innocampus (CEI-2010- 1-0010)
Table 1. Patients basic characteristics and comparison between centers

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Graphic 1. Left, IFNG rs2069727 aGVHD grades III-IV cumulative incidence. Right, CD86 rs17281995 aGVHD grades II-IV cumulative incidence.

Disclosure of conflict of interest

None
Impact of graft-versus-host disease on outcomes after pediatric single cord blood transplantation: A retrospective analysis from the JSHCT GVHD Working Group

Junya Kanda\(^1\), Katsusugu Umeda\(^2\), Koji Kato\(^3\), Makoto Murata\(^4\), Junichi Sugita\(^5\), Souichi Adachi\(^6\), Katsuyoshi Koh\(^7\), Jiro Inagaki\(^8\), Hiroaki Goto\(^9\), Nao Yoshida\(^1\), Masahiro Yasui\(^10\), Yuhki Koga\(^11\), Tsukasa Hori\(^12\), Masami Inoue\(^10\), Yoshiko Hashii\(^13\), Yoshiko Atsuta\(^14,15\), Takanori Teshima\(^5\)

\(^1\)Department of Hematology and Oncology, Graduate School of Medicine, Kyoto University, Kyoto, Japan; \(^2\)Department of Pediatrics, Graduate School of Medicine, Kyoto University, Kyoto, Japan; \(^3\)Department of Hematology/Oncology, Children’s Medical Center, Japanese Red Cross Nagoya First Hospital, Nagoya, Japan; \(^4\)Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; \(^5\)Department of Hematology, Hokkaido University Graduate School of Medicine, Sapporo, Japan; \(^6\)Department of Hematology and Oncology, Nagoya University Graduate School of Medicine, Nagoya, Japan; \(^7\)Department of Hematology/Oncology, Saitama Children’s Medical Center, Saitama, Japan; \(^8\)Department of Pediatrics, National Kyushu Cancer Center, Fukuoka, Japan; \(^9\)Division of Hemato-Oncology/Regenerative Medicine, Kanagawa Children’s Medical Center, Yokohama, Japan; \(^10\)Department of Hematology/Oncology, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan; \(^11\)Department of Pediatrics, Kyushu University Hospital, Fukuoka, Japan; \(^12\)Department of Pediatrics, Sapporo Medical University School of Medicine, Sapporo, Japan; \(^13\)Department of Pediatrics, Osaka University Graduate School of Medicine, Suita, Japan; \(^14\)Japanese Data Center for Hematopoietic Cell Transplantation; \(^15\)Department of Healthcare Administration, Nagoya University Graduate School of Medicine, Nagoya, Japan

Abstract

Background: The effect of graft-versus-host disease (GVHD) on transplant outcomes after cord blood transplantation (CBT) is not yet fully understood. We recently reported that mild acute GVHD (aGVHD) or chronic GVHD (cGVHD) was associated with not only a low risk of relapse but also a low risk of non-relapse mortality (NRM) and provides a survival benefit in adult single CBT (Kanda J, et al. Leukemia 2016). However, the number of total nucleated cells and T cells per recipient weight in cord blood differs considerably between pediatric and adult patients, which may lead to different effects of GVHD on transplant outcomes. Therefore, using registry data from the Japan Society for Hematopoietic Cell Transplantation (JSHCT), we analyzed the impact of GVHD on outcomes in pediatric single CBT. Methods: We included pediatric patients, aged 0 to 15 years, with acute leukemia or myelodysplastic syndrome who underwent their first CBT (n = 740) between 2000 and 2014. The effect of aGVHD on outcomes was analyzed after adjusting for other significant variables among all engrafted patients, and the effect of cGVHD was analyzed among the engrafted patients who survived without relapse for at least 100 days. The occurrence of GVHD was treated as a time-dependent covariate. Results: The median age of the recipients at transplant was 5 (range, 0–15) years. Two-thirds of the patients had standard-risk disease. Calcineurin inhibitor and methotrexate were used in 83% of patients. Only single unit grafts were included in the cohort. Fifty percent received a UCB unit containing more than 5.0 × 107/kg TNCs. Acute GVHD of grades I-II (GI-II) and III-IV (GIII-IV) occurred in 56% and 14% of the patients, respectively. The occurrence of GIII-IV aGVHD was associated with a higher risk of NRM (hazard ratio [HR] 4.07, P < 0.001) when compared with no aGVHD. GI-II aGVHD was not associated with NRM. The occurrence of GI-II or GIII-IV aGVHD was not associated with a low relapse risk. These results in no survival benefit of GI-II aGVHD (HR 1.04, P = 0.789) and an adverse effect of GIII-IV aGVHD (HR 1.68, P = 0.007; Figure 1A). Limited and extensive chronic GVHD occurred in 16% and 7% of the evaluable patients, respectively. The occurrence of limited cGVHD (HR 0.82, P = 0.410) or extensive cGVHD (HR 0.62, P = 0.199) was not associated with a low relapse risk as compared to no cGVHD. The occurrence of limited cGVHD was marginally associated with a lower risk of NRM (HR 0.16, P = 0.077), whereas the occurrence of extensive cGVHD was associated with a higher risk of NRM (HR 2.69, P = 0.027). These resulted in the significant association between limited cGVHD and a low risk of overall mortality (HR 0.60, P = 0.045), but no significant association between extensive cGVHD and overall mortality (Figure 1B). Conclusions: Consistent with the observations in adult cohorts, our results
indicate that severe aGVHD should be prevented because of its association with high overall mortality and NRM. However, unlike adult cohorts, mild acute GVHD provides no overall benefit for relapse, NRM, and overall mortality. Consistent with adult cohorts, mild cGVHD may be beneficial for survival, probably due to the low risk of NRM when compared to no cGVHD. Severe cGVHD is associated with a higher risk of NRM.

Disclosure of conflict of interest

There is no conflict of interest regarding this abstract.
Interleukin-6 is an Early Biomarker for Acute GvHD and Survival after Allogeneic Transplant

Greco, Raffaella 1; Lorentino, Francesca 1; Lupo Stanghellini, Maria Teresa 1; Nitti, Rosamaria 1; Vaccari, Linda Cheyenne 2; Forcina, Alessandra 1; Morelli, Mara 1; Giglio, Fabio 1; Xue, Elisabetta 1; Perini, Tommaso 1; Dalto, Serena 1; Mastaglio, Sara 1; Piemontese, Simona 1; Assanelli, Andrea 1; Marktel, Sarah 1; Carrabba, Matteo 1; Corti, Consuelo 1; Bernardi, Massimo 1; Bondanza, Attilio 3; Bonini, Chiara 4; Ciceri, Fabio 1; Peccatori, Jacopo 1

1Haematology and Bone Marrow Transplant Unit, IRCCS San Raffaele Scientific Institute, Milano, Italy, 2College of Medicine and Veterinary Medicine, University of Edinburgh, Edinburgh, United Kingdom, 3Innovative Immunotherapies Unit, T Division of Immunology, Transplantation and Infectious Diseases, Program In Immunology and Bio-immunotherapy of Cancer (PIBIC), San Raffaele Scientific Institute, Milan, Italy, 4Experimental Hematologic Unit, Division of Immunology, Transplantation and Infectious Disease, San Raffaele Scientific Institute, Vita-Salute San Raffaele University, Milano, Italy

Abstract

Background: Acute graft-versus-host disease (aGvHD) is a leading cause of transplant-related mortality (TRM) after allogeneic HSCT (allo-HSCT). We conducted a prospective observational study to ascertain the potential of interleukin-6 (IL6), measured in patient blood samples before conditioning and 7 days after allo-HSCT, in predicting aGvHD, TRM and survival after transplant. Material and Methods: We collected samples from 151 consecutive patients (93 males; median age 52) who underwent allo-HSCT at our institution between April 2014 and June 2016. Most patients were affected by myeloid malignancies (AML=50%, MDS=14%). Revised DRI (Armand et al.) was low-intermediate in 48%, high in 45% and very high in 7% of patients. Most patients (88%) received PBSC. Conditioning was myeloablative in 118 patients. Stem cell donors were unrelated (n=59), family haploidalentical (n=63), HLA-identical sibling (n=29). Post-transplant GvHD prophylaxis was PT-Cy in 96 patients, ATG in 31 patients, both agents in 17 cases. Sirolimus and MMF were used as additional prophylaxis. Results: All patients included in this analysis were tested for IL6 baseline and at day +7 after HSCT. Median follow-up on survivors was 14 months (range 2-31). The 100-d cumulative incidence (CI) of grade 2-4 aGvHD was 26% (14% grade 3-4 aGvHD). The median day of aGVHD onset was 30 days. The 100-d CI of TRM was of 4% with a 1-year OS of 72%. ROC analysis identified a threshold of 2.5 pg/ml for IL6 baseline levels as predictor for 100-d TRM (AUC 0.83; sens 83%, spec 67%, p=0.006). IL6 concentrations maintained diagnostic utility also in patients experiencing grade 3 or 4 aGvHD, when measured 7 days after HSCT: in this setting, ROC analysis allowed us to identify a threshold of 14.5 pg/ml (AUC 0.75; sens 90%, spec 65%, p 0.001). Moreover, we divided patients into groups according to whether biomarker concentrations were above (high) or below (low) the identified thresholds. At baseline, IL6 levels above 2.5 pg/ml were significantly associated with a higher 100-days CI of TRM (10% vs 1%; p<0.01) and a worse 1-year OS (52% vs 86%; p<0.01). When measured 7 days after HSCT, IL6 levels equal or superior to 14.5 pg/ml identified patients with a higher 100-days CI of grade 2-4 aGvHD (32% vs 18%; p 0.05), and grade 3-4 aGvHD (26% vs 3%; p<0.01). Moreover, higher IL6 concentrations at day +7 correlated with higher TRM (7% vs 1%; p 0.06) and worse 1-year OS (52% vs 87%; p<0.01). Interestingly, IL6 concentrations were able to better stratify OS in patients with the same DRI class. By multivariate analysis (adjusting for age, DRI, Sorror comorbidity index, type of donor, source of stem cells, conditioning regimen and GvHD prophylaxis) pre-transplant IL6 concentrations were significantly associated to grade 2-4 aGvHD (HR 2.2, 95% CI 1.4-8.8; p 0.03), grade 3-4 aGvHD (HR 3.8, 95% CI 1.1-13; p 0.03), TRM (HR 4.2, 95% CI 1.7-17.2; p 0.04), and OS (HR 2.8, 95% CI 1.3-6.4; p 0.01); post-transplant IL6 levels correlated with grade III-IV aGvHD (HR 9.1, 95% CI 1.7-50; p 0.01), and OS (HR 3.5, 95% CI 1.4-8.7; p<0.01). Conclusion: In this prospective observational study, measurement of plasma IL6 resulted a valuable biomarker in predicting the risk of aGvHD and TRM, providing a window for additional prophylactic or preemptive strategies, and potentially improving the final outcome of allo-HSCT. These findings should be validated in a multicenter study.
IL6 at 7 days after HSCT:

\[ \begin{align*}
&\text{----- } \geq 14.5 \text{ pg/ml} \\
&\text{----- } < 14.5 \text{ pg/ml}
\end{align*} \]

Cl of grade 3-4 aGvHD (26% vs 3%; p < 0.01)

Disclosure of conflict of interest

The authors declare no competing financial interests.