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Comprehensive plasma profiling for the characterization of graft-versus-host disease biomarkers

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ABSTRACT

Acute graft-versus-host disease (aGVHD) remains a life-threatening complication of hematopoietic stem cell transplantation (HSCT) therefore limiting its application. To optimize the management of aGVHD and reduce therapy-related toxicity, early specific markers are needed. The main objective of this study was to uncover diagnostic biomarkers by comparing plasma protein profiles of patients at the time of acute GVHD diagnosis with those of patients undergoing HSCT without aGVHD. Additional analysis of samples taken 15 days before aGVHD diagnosis was also performed to evaluate the potential of our newly discovered biomarkers for early diagnosis. To get complementary information from plasma samples, we used three different proteomic approaches, namely 2D-DIGE, SELDI-TOF-MS and 2D-LC-MS^E.

We identified and confirmed by the means of independent techniques, the differential expression of several proteins indicating significantly increased inflammation response and disturbance in the coagulation cascade. The variation of these proteins was already observed 15 days before GVHD diagnosis, suggesting the potential early detection of the disease before symptoms appearance.

Finally, logistic regression analysis determined a composite biomarker panel comprising fibrinogen, fragment of fibrinogen beta chain, SAA, prothrombin fragments, apolipoprotein A1 and hepcidin that optimally discriminated patients with and without GVHD. The area under the receiver operating characteristic curve distinguishing these 2 groups was 0.95.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) has been included in the therapeutic arsenal of hematological malignancies and genetic disorders for many years. Although this therapeutic approach has demonstrated good rates of success for disease eradication, life-threatening complications such as severe infections or graft-versus-host disease (GVHD) remain a major problem after HSCT.

GVHD can be defined as an exacerbated immune reaction mediated by the infused donor immunocompetent cells present in a genetically different and immunosuppressed host. Damaged host cells and bacterial products such as bacterial lipopolysaccharides induce the secretion of proinflammatory chemokines and cytokines, such as TNF-alpha, IL-1 and IL-6 that activate antigen-presenting cells. Presentation of host alloantigens to donor T cells leads to their proliferation and differentiation, thereby inducing a “cytokine storm” leading finally to the activation of cellular effectors amplifying host tissue injury [1–3]. Skin, liver and gastrointestinal tract are the main organs affected by acute GVHD. The staging of this pathology is based on the localization and the severity of injury.

Although improvements have been achieved in the prevention of GVHD through the use of new immunosuppressive drugs or changes in the source of cells and graft manipulation [4–6], prophylactic approaches appear to be insufficient to avoid any

Abbreviations: aGVHD, acute graft-versus-host disease; Apo, apolipoprotein; APP, acute phase protein; ASB-14, amidosulfobetaine-14; CRP, C-reactive protein; F13B, coagulation factor XIII beta chain; FBG, fibrinogen; FGB, fibrinogen beta chain; HAMP, hepcidin antimicrobial peptide; HRG, histidine-rich glycoprotein; HSCT, hematopoietic stem cell transplantation; LC-MS, liquid chromatography–mass spectrometry; PLG, plasminogen; SAA, serum amyloid A; SELDI-TOF-MS, surface enhanced laser desorption ionisation-time of flight-mass spectrometry

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complications. Moreover, such improvements are accompanied by an increased rate of relapse due to the close correlation between GVHD and graft-versus-tumor effects [7,8], thereby compromising HSCT efficacy. Although consensus has emerged supporting the use of high-dose (methyl)prednisolone or prednisone for initial treatment of acute GVHD, practices differ among centers with respect to the initial glucocorticoid dose to be applied, the use of additional immunosuppressive agents, the management of treatment withdrawal after initial improvement, and the treatment of patients who failed to respond to steroids [9]. Second line of treatment for steroid-refractory aGVHD includes increased dose of immunosuppressive agent (cyclosporine, mycophenolate mofetil or tacrolimus), antithymocyte globulin, monoclonal antibodies as well as extracorporeal photophoresis or mesenchymal stem cell infusion [10]. For all these reasons, GVHD remains a challenge for clinicians in the application of HSCT.

Currently, diagnosis and grading of acute GVHD are based on clinical manifestations and histopathological analysis of involved organ biopsies [11]. Those are time-consuming, invasive and poorly specific practices. Measurement of biomarkers from fluids such as blood or urine could be a useful tool to diagnose and even predict the GVHD onset allowing an earlier initiation of treatment and a better management of this complication. Moreover, the identification of new biomarkers of GVHD could give novel insights on the underlying mechanisms and physiological processes of this pathology.

Although many studies report the monitoring of chemokines and cytokines as potential acute GVHD (aGVHD) biomarkers [12–17], only few investigations based on non-targeted proteomic approaches have been performed [18–25]. Among those, Weissinger et al. identified in urine modulation of peptide expression generated from collagen, albumin, beta2-microglobulin and CD99, indicating significant disturbances in collagen metabolism and T-cell activation [18,22,26]. Moreover, recent studies from Ferrara's group by cytokine antibody microarrays and Intact Protein Analysis System identified a panel of GVHD plasma biomarkers (namely IL-2 alpha, TNFR1, HGF, IL-8, elafin and reg3alpha) [15,19,23]. Those markers were validated and could discriminate therapy responsive from non-responsive patients and predict survival in patients receiving GVHD therapy [27,28].

Non-targeted proteomic approaches present the advantage to examine in a single experiment a large panel of peptides and proteins, providing a fingerprint of a pathophysiological situation at a given time. As a single biomarker could be the indicator of many unrelated pathological changes, the simultaneous detection of several markers is a key to improve specificity [29]. In this particular disease and considering the diversity of complications after HSCT, combination of biomarkers should assure a more specific diagnosis.

In this study, a non-targeted proteomic approach was used with the objective to find diagnostic markers of GVHD and to highlight physiopathological mechanisms of the disease. As there are numerous factors of variability associated to HSCT, very well characterized (GradeII aGVHD) and homogeneous groups (conditioning regimen intensity, donor, preventive treatment therapy,…) were build. Plasma protein profiles from patients with aGVHD were compared with those of patients undergoing HSCT without any aGVHD symptoms. Additional analysis of samples taken 15 days before aGVHD diagnosis was also performed to evaluate the potential of our newly discovered biomarkers for early diagnosis. To extract a maximum of information from plasma samples, three complementary proteomic approaches: a gel-based (2D-DIGE) and two MS-based (SELDI-TOF-MS and 2D-LC-MS^E) approaches were undertaken.

2. Materials and methods

2.1. Patients and sample collection

2.1.1. Ethics

Written informed consent was obtained from each patient to undergo allo-HSCT and to collect, store and analyze blood samples

Table 1
Patient characteristics: control vs Grade II aGVHD.

Characteristics	2D-DIGE		SELDI-TOF-MS		2D-LC-MS^E		Western blot	
	***Control* (*n*=16)**	***aGVHD* (*n*=16)**	***Control* (*n*=16)**	***aGVHD* (*n*=16)**	***Control* (*n*=23)**	***aGVHD* (*n*=23)**	***Control* (*n*=28)**	***aGVHD* (*n*=28)**
Median age, years (range)	58 (16–66)	57 (21–67)	63 (30–66)	61 (23–70)	61 (30–72)	61 (23–70)	60 (30–72)	60 (21–70)
Gender								
Male	13	14	11	14	15	16	20	20
Female	3	2	5	2	8	7	8	8
Diagnosis								
Acute myeloblastic leukemia	6	4	7	4	9	5	13	7
Lymphoma	2	5	3	6	3	7	4	9
Multiple myeloma	4	5	1	2	4	4	5	5
Myelodysplastic syndrome	0	0	1	3	2	5	1	4
Other malignancies	4	2	4	1	5	2	5	3
Donor								
Related	4	7	3	3	4	5	6	8
Unrelated	12	9	13	13	19	18	22	20
Conditioning regimen intensity								
Myeloablative	4	5	0	0	0	0	4	5
Reduced	12	11	16	16	23	23	24	23
ATG administration	6	6	0	0	0	0	6	5
Acute GVHD								
Skin		15		12		19		22
Gut		7		7		7		9
Liver		1		0		0		2
Combined		7		3		3		5
Day of onset of acute GVHD, median (range)		34 (13–139)		46 (15–245)		47 (15–245)		44 (13–245)
Post-HSCT day of samples, median (range)	34 (10–139)	36 (14–139)	46 (21–259)	46 (18–248)	47 (21–259)	47 (18–248)	45 (10–259)	44.5 (14–248)