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Biology: Biomarkers

Biomarkers for Diagnosis and Prognosis of Sinusoidal Obstruction Syndrome after Hematopoietic Cell Transplantation

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A B S T R A C T

Reliable, noninvasive methods for diagnosing and prognosing sinusoidal obstruction syndrome (SOS) early after hematopoietic cell transplantation (HCT) are needed. We used a quantitative mass spectrometry–based proteomics approach to identify candidate biomarkers of SOS by comparing plasma pooled from 20 patients with and 20 patients without SOS. Of 494 proteins quantified, we selected 6 proteins (L-Ficolin, vascular cell adhesion molecule-1 [VCAM1], tissue inhibitor of metalloproteinase-1, von Willebrand factor, intercellular adhesion molecule-1, and CD97) based on a differential heavy/light isotope ratio of at least 2 fold, information from the literature, and immunoassay availability. Next, we evaluated the diagnostic potential of these 6 proteins and 5 selected from the literature (suppression of tumorigenicity-2 [ST2], angiopoietin-2 (ANG2), hyaluronic acid [HA], thrombomodulin, and plasminogen activator inhibitor-1) in samples from 80 patients. The results demonstrate that together ST2, ANG2, L-Ficolin, HA, and VCAM1 compose a biomarker panel for diagnosis of SOS. L-Ficolin, HA, and VCAM1 also stratified patients at risk for SOS as early as the day of HCT. Prognostic Bayesian modeling for SOS onset based on L-Ficolin, HA, and VCAM1 levels on the day of HCT and clinical characteristics showed >80% correct prognosis of SOS onset. These biomarkers may provide opportunities for preemptive intervention to minimize SOS incidence and/or severity.

INTRODUCTION

Hematopoietic cell transplantation (HCT) is a potentially life-saving treatment for many patients with inherited disorders and hematologic malignancies. However, its practical use is impeded by the risk of serious adverse events, including sinusoidal obstruction syndrome (SOS, the now-preferred name for veno-occlusive disease occurring after HCT or chemotherapy). Although the overall incidence and severity after allogeneic HCT have decreased in recent years, SOS is still a life-threatening liver injury complication with greater than 80% mortality in severe cases, and SOS affects up to 20% of allogeneic HCT recipients in some centers [1-5]. SOS can also occur after intense chemotherapy when either the chemotherapy or radiation induces both systemic inflammation and tissue damage, particularly to the sinusoidal

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endothelial cells of the hepatic acinus [6-8]. In addition, SOS can occur after the use of drugs, such as gemtuzumab ozogamicin, inotuzumab ozogamicin, and after the combination of tacrolimus and sirolimus under certain circumstances [9-12].

SOS typically occurs between the first and third weeks after HCT but it may occur later, and it is often clinically indistinguishable from other causes of weight gain and respiratory distress, particularly in children (eg, cytokine storm syndrome and idiopathic pneumonia syndrome), or other causes of abdominal pain and jaundice (eg, graft-versus-host disease [GVHD] of the gastrointestinal tract or liver) [4]. Diagnosis of SOS is made according to 2 clinical criteria scales (Baltimore [13] and Seattle [6]) that measure different degrees of liver dysfunction and weight gain. Abdominal ultrasound showing a reversal of the sinusoidal flow is commonly used to confirm the diagnosis. However, these clinical criteria and reversal of the sinusoidal flow are late events in the pathology of the disease. The investigational drug defibrotide (Gentium/Jazz Pharmceutical, Palo Alto, CA) has shown the most promising results in several clinical trials [5,14]. However, treatment with defibrotide carries significant risks, particularly of severe hemorrhage, when given late in the disease course. Therefore, a noninvasive method for early and accurate diagnosis of SOS is urgently needed [15].

Although a few potential biomarkers for SOS have been identified based on hypothesis-driven testing, there is still no validated blood test for SOS. Therefore, in the present study, we applied a quantitative mass spectrometry (MS)–based proteomics discovery approach to identify potential biomarkers for SOS and then used immunoassays to test the diagnostic value of 11 candidate biomarkers. These analyses led to the identification of a reliable biomarker panel specific for SOS that can be used in the diagnosis and management of patients with this disorder. Most importantly, given the high mortality rate associated with severe SOS and the lack of a therapeutic measure with 100% efficacy for this life-threatening disease, we next focused on prognostic markers that will afford opportunities for early preventative care. Therefore, this study focused on both diagnostic and prognostic markers, and although they are potentially interesting, markers predictive of disease severity, response to treatment, and nonrelapse mortality are beyond the scope of this study.

MATERIALS AND METHODS

Patients and Samples

Three cohorts of HCT patients were included in this study (discovery, training, and independent verification cohorts). Patients were treated at the University of Michigan, Indiana University, and University of Barcelona. All patients or their legal guardians provided written informed consent, and the collection of samples for studying post-HCT complications was approved by the institutional review boards of the University of Michigan, Indiana University, and Hospital Clinic of the University of Barcelona. Heparinized blood samples were collected before or on the day of HCT, then weekly for 2 to 4 weeks after allogeneic HCT, and, in some centers, at the time of the onset of symptoms consistent with SOS.

Proteomics Analysis

The methods used for sample preparation, protein fractionation, MS analysis, protein identification, and quantitative analysis of protein concentrations during the intact protein analysis system have been previously reported [16-18].

Immunoassays

Suppression of tumorigenicity-2 (ST2), angiopoietin2 (ANG2), L-Ficolin, hyaluronic acid (HA), vascular cell adhesion molecule-1 (VCAM1), tissue inhibitor of metalloproteinase-1 (TIMP1), thrombomodulin, intercellular adhesion molecule-1 (ICAM1), plasminogen activator inhibitor-1 (PAI-1), von Willebrand factor (vWF), and CD97 concentrations were measured by enzyme-linked immunosorbent assays (ELISAs). The antibodies pairs used for these ELISAs were as follows: anti-ST2 (R&D Systems, Minneapolis, MN), anti-ANG2 (R&D Systems), anti-L-Ficolin (Hycult Biotech, Plymouth Meeting, PA), anti-HA (Corgenix, Broomfield, CO), anti-VCAM1 (R&D Systems), anti-TIMP1 (R&D Systems), anti-thrombomodulin (Diaclone, Besancon, France), anti-ICAM1 (R&D Systems), anti-PAI-1 (eBioscience, San Diego, CA), anti-vWF (American Diagnostica, Stamford, CT), and anti-CD97 (R&D Systems).

For analysis, plasma samples were thawed and centrifuged at 12,000 rpm for 10 minutes to separate the clots at the bottom and lipids on top from the plasma. Then 150-μL aliquots of each undiluted plasma sample were transferred to individual wells of 96-well V-bottom plates. The plates were wrapped in parafilm and kept in a humid chamber at 4°C throughout the entire process, which did not exceed 96 hours. Capture antibodies were reconstituted and diluted per manufacturers' specifications or precoated plates were used as recommended by the manufacturer. Then, 50 μL of diluted antibodies were added to wells of 96-well high-binding half-well plates, which were then sealed and incubated overnight. The next day, the test plates containing the capture antibodies were washed and blocked with specific manufacturer's recommended blocking buffer. After additional wash steps, 50-μL or 100-μL aliquots of plasma samples (dilutions listed in Supplemental Table 1) were added in duplicate to the ELISA test plates. In addition, 50-μL or 100-μL aliquots of reconstituted standard at different concentrations (see Supplemental Table 1) were added in duplicate for the preparation of 8-point standard curves, per the manufacturers' protocols. After addition of samples and standard solutions, the plates were sealed and incubated for 2 hours at room temperature on a plate rotator at 300 rpm. The ELISAs were completed by adding biotinylated detection antibodies specific for each target followed by the enzyme horseradish peroxidase and horseradish peroxidase substrate. The optical density of each well was read using a plate reader set to 450 to 570 nm. For ELISA kits with precoated plates, the manufacturers' protocols were applied. The ELISAs were performed in duplicate and sequentially, as previously reported [18-22].

Statistical Analysis

The statistical methods used for the Intact Protein Analysis System (IPAS) were previously described [16-18]. Differences in characteristics between patient groups were assessed with Kruskal-Wallis tests for continuous values and chi-squared tests of association for categorical values. Protein concentrations from individual samples in the training and independent sets were compared using unpaired *t*-tests. Receiver operating characteristic (ROC) areas under the curves (AUCs) were estimated nonparametrically. Differences in median pre-HCT, day 0, +7, and +14 biomarker levels between SOS− and SOS + patients were assessed using a Wilcoxon rank-sum test. Additionally, we examined the differences in biomarkers trajectories over time using a modeling approach (see Supplementary Methods).

Prognostic Bayesian Modeling

The plasma concentrations of 3 proteomic biomarkers (L-Ficolin, HA, and VCAM1) on the day of HCT were used to evaluate their prognostic performance for future occurrence of SOS onset. The clinical characteristics also included in the analysis were age, gender, donor type (related or unrelated), donor match (matched or mismatched), transplantation period (before or in 2005 or after 2005), transplantation number (1 or >1), conditioning regimen (chemotherapy only or combined with irradiation), busulfan (16 mg/kg) use in the conditioning (yes or no), and cyclophosphamide use in the conditioning (yes or no). Plasma protein concentrations and clinical characteristics were used as attributes for the prognosis of SOS onset. The naïve Bayes classifier was selected for SOS onset prognosis because of its simplicity and high classification performance. Ten-fold cross-validation was used to avoid over training, bias, and/or artifacts (see Supplemental Methods). This naïve Bayes classifier was developed with Waikato Environment for Knowledge Analysis software v3.6.10 [23].

RESULTS

Proteomic Biomarker Discovery

We first performed discovery proteomic analysis comparing plasma pooled from 20 patients with SOS to plasma pooled from 20 patients without SOS. The clinical characteristics of patients in this discovery cohort are provided in Table 1. Of 494 proteins identified and quantified, 151 proteins showed at least a 2-fold increase in the heavy-light isotope ratio, and 77 proteins showed a heavy-light