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(54) **METHODS FOR DETECTING SINUSOIDAL OBSTRUCTIVE SYNDROME (SOS)**

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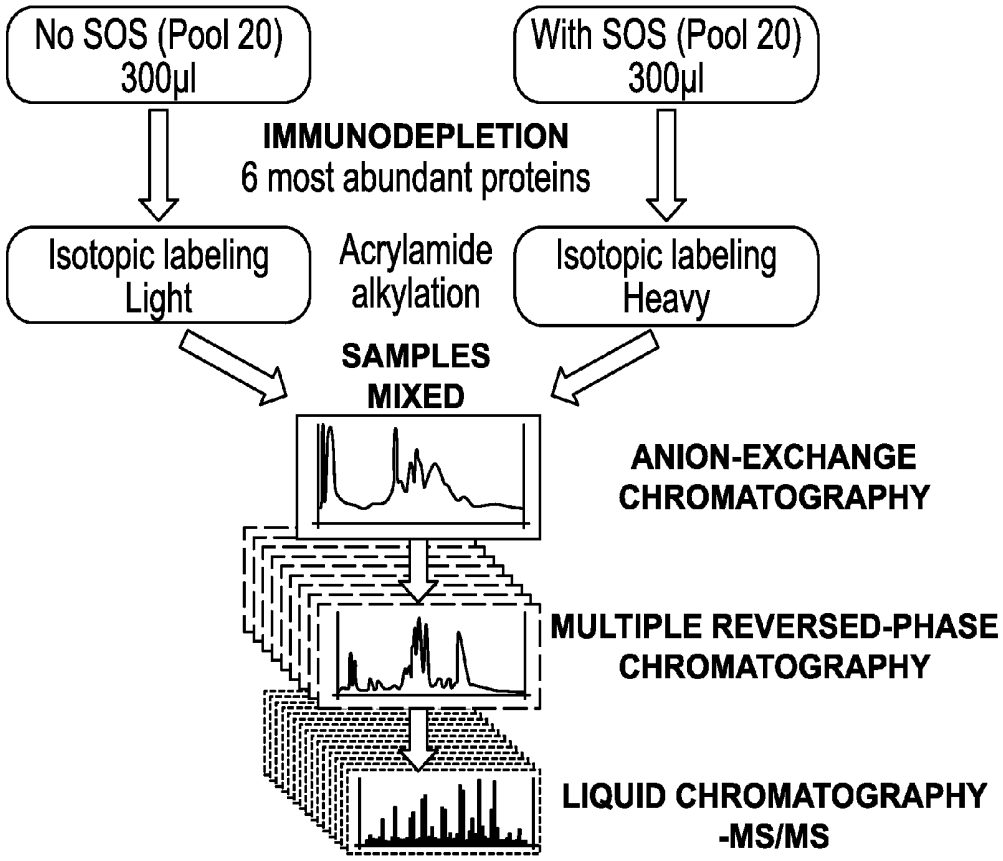
(57) **ABSTRACT**

Disclosed are biomarker panels for evaluating subjects at risk of sinusoidal obstruction syndrome (SOS) early after hematopoietic stem cell transplantation (HSCT). In particular, the present disclosure relates to the use of one or more of ST2, ANG2, L-Ficolin, HA, and VCAM1 for prognosing, diagnosing, and/or treating SOS.



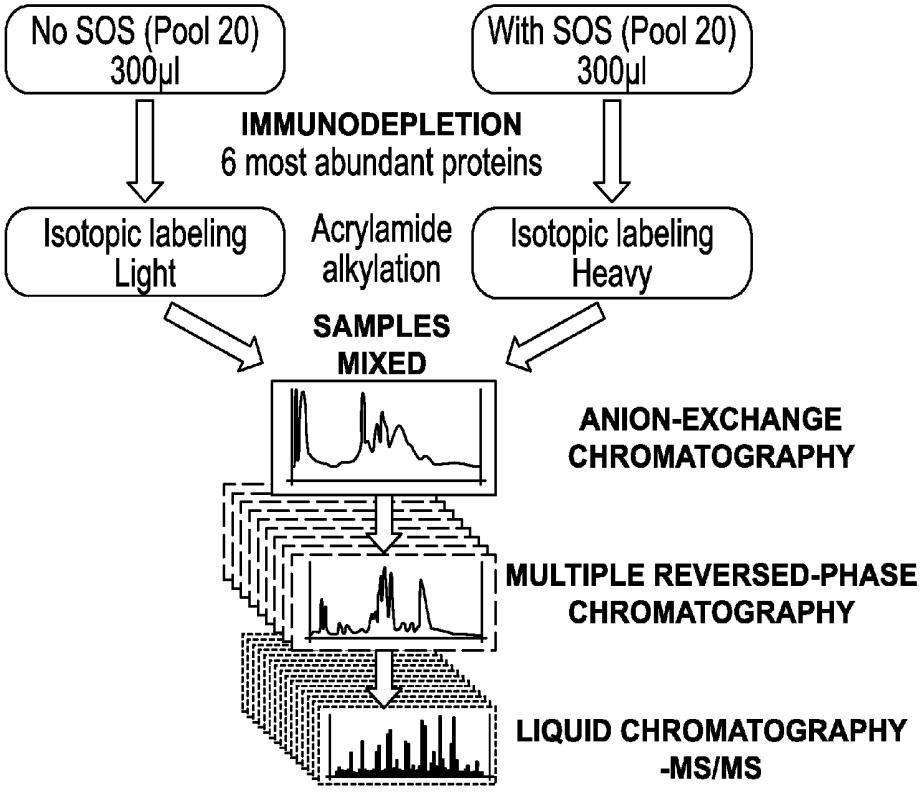

No SOS (Pool 20)
300µl
With SOS (Pool 20)
300µl
IMMUNODEPLETION
6 most abundant proteins
Isotopic labeling
Light
Acrylamide
alkylation
Isotopic labeling
Heavy
SAMPLES
MIXED
ANION-EXCHANGE
CHROMATOGRAPHY
MULTIPLE REVERSED-PHASE
CHROMATOGRAPHY
LIQUID CHROMATOGRAPHY
-MS/MS

FIG. 1

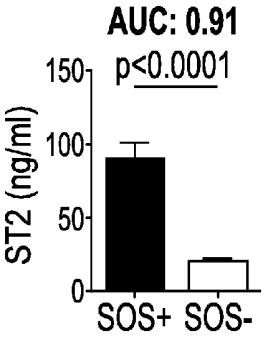


FIG. 2A

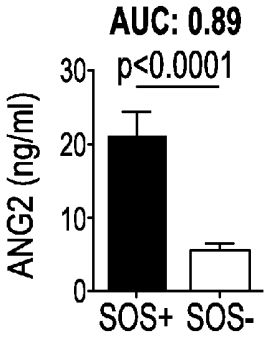


FIG. 2B

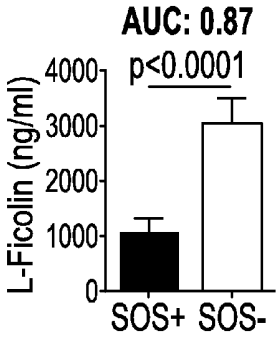


FIG. 2C

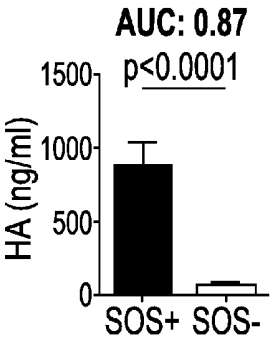


FIG. 2D

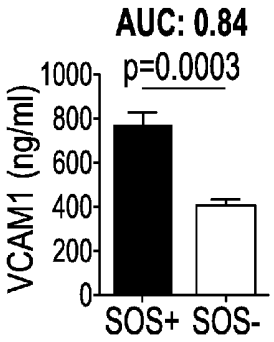


FIG. 2E

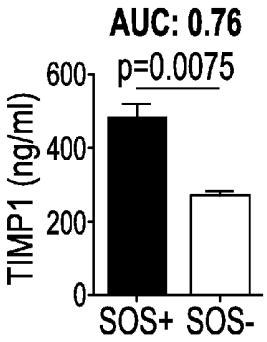


FIG. 2F

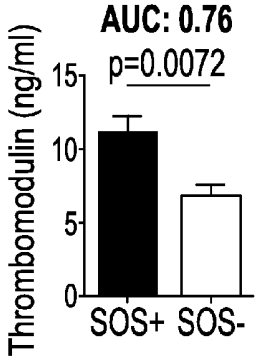


FIG. 2G

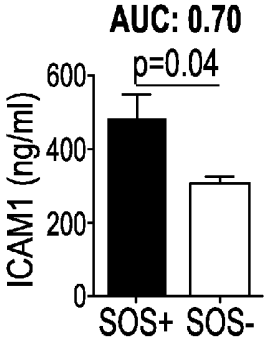


FIG. 2H

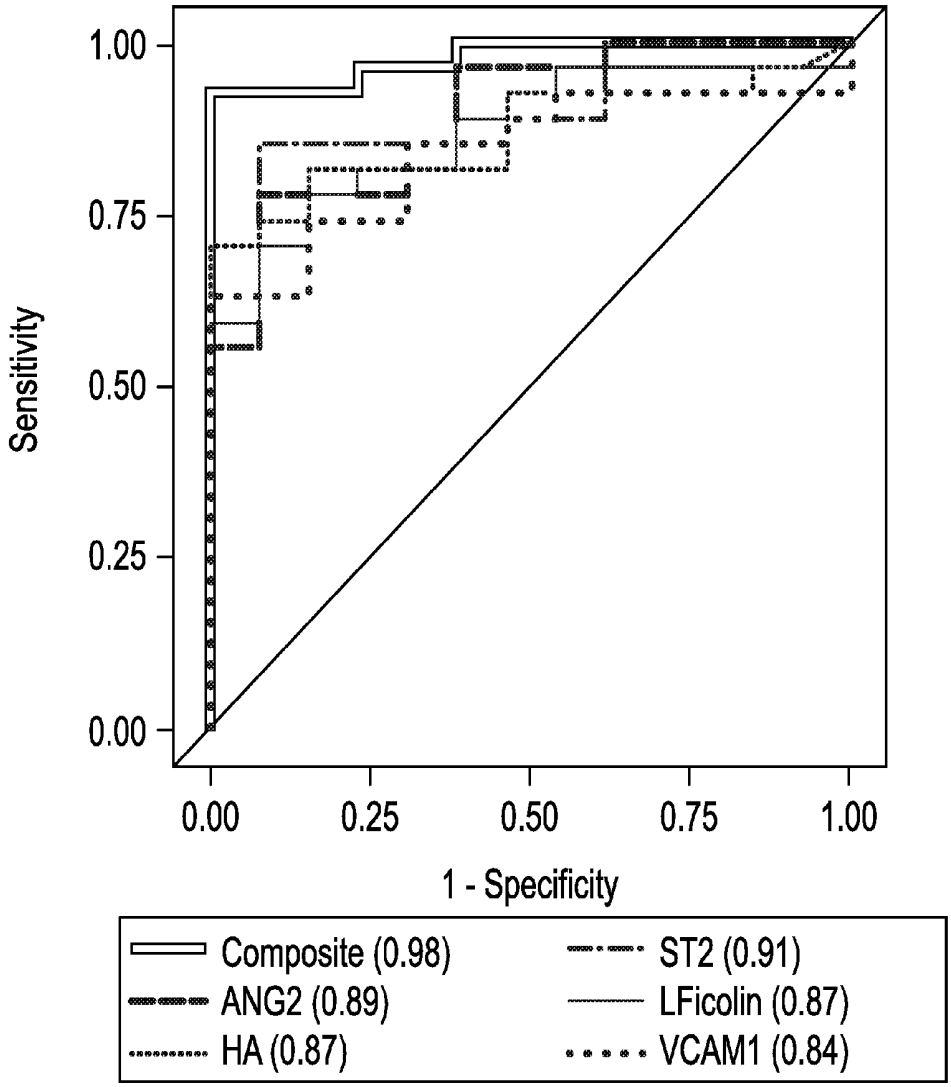

1.00
0.75
0.50
0.25
0.00
Sensitivity
0.00
0.25
0.50
0.75
1.00
1 - Specificity
Composite (0.98)
ANG2 (0.89)
HA (0.87)
ST2 (0.91)
LFicolin (0.87)
VCAM1 (0.84)

FIG. 3

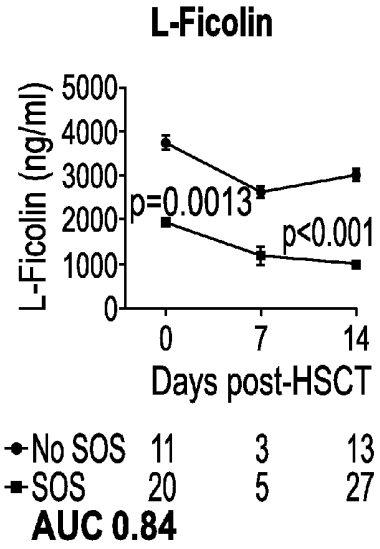


FIG. 4A

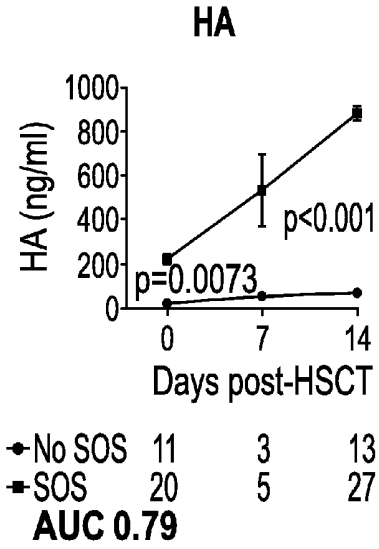


FIG. 4B

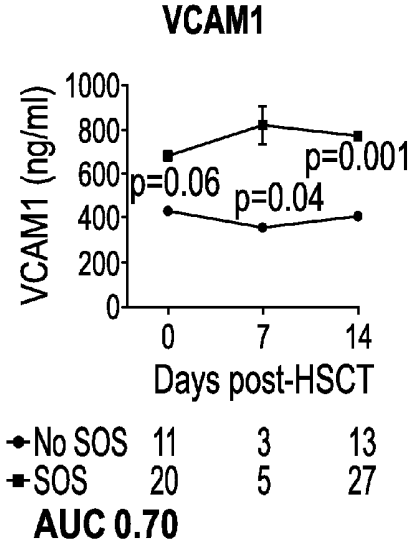


FIG. 4C

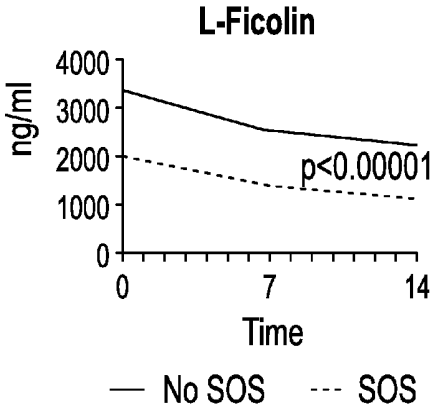


FIG. 5A

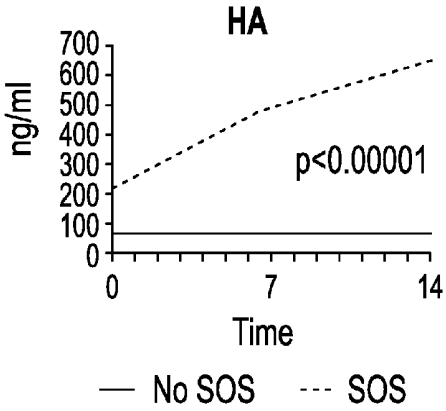


FIG. 5B

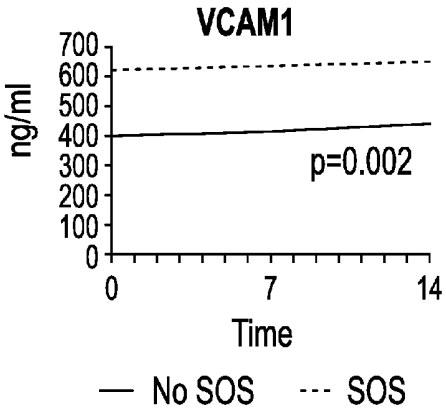


FIG. 5C

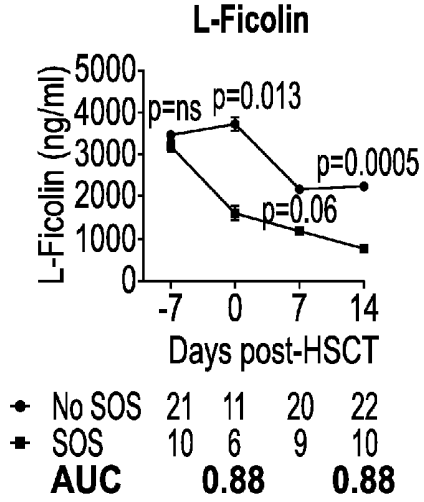


FIG. 6A

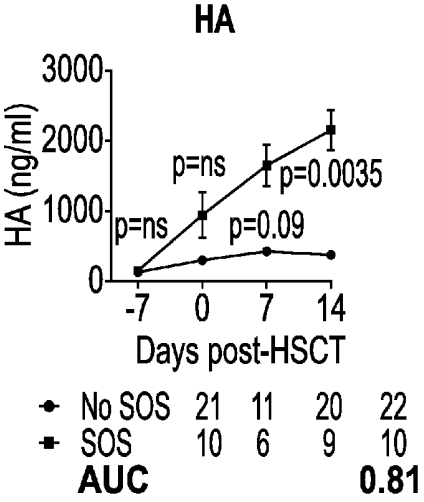


FIG. 6B

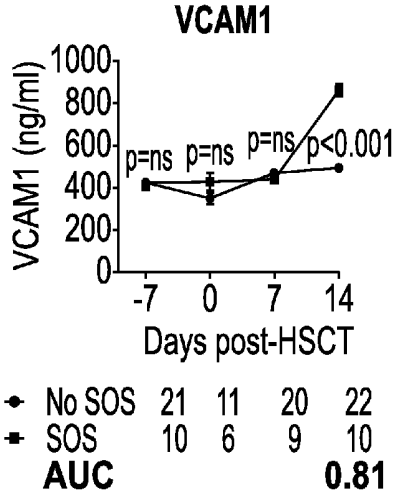


FIG. 6C

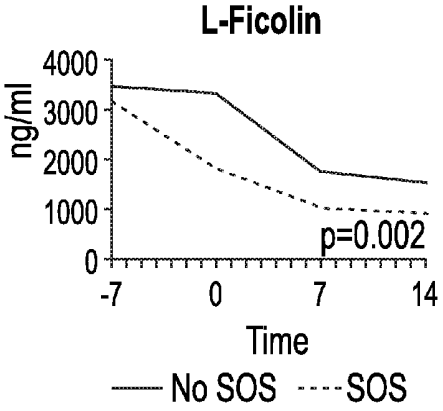


FIG. 7A

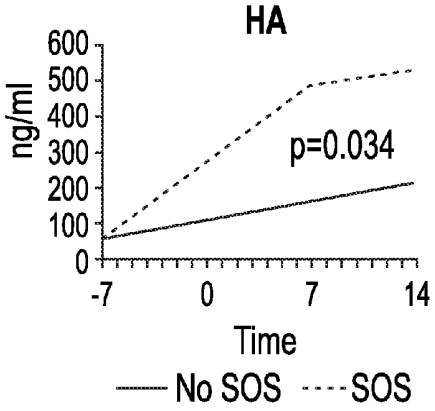


FIG. 7B

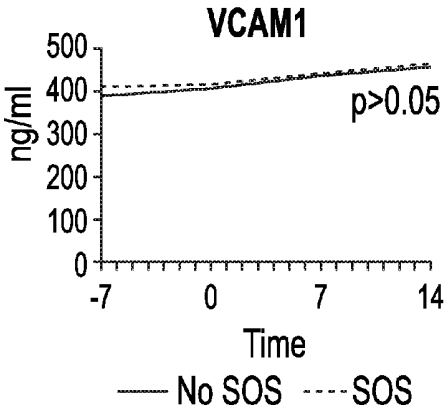


FIG. 7C

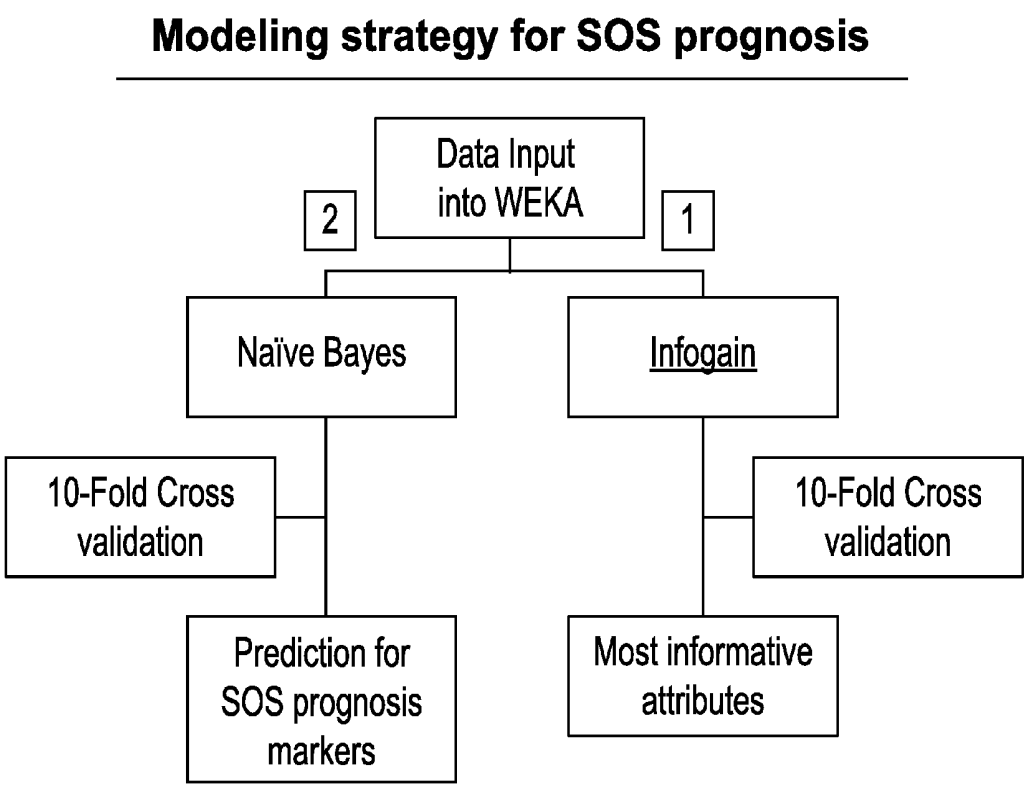

Modeling strategy for SOS prognosis
Data Input into WEKA
2
1
Naïve Bayes
Infogain
10-Fold Cross validation
10-Fold Cross validation
Prediction for SOS prognosis markers
Most informative attributes

FIG. 8

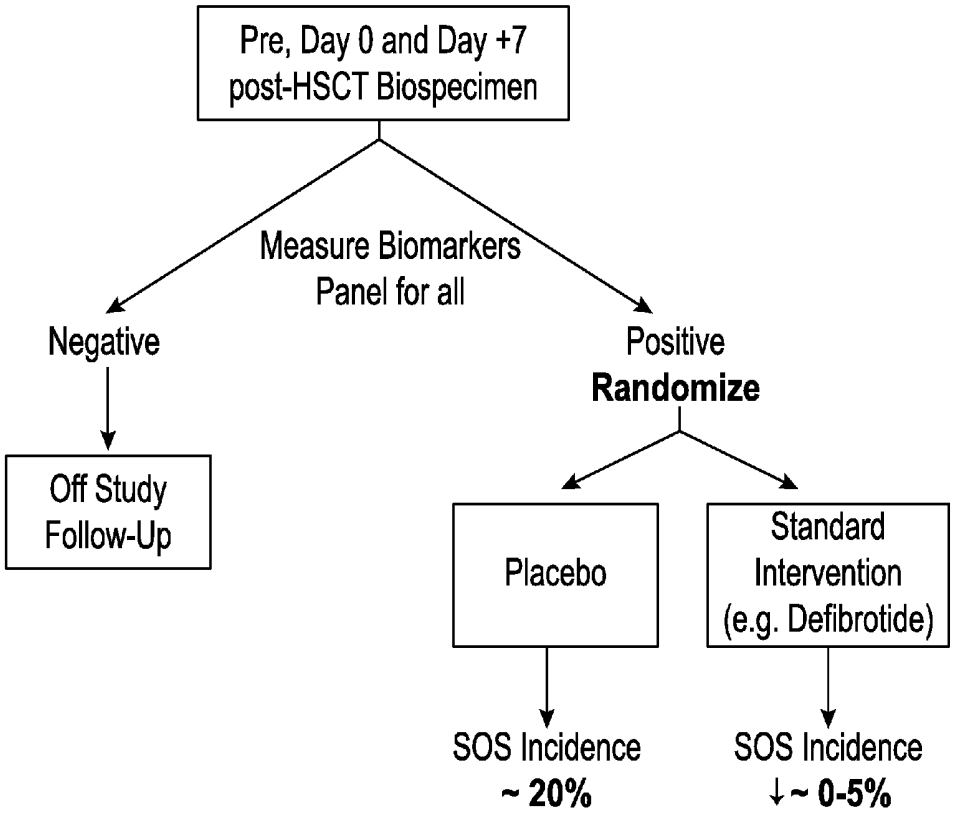

Pre, Day 0 and Day +7
post-HSCT Biospecimen
Measure Biomarkers
Panel for all
Negative
Off Study
Follow-Up
Positive
Randomize
Placebo
Standard
Intervention
(e.g. Defibrotide)
SOS Incidence
~ 20%
SOS Incidence
↓~ 0-5%

FIG. 9

METHODS FOR DETECTING SINUSOIDAL OBSTRUCTIVE SYNDROME (SOS)

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to International Application Number PCT/US2015/057393, filed on 26 Oct. 2015, which claims priority to U.S. Provisional Patent Application Ser. No. 62/069,394, filed Oct. 28, 2014, the disclosures of which are hereby expressly incorporated by reference in their entireties.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH & DEVELOPMENT

[0002] This invention was made with government support under HD071598, HL101102, and CA168814 awarded by the National Institutes of Health. The government has certain rights in the invention.

FIELD OF THE DISCLOSURE

[0003] The present disclosure relates generally to biomarkers for evaluating subjects at risk of sinusoidal obstruction syndrome (SOS) early after hematopoietic stem cell transplantation (HSCT). In particular, the present disclosure relates to the use of ST2, ANG2, L-Ficolin, HA, and VCAM1 as a biomarker panel for prognosing, diagnosing, and/or treating SOS (also referred to as veno-occlusive disease (VOD)). The present disclosure is further directed to the use of this biomarker panel for preemptive intervention to minimize the incidence and severity of SOS.

BACKGROUND OF THE DISCLOSURE

[0004] Hematopoietic stem cell transplantation (HSCT) 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 (VOD), occurring after stem cell transplantation or chemotherapy). Although the overall incidence and severity has fallen in the recent years, SOS is still a life-threatening liver injury complication with greater than 80% mortality in severe cases that affects up to 20% of allogeneic HSCT recipients in some centers. SOS can also occur after intense chemotherapy when either the chemotherapy or radiation induces both systemic inflammation and tissue damage particularly to the sinusoidal endothelial cells of the hepatic acinus. In addition, SOS can also occur after use of drugs such as gemtuzumab ozogamicin and the combination of tacrolimus and sirolimus under certain circumstances.

[0005] The pathogenesis of SOS is complex, involving cytokine release, endothelial injury, hemostatic activation, and hepatic drug detoxification through the glutathione pathway. Hepatocellular necrosis, fibrosis, and vascular occlusion ultimately lead to liver failure, hepatorenal syndrome, multiorgan failure, and death. Patients with SOS may present with the classical triad of unexplained weight gain and ascites and, in more severe cases, respiratory distress due to fluid overload, elevated bilirubin, and right upper quadrant pain in severe cases. However, the presentation may be variable in less severe cases. Thus, the etiology of abdominal pain and weight gain following HSCT presents a diagnostic challenge. SOS typically occurs between the first and third weeks after HSCT, but may occur later, and is often clinically indistinguishable from other causes of weight gain and respiratory distress particularly in children (e.g., cytokine storm syndrome and idiopathic pneumonia syndrome) or other causes of abdominal pain and jaundice (e.g., graft-versus-host disease of the gastrointestinal tract or liver). Diagnosis of SOS is assessed according to two clinical scales (Baltimore (Jones R J et al., "Venoocclusive disease of the liver following bone marrow transplantation," Transplantation, 1987: 44(6):778-783) and Seattle (Shulman H M et al., "Hepatic veno-occlusion disease—liver toxicity syndrome after bone marrow transplantation," Bone Marrow Transplant. 1992: 10(3):197-214)) that measure different degrees of liver dysfunction and weight gain, and 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, and ultrasound examination for this phenomenon is not standardized and varies according to operator-dependent practices. Histological evaluation is not routinely performed to confirm the diagnosis in these patients due to their increased risk for bleeding complications with liver biopsy.

[0006] Although there is general agreement on the use of clinical criteria for diagnosing SOS, no definitive consensus has been reached regarding a suitable classification system for disease severity beyond the Bearman scale (Bearman S I et al., "Venoocclusive disease of the liver: development of a model for predicting fatal outcome after marrow transplantation," J Clin Oncol. 1993: 11(9):1729-1736). Consequently, a diagnosis of severe SOS is associated with multiorgan failure and a high mortality rate.

[0007] Although no agents have been approved for SOS treatment in the United States, the investigational drug defibrotide has shown the most promising results in several clinical trials and is approved in the European Union for treatment of SOS. Defibrotide is a polydisperse oligonucleotide with fibrinolytic properties and protective effects on vascular endothelium. However, treatment with defibrotide therapy carries significant risks when given late in the disease course, particularly severe hemorrhage. Therefore, a noninvasive method for early and accurate diagnosis of SOS is urgently needed.

[0008] Further, although a few potential biomarkers for SOS have been identified based on hypothesis-driven testing, there is still no validated blood test for SOS. Accordingly, there exists a need to identify non-invasive biomarkers for use in diagnosing and prognosing SOS early after HSCT. It would further be advantageous if these methods can be used to provide preemptive intervention to minimize the incidence and severity of SOS.

BRIEF DESCRIPTION OF THE DISCLOSURE

[0009] In one aspect, the present disclosure is directed to a diagnostic biomarker panel comprising suppressor of tumorigenicity 2 (ST2), angiopoietin 2 (ANG2), L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

[0010] In another aspect, the present disclosure is directed to a prognosis biomarker panel comprising L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

[0011] In another aspect, the present disclosure is directed to a method of diagnosing or of aiding diagnosis of sinu-

soidal obstructive syndrome (SOS) in a subject receiving hematopoietic stem cell transplantation (HSCT). The method comprises measuring in a biological sample from the subject the expression of at least one biomarker selected from the group consisting of ST2, ANG2, L-Ficolin, HA, and VCAM1 by contacting the biological sample obtained from the subject with a specific binding agent that specifically binds to the biomarker, wherein the specific binding agent forms a complex with the biomarker; and detecting the agent-biomarker complex, thereby determining the biomarker expression level; wherein an elevated biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of SOS.

[0012] In another aspect, the present disclosure is directed to a method of prognosing or of aiding prognosis of sinusoidal obstructive syndrome (SOS) in a subject receiving hematopoietic stem cell transplantation (HSCT). The method comprises: measuring in a biological sample from the subject the expression of at least one biomarker selected from the group consisting of ST2, ANG2, L-Ficolin, HA, and VCAM1 by contacting the biological sample obtained from the subject with a specific binding agent that specifically binds to the biomarker, wherein the specific binding agent forms a complex with the biomarker; and detecting the agent-biomarker complex, thereby determining the biomarker expression level; wherein an elevated biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of a prognosis for shortened survival compared to median survival in a subject having SOS, and wherein a reduced biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of a prognosis for increased survival compared to median survival in a subject having SOS.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] FIG. **1** depicts a flow diagram for the proteomics analysis used in the Examples below.

[0014] FIGS. **2**A-**2**H depict eight diagnostic biomarkers of SOS according to the highest AUCs (0.91-0.70).

[0015] FIG. **3** depicts a composite ROC curve compared to the individual ROC curves for the five best SOS diagnostic markers (ST2, ANG2, L-Ficolin, HA, and VCAM1).

[0016] FIGS. **4**A-**4**C depict curves for SOS markers measured at different times post-HSCT (0 days, 7 days, and SOS onset).

[0017] FIGS. **5**A-**5**C depict the trajectories of L-Ficolin, HA, and VCAM1 in the training set as modeled by population mixed effects approach as analyzed in Example 3. Shown is the population median for each biomarker with the p-value comparing the trajectories of the two groups.

[0018] FIGS. **6**A-**6**C depict curves for SOS markers measured at different times pre- and post-HSCT (−7 days, 0 days, 7 days, and 14 days). Shown is the population median for each biomarker with the p-value comparing the trajectories of the two groups.

[0019] FIGS. **7**A-**7**C depict the trajectories of L-Ficolin, HA, and VCAM1 in the independent set as modeled by population mixed effects approach as analyzed in Example 4. Shown is the population median for each biomarker with the p-value comparing the trajectories of the two groups.

[0020] FIG. **8** depicts the modeling strategy for SOS prognosis as used in Example 5.

[0021] FIG. **9** depicts a preemptive SOS trial based on prognostic biomarker model.

DETAILED DESCRIPTION OF THE DISCLOSURE

[0022] It has been discovered herein that suppressor of tumorigenicity 2 (ST2), angiopoietin 2 (ANG2), L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1) can be employed in biomarker panels to diagnosis SOS. Further, in one embodiment, a biomarker panel can be employed to provide opportunities for preemptive intervention to minimize the incidence and severity of SOS clinical symptoms, and thereby increase survival. The present disclosure further relates to the use of these biomarkers and biomarker panels for prognosing, diagnosing, and/or treating SOS in a subject that has received or is receiving hematopoietic stem cell transplantation (HSCT).

[0023] The present disclosure uses examples to disclose the invention to enable any person skilled in the art to practice the invention, including making and using any panels or devices and performing any incorporated methods. The patentable scope of the invention is defined by the claims, and may include other examples that occur to those skilled in the art. Such other examples are intended to be within the scope of the claims if they have structural elements that do not differ from the literal language of the claims, or if they include equivalent structural elements with insubstantial differences from the literal language of the claims.

[0024] Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the ordinary meanings commonly understood by those of ordinary skill in the art to which this invention pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

A. Definitions

[0025] As used herein, the term "biomarker" refers to an indicator of, for example, a pathological state of a subject, which can be detected in a biological sample of the subject. Biomarkers include DNA-based, RNA-based and protein-based molecular markers.

[0026] As used herein, the term "diagnosis" refers to the identification or classification of a molecular or pathological state, disease or condition. For example, "diagnosis" can refer to identification of a particular type of a condition (such as sinusoidal obstruction syndrome ("SOS")).

[0027] As used herein, the term "aiding diagnosis" refers to methods that assist in making a clinical determination

regarding the presence, or nature, of a particular type of symptom of a condition (such as SOS). For example, a method of aiding diagnosis of a condition (such as SOS) can include measuring the expression of certain genes in a biological sample from an individual.

[0028] As used herein, the term "prognosis" is used herein to refer to the categorization of patients by degree of risk for a disease (such as SOS) or progression of such disease. A "prognostic marker" refers to an assay that categorizes patients by degree of risk for disease occurrence or progression.

[0029] As used herein, the term "sample" refers to a composition that is obtained or derived from a subject of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. For example, the phrase "disease sample" and variations thereof refers to any sample obtained from a subject of interest that would be expected or is known to contain the cellular and/or molecular entity that is to be characterized. A "tissue" or "cell sample" refers to a collection of similar cells obtained from a tissue of a subject or patient. The source of the tissue or cell sample may be blood or any blood constituents (e.g., whole blood, plasma, serum) from the subject. The tissue sample can also be primary or cultured cells or cell lines. Optionally, the tissue or cell sample is obtained from a disease tissue/organ. The tissue sample can contain compounds which are not naturally intermixed with the tissue in nature such as preservatives, anticoagulants, buffers, fixatives, nutrients, antibiotics, and the like.

[0030] As used herein, the terms "control", "control cohort", "reference sample", "reference cell", "reference tissue", "control sample", "control cell", and "control tissue" refer to a sample, cell or tissue obtained from a source that is known, or believed, to not be afflicted with the disease or condition for which a method or composition of the invention is being used to identify. The control can include one control or multiple controls. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy part of the body of the same subject or patient in whom a disease or condition is being identified using a composition or method of the invention. In one embodiment, a reference sample, reference cell, reference tissue, control sample, control cell, or control tissue is obtained from a healthy part of the body of an individual who is not the subject or patient in whom a disease or condition is being identified using a composition or method of the invention.

[0031] The term "antibody" is used in its broadest sense and specifically covers, for example, monoclonal antibodies, polyclonal antibodies, antibodies with polyepitopic specificity, single chain antibodies, multi-specific antibodies and fragments of antibodies. Such antibodies can be chimeric, humanized, human and synthetic.

[0032] The term "subject" is used interchangeably herein with "patient" to refer to an individual to be treated. The subject is a mammal (e.g., human, non-human primate, rat, mouse, cow, horse, pig, sheep, goat, dog, cat, etc.). The subject can be a clinical patient, a clinical trial volunteer, an experimental animal, etc. The subject can be suspected of having or at risk for having a condition (such as SOS) or be diagnosed with a condition (such as SOS). The subject can also be suspected of having or at risk for having SOS. According to one embodiment, the subject to be treated according to this invention is a human.

[0033] As used herein, "treating", "treatment" and "alleviation" refer to measures, wherein the object is to prevent or slow down (lessen) the targeted pathologic condition or disorder or relieve some of the symptoms of the disorder. Those in need of treatment can include those already with the disorder as well as those prone to have the disorder, those at risk for having the disorder and those in whom the disorder is to be prevented.

[0034] "Elevated expression level" and "elevated levels" refer to an increased expression of a mRNA or a protein in a patient (e.g., a patient suspected of having or diagnosed as having SOS) relative to a control, such as subject or subjects who are not suffering from SOS.

B. Methods of Prognosing

[0035] In one embodiment, the present disclosure is directed to a method of prognosing or of aiding in the prognosis of sinusoidal obstructive syndrome (SOS) in a subject receiving hematopoietic stem cell transplantation (HSCT). The method comprises: obtaining a biological sample from the subject; measuring in a biological sample from the subject, the expression of at least one biomarker selected from the group consisting of ST2, ANG2, L-Ficolin, HA, and VCAM1 by contacting the biological sample obtained from the subject with a specific binding agent that specifically binds to the biomarker, wherein the specific binding agent forms a complex with the biomarker; and detecting the agent-biomarker complex, thereby determining the biomarker expression level; wherein an elevated biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of a prognosis for shortened survival compared to median survival in a subject having SOS, and wherein a reduced biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of a prognosis for increased survival compared to median survival in a subject having SOS.

[0036] The specific binding agent can be selected from a nucleic acid, an antibody, a receptor, and a lectin.

[0037] The sample can be selected from liver tissue, whole blood, plasma and serum.

[0038] In some embodiments, the step of measuring includes contacting the biological sample with a biomarker panel comprising L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

[0039] The specific binding agent-biomarker complex can be detected using methods known to those skilled in the art such as, for example, microarray analysis, immunoassay, immunohistochemistry, and mass spectrometry. Representative immunoassays include Western blot analysis and ELISA.

[0040] It has been advantageously found that the biomarker panels used in the methods of the present disclosure can be used for prognosing SOS early after HSCT. Particularly, in some embodiments, the methods can be used to prognosis SOS the same day as HSCT. In other embodiments, prognosis can be made one week, two weeks, or three weeks from HSCT. Accordingly, the methods of prognosing SOS can include obtaining the biological sample at day 0 from HSCT, including obtaining the sample from day 0 to day 7 from HSCT, including obtaining the sample from day

0 to day 14 from HSCT, and including obtaining the sample from day 0 to day 21 from HSCT.

C. Methods of Diagnosing

[0041] In another embodiment, the present disclosure is directed to a method for diagnosing SOS in a subject, particularly a subject receiving hematopoietic stem cell transplantation (HSCT). The method comprises: measuring in a biological sample from the subject the expression of at least one biomarker selected from the group consisting of ST2, ANG2, L-Ficolin, HA, and VCAM1 by contacting the biological sample obtained from the subject with a specific binding agent that specifically binds to the biomarker, wherein the specific binding agent forms a complex with the biomarker; and detecting the agent-biomarker complex, thereby determining the biomarker expression level; wherein an elevated biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of SOS.

[0042] The specific binding agent can be selected from a nucleic acid, an antibody, a receptor, and a lectin.

[0043] The sample can be selected from whole blood and plasma.

[0044] In some embodiments, the step of measuring includes contacting the biological sample with a biomarker panel comprising tumorigenicity 2 (ST2), angiopoietin 2 (ANG2), L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

[0045] The specific binding agent-biomarker complex can be detected using methods known to those skilled in the art such as, for example, microarray analysis, immunoassay, immunohistochemistry, and mass spectrometry. Representative immunoassays include Western blot analysis and ELISA.

[0046] It has been advantageously found that the biomarker panels used in the methods of the present disclosure can be used for diagnosing SOS early after HSCT. Particularly, in some embodiments, the methods can be used to diagnose SOS the same day as HSCT. In other embodiments, diagnosis can be made one week, two weeks, or three weeks from HSCT. Accordingly, the methods of diagnosing SOS can include obtaining the biological sample at day 0 from HSCT, including obtaining the sample from day 0 to day 7 from HSCT, including obtaining the sample from day 0 to day 14 from HSCT, and including obtaining the sample from day 0 to day 21 from HSCT.

G. Biological Sample

[0047] The biological sample used in the methods of the present disclosure can be obtained using certain methods known to those skilled in the art. Biological samples may be obtained from vertebrate animals, and in particular, mammals. In certain instances, a biological sample is whole blood, plasma, or serum. By screening such body samples, a prognosis or diagnosis can be achieved for SOS.

[0048] As used in the various methods of the present disclosure, the terms "control", "control value", "reference" and "reference value" refer to an expression level value obtained from "control sample", "control cell", and "control tissue" "reference sample", "reference cell", and "reference tissue" obtained from a source that is known, or believed, to not be afflicted with the condition for which a method or composition is being used to identify. It is to be understood that the control need not be obtained at the same time as the biological sample of the subject is obtained. Thus, a control value for an expression level can be determined and used for comparison of the expression level for the biological sample of the subject or the biological samples of multiple subjects.

H. Detection of Biomarkers

[0049] Expression levels of proteins may be detected in samples of whole blood, plasma, or serum. Various methods are known in the art for detecting protein expression levels in such biological samples, including various immunoassay methods.

EXAMPLES

[0050] Materials and Methods

[0051] A. Patients and Samples

[0052] Three sets of HSCT patients were included in these Examples. Patients were treated at the University of Michigan, at Indiana University, and at University of Barcelona. All patients or their legal guardians provided written informed consent, and the study for post-HSCT complications samples collection was approved by the institutional review boards of the University of Michigan, Indiana University, and Hospital Clinic, University of Barcelona.

[0053] Heparinized blood samples were collected before or on the day of HCT, then weekly for 2 or 4 weeks after allogeneic HSCT, then monthly for 2 months, as well as at the time of key clinical events, including the onset of symptoms consistent with SOS. Plasma samples were collected prospectively per institutional guidelines.

[0054] 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 plated in 96-well V-bottom plates by manual pipetting. The plates were wrapped in parafilm and kept in a humid chamber at 4° C. during the entire process, which did not exceed 96 hours.

[0055] B. Proteomics Analysis

[0056] 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 in Faca V. et al., "Quantitative analysis of acrylamide labeled serum proteins by LC-MS/MS," J. Proteome Res. 2006: 5(8):2009-2018; Faca V. et al., "Contribution of protein fractionation to depth of analysis of the serum and plasma proteomes," J Proteome Res. 2007: 6(9):3558-3565; and Paczesny S. et al., "Elafin is a biomarker of graft-versus-host disease of the skin," Science Translational Medicine, 2010: 2(13):13ra12. The MS-based proteomics approach used in these Examples is illustrated in FIG. **1**.

[0057] C. Immunoassays

[0058] Suppressor of tumorigenicity 2 (ST2), angiopoietin2 (ANG2), L-Ficolin, hyaluronic acid (HA), vascular cell adhesion molecule 1 (VCAM1), tissue inhibitor of metalloproteinase 1 (TIMP1), thrombomodulin (sCD141), 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 antibody pairs used for these ELISAs were as follows: anti-ST2 (R&D Systems, Minneapolis, Minn.), anti-ANG2 (R&D Systems), anti-L-Ficolin (Hycult Biotech, Plymouth Meeting, Pa.), anti-HA (Corgenix, Broomfield, Colo.), 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, Calif.), anti-vWF (American Diagnostica, Stamford, Conn.), and anti-CD97 (R&D Systems).

[0059] Capture antibodies were reconstituted and diluted per manufacturers' specifications or pre-coated 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-μ1 or 100-μ1 aliquots of plasma samples (dilutions listed in Table 1) were added in duplicate to the ELISA test plates. In addition, 50-μ1 or 100-μ1 aliquots of reconstituted standard at different concentrations (see 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 (HRP) and HRP substrate. The optical density of each well was read using a plate reader set to 450-570 nm. The ELISAs were performed in duplicate and sequentially.

TABLE 1

ELISA parameters for the 11 tested proteins

	Standard curve range	Dilution factor	CV %	LLOD (optical density)	LLOD (concentration)
ST2	2000-31 pg/ml	1/50	3.30	0.06	6 pg/ml
ANG2	3000-47 pg/ml	1/10	10.95	0.11	18 pg/ml
L-Ficolin	1000-15 pg/ml	1/100	2.90	0.04	7 pg/ml
HA	800-50 ng/ml	NEAT	2.67	0.06	9 ng/ml
VCAM	1000-15 pg/ml	1/2000	4.41	0.01	12 pg/ml
TIMP1	2000-31 pg/ml	1/250	4.70	0.02	7 pg/ml
Thrombomodulin	20-0.62 ng/ml	1/4	6.60	0.21	0.50 ng/ml
ICAM	2000-31 pg/ml	1/500	0.93	0.03	23 pg/ml
PAI-1	5000-78 pg/ml	1/100	3.60	0.03	14 pg/ml
vWF	10-0.5 mU/ml	1/250	10.66	0.05	0.67 mU/ml
CD97	8000-125 pg/ml	1/100	12.73	0.03	100 pg/ml

CV: coefficient of variation; LLOD: lower limit of detection.

[0060] D. Statistical Analysis

[0061] The statistical methods used for the IPAS were previously described in Faca V. et al., J Proteome Res. 2006: 5(8):2009-2018; Faca V. et al., J Proteome Res. 2007: 6(9):3558-3565; and Paczesny S. et al., Science Translational Medicine. 2010: 2(13):13ra12. Differences in characteristics between patient groups were assessed with Kruska-Wallis tests for continuous values and chi-squared tests of association for categorical values. Protein concentrations from individual samples in the discovery and validation sets were compared using two sample t-tests. Receiver operating characteristic (ROC) areas under the curves (AUCs) were estimated nonparametrically. A ROC curve is a plot of the false positive rate on the x axis and true positive rate on the y axis for every possible level of a marker. A perfect test would have a ROC curve that is a right angle demonstrating 100% of true positives and no false positives. In this case, the corresponding Area Under the Curve (AUC) will equal to 1. A random test will have an AUC of 0.5 meaning there is one false positive for every true positive. 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, the differences in biomarkers trajectories were examined over time using a modeling approach.

[0062] E. Prognostic Bayesian Modeling

[0063] 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. This Naïve Bayes classifier was developed with Waikato Environment for Knowledge Analysis software v3.6.10.

Example 1

[0064] In this Example, proteomic analysis was conducted to compare plasma pooled from 20 patients with SOS to plasma pooled from 20 patients without SOS. The clinical characteristics of patients are provided in Table 2.

TABLE 2

Clinical characteristics of patients in the discovery set

Characteristic		Discovery Cohort SOS− (N = 20)	Discovery Cohort SOS+ (N = 20)	P	Training Cohort SOS− (N = 13)	Training Cohort SOS+ (N = 32)	P	Independent Verification Cohort SOS− (N = 22)	Independent Verification Cohort SOS+ (N = 13)	P
Age, years	Median	43	43	ns	45	16	0.02	29	8	0.06
	Range	3-56	1-58		3-55	1-58		1-66	1-48	
Disease, n (%)	Malignant*	19 (95)	17 (90)	ns	12 (92)	27 (84)	ns	22 (100)	13 (100)	ns
	Non-malignant†	1 (5)	2 (10)		1 (8)	5 (16)		0 (0)	0 (0)	
Donor type, n (%)	Related	18 (90)	17 (85)	ns	12 (92)	17 (53)	0.02	14 (64)	3 (33)	0.02
	Unrelated	2 (10)	3 (15)		1 (8)	5 (16)		8 (36)	10 (77)	
Donor match, n (%)	Matched	20 (100)	20 (100)	ns	13 (100)	25 (78)	0.08	18 (82)	47 (54)	ns
	Mismatched	0 (0)	0 (0)		0 (0)	7 (22)		4 (18)	6 (46)	
Conditioning regimen intensity, n (%)‡	Full	20 (100)	20 (100)	ns	13 (100)	32 (100)	ns	16 (73)	13 (100)	ns
	With Busulfan (16 mg/kg, 4 days)	14 (74)	17 (90)		9 (69)	26 (81)		1 (5)	3 (23)	
	With TBI	2 (10)	1 (5)		2 (15)	4 (12)		8 (36)	6 (46)	
GVHD prophylaxis regimen, n (%)	Tacro or CsA/MTX	19 (95)	18 (90)	ns	12 (92)	23 (72)	ns	5 (23)	5 (38)	ns
	With rapamycin	0 (0)	0 (0)		0 (0)	1 (3)		6 (27)	1 (8)	
	With MMF	0 (0)	0 (0)		0 (0)	7 (22)		4 (18)	4 (31)	
	Other§	1 (5)	2 (10)		1 (8)	1 (3)		1 (5)	0 (0)	
	NA	0 (0)	0 (0)		0 (0)	0 (0)		6 (27)	3 (23)	
Time after HCT to SOS onset, day	Median	na	14	na	na	11	na	na	9	na
	Range	na	4-37		na	4-63		na	5-23	
Time after HCT to SOS sample acquisition, day	Median	14	14	ns	14	11	ns	14	11	ns
	Range	7-41	7-37		7-41	4-63		7-14	5-23	
Future acute GVHD 2-4, n (%)	Yes	0 (0)	0 (0)	ns	0 (0)	14 (44)	0.004	0 (0)	6 (46)	0.0005
	No	20 (100)	20 (100)		13 (100)	18 (56)		22 (100)	7 (54)	
Time after HCT to GVHD onset, day	Median	na	na	na	na	33	na	na	21	na
	Range					14-75			(11-46)	

na: not applicable,

ns: not significant;

TBI: total body irradiation;

Tacro: tacrolimus;

CsA: cyclosporine A;

MTX: methotrexate;

MMF: mycophenolate mofetil

*Malignant disease includes acute leukemia/myelodysplastic syndrome (n = 69), lymphoma (n = 18), multiple myeloma (n = 2), chronic leukemia (n = 13), myelofibrosis (n = 2), and paroxysmal nocturnal hemoglobinuria (PNH) (n = 2), neuroblastoma (n = 3), rhabdoid tumor (n = 1), and carcinoid tumor (n = 1).

†Non-malignant disease includes severe aplastic anemia (n = 2), thalassemia (n = 3), sickle cell disease (n = 2), chronic granulomatous disease (n = 1), and familial lymphohistiocytosis (n = 1).

‡Full-intensity conditioning regimens include: cyclophosphamide/etoposide/carmustine (CVB) (n = 7), busulfan (Bu)/cyclophosphamide (Cy) (n = 35), BAC (Bu [16 mg/kg], cytarabine [8000 mg/m^2], and Cy [120 mg/kg] (n = 31), CyTBI (n = 21), fludarabine (Flu) or Clo + Bu (16 mg/kg) (n = 6), Busufan/Melphalan (n = 1), Flu/melphalan (n = 1), carboplatin/etoposide/melphalan (n = 4), carboplatin/thiotepa (n = 2), CyFlu (n = 4), and CyThiotepa (n = 2).

§Other GVHD prophylaxis included Tacro/corticosteroids (n = 3), MTX/corticosteroids (n = 2), Tacro/MTX/corticosteroids (n = 1).

[0065] 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 isotope ratio of 0.5 or less (see Table 3 for complete summary) From the identified proteins, six proteins were selected for further analysis: L-Ficolin, VCAM1, TIMP1, vWF, and CD97. These proteins were selected based on the observation of at least a 2-fold increase or decrease in the heavy/light isotope ratio and their involvement in networks possibly involved in the pathogenesis of SOS. In addition, five endothelial markers (ST2, ANG2, HA, thrombomodulin, and PAI-1) were analyzed based on their involvement in SOS.

TABLE 3

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00295857	COPA	coatomer subunit alpha.	87.5
IPI00028318	PHACTR1	isoform 1 of phosphatase and actin regulator 1.	74.9
IPI00004922	CMA1	chymase.	67.3

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00410333	TREML1	isoform 1 of trem-like transcript 1 protein precursor.	33.1
IPI00290035	PCDH15	protocadherin 15.	23.7
IPI00107155	TMEM103	isoform 1 of upf0405 protein tmem103.	21.8
IPI00410143	CENPM	isoform 2 of centromere protein m.	18.8
IPI00787049	DDT	similar to d-dopachrome decarboxylase.	15.7
IPI00012007	AHCY	adenosylhomocysteinase.	14.3
IPI00218407	ALDOB	fructose-bisphosphate aldolase b.	13.9
IPI00654755	HBB	hemoglobin subunit beta.	12.9
IPI00010290	FABP1	fabp1 protein (fragment).	11.8
IPI00307781	CHRDL2	isoform 2 of chordin-like protein 2 precursor.	10.5
IPI00793758	DCTN2	9 kda protein.	10.1
IPI00759493	SUCLG1	succinate-coa ligase, gdp-forming, alpha subunit.	9.9
IPI00400903	C2orf46	putative uncharacterized protein c2orf46.	9.7
IPI00306322	COL4A2	collagen alpha-2(iv) chain precursor.	9.7
IPI00792459	HSPA8	23 kda protein.	9.3
IPI00016832	PSMA1	isoform short of proteasome subunit alpha type 1.	9.0
IPI00152591	PGR	delta 4 progesterone receptor.	8.9
IPI00012828	ACAA1	3-ketoacyl-coa thiolase, peroxisomal precursor.	8.1
IPI00219446	PEBP1	phosphatidylethanolamine-binding protein 1.	8.0
IPI00218733	SOD1	16 kda protein.	7.0
IPI00301288	SVEP1	polydom.	6.9
IPI00216085	COX6B1	cytochrome c oxidase subunit vib isoform 1.	6.6
IPI00465436	CAT	catalase.	6.6
IPI00031008	TNC	isoform 1 of tenascin precursor.	6.5
IPI00024993	ECHS1	enoyl-coa hydratase, mitochondrial precursor.	6.2
IPI00020977	CTGF	isoform 1 of connective tissue growth factor precursor.	6.2
IPI00029039	REG3A	regenerating islet-derived protein 3 alpha precursor.	6.1
IPI00025698	LIMK2	lim domain kinase 2 isoform 1.	6.1
IPI00291006	MDH2	malate dehydrogenase, mitochondrial precursor.	6.1
IPI00219038	H3F3B	histone h3.3.	5.7
IPI00009440	CYP8B1	cytochrome p450 8b1.	5.6
IPI00029723	FSTL1	follistatin-related protein 1 precursor.	5.5
IPI00304962	COL1A2	collagen alpha-2(i) chain precursor.	5.4
IPI00299547	LCN2	neutrophil gelatinase-associated lipocalin precursor.	5.3
IPI00171516	PLVAP	plasmalemma vesicle-associated protein.	5.2
IPI00031086	IGFBP1	insulin-like growth factor-binding protein 1 precursor.	5.2
IPI00032292	TIMP1	metalloproteinase inhibitor 1 precursor.	4.8
IPI00011522	ACAA1	*homo sapiens* clone 23623.	4.7
IPI00306543	GDF15	growth/differentiation factor 15 precursor.	4.7
IPI00005038	HRSP12	ribonuclease uk114.	4.4
IPI00022417	LRG1	leucine-rich alpha-2-glycoprotein precursor.	4.4
IPI00216138	TAGLN	transgelin.	4.4
IPI00219025	GLRX	glutaredoxin-1.	4.4
IPI00028911	DAG1	dystroglycan precursor.	4.3
IPI00549467	NIT2	nitrilase family member 2.	4.3
IPI00418471	VIM	vimentin.	4.2
IPI00020687	SPINK1	pancreatic secretory trypsin inhibitor precursor.	4.1
IPI00745729	SELENBP1	53 kda protein.	4.1
IPI00027038	VSIG4	isoform 1 of v-set and immunoglobulin domain-containing protein 4precursor.	4.1
IPI00026154	PRKCSH	glucosidase 2 subunit beta precursor.	4.0
IPI00412546	CR1	complement receptor type 1 precursor.	4.0
IPI00000874	PRDX1	peroxiredoxin-1.	3.9
IPI00009027	REG1A	lithostathine 1 alpha precursor.	3.9
IPI00015881	CSF1	isoform 1 of macrophage colony-stimulating factor 1 precursor.	3.8
IPI00647950	DLG2	isoform 2 of discs large homolog 2.	3.8
IPI00549411	OR51E1/PSGR2	dresden-g-protein-coupled receptor.	3.8
IPI00019038	LYZ	lysozyme c precursor.	3.7
IPI00215746	FABP4	fatty acid-binding protein, adipocyte.	3.6
IPI00383032	HAVCR2	isoform 2 of hepatitis a virus cellular receptor 2 precursor.	3.6
IPI00305975	SPON2	spondin-2 precursor.	3.6
IPI00297646	COL1A1	collagen alpha-1(i) chain precursor.	3.4
IPI00020356	MAP1A	331 kda protein.	3.3

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00465248	ENO1	isoform alpha-enolase of alpha-enolase.	3.3
IPI00293276	MIF	macrophage migration inhibitory factor.	3.3
IPI00022892	THY1	thy-1 membrane glycoprotein precursor.	3.3
IPI00298388	PIK3IP1	isoform 1 of phosphoinositide-3-kinase-interacting protein 1precursor.	3.3
IPI00003933	HAGH	hydroxyacyl glutathione hydrolase isoform 1.	3.2
IPI00002324	MAT2B	isoform 1 of methionine adenosyltransferase 2 subunit beta.	3.2
IPI00011229	CTSD	cathepsin d precursor.	3.1
IPI00001528	IL18BP	isoform c of interleukin-18-binding protein precursor.	3.1
IPI00295741	CTSB	cathepsin b precursor.	3.1
IPI00298547	PARK7	protein dj-1.	3.0
IPI00039050	FOLR2	folate binding protein.	3.0
IPI00216318	YWHAB	isoform long of 14-3-3 protein beta/alpha.	3.0
IPI00022200	COL6A3	alpha 3 type vi collagen isoform 1 precursor.	3.0
IPI00465028	TPI1	triosephosphate isomerase 1 variant.	3.0
IPI00419990	PTCRA	pre-t-cell antigen receptor alpha.	2.9
IPI00219910	BLVRB	23 kda protein.	2.9
IPI00514310	F11R	isoform 4 of putative thiosulfate sulfurtransferase kat.	2.9
IPI00004656	B2M	beta-2-microglobulin precursor.	2.9
IPI00008298	DEFA5	defensin 5 precursor.	2.9
IPI00299412	CD97	isoform 2 of cd97 antigen precursor.	2.9
IPI00000144	OXT	oxytocin-neurophysin 1 precursor.	2.9
IPI00000335	HINT2	histidine triad nucleotide-binding protein 2.	2.8
IPI00008494	ICAM1	intercellular adhesion molecule 1 precursor.	2.7
IPI00002535	FKBP2	fk506-binding protein 2 precursor.	2.7
IPI00029658	EFEMP1	isoform 1 of egf-containing fibulin-like extracellular matrix protein1 precursor.	2.7
IPI00011302	CD59	cd59 glycoprotein precursor.	2.7
IPI00219018	GAPDH	glyceraldehyde-3-phosphate dehydrogenase.	2.7
IPI00018136	VCAM1	isoform 1 of vascular cell adhesion protein 1 precursor.	2.7
IPI00414784	CD300A	isoform 1 of cmrf35-h antigen precursor.	2.6
IPI00104074	CD163	isoform 1 of scavenger receptor cysteine-rich type 1 protein m130precursor.	2.6
IPI00032876	CYTL1	cytokine-like protein 1 precursor.	2.6
IPI00289275	CILP	cartilage intermediate layer protein 1 precursor.	2.6
IPI00013895	S100A11	protein s100-a11.	2.6
IPI00304483	PAIP2	polyadenylate-binding protein-interacting protein 2.	2.5
IPI00291170	KIAA1199	isoform 2 of protein kiaa1199 precursor.	2.5
IPI00023648	ISLR	immunoglobulin superfamily containing leucine-rich repeat.	2.5
IPI00022284	PRNP	major prion protein precursor.	2.5
IPI00014048	RNASE1	ribonuclease pancreatic precursor.	2.5
IPI00216298	TXN	thioredoxin.	2.5
IPI00472035	MICA	isoform 2 of hla class i histocompatibility antigen, cw-16 alpha chainprecursor.	2.5
IPI00334254	EGFLAM	egf-like, fibronectin type iii and laminin g domains isoform 2.	2.5
IPI00642816	SRP9	signal recognition particle 9 kda protein.	2.5
IPI00291488	WFDC2	isoform 1 of wap four-disulfide core domain protein 2 precursor.	2.5
IPI00375441	FUBP1	isoform 1 of far upstream element-binding protein 1.	2.4
IPI00016915	IGFBP7	insulin-like growth factor-binding protein 7 precursor.	2.4
IPI00103871	ROBO4	isoform 1 of roundabout homolog 4 precursor.	2.4
IPI00784257	FOLR2	folate receptor beta precursor.	2.4
IPI00301579	NPC2	epididymal secretory protein e1 precursor.	2.4
IPI00003440	CCL15	small inducible cytokine a15 precursor.	2.4
IPI00328550	THBS4	thrombospondin-4 precursor.	2.4
IPI00014953	NHLRC2	cdna flj20147 fis, clone col07954.	2.4
IPI00023014	VWF	von willebrand factor precursor.	2.3
IPI00062700	TIMD4	t-cell immunoglobulin and mucin domain-containing protein 4 precursor.	2.3
IPI00029863	SERPINF2	alpha-2-antiplasmin precursor.	2.3
IPI00294615	FBLN5	fibulin-5 precursor.	2.3

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00465439	ALDOA	fructose-bisphosphate aldolase a.	2.3
IPI00025846	DSC2	isoform 2a of desmocollin-2 precursor.	2.2
IPI00022426	AMBP	ambp protein precursor.	2.2
IPI00791848	SIPA1L3	similar to signal-induced proliferation-associated 1 like protein 3.	2.2
IPI00003375	CCL14	isoform hcc-1 of small inducible cytokine a14 precursor.	2.2
IPI00014263	EIF4H	isoform long of eukaryotic translation initiation factor 4h.	2.2
IPI00167498	C9orf93	isoform 2 of uncharacterized protein c9orf93.	2.2
IPI00021439	ACTB	actin, cytoplasmic 1.	2.2
IPI00215760	FMO5	dimethylaniline monooxygenase [n-oxide-forming] 5.	2.2
IPI00019449	RNASE2	nonsecretory ribonuclease precursor.	2.1
IPI00022974	PIP	prolactin-inducible protein precursor.	2.1
IPI00101678	KAZALD1	isoform 1 of kazal-type serine protease inhibitor domain-containingprotein 1 precursor.	2.1
IPI00297284	IGFBP2	insulin-like growth factor-binding protein 2 precursor.	2.1
IPI00179851	C11orf9	imp dehydrogenase/gmp reductase family protein.	2.1
IPI00296777	SPARCL1	sparc-like protein 1 precursor.	2.1
IPI00009521	MARCO	macrophage receptor marco.	2.1
IPI00293487	REC8	meiotic recombination protein rec8-like 1.	2.1
IPI00009822	SRP54	signal recognition particle 54 kda protein.	2.1
IPI00011385	LOXL3	lysyl oxidase homolog 3 precursor.	2.1
IPI00025426	PZP	pregnancy zone protein precursor.	2.1
IPI00027827	SOD3	extracellular superoxide dismutase [cu—zn] precursor.	2.1
IPI00031490	COLEC11	collectin sub-family member 11 isoform a.	2.1
IPI00299485	CD93	complement component c1q receptor precursor.	2.1
IPI00290856	XLKD1	lymphatic vessel endothelial hyaluronic acid receptor 1 precursor.	2.1
IPI00020990	OMD	osteomodulin precursor.	2.0
IPI00024915	PRDX5	isoform mitochondrial of peroxiredoxin-5, mitochondrial precursor.	2.0
IPI00019579	CFD	complement factor d precursor.	2.0
IPI00386854	HNRPA2B1	hnrpa2b1 protein.	2.0
IPI00008780	STC2	stanniocalcin-2 precursor.	2.0
IPI00025204	CD5L	cd5 antigen-like precursor.	2.0
IPI00299181	OR2F1	olfactory receptor 2f1.	2.0
IPI00007797	FABP5	fatty acid-binding protein, epidermal.	1.9
IPI00004480	ADAMDEC1	adam dec1 precursor.	1.9
IPI00215767	B4GALT1	isoform long of beta-1,4-galactosyltransferase 1.	1.9
IPI00018880	TNFRSF1A	tumor necrosis factor receptor superfamily member 1a precursor.	1.9
IPI00006608	APP	isoform app770 of amyloid beta a4 protein precursor (fragment).	1.9
IPI00024284	HSPG2	basement membrane-specific heparan sulfate proteoglycan core proteinprecursor.	1.9
IPI00328703	OAF	oaf homolog.	1.9
IPI00032293	CST3	cystatin-c precursor.	1.9
IPI00029260	CD14	monocyte differentiation antigen cd14 precursor.	1.9
IPI00011155	ASGR2	isoform 1 of asialoglycoprotein receptor 2.	1.9
IPI00218803	FBLN1	isoform b of fibulin-1 precursor.	1.8
IPI00419966	ABI3BP	isoform 2 of target of nesh-sh3 precursor.	1.8
IPI00029235	IGFBP6	insulin-like growth factor-binding protein 6 precursor.	1.8
IPI00292150	LTBP2	latent-transforming growth factor beta-binding protein 2 precursor.	1.8
IPI00550991	SERPINA3	isoform 1 of alpha-1-antichymotrypsin precursor.	1.8
IPI00023505	FCGR2A	low affinity immunoglobulin gamma fc region receptor ii-a precursor.	1.8
IPI00022810	CTSC	dipeptidyl-peptidase 1 precursor.	1.8
IPI00009802	VCAN	isoform v0 of versican core protein precursor.	1.8
IPI00305380	IGFBP4	insulin-like growth factor-binding protein 4 precursor.	1.8
IPI00015525	MMRN2	multimerin-2 precursor.	1.8
IPI00021891	FGG	isoform gamma-b of fibrinogen gamma chain precursor.	1.8

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00007067	C9orf19	golgi-associated plant pathogenesis-related protein 1.	1.8
IPI00219219	LGALS1	galectin-1.	1.7
IPI00028413	ITIH3	inter-alpha-trypsin inhibitor heavy chain h3 precursor.	1.7
IPI00023673	LGALS3BP	galectin-3-binding protein precursor.	1.7
IPI00003648	PVRL1	isoform delta of poliovirus receptor-related protein 1 precursor.	1.7
IPI00303161	ESAM	endothelial cell-selective adhesion molecule precursor.	1.7
IPI00332887	SIRPA	signal-regulatory protein alpha precursor.	1.7
IPI00021923	FAM3C	protein fam3c precursor.	1.7
IPI00011876	MTAP	s-methyl-5-thioadenosine phosphorylase.	1.7
IPI00019954	CST6	cystatin-m precursor.	1.7
IPI00022620	SLURP1	secreted ly-6/upar-related protein 1 precursor.	1.7
IPI00418262	ALDOC	fructose-bisphosphate aldolase c.	1.7
IPI00008580	SLPI	antileukoproteinase precursor.	1.7
IPI00298497	FGB	fibrinogen beta chain precursor.	1.7
IPI00294705	PAPLN	papilin.	1.7
IPI00296083	SFTPB	pulmonary surfactant-associated protein b precursor.	1.6
IPI00179164	KIAA1244	sec7-like domain containing protein.	1.6
IPI00294193	TMEM110	isoform 1 of inter-alpha-trypsin inhibitor heavy chain h4 precursor.	1.6
IPI00027983	CDA	cytidine deaminase.	1.6
IPI00299435	APOF	apolipoprotein f precursor.	1.6
IPI00022933	CD74	isoform long of hla class ii histocompatibility antigen gamma chain.	1.6
IPI00328746	RTN4RL2	reticulon-4 receptor-like 2 precursor.	1.6
IPI00026199	GPX3	glutathione peroxidase 3 precursor.	1.6
IPI00376353	ANKRD37	ankyrin repeat domain-containing protein 37.	1.6
IPI00013303	LSAMP	limbic system-associated membrane protein precursor.	1.6
IPI00297160	CD44	isoform 12 of cd44 antigen precursor.	1.6
IPI00007425	DSC1	desmocollin 1 isoform dsc1b preproprotein.	1.6
IPI00297444	CD177	isoform 1 of cd177 antigen precursor.	1.6
IPI00419585	PPIA	peptidyl-prolyl cis-trans isomerase a.	1.5
IPI00029699	RNASE4	ribonuclease 4 precursor.	1.5
IPI00013894	STIP1	stress-induced-phosphoprotein 1.	1.5
IPI00217481	GPR126	developmentally regulated g-protein-coupled receptor beta 1.	1.5
IPI00010295	CPN1	carboxypeptidase n catalytic chain precursor.	1.5
IPI00030871	VNN1	pantetheinase precursor.	1.5
IPI00303966	C6orf155	uncharacterized protein c6orf155.	1.5
IPI00021834	TFPI	isoform alpha of tissue factor pathway inhibitor precursor.	1.5
IPI00478816	SPINK5	serine protease inhibitor kazal-type 5 precursor.	1.5
IPI00148061	LDHAL6A	l-lactate dehydrogenase a-like 6a.	1.5
IPI00005142	FGFR1	isoform 1 of basic fibroblast growth factor receptor 1 precursor.	1.5
IPI00022429	ORM1	alpha-1-acid glycoprotein 1 precursor.	1.5
IPI00006988	RETN	resistin precursor.	1.5
IPI00030075	FGL2	fibroleukin precursor.	1.5
IPI00021885	FGA	isoform 1 of fibrinogen alpha chain precursor.	1.5
IPI00015102	ALCAM	isoform 1 of cd166 antigen precursor.	1.5
IPI00028030	COMP	cartilage oligomeric matrix protein precursor.	1.4
IPI00016112	PXDN	peroxidasin homolog.	1.4
IPI00334238	NPTXR	neuronal pentraxin receptor.	1.4
IPI00297412	CADPS	isoform 1 of calcium-dependent secretion activator 1.	1.4
IPI00220857	CAST	isoform 2 of calpastatin.	1.4
IPI00045600	DAB2IP	dab2 interacting protein isoform 1.	1.4
IPI00470535	CACNA2D1	dihydropyridine receptor alpha 2 subunit.	1.4
IPI00395488	VASN	vasorin precursor.	1.4
IPI00017601	CP	ceruloplasmin precursor.	1.4
IPI00176221	NEGR1	neuronal growth regulator 1 precursor.	1.4
IPI00374316	C6orf115	similar to protein c6orf115.	1.4
IPI00026183	CCL18	small inducible cytokine a18 precursor.	1.4
IPI00290283	MASP1	mannan-binding lectin serine protease 1 isoform 2 precursor.	1.4

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00027972	LILRA2	isoform 1 of leukocyte immunoglobulin-like receptor subfamily a member2 precursor.	1.4
IPI00299738	PCOLCE	procollagen c-endopeptidase enhancer 1 precursor.	1.4
IPI00303963	C2	complement c2 precursor (fragment).	1.4
IPI00374068	ADAMTSL4	isoform 1 of adamts-like protein 4 precursor.	1.4
IPI00291866	SERPING1	plasma protease c1 inhibitor precursor.	1.4
IPI00027507	CFHR3	complement factor h-related protein 3 precursor.	1.4
IPI00791350	CLEC3B	11 kda protein.	1.4
IPI00301143	PI16	isoform 1 of peptidase inhibitor 16 precursor.	1.4
IPI00020986	LUM	lumican precursor.	1.4
IPI00021842	APOE	apolipoprotein e precursor.	1.4
IPI00021578	CFHR4	complement factor h-related protein 4 precursor.	1.3
IPI00022418	FN1	isoform 1 of fibronectin precursor.	1.3
IPI00027166	TIMP2	metalloproteinase inhibitor 2 precursor.	1.3
IPI00644346	ADAMTSL2	adamts-like protein 2 precursor.	1.3
IPI00032258	C4A	complement c4-a precursor.	1.3
IPI00011651	PTPRG	isoform 1 of receptor-type tyrosine-protein phosphatase gammaprecursor.	1.3
IPI00396077	TOPORS	isoform 1 of e3 ubiquitin-protein ligase topors.	1.3
IPI00008433	RPS5	40s ribosomal protein s5.	1.3
IPI00029168	LPA	apolipoprotein.	1.3
IPI00216882	MASP1	mannan-binding lectin serine protease 1 isoform 3.	1.3
IPI00299150	CTSS	cathepsin s precursor.	1.3
IPI00003351	ECM1	extracellular matrix protein 1 precursor.	1.3
IPI00465322	BOC	121 kda protein.	1.3
IPI00218795	SELL	l-selectin precursor.	1.3
IPI00293565	FLT4	fms-related tyrosine kinase 4 isoform 1.	1.3
IPI00397717	SYCN	syncollin.	1.3
IPI00299307	MASP1	complement-activating component of ra-reactive factor precursor.	1.3
IPI00020091	ORM2	alpha-1-acid glycoprotein 2 precursor.	1.3
IPI00294713	MASP2	isoform 1 of mannan-binding lectin serine protease 2 precursor.	1.3
IPI00291316	ARHGEF2	rho/rac guanine nucleotide exchange factor (gef) 2.	1.3
IPI00478414	CHRDL1	ventroptin (fragment).	1.2
IPI00022395	C9	complement component c9 precursor.	1.2
IPI00004084	CREBL1	isoform 2 of cyclic amp-dependent transcription factor atf-6 beta.	1.2
IPI00329104	LILRA3	leukocyte immunoglobulin-like receptor subfamily a member 3 precursor.	1.2
IPI00296165	C1R	complement c1r subcomponent precursor.	1.2
IPI00015029	PTGES3	prostaglandin e synthase 3.	1.2
IPI00296608	C7	complement component c7 precursor.	1.2
IPI00006717	CCL16	small inducible cytokine a16 precursor.	1.2
IPI00478003	A2M	alpha-2-macroglobulin precursor.	1.2
IPI00006662	APOD	apolipoprotein d precursor.	1.2
IPI00025285	ATP6V1G1	vacuolar atp synthase subunit g 1.	1.2
IPI00009793	C1RL	complement c1r-like protein.	1.2
IPI00219861	ACP1	isoform 1 of low molecular weight phosphotyrosine protein phosphatase.	1.2
IPI00796830	A2M	13 kda protein.	1.2
IPI00604691	GPR157	hypothetical protein (fragment).	1.2
IPI00025864	BCHE	cholinesterase precursor.	1.2
IPI00003817	ARHGDIB	rho gdp-dissociation inhibitor 2.	1.2
IPI00006114	SERPINF1	pigment epithelium-derived factor precursor.	1.2
IPI00004373	MBL2	mannose-binding protein c precursor.	1.2
IPI00742705	MAP3K14	6 kda protein.	1.2
IPI00477992	C1QB	complement component 1, q subcomponent, b chain precursor.	1.2
IPI00011036	INHBE	inhibin beta e chain precursor.	1.2
IPI00019591	CFB	isoform 1 of complement factor b precursor (fragment).	1.2
IPI00007047	S100A8	protein s100-a8.	1.2
IPI00022895	A1BG	alpha-1b-glycoprotein precursor.	1.2
IPI00555812	GC	vitamin d-binding protein precursor.	1.2
IPI00000075	TGFB1	transforming growth factor beta-1 precursor.	1.1
IPI00027780	MMP2	72 kda type iv collagenase precursor.	1.1
IPI00414283	FN1	fibronectin 1 isoform 4 preproprotein.	1.1
IPI00000879	TXK	tyrosine-protein kinase txk.	1.1

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00298003	SEMA3F	semaphorin-3f precursor.	1.1
IPI00387168	PCSK9	isoform 1 of proprotein convertase subtilisin/kexin type 9 precursor.	1.1
IPI00029739	CFH	isoform 1 of complement factor h precursor.	1.1
IPI00011252	C8A	complement component c8 alpha chain precursor.	1.1
IPI00292530	ITIH1	inter-alpha-trypsin inhibitor heavy chain h1 precursor.	1.1
IPI00299059	CHL1	isoform 2 of neural cell adhesion molecule l1-like protein precursor.	1.1
IPI00022394	C1QC	complement c1q subcomponent subunit c precursor.	1.1
IPI00027774	THAP2	thap domain-containing protein 2.	1.1
IPI00006154	CFHR2	isoform long of complement factor h-related protein 2 precursor.	1.1
IPI00032328	KNG1	isoform hmw of kininogen-1 precursor.	1.1
IPI00738433	CPN2	similar to carboxypeptidase n subunit 2 precursor.	1.1
IPI00026314	GSN	isoform 1 of gelsolin precursor.	1.1
IPI00022371	HRG	histidine-rich glycoprotein precursor.	1.1
IPI00009028	CLEC3B	tetranectin precursor.	1.1
IPI00022488	HPX	hemopexin precursor.	1.1
IPI00294469	COQ4	ubiquinone biosynthesis protein coq4 homolog.	1.1
IPI00041065	HABP2	hyaluronan-binding protein 2 precursor.	1.1
IPI00017696	C1S	complement c1s subcomponent precursor.	1.0
IPI00742696	GC	vitamin d-binding protein precursor.	1.0
IPI00027396	HN1L	isoform 1 of protein cramped-like.	1.0
IPI00220327	KRT1	keratin, type ii cytoskeletal 1.	1.0
IPI00007244	MPO	isoform h17 of myeloperoxidase precursor.	1.0
IPI00218732	PON1	serum paraoxonase/arylesterase 1.	1.0
IPI00019576	F10	coagulation factor x precursor.	1.0
IPI00215894	KNG1	isoform lmw of kininogen-1 precursor.	1.0
IPI00291867	CFI	complement factor i precursor.	1.0
IPI00218192	ITIH4	isoform 2 of inter-alpha-trypsin inhibitor heavy chain h4 precursor.	1.0
IPI00792115	CLEC3B	hypothetical protein dkfzp686h17246.	1.0
IPI00375682	NRK	isoform 1 of nik-related protein kinase.	1.0
IPI00479116	CPN2	carboxypeptidase n subunit 2 precursor.	1.0
IPI00760855	TMEM110	101 kda protein.	1.0
IPI00019176	RARRES2	retinoic acid receptor responder protein 2 precursor.	1.0
IPI00064534	CIZ1	cdna flj14381 fis, clone hemba1001824, highly similar to *homo sapiens*nuclear protein np94 mrna.	1.0
IPI00021085	PGLYRP1	peptidoglycan recognition protein precursor.	1.0
IPI00303292	KPNA1	importin alpha-1 subunit.	1.0
IPI00654888	KLKB1	kallikrein b, plasma (fletcher factor) 1.	1.0
IPI00017841	OLFM1	isoform 1 of noelin precursor.	1.0
IPI00023314	INHBC	inhibin beta c chain precursor.	1.0
IPI00298860	LTF	growth-inhibiting protein 12.	1.0
IPI00305461	ITIH2	inter-alpha-trypsin inhibitor heavy chain h2 precursor.	1.0
IPI00004944	SLC4A10	isoform 1 of sodium-driven chloride bicarbonate exchanger.	1.0
IPI00296176	F9	coagulation factor ix precursor.	1.0
IPI00011264	CFHR1	complement factor h-related protein 1 precursor.	1.0
IPI00291262	CLU	clusterin precursor.	1.0
IPI00218413	BTD	biotinidase precursor.	1.0
IPI00007199	SERPINA10	protein z-dependent protease inhibitor precursor.	1.0
IPI00005721	DEFA1	neutrophil defensin 1 precursor.	1.0
IPI00009920	C6	complement component c6 precursor.	1.0
IPI00006543	CFHR5	complement factor h-related 5.	1.0
IPI00019568	F2	prothrombin precursor (fragment).	1.0
IPI00011261	C8G	complement component c8 gamma chain precursor.	1.0
IPI00783987	C3	complement c3 precursor (fragment).	1.0
IPI00235003	FAS	tumor necrosis factor receptor superfamily, member 6 isoform 1 variant	1.0
IPI00022431	AHSG	alpha-2-hs-glycoprotein precursor.	0.9
IPI00032179	SERPINC1	antithrombin iii variant.	0.9
IPI00643525	C4A	complement component 4a.	0.9
IPI00164623	C3	187 kda protein.	0.9
IPI00171678	DBH	dopamine beta-hydroxylase precursor.	0.9

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00795830	AHSG	29 kda protein.	0.9
IPI00010402	SH3BGRL3	hypothetical protein.	0.9
IPI00293925	FCN3	isoform 1 of ficolin-3 precursor.	0.9
IPI00479186	PKM2	isoform m2 of pyruvate kinase isozymes m1/m2.	0.9
IPI00027235	ATRN	isoform 1 of attractin precursor.	0.9
IPI00029061	SEPP1	selenoprotein p precursor.	0.9
IPI00012503	PSAP	isoform sap-mu-0 of proactivator polypeptide precursor.	0.9
IPI00298828	APOH	beta-2-glycoprotein 1 precursor.	0.9
IPI00007240	F13B	coagulation factor xiii b chain precursor.	0.9
IPI00031392	CARD14	caspase recruitment domain protein 14 isoform 2.	0.9
IPI00019530	TIE1	tyrosine-protein kinase receptor tie-1 precursor.	0.9
IPI00032291	C5	complement c5 precursor.	0.9
IPI00418163	C4B	complement component 4b preproprotein.	0.9
IPI00004372	MEP1A	meprin a subunit alpha precursor.	0.9
IPI00294395	C8B	complement component c8 beta chain precursor.	0.9
IPI00029236	IGFBP5	insulin-like growth factor-binding protein 5 precursor.	0.9
IPI00022229	APOB	apolipoprotein b-100 precursor.	0.9
IPI00030739	APOM	apolipoprotein m.	0.9
IPI00242956	FCGBP	iggfc-binding protein precursor.	0.9
IPI00008556	F11	isoform 1 of coagulation factor xi precursor.	0.9
IPI00645051	BBS1	bbs1 protein.	0.8
IPI00789477	LTF	73 kda protein.	0.8
IPI00022331	LCAT	phosphatidylcholine-sterol acyltransferase precursor.	0.8
IPI00298971	VTN	vitronectin precursor.	0.8
IPI00009938	CEACAM1	isoform a of carcinoembryonic antigen-related cell adhesion molecule 1precursor.	0.8
IPI00744286	B3GALNT2	isoform 2 of udp-galnac: beta-1,3-n-acetylgalactosaminyltransferase 2.	0.8
IPI00022420	RBP4	plasma retinol-binding protein precursor.	0.8
IPI00021727	C4BPA	c4b-binding protein alpha chain precursor.	0.8
IPI00019580	PLG	plasminogen precursor.	0.8
IPI00296840	POLI	dna polymerase iota.	0.8
IPI00297655	NOTCH2	neurogenic locus notch homolog protein 2 precursor.	0.8
IPI00021364	CFP	properdin precursor.	0.8
IPI00001754	F11R	junctional adhesion molecule a precursor.	0.8
IPI00025862	C4BPB	isoform 1 of c4b-binding protein beta chain precursor.	0.8
IPI00328113	FBN1	fibrillin-1 precursor.	0.8
IPI00292218	MST1	hepatocyte growth factor-like protein precursor.	0.8
IPI00163207	PGLYRP2	isoform 1 of n-acetylmuramoyl-l-alanine amidase precursor.	0.8
IPI00024825	PRG4	isoform a of proteoglycan-4 precursor.	0.8
IPI00023019	SHBG	isoform 1 of sex hormone-binding globulin precursor.	0.8
IPI00220249	LTBP1	latent-transforming growth factor beta-binding protein, isoform 1lprecursor.	0.8
IPI00013418	BIRC2	baculoviral iap repeat-containing protein 2.	0.7
IPI00019943	AFM	afamin precursor.	0.7
IPI00216691	PFN1	profilin-1.	0.7
IPI00011255	GP1BA	platelet glycoprotein ib alpha chain precursor.	0.7
IPI00382606	F7	factor vii active site mutant immunoconjugate.	0.7
IPI00007634	LIMS1	lim and senescent cell antigen-like-containing domain protein 1.	0.7
IPI00004798	CRISP3	cysteine-rich secretory protein 3 precursor.	0.7
IPI00657788	LAIR1	30 kda protein.	0.7
IPI00655676	PRG4	isoform d of proteoglycan-4 precursor.	0.7
IPI00220644	PKM2	isoform m1 of pyruvate kinase isozymes m1/m2.	0.7
IPI00001611	IGF2	isoform 1 of insulin-like growth factor ii precursor.	0.7
IPI00432707	CASP12	caspase-12.	0.7
IPI00021854	APOA2	apolipoprotein a-ii precursor.	0.7
IPI00294250	EPHA1	ephrin type-a receptor 1 precursor.	0.7
IPI00168459	PHLDB2	isoform 2 of pleckstrin homology-like domain family b member 2.	0.7
IPI00294004	PROS1	vitamin k-dependent protein s precursor.	0.7
IPI00021817	PROC	vitamin k-dependent protein c precursor.	0.7

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00217405	UBR1	isoform 1 of e3 ubiquitin-protein ligase ubr1.	0.7
IPI00005439	FETUB	fetuin-b precursor.	0.6
IPI00012011	CFL1	cofilin-1.	0.6
IPI00170692	VAPA	vesicle-associated membrane protein-associated protein a.	0.6
IPI00018305	IGFBP3	insulin-like growth factor-binding protein 3 precursor.	0.6
IPI00002714	DKK3	dickkopf-related protein 3 precursor.	0.6
IPI00220257	TTLL1	isoform 3 of probable tubulin polyglutamylase.	0.6
IPI00296713	GRN	isoform 1 of granulins precursor.	0.6
IPI00220901	TBC1D4	tbc1 domain family member 4.	0.6
IPI00027255	MYL6B	myosin light polypeptide 6b.	0.6
IPI00168262	GLT25D1	cdna psec0241 fis, clone nt2rp3000234, moderately similar to *homosapiens* cerebral cell adhesion molecule mrna.	0.6
IPI00011194	FGFBP2	fibroblast growth factor-binding protein 2 precursor.	0.6
IPI00001610	IGF1	insulin-like growth factor ia precursor.	0.6
IPI00011832	SPP2	secreted phosphoprotein 24 precursor.	0.5
IPI00008603	ACTA2	actin, aortic smooth muscle.	0.5
IPI00232895	DGAT2L6	diacylglycerol o-acyltransferase 2-like protein 6.	0.5
IPI00550363	TAGLN2	transgelin-2.	0.5
IPI00020996	IGFALS	insulin-like growth factor-binding protein complex acid labile chainprecursor.	0.5
IPI00550533	MLLT11	uncharacterized protein c1orf56.	0.5
IPI00292532	CAMP	antibacterial protein fall-39 precursor.	0.5
IPI00019581	F12	coagulation factor xii precursor.	0.5
IPI00017530	FCN2	ficolin-2 precursor or LFicolin	0.5
IPI00385595	TMPRSS12	transmembrane protease, serine 12.	0.5
IPI00395667	IFRD2	interferon-related ifrd2 (pc4-b) protein.	0.5
IPI00027843	PROZ	isoform 1 of vitamin k-dependent protein z precursor.	0.5
IPI00655976	PRG4	isoform c of proteoglycan-4 precursor.	0.4
IPI00301058	VASP	vasodilator-stimulated phosphoprotein.	0.4
IPI00328748	ARMET	armet protein precursor.	0.4
IPI00477597	HPR	isoform 1 of haptoglobin-related protein precursor.	0.4
IPI00473014	DSTN	destrin.	0.4
IPI00102923	FAM108A1	protein fam108a1.	0.4
IPI00060181	EFHD2	ef-hand domain-containing protein 2, swiprosin-1	0.4
IPI00302592	FLNA	filamin a, alpha.	0.4
IPI00401283	MEGF9	multiple epidermal growth factor-like domains 9 precursor.	0.4
IPI00019848	HCFC1	isoform 1 of host cell factor.	0.4
IPI00022445	PPBP	platelet basic protein precursor.	0.4
IPI00007750	TUBA4A	tubulin alpha-1 chain.	0.4
IPI00335280	RPE	isoform 1 of ribulose-phosphate 3-epimerase.	0.4
IPI00010414	PDLIM1	pdz and lim domain protein 1.	0.4
IPI00022731	APOC4	apolipoprotein c-iv precursor.	0.4
IPI00022295	PF4V1	platelet factor 4 variant precursor.	0.4
IPI00289876	STX7	isoform 1 of syntaxin-7.	0.3
IPI00017891	APC2	adenomatosis polyposis coli 2 protein.	0.3
IPI00009309	CCL5	small inducible cytokine a5 precursor.	0.3
IPI00253323	ANKRD57	ankyrin repeat domain-containing protein 57.	0.3
IPI00790010	GULP1	gulp, engulfment adaptor ptb domain containing 1.	0.3
IPI00168877	HELB	helicase (dna) b.	0.3
IPI00022446	PF4	platelet factor 4 precursor.	0.3
IPI00217537	ASXL1	isoform 1 of putative polycomb group protein asxl1.	0.3
IPI00029193	HGFAC	hepatocyte growth factor activator precursor.	0.3
IPI00746107	TRIM35	isoform 2 of tripartite motif-containing protein 35.	0.3
IPI00786924	MFSD7	similar to b0416.5a.	0.3
IPI00641826	THOC6	isoform 2 of the complex subunit 6 homolog.	0.3
IPI00179589	MTPN	myotrophin.	0.3
IPI00185326	FBXL10	isoform 1 of jmjc domain-containing histone demethylation protein 1b.	0.3
IPI00010164	C21orf91	protein eurl homolog.	0.3
IPI00298994	TLN1	271 kda protein.	0.3
IPI00794328	TPD52	8 kda protein.	0.3
IPI00554497	NHS	isoform 3 of nance-horan syndrome protein.	0.3
IPI00552243	TMEM1/TRAPPC1	hypothetical protein dkfzp667i0321 (fragment).	0.3

TABLE 3-continued

Complete list of genes identified by MS-based proteomics in pooled plasma from SOS patients

International Protein Index	Gene name	Gene Description	Ratio (mean)
IPI00386763	ADAMTS9	isoform 1 of adamts-9 precursor.	0.3
IPI00008453	CORO1C	coronin-1c.	0.2
IPI00155729	PLXNB3	plexin-b3 precursor.	0.2
IPI00334190	STOML2	stomatin-like protein 2.	0.2
IPI00292056	PIK3C2B	phosphatidylinositol-4-phosphate 3-kinase c2 domain-containing betapolypeptide.	0.2
IPI00656092	PRG4	isoform f of proteoglycan-4 precursor.	0.2
IPI00017921	BICC1	isoform 2 of protein bicaudal c homolog 1.	0.2
IPI00008274	CAP1	adenylyl cyclase-associated protein 1.	0.2
IPI00292817	KIAA1462	novel protein.	0.2
IPI00022432	TTR	transthyretin precursor.	0.2
		similar to calcium/calmodulin-dependent protein kinase type 1bki beta) (pregnancy upregulated non-ubiquitously expressed cam kinasehomolog).	
IPI00550276	PNCK	splice isoform 2.	0.2
IPI00164719	KIAA1432	protein kiaa1432.	0.2
IPI00329345	SPATS2	spats2 protein.	0.2
IPI00647939	C6orf148	cdna flj30329 fis, clone brace2007201.	0.2
IPI00783169	F12	coagulation factor xii.	0.2
IPI00019383	GALK1	galactokinase.	0.1
IPI00298347	PTPN11	isoform 2 of tyrosine-protein phosphatase non-receptor type 11.	0.1
IPI00299608	PSMD1	isoform 1 of 26s proteasome non-atpase regulatory subunit 1.	0.1
IPI00015983	EDG3	sphingosine 1-phosphate receptor edg-3.	0.1
IPI00442264	ZNF195	cdna flj16258 fis, clone hsyra2005628, moderately similar to zincfinger protein 195.	0.1
IPI00642639	LAMA3	5 kda protein.	0.1
IPI00168627	CXorf20	uncharacterized protein cxorf20.	0.1
IPI00023456	CHRM3	muscarinic acetylcholine receptor m3.	0.1
IPI00480027	KIAA0649	1a6/drim (down-regulated in metastasis) interacting protein.	0.1
IPI00646555	ZNF452	protein znf452.	0.1
IPI00027193	CLIC5	isoform 2 of chloride intracellular channel protein 5.	0.1
IPI00014287	FOLR3	folate receptor 3 precursor.	0.0
IPI00747210	NBPF1	conserved hypothetical protein.	0.0
IPI00032534	GTPBP2	21 kda protein.	0.0
IPI00106882	ZNF692	isoform 1 of zinc finger protein 692.	0.0

Example 2

[0066] In this Example, the biomarkers identified in Example 1 were further analyzed in plasma using sequential ELISAs from a validation set of 45 patients: 32 SOS patients at disease onset (days+14 to +21 post-HSCT) and from 13 time-matched controls.

[0067] The clinical characteristics of patients in this validation set are described in Table 1. Further, diagnosis samples from SOS+ patients that were taken at the time of SOS onset were used and samples from SOS− patients were selected so that both groups of samples were balanced according to time of acquisition. The clinical characteristics of patients in this training cohort are described in Table 1. The SOS− and SOS+ groups were balanced for age, primary disease, donor type (related versus unrelated), donor match, and intensity of the conditioning regimen (all full intensity with most receiving 16 mg/kg busulfan for 4 days or total body irradiation). More than 90% of patients received GVHD prophylaxis of methotrexate and tacrolimus (or cyclosporine) of standard duration. The value of these proteins as diagnostic biomarkers of SOS were analyzed using two-sample t-tests and by calculating the AUCs of the ROCs, which represent the false positive and true positive rates for every possible level of a marker.

[0068] ST2, ANG2, L-Ficolin, HA, VCAM1, TIMP1, sCD141, ICAM1, and PAI-1 were identified as diagnostic biomarkers of SOS with p-values ranging from <0.001 to 0.04 and with AUCs between 0.91 and 0.70 (FIGS. **2**A-**2**H). The composite ROC of markers ST2, ANG2, L-Ficolin, HA, and VCAM1 had an AUC of 0.98 (95% confidence interval, 0.94-1.00; FIG. **3**). Addition of TIMP1, thrombomodulin, and ICAM1 to the biomarker panel did not improve this AUC value (data not shown). Because ST2 has been shown to correlate with the development of acute GVHD, its prognostic value in the training and independent cohorts was evaluated. In these 2 cohorts, approximately 45% of SOS patients later developed GVHD (median number of days to onset of 33 and 21 versus 11 and 9 for SOS in the training and independent cohorts, respectively). ST2 plasma concentrations at day 14 after HCT (when almost all SOS patients have already developed clinical signs of SOS) did not differ between the SOS+ GVHD− and SOS+ GVHD+ groups, meaning that for SOS cases, ST2 is a diagnostic marker of SOS and this is more important than its prognostic value for future GVHD.

Example 3

[0069] In this Example, the prognostic significance of the biomarkers identified in Example 2 was analyzed using Wicoxon Rank-Sum analysis of protein levels measured before presentation of the clinical signs (days 0 and +7 post-HSCT). Three diagnostic biomarkers were also determined to be prognostic before clinical signs were apparent (L-Ficolin, HA, and VCAM1; AUC: 0.83-0.69), and the corresponding AUC values for biomarker values on the day of HCT were between 0.84 and 0.70 (FIGS. **4**A-**4**C). Modeling of these biomarkers' trajectories showed significant differences between the SOS− and SOS+ groups (FIGS. **5**A-**5**C). These results indicated that biomarkers of innate immune response, mitochondrial clearance, and leukocyte-endothelial cell adhesion in the sinusoidal endothelial cells of the liver are altered prior to the clinical signs of SOS and can be detected as early as the day of HSCT (day 0).

Example 4

[0070] In this Example, three biomarkers (L-Ficolin, HA, and VCAM1) identified as prognostic biomarkers in Example 3 were validated as prognostic biomarkers in an independent set of 35 patients from the Indiana University HSCT biobank (13 patients with SOS; 22 patients without SOS). The prognostic significance of these biomarkers was analyzed using Wilcoxon Rank-Sum analysis of their plasma levels measured before the clinical signs (days 0, +7 post-HSCT, and +14 post-HSCT). Further, plasma levels of these markers measured pre-transplant showed no difference suggesting that the conditioning regimen (i.e., intense chemotherapy+/−total body irradiation to prepare the subject for its graft) explain the levels seen at day 0. Particularly, the conditioning regimen is conducted between day −7 (pre-sample (i.e., samples taken before the conditioning regimen)) and day −1. At day 0, the donor cells were injected before the graft was injected. Thus, the only difference between the day −7 and day 0 samples is the conditioning regimen.

[0071] In this smaller set, L-Ficolin remained a strong prognosis marker as early as the day of transplant with an AUC of 0.88. Of note, the three markers were highly significant at day 14 (median day of onset) (see FIGS. **6**A-**6**C).

[0072] L-Ficolin, HA, and VCAM1 were then tested as prognostic markers of SOS with samples taken before the appearance of clinical signs of SOS. L-Ficolin and HA also stratified patients at risk for SOS as early as the day of HCT in this independent cohort (FIGS. **6**A-**6**C. Modeling of these biomarkers trajectories showed significant differences between the SOS+ and SOS− groups for L-Ficolin and HA but not for VCAM1 (FIGS. **7**A-**7**C). Notably, for most patients in this cohort, in addition to the day 0 and day 7 samples, samples collected before the conditioning were included, and plasma levels of L-Ficolin, and HA measured before transplantation did not differ between the SOS− and SOS+ groups. Therefore, these results strongly suggest that levels of these biomarkers are altered during the conditioning regimen and before the appearance of clinical signs of SOS, as they can be detected as early as the day of HCT.

Example 5

[0073] In this Example, a Naïve Bayes classifier implemented in Waikato Environment for Knowledge Analysis (WEKA) was developed for SOS prognosis based on a balanced subset of 24 patients (11 SOS−; 13 SOS+). The classifier performance was evaluated by doing a 10-fold cross-validation.

[0074] Naïve Bayes is an algorithm that is based on Bayes rule of probability. It combines all attributes to maximize the probability of a correct prediction for an outcome. It works by calculating the probabilities for each attribute and then multiplying them.

[0075] Infogain is an attribute selection algorithm that evaluates each attribute separately by calculating their information gain with respect to the outcome.

[0076] 10-fold Cross-Validation: a technique that partitions the dataset into 10-folds. Each fold is held out for testing or validating the model and the remainder is used for learning or building the model.

[0077] The modeling strategy used both models (see FIG. **8**):

[0078] 1) The infogain algorithm with 10-fold cross-validation that resulted in the selection of the most informative attributes.

[0079] 2) The Naïve Bayes model with 10-fold cross-validation that resulted in the generation of the final prediction.

[0080] The attributes tested were:

[0081] 1) VCAM-1, L-Ficolin, HA (day 0 and slope)

[0082] 2) Age at SOS Onset

[0083] 3) Gender

[0084] 4) Donor Type (RD, URD)

[0085] 5) Match (yes, no)

[0086] 6) Transplant Period (≤2005=0, >2005=1)

[0087] 7) Transplant number out of total transplantation (one=0, more than one=1)

[0088] 8) Conditioning Regimen:

[0089] a. TBI inclusion

[0090] b. Busulfan inclusion

[0091] c. Cyclophosphamide inclusion

[0092] Three different groups of patients were evaluated:

[0093] 1) Subset 1 was an imbalanced dataset (8 SOS− versus 20 SOS+) that included some missing day 0 biomarker information,

[0094] 2) Subset 2 was a balanced dataset (11 SOS− versus 13 SOS+) that included complete clinical and biomarker information, and

[0095] 3) subset 3 was a balanced dataset (21 SOS− versus 20 SOS+) that included some missing day 0 biomarker information.

[0096] The balanced subset 2 with no missing attribute information was selected to build the prognostic model. This selection was based on results comparing the correct prognosis between the 3 subsets tested and their corresponding ROC AUCs (Table 4).

TABLE 4

Naïve Bayes classifier results stratified by 10-fold cross-validation (subset comparison)

	Subset One* (n = 28)	Subset Two** (n = 24)	Subset Three*** N = 42)
Correct Prediction	71.43%	83.33%	73.17%
ROC AUC (Yes)	0.856	0.902	0.831
False Positive	1	1	2
False Negative	7	3	9

*Dataset was imbalanced (8 SOS− vs 20 SOS+). Includes some missing biomarker day 0 plasma concentrations.

**Dataset was balanced (11 SOS− vs 13 SOS+). Attribute information is complete (i.e., no missing data for any attribute).

The clinical characteristics of patients in this set are presented in Table 5.

TABLE 5

Clinical characteristics of patients in the Bayesian model development set

Characteristic		SOS− (n = 11)	SOS+ (n = 13)
Age, years	Median	49	14
	Range	3-55	2-58
Gender	Male	5 (45)	10 (77)
	Female	6 (55)	3 (23)
Transplantation period, n (%)	2005 or before	8 (73)	9 (69)
	After 2005	3 (27)	4 (31)
Transplantation number, n (%)	1	10 (91)	11 (85)
	>1	1 (9)	2 (15)
Donor type, n (%)	Related/Auto	10 (91)	8 (62)
	Unrelated	1 (9)	5 (38)
Donor match, n (%)	Matched/Auto	11 (100)	9 (69)
	Mismatched	0 (0)	4 (31)
Conditioning regimen type, n (%)	Chemotherapy only	9 (82)	11 (85)
	Chemotherapy + TBI	2 (18)	2 (15)
Busulfan in conditioning regimen, n (%)	Yes	9 (82)	10 (77)
	No	2 (18)	3 (23)
Cyclophosphamide in conditioning regimen, n (%)	Yes	9 (82)	11 (85)
	No	2 (18)	2 (15)

The model was evaluated using plasma concentrations of biomarkers on day 0 with and without the addition of the clinical characteristics. Table 6 shows the results (correct prognosis and false negatives and positives) of the model building using the selected data subset. The correct prognosis was achieved in 83.3% of patients using the day 0 plasma biomarker concentrations in addition to clinical attributes (ROC AUC=0.90).

TABLE 6

Naïve Bayes Classifier Results Stratified by Ten-fold Cross-Validation

	Clinical Characteristics + Biomarkers	Biomarkers	Clinical Characteristics
Correct prognosis	83.3%	70.8%	58.3%
ROC AUC (yes)	.90	.83	.61
False positive	1	1	4
False negative	3	6	6

[0097] The results of the infogain (Table 7) showed that in all groups the biomarkers at day 0 or the biomarker slopes provided the best infogain.

TABLE 7

Infogain

Unbalanced (with missing values, only validation set)	Balanced (no missing values, only validation set)	Balanced (validation and independent sets)
L-Ficolin imputed day 0	HA slope	HA slope
L-Ficolin day 0	HA day 0	L-Ficolin imputed day 0
VCAM-1 day 0	VCAM-1 day 0	HA day 0
HA slope	VCAM-1 slope	L-Ficolin day 0
HA imputed day 0		VCAM-1 day 0
HA day 0		
Match	Match	Match
BU in CONREG	Donor type	Donor type
Transplantation number	Gender	CONREG
Donor type	Transplantation number	BU in CONREG
Gender	BU in CONREG	Transplantation period
CONREG	CY in CONREG	Gender
CY in CONREG	CONREG	Transplantation number
Transplantation period	Transplantation period	CY in CONREG
		Age at BMT

Example 6

[0098] In this Example, the diagnostic and prognostic values of the biomarkers were analyzed in an independent prospective set of 16 patients from the Indiana University HSCT biobank (6 patients with SOS; 10 patients without SOS). The basic and clinical characteristics of patients in this independent set are presented in Table 8. Despite the small sample size, the results further validated L-Ficolin and HA as diagnostic (AUC: 0.83 and 0.75, respectively) and prognostic markers of SOS.

TABLE 8

Clinical characteristics of patients in the independent set

Characteristic		SOS− (N = 10)	SOS+ (N = 6)	P
Age, years	Median	37	5	0.06
	Range	1-66	1-19	
Disease, n (%)	Malignant*	10 (100)	6 (100)	ns
	Non-malignant§	0 (0)	0 (0)	
Donor type, n (%)	Related/Auto	8 (80)	3 (50)	ns
	Unrelated/Cord	2 (20)	3 (50)	
Donor match, n (%)	Matched/Auto	10 (100)	4 (66)	ns
	Mismatched	0 (0)	2 (34)	
Conditioning regimen intensity, n (%)	Full‡	10 (100)	6 (100)	ns
	With Busulfan	0 (0)	2 (34)	
	With TBI	0 (0)	2 (34)	
SOS onset day	Median	na	11	na
	Range	na	7-23	
Sample day post-HSCT	Median	14	11	ns
	Range	na	7-23	

na: not applicable,

ns: not significant

*Malignant disease includes acute leukemia/MDS (n = 7), lymphoma (n = 1), chronic leukemia (n = 1), neuroblastoma (n = 3), rhabdoid tumor (n = 1) and carcinoid tumor (n = 1)

‡Full-Intensity conditioning regimens include: BuCy (n = 1), BAC (n = 15), CyTBI (n = 2), FluBu (n = 1), Fludarabine/Melphalan (n = 1), Carboplatin/Etoposide/Melphalan (n = 4), Carboplatin/Thiotepa (n = 2), CyFlu (n = 4), and CyThiotepa (n = 2)

[0099] Based on the foregoing Examples, for the first time, biomarkers of SOS in plasma samples from patients undergoing allogeneic HSCT were identified. In addition to identifying a panel of biomarkers that can be used for SOS diagnosis (i.e., together ST2, ANG2, L-Ficolin, HA, and VCAM1 represent a biomarker panel for reliable, non-invasive diagnosis of SOS (AUC=0.98)), a panel of three biomarkers (L-Ficolin, HA, and VCAM1) were identified

that can be used to evaluate the risk of developing SOS before clinical signs appear, even as early as the day of HSCT. L-Ficolin, HA, and VCAM1 can stratify patients at risk of SOS as early as the day of HSCT, which has therapeutic consequences including potential preemptive interventions. L-Ficolin's mechanism of action implicates pathways in SOS other than those related to hemostasis and endothelial injury.

[0100] These results demonstrate that SOS can be diagnosed based on a panel of biomarkers in plasma as well as predicted as early as the day of HSC infusion in patients. The identified markers represent several pathways, including pathways suspected to be involved in hemostasis and endothelial injury, as well as novel pathways related to innate immunity and homeostatic clearance of mitochondria. Analyses using the biomarker panels provide preemptive intervention to minimize the incidence and severity of SOS clinical symptoms, and thereby increase survival.

[0101] Bayesian Modeling Discussion

[0102] Bayesian modeling infers causal relationships between molecular interactions by randomly generating many possible network models and using statistical techniques to select a consensus model that best fits the data. Thus, these methods balance the trade-off between prior knowledge and the data. A Bayesian model was developed to confirm the value of the prognostic biomarker panels to risk-stratify the patients for SOS with a more unbiased approach. The high sensitivity and specificity of the biomarkers identified in the present disclosure make them useful for real-time clinical testing and early clinical intervention.

[0103] A proposed SOS preemptive clinical study is presented in FIG. **9**. Biomarker cutoffs can be used to risk-stratify patients at low- or high-risk for developing SOS before presentation of the clinical signs. Low-risk patients will receive no preemptive intervention, whereas high-risk patients will be randomized to receive either a standard SOS intervention (defibrotide) or no intervention. A comparison of outcomes from the randomized high-risk groups will show whether the preemptive intervention reduces the incidence of SOS in high-risk patients identified according to the developed biomarker panel. The expectation is that subclinical SOS can be effectively managed via early treatment.

What is claimed is:

1. A diagnostic biomarker panel comprising suppressor of tumorigenicity 2 (ST2), angiopoietin 2 (ANG2), L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

2. A prognosis biomarker panel comprising L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

3. A method of diagnosing or of aiding diagnosis of sinusoidal obstructive syndrome (SOS) in a subject receiving hematopoietic stem cell transplantation (HSCT), the method comprising:

measuring in a biological sample from the subject the expression of at least one biomarker selected from the group consisting of ST2, ANG2, L-Ficolin, HA, and VCAM1 by contacting the biological sample obtained from the subject with a specific binding agent that specifically binds to the biomarker, wherein the specific binding agent forms a complex with the biomarker; and

detecting the agent-biomarker complex, thereby determining the biomarker expression level; wherein an elevated biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of SOS.

4. The method of claim **3** wherein the biological sample is obtained at day 0 from HSCT.

5. The method of claim **3** wherein the biological sample is obtained from day 0 to day 7 from HSCT.

6. The method of claim **3** wherein the biological sample is obtained from day 0 to day 14 from HSCT.

7. The method of claim **3** wherein the biological sample is obtained from day 0 to day 21 from HSCT.

8. The method of claim **3** wherein the specific binding agent is selected from the group consisting of a nucleic acid, an antibody, a receptor, and a lectin.

9. The method of claim **3** wherein the biological sample is selected from the group consisting of whole blood and plasma.

10. The method of claim **3** wherein detecting the specific binding agent-biomarker complex is selected from the group consisting of microarray analysis, immunoassay, immunohistochemistry, and mass spectrometry.

11. The method of claim **3** wherein the measuring comprises contacting the biological sample with a biomarker panel comprising tumorigenicity 2 (ST2), angiopoietin 2 (ANG2), L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

12. A method of prognosing or of aiding prognosis of sinusoidal obstructive syndrome (SOS) in a subject receiving hematopoietic stem cell transplantation (HSCT), the method comprising:

measuring in a biological sample from the subject the expression of at least one biomarker selected from the group consisting of ST2, ANG2, L-Ficolin, HA, and VCAM1 by contacting the biological sample obtained from the subject with a specific binding agent that specifically binds to the biomarker, wherein the specific binding agent forms a complex with the biomarker; and

detecting the agent-biomarker complex, thereby determining the biomarker expression level; wherein an elevated biomarker expression level compared to biomarker expression obtained from a biological sample obtained from a control is indicative of a prognosis for a subject having SOS.

13. The method of claim **12** wherein the biological sample is obtained at day 0 from HSCT.

14. The method of claim **12** wherein the biological sample is obtained from day 0 to day 7 from HSCT.

15. The method of claim **12** wherein the biological sample is obtained from day 0 to day 14 from HSCT.

16. The method of claim **12** wherein the biological sample is obtained from day 0 to day 21 from HSCT.

17. The method of claim **12** wherein the specific binding agent is selected from the group consisting of a nucleic acid, an antibody, a receptor, and a lectin.

18. The method of claim **12** wherein the biological sample is selected from the group consisting of whole blood, plasma and serum.

19. The method of claim **12** wherein detecting the specific binding agent-biomarker complex is selected from the group consisting of microarray analysis, immunoassay, immunohistochemistry, and mass spectrometry.

20. The method of claim **12** wherein the measuring comprises contacting the biological sample with a biomarker panel comprising L-Ficolin, hyaluronic acid (HA) and vascular cell adhesion molecule 1 (VCAM1).

* * * * *

专利名称(译)	检测窦状隙阻塞综合征（SOS）的方法		
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申请(专利权)人(译)	印第安纳大学研究与科技股份有限公司		
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[标]发明人	PACZESNY SOPHIE		
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摘要(译)

公开了用于在造血干细胞移植（HSCT）后早期评估具有窦性阻塞综合征（SOS）风险的受试者的生物标志物组。特别地，本公开涉及ST2，ANG2，L-纤维胶凝蛋白，HA和VCAM1中的一种或多种用于预后，诊断和/或治疗SOS的用途。