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Determination of burn patient outcome by large-scale quantitative discovery proteomics

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Abstract

Objective—Emerging proteomics techniques can be used to establish proteomic outcome signatures and to identify candidate biomarkers for survival following traumatic injury. We

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applied high-resolution liquid chromatography-mass spectrometry (LC-MS) and multiplex cytokine analysis to profile the plasma proteome of survivors and non-survivors of massive burn injury to determine the proteomic survival signature following a major burn injury.

Design—Proteomic discovery study.

Setting—Five burn hospitals across the U.S.

Patients—Thirty-two burn patients (16 non-survivors and 16 survivors), 19–89 years of age, were admitted within 96 h of injury to the participating hospitals with burns covering >20% of the total body surface area and required at least one surgical intervention.

Interventions—None.

Measurements and Main Results—We found differences in circulating levels of 43 proteins involved in the acute phase response, hepatic signaling, the complement cascade, inflammation, and insulin resistance. Thirty-two of the proteins identified were not previously known to play a role in the response to burn. IL-4, IL-8, GM-CSF, MCP-1, and β 2-microglobulin correlated well with survival and may serve as clinical biomarkers.

Conclusions—These results demonstrate the utility of these techniques for establishing proteomic survival signatures and for use as a discovery tool to identify candidate biomarkers for survival. This is the first clinical application of a high-throughput, large-scale LC-MS-based quantitative plasma proteomic approach for biomarker discovery for the prediction of patient outcome following burn, trauma or critical illness.

Keywords

burn; inflammation; proteomic profiling; plasma proteins; LC-MS; biomarker

INTRODUCTION

Burn injury is the most severe form of trauma, accounting for ~330,000 deaths per year worldwide (1); this devastating injury affects nearly every organ system and leads to significant morbidity and mortality (2). The dearth of biomarkers for early assessment of outcome following burn, trauma, or critical illness forces clinicians to rely on parameters such as heart rate, hypotension, and hyperglycemia to monitor the patient's clinical trajectory. These measures do not allow prediction of patient outcome. Discovery of a biomarker or proteomic signature comprised of the expression of a series of proteins would enable early assessment of patient risk for succumbing to burn, trauma, or critical illness. This signature would facilitate early identification of patients requiring aggressive therapy to improve their chances of survival.

The development of new high-throughput proteomic technologies facilitates the identification of protein profiles that can serve as diagnostic biomarkers or expression signatures for early assessment of predicted outcome as a result of disease or injury. Because burns, trauma, and critical illness involve multiple organs, a reliable high-throughput method to assess the systemic response—such as profiling the plasma proteome—would aid in identification of biomarkers, therapeutic targets, proteins with unexpected participation in outcome, and outcome signatures. Measurement of these proteins may be complicated by variable protein abundance due to biological heterogeneity, a broad dynamic range covering more than 10 orders of magnitude, and the complexity of the plasma proteome (3). Despite recent advances in liquid chromatography-mass spectrometry (LC-MS) approaches that have the potential to compensate for these confounders, this platform is often not robust enough to offer the high reproducibility required for large-scale clinical studies (3). Therefore, an effective method for evaluating the reproducibility among the large number of biological

analytes is essential. Here, we describe the novel application of quantitative, large-scale proteomic profiling via LC-MS coupled with multiplex cytokine measurement to establish a plasma proteome survival signature and to discover new candidate plasma biomarkers for survival of a severe burn injury. The use of an ^{18}O -labeled “universal” reference sample enables quantitation with concurrent evaluation of reproducibility, while allowing the simultaneous integration of a labeled approach with the label-free approach for quantitation (4). Furthermore, we propose that proteomic outcome signatures may reveal new drug targets in the form of proteins or pathways previously unknown to play a role in the disease or injury process and that a patient's proteomic survival signature and outcome trajectory may be altered via therapeutic intervention.

MATERIALS AND METHODS

Patients

The study, a part of the Inflammation and the Host Response Glue Grant, was approved by the Institutional Review Boards of the University of Texas Medical Branch (Galveston, TX), Loyola University Medical College (Chicago, IL), University of Texas Southwestern (Dallas, TX), University of Washington Seattle (Seattle, WA), and Massachusetts General Hospital (Boston, MA). Seventy-five patients who were enrolled from 2000–2005 and met the following criteria were considered for inclusion in this study: 19–89 years of age, admitted within 96 h after injury to the participating hospitals, and had burns covering more than 20% of the total body surface area requiring at least one surgical intervention. After admission, patients were treated according to the standard of burn care established by this consortium (5). Each subject or family member signed a written informed consent form. Blood was drawn between post-burn days 0 and 19 for plasma isolation. Demographics (age, dates of burn and admission, gender, burn size, and depth of burn) and concomitant injuries such as inhalation injury, infection, morbidity, and mortality were recorded prospectively throughout hospital course. The patients were retrospectively divided into two groups—survivors and non-survivors. Survivors and non-survivors were matched based on age, burn size, burn mechanism, sex, and ethnicity. Patients in both groups arrived at the participating hospitals at similar post-burn times, received similar hospital care, and had a similar time from burn to resuscitation.

Sample Handling

The protocol calls for blood drawn at the time of surgical intervention on an approximate weekly basis. Peripheral blood samples were collected prior to the induction of anesthesia and processed within one hour of being drawn as previously described (6, 7). To minimize the effect of temporal expression, we selected blood samples drawn 7 days or more prior to death and chose a sample from a similar time point (post-burn days 0–19) for the matched surviving patient. One non-surviving patient had a single plasma sample at post-burn day 19, so that sample was used.

Quantitative Proteomic Strategy

An integrated LC-MS-based proteomic strategy that incorporates an ^{18}O -labeled “universal” reference sample for achieving reliable quantitation was used to profile patient plasma (4) (Supplemental Fig. 1 [Supplemental Digital Content 1]). The ^{18}O -labeled “universal” reference sample served as a comprehensive internal standard in each sample, enabling isotope labeling-based quantification of relative protein abundances in patients relative to the reference and facilitating better evaluation of the platform reproducibility for normalization of the label-free abundance data. The label-free and isotope labeling-based dual quantitation approaches can be applied simultaneously to take advantage of their complementarity to achieve robust quantitation.

The overall dynamic measurement range was enhanced by integrating immunoaffinity chromatography for removing the 12 most abundant proteins in human plasma (8) and a cysteinyl-peptide enrichment-based fractionation at the peptide level (9) with an automated high-resolution LC-MS system. The overall throughput of analysis is only slightly compromised since the strategy only produces two fractions per patient sample. Peptide features detected by LC-MS are identified by matching the detected accurate masses and normalized elution times to a pre-established accurate mass and time (AMT) tag database applying the AMT tag strategy (10). The peptide AMT tag database was established based on an extensive LC-MS/MS survey of pooled trauma patients as previously described (11); the database includes the calculated masses and normalized elution times for all identified peptides so that the database serves as a “look-up table” for identifying peptides from LC-MS analyses using the AMT tag approach. Quantitative information for each identified feature was extracted based on either isotope $^{16}\text{O}/^{18}\text{O}$ ratios or label-free ^{16}O MS-intensities.

Plasma Protein Processing and Liquid Chromatography-Mass Spectrometric Analysis

The initial protein concentration for each sample was determined by Coomassie protein assay (Pierce). A reference peptide sample was generated by pooling 100 μL of plasma from each patient. The patient samples and the pooled reference sample were individually depleted of the 12 most abundant plasma proteins (albumin, IgG, α 1-antitrypsin, IgA, IgM, transferrin, haptoglobin, α 1-acid glycoprotein, α 2-macroglobulin, apolipoprotein A-I, apolipoprotein A-II, and fibrinogen) using a prepacked ProteomeLabTM IgY-12 affinity LC-10 column (Beckman Coulter) on an Agilent 1100 series HPLC system (Agilent). The flow-through fractions were then individually concentrated using iCON concentrators (Pierce) and digested with trypsin. Immunoaffinity depletion and plasma protein digestion were performed as previously described (8).

The peptides from the reference sample were labeled by stable isotope ^{18}O via trypsin-catalyzed ^{18}O labeling as previously described (10). Identical aliquots of labeled reference peptides were then mixed with an equal amount of each individual patient sample so that each final processed patient sample contained an identical reference sample. Each mixed patient sample was then fractionated as described (11). All final peptide fractions were then analyzed using a fully automated custom-built capillary LC platform coupled on-line using an in-house-manufactured ESI interface to an 11.5 Tesla Fourier transform ion cyclotron resonance (FTICR) mass spectrometer similar to as previously described (10).

Proteomic Data Processing

Analyses of datasets obtained from quantitative LC-Fourier transform ion cyclotron resonance were performed as previously described (10, 12). The AMT tag database was established based on those peptides and proteins previously identified in the trauma patient plasma proteome (11). Peptides were identified in the AMT tag database based on the normalized elution times and accurate mass measurements within a 2.5 ppm mass error and a 2% normalized elution time error. All identified peptides were reported for their $^{16}\text{O}/^{18}\text{O}$ isotopic ratios in addition to the label-free ^{16}O -MS intensities. The $^{16}\text{O}/^{18}\text{O}$ ratio data and label-free intensity data were processed separately. The median of $^{16}\text{O}/^{18}\text{O}$ ratios (log2 scale) was normalized to zero to correct potential errors in mixing the labeled reference and patient samples. The label-free intensity data were also normalized across the patients using the central tendency global normalization technique (13). The data from cysteinyl and non-cysteinyl fractions for each patient were combined, and the corresponding proteins or protein groups determined using the software tool ProteinProphet (14). Two separate datasets were generated for statistical analysis of the proteomic difference. One dataset

contained $^{16}\text{O}/^{18}\text{O}$ ratios for each detected peptide from each patient, and the other dataset contained normalized label-free intensities.

Orthogonal Validation

β 2-Microglobulin, Apolipoprotein A, Antithrombin, and Plasminogen were analyzed using a nephelometric technique on the Plasma Protein Analyzer BN II (Dade Behring) according to the manufacturer's instructions. Complement C8 and C9 were analyzed using a radial immunodiffusion technique (The Binding Site) according to the manufacturer's instructions. Factor X and prothrombin were analyzed by ELISA (Hyphen Biomed) according to the manufacturer's instructions.

Statistical Analysis of Proteomic Data

Patient (label-free ^{16}O intensity) data were filtered to remove peptides that mapped to more than one protein group and peptides that were not observed in more than half of the samples from at least one of the patient groups. The intensities were then scaled to the protein concentration and normalized to the reference intensities. The resulting matrix was then permuted 1,000 times, and the intensity difference between non-survivors and survivors was calculated in each permutation. The number of times the observed ranked difference exceeded the null difference was used to generate a p value. Principal components analysis was performed using peptide features from the significant proteins.

Plasma Cytokine Measurement

Plasma concentrations of 22 cytokines were measured using the Linco Research multiplex bead array and analyzed using MiraiBio software. Unpaired Student's t-tests were used to compare differences in cytokine expression.

Pathway Analysis

Protein annotations were obtained through a review of National Center for Biotechnology Information databases and the Ingenuity Pathways Knowledge Base (www.ingenuity.com). The Ingenuity Pathways Knowledge Base was used to overlay the concentration changes of significant proteins on the genomic and proteomic networks of previously known interactions between human orthologs (6).

RESULTS

Patients

Of the 75 patients meeting the study criteria, 19 were non-survivors. Two who died in nursing homes after discharge and one who died the day of injury were excluded from the analysis to minimize variation in time after injury and prior to death. There were no significant differences between non-survivors (n=16) and matched survivors (n=16) in age, percent third-degree burn, burn mechanism, or sex (Table 1). Although there was a small but significant difference in total burn size (total body surface area burned: $63 \pm 21\%$, non-survivors vs. $49 \pm 16\%$, survivors, $p=0.03$), the full thickness burn extent was comparable. Non-surviving patients died within 32 ± 19 days of burn. Causes of death are shown in Supplemental Table 1 (Supplemental Digital Content 1). Eight patients died of multiple organ failure, and 8 patients of cardiac dysfunction; sepsis was a cause or co-cause of death in 9 patients.

Liquid Chromatography-Mass Spectrometry Platform Performance

We performed a total of 64 LC-MS analyses. The assessment of platform reproducibility based on the pair-wise correlation of ^{18}O -labeled reference sample is shown in

Supplemental Fig. 2 (Supplemental Digital Content 1). Pair-wise correlation of ^{18}O -labeled reference peptide intensities between any two patient samples had an average correlation of 0.93 ± 0.03 , indicating good reproducibility (Supplemental Fig. 2a [Supplemental Digital Content 1]). Significantly higher variations were observed between patient peptide intensities with an average correlation of 0.75 ± 0.11 . Comparison of the results from isotope labeling ($^{16}\text{O}/^{18}\text{O}$) and the label-free approach revealed good overall correlation (Supplemental Fig. 2b [Supplemental Digital Content 1]).

The estimated dynamic range for this platform is $\sim 10^5$, allowing the detection of concentrations of ~ 500 ng/mL (3). Low-abundance proteins (1–100 ng/mL) were detected and included platelet factor 4, cathepsin d, metalloproteinase inhibitor 1, insulin-like growth factor binding protein 2, insulin-like growth factor 1, and hepatocyte growth factor-like protein.

Identification of Proteins through Liquid Chromatography-Mass Spectrometry

A total of 4,163 different peptides corresponding to 602 different plasma proteins were identified (strategy outlined in Supplemental Fig. 1 [Supplemental Digital Content 1]). Thirty-nine proteins were differentially detected in survivors and non-survivors (Table 2). Fold changes (non-survivors to survivors) were calculated for the 201 proteins that were detected in samples from both survivors and non-survivors (Table 3).

Unsupervised methods (e.g., hierarchical clustering or principal components analyses) did not demonstrate separation according to survival, indicating that other factors aside from survival determine the majority of differences in protein abundance in the dataset. The 39 proteins that were significantly different between the cohorts clustered according to survival.

Cytokine Differences between Non-survivors and Survivors Detected by Multiplex Technology

Cytokine measurement revealed that IL-4, IL-8, GM-CSF, and MCP-1 were significantly higher in non-survivors than in survivors ($p < 0.05$ for all, Fig. 1a–d). There were no differences in circulating levels of the 18 additional cytokines (Supplemental Table 2 [Supplemental Digital Content 1]).

Biological Implications of the Proteomic Survival Signature

The 43 proteins (39 identified by mass spectrometry and 4 by multiplex cytokine analysis) were used to create a proteomic survival signature. To determine which biological pathways are implicated by the proteins expressed differentially between the two patient populations, we examined the 43 proteins using the Ingenuity Pathway Knowledge Base. The proteins were associated with the following biological functions (n): inflammatory disease (13), cell movement (15), hematological system development and function (17), immune response (16), cell death (13), immune and lymphatic system function and development (12), tissue development (14), molecular transport (15), and cell signaling (16). We further examined signaling pathways to identify the tissues involved in the systemic response to burn. The main canonical pathways that were affected were the coagulation cascade, the complement response, hepatic acute-phase response signaling, and inflammatory cytokine pathways. Proteins in the coagulation cascade were down-regulated (factor 2, factor 13B, plasminogen, SERPINC1), while factor 10 was up-regulated in non-survivors. Proteins active in the complement cascade were down-regulated in non-survivors (Complement components 9, 8A, 8B, and complement factor 1). Proteins active in acute phase response signaling and/or inflammatory cytokine signaling were detected at higher levels in non-survivors (IL-4, IL-8, CCL2), while SERPINA3, factor 2, plasminogen, and complement component 9 were more abundant in survivors. In the glucocorticoid receptor signaling pathways, CCL2, CSF2,

IL-4, and IL-8 were up-regulated in non-survivors. The activity of the proteins in the complement cascade, coagulation cascade, acute phase response pathway, and cytokine signaling suggest that the response of the liver may be crucial to survival following a burn injury. Use of a networking tool that permits visualization of interactomes revealed that 13 of the 43 proteins interact directly with each other; IL-8, CCL-2, and F2 are the central molecules through which IL-4, LPA, CD44, plasminogen, SERPINC1, PPBP, HAP2, F10, CSF2, and B2M are linked (Supplemental Fig. 3 [Supplemental Digital Content 1]), suggesting that therapies designed to modulate the expression or interactions of these proteins might affect patient outcome.

Orthogonal Candidate Biomarker Measurement

We measured a subset of the LS-MS-identified proteins to determine whether standard clinical laboratory techniques could be used to detect differences in protein abundance between surviving and non-surviving patients. β 2-microglobulin, apolipoprotein A, factor X, prothrombin, complement C8 and C9, anti-thrombin 3, and plasminogen were selected as candidates for orthogonal validation based on the p values and peptide-fold change data. Beta-2 microglobulin was found to be decreased in non-survivors using nephelometry ($p < 0.05$), confirming the LC-MS data. Given the small sample sizes and sample preprocessing steps in the LC-MS not used in the clinical laboratory, confirmation of one of the proteins to a statistically significant degree was notable.

DISCUSSION

After severe burn, the major causes of death, particularly in adult and elderly patients, are infectious and septic complications resulting from organ dysfunction. The post-burn hypermetabolic response frequently leads to multi-organ failure and septic complications, increasing morbidity and mortality in the burn patient population. The hypermetabolic response is driven by systemic inflammation mediated by cytokines and acute-phase proteins. Although the burn size differed between the patient groups, the severity of the burn was the same. Several studies have demonstrated that the third-degree component is more important for the prediction of outcome than is the total body surface area burned (1, 16–18). Our data confirm this and strongly support the central role of the liver in survival from a severe burn injury.

Forty-three proteins were significantly altered in the plasma proteome of non-survivors compared to survivors, including coagulation proteins, adhesion molecules, inflammatory markers, metabolic markers, and hepatic acute-phase proteins. Although this is the first time that many of these proteins have been associated with a burn injury, the physiological processes that they represent are required for recovery from any disease or traumatic injury. The response to a burn injury requires an intact coagulation cascade for survival. Hypercoagulation with subsequent increased consumption of coagulation factors leads to disseminated intravascular coagulation, which is characterized by decreased levels of plasminogen and coagulation factor XIII in non-survivors. Therapeutic modulation of hypercoagulability may improve burn survival, as suggested by the clinical trial of drotrecogin alfa, recombinant activated C in patients with septic shock (19, 20). Hypercoagulation and disseminated intravascular coagulation occur alongside increased inflammation and abundance of adhesion molecules. Vascular cell adhesion molecule 1, a major cell adhesion molecule, is markedly increased in non-survivors immediately post-burn. White blood cells adhere to the endothelium and subsequently diapedese through the blood vessel wall, releasing inflammatory molecules such as metalloproteinases and cytokines into the tissue. Inflammatory markers such as TIMP-1, GM-CSF, IL-4, IL-8, and MCP-1 are up-regulated in non-survivors, indicating a greater inflammatory response in non-survivors and confirming our results from a genomic analysis of white blood cells

(*Lancet*, under review). These markers are also associated with insulin resistance and metabolic derangements. Markers of insulin resistance such as retinol binding protein 4, sex hormone binding globulin, CD-14, glycosylphosphatidylinositol specific phospholipase D1, and pro-platelet basic protein were increased in non-survivors. The complement system is also deranged in non-survivors. Significant decreases in complement component 1, complement factor D, complement 8, and complement 9 in non-survivors further support the notion that hyperinflammatory and hyperimmune responses are modulated differently in non-survivors, ultimately contributing to immune compromise, subsequent infection, and septic complications.

The majority of the 43 proteins associated with burn survival are produced in the liver, supporting the hypothesis that the liver plays a central role in the post-burn response as well as in determining survival. The liver's metabolic, inflammatory, immune, and acute phase functions play a pivotal role in recovery from injury by modulating multiple pleiotropic pathways. The hepatic acute-phase response is characterized by an increase in production of acute phase proteins coupled with a failure to produce constitutive proteins. This shift in hepatic protein synthesis leads to physiologic alterations of transport, metabolic, and immune functions, which may prove to be useful for predicting survival. A study of 31,338 burn patients demonstrated that healthy liver function is essential for survival following traumatic injury (21). A sub-group analysis of 180 patients with liver disease prior to the burn injury revealed a 6–8-fold increase in mortality with pre-existing liver dysfunction was. Our study supports this conclusion and further suggests that the secretion of liver-derived proteins can be used as predictive markers for survival or as part of the proteomic survival signature in severely burned patients. Furthermore, the differentially expressed proteins indicate that hepatic insulin resistance accompanies post-burn peripheral insulin resistance. That insulin therapy may be acting to overcome hepatic insulin resistance may explain why insulin therapy improves morbidity and mortality in severely burned as well as critically ill patients (22–24).

Our study demonstrates that the integrated LC-MS-based quantitative proteomic discovery approach can be used to successfully identify candidate proteins for classification of patient outcomes, whether for use in establishing proteomic outcomes signatures or to distinguish proteins that will serve as potential biomarkers or targets for therapeutic intervention. The plasma proteomics-based discovery efforts have traditionally been challenged by lack of reproducibility of quantitative results, low dynamic range of detection, and low sample throughput. The present large-scale quantitative strategy was enabled by the incorporation of a stable isotope labeled “universal” reference sample into each patient sample, which provided an effective means for assessing the reproducibility of large-scale sample analyses (4). The labeled reference sample also enabled the application of isotope-labeling-based quantitation across an unlimited number of samples, distinguishing this technique from traditional labeling approaches, which are always limited to pair-wise comparisons or comparison between a few conditions. The simultaneous integration of the two quantitative approaches into a single strategy also enhanced the overall confidence of the quantitative data by providing cross-validation between the two approaches. The current quantitative strategy can also be effectively integrated with the immunoaffinity chromatography and cysteinyl-peptide enrichment-based limited fractionation with high resolution LC-MS to offer relatively high dynamic range measurements ($>10^5$) for achieving good proteome coverage and the throughput necessary for analyzing a large number of samples in a relatively short time. Low molecular weight and low abundance proteins such as cytokines cannot be effectively detected via LC-MS methods. By augmenting the LCMS technique with multiplex cytokine analysis, we can reliably detect the plasma proteome alterations that accompany the response to injury. However, because our cytokine assay only measures a select number of known cytokines, the likelihood of missing expression differences for other

non-measured cytokines, or additional low molecular weight or low abundance proteins, is highly likely. Development of techniques to adequately assess these classes of proteins would allow full interrogation of the post-burn plasma proteome.

Because a huge immuno-inflammatory response accompanies a burn, trauma, or critical illness, high-throughput multiplex cytokine measurement was performed to determine whether complementary information regarding protein abundance could be used to build the proteomic survival signature. Pro-inflammatory cytokines have been suggested as potential biomarkers for outcome and as targets for interventional trials. Multiplex cytokine measurement has been used to successfully predict patient outcomes as well (25, 26), although attempts to alter cytokine expression to date have failed to improve patient outcomes. This combination of quantitative proteomic strategies has allowed us to quantitatively measure more than 600 proteins in samples from 32 patients; 43 proteins were identified as significantly differing between the patient cohorts, demonstrating a role for discovery proteomics in treating critical illness.

One drawback to this study was the small patient numbers. This was due to the lack of non-survivors enrolled in the program, as the intent to treat the patient is one of the enrollment criteria. Additional studies with larger patient numbers will be necessary to confirm the results reported here and to further develop this LC-MS approach. Future efforts may focus on determining whether the plasma protein profiles vary based on the cause of death. At this point, however, the numbers of patients are too small. Moreover, in light of the potential demonstrated in our small patient cohort, studies are being currently conducted with larger patient numbers to investigate outcomes that are not as clearly determined such as multi-organ failure. A further concern about this study is the difficulty in orthogonally validating the proteins identified by LC-MS. This may be due to the following: large biological variation in a small data set; removal of binding proteins (*e.g.*, albumin) prior to LC-MS, which leads to the removal of additional proteins that can still be detected via clinical assays; the inability of antibody-based technologies to recognize the protein identified by LC-MS due to epitope masking; or the inability of the peptide database to distinguish between the presence of multiple isoforms of a particular protein. That β 2-microglobulin was confirmed as a potential biomarker using traditional techniques demonstrated that the LC-MS platform can be used to identify targets for confirmation in further studies with larger patient numbers.

Conclusions

This study supports the utility of the quantitative LC-MS technique used here in discovering proteins relevant to critical illness, defining proteome signatures, and identifying potential biomarkers. High-throughput proteomic screening techniques such as the quantitative LC-MS technique may provide mechanistic insight into the clinical phenomenon that stress, injury, or acute illness can induce insulin resistance. Considering that post injury loss of more than 30–40% of lean body mass is associated with 100% mortality (27), the proteomic survival signature may also yield important clues regarding the mechanisms of survival. Finally, the finding that the majority of the 43 proteins with differential abundances between non-survivors and survivors had not been previously associated with the burn response let alone with survival illustrates the applicability of LC-MS and multiplex cytokine analysis for detecting potential biomarkers and concurrently defining a proteomic outcome signature. This provides for the possibility of improving clinical management and enabling development of successful interventions that alter outcome trajectories and decrease mortality after a severe burn.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Comparison of serum levels of GM-CSF (a), IL-8 (b), IL-4 (c), and MCP-1 (d) between surviving and non-surviving patients. Data are expressed as the mean $\pm$ SEM. * $p < 0.05$.

Table 1

Patient characteristics

Characteristic ^a	Non-Survivors (n=16)	Survivors (n=16)	p<0.05 ^c
Age (years)	49±19	39±13	No
Sex (F/M)	6/10	6/10	No
Total body surface area burned (%)	63±21	49±17	Yes
3 rd degree (%)	48±29	34±19	No
Inhalation injury (yes/no)	6/10	8/8	No
Days from injury to admit	0.4±0.5	0.3±0.4	No
Length of hospital stay (days)		68±48	
Post-burn days until death	32±19	N/A ^b	
Type burn (flame/scald/other)	14/1/1	14/1/1	No

^aData are expressed as percentage or mean ± standard deviation.

^bNot applicable.

^cStudent's *t* test or Chi-squared test.

Table 2

Proteins differentially detected in survivors versus non-survivors

IPI ^a	Protein	Reported active in	Direction of change in NS vs. S
IPI00004656.1	Beta-2-microglobulin	burn not survival	Up
IPI00007240.1	coagulation factor XIII, B polypeptide	burn not survival	Down
IPI00029168.1	Lipoprotein A	burn not survival	Up
IPI00166930.4	CARBOXYPEPTIDASE N SUBUNIT 2 PRECURSOR	burn response	Down
IPI00219018.4	Glyceraldehyde-3-phosphate dehydrogenase	burn response	Down
IPI00020996.3	Insulin-like growth factor binding protein complex acid labile subunit	burn response	Down
IPI00550991.1	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 3	burn response	Down
IPI00032179.2	serpin peptidase inhibitor, clade C (antithrombin), member 1	burn response	Down
IPI00019580.1	plasminogen	burn/survival	Down
IPI00006662.1	Apolipoprotein D	none	Up
IPI00451624.1	cartilage acidic protein 1	none	Down
IPI00297160.2	CD44	none	Down
IPI00019576.1	Coagulation factor X precursor	none	Up
IPI00015618.1	coiled-coil domain containing 49	none	Down
IPI00022395.1	complement component 9	none	Down
IPI00011252.1	complement component C8 alpha chain	none	Down
IPI00294395.1	Complement component C8 beta chain precursor	none	Down
IPI00291867.3	Complement factor I precursor	none	Down
IPI00029751.1	Contactin 1	none	Up
IPI00013452.7	glutamyl-prolyl-tRNA synthetase	none	Down
IPI00041065.3	HGF activator like protein	none	Down
IPI00018891.5	HKR1 GLI-Kruppel family member HKR1	none	Down
IPI00328089.2	human immunodeficiency virus type I enhancer binding protein 3	none	Down
IPI00292530.1	inter-alpha (globulin) inhibitor H1	none	Down
IPI00021304.1	Keratin, type II cytoskeletal 2 epidermal	none	Up
IPI00011876.1	methylthioadenosine phosphorylase	none	Up
IPI00414037.1	Myosin tail domain-containing protein /UNCHARACTERIZED PROTEIN C9ORF93	none	Down
IPI00022640.1	neurogranin	none	Up
IPI00218732.2	paraoxonase 1	none	Down
IPI00419262.1	PEPTIDYLPROLYL ISOMERASE B / Cyclophilin B	none	Down
IPI00219425.3	Poliovirus receptor beta	none	Down
IPI00397137.2	PREDICTED: hypothetical protein XP_374121	none	Down
IPI00176169.1	PREDICTED: similar to RIKEN cDNA 2510009E07	none	Up
IPI00022445.1	Pro-platelet basic protein	none	Down
IPI00019568.1	Prothrombin precursor	none	Down
IPI00465275.2	RAN binding protein 9	none	Down
IPI00010317.3	Ras-related GTP binding B	none	Down
IPI00239405.3	spectrin repeat containing, nuclear envelope 2	none	Down

IPI^a	Protein	Reported active in	Direction of change in NS vs. S
IPI00020165.1	Splice Isoform 1 of Chitotriosidase 1 precursor	none	Down

^aInternational Protein Index ID number

Table 3

Fold-change in protein between survivors and non-survivors

IPI^d	Protein Name	Fold Change NS:S
IPI00020165.1	Splice Isoform 1 of Chitotriosidase 1 precursor	-1.7965
IPI00215894.1	Splice Isoform LMW of Kininogen precursor	-1.5808
IPI00015618.1	Hypothetical protein FLJ20291	-1.0383
IPI00451624.1	Splice Isoform 1 of Cartilage acidic protein 1 precursor	-0.9083
IPI00013452.7	Hypothetical protein DKFZp313B047	-0.8405
IPI00007240.1	Coagulation factor XIII B chain precursor	-0.7962
IPI00397137.2	PREDICTED: hypothetical protein XP_374121	-0.7851
IPI00019580.1	Plasminogen precursor	-0.7790
IPI00010317.3	RagB	-0.7482
IPI00419262.1	Peptidyl-prolyl cis-trans isomerase	-0.7367
IPI00011252.1	Complement component C8 alpha chain precursor	-0.6699
IPI00022395.1	Complement component C9 precursor	-0.6519
IPI00021841.1	Apolipoprotein A-I precursor	-0.6517
IPI00022417.4	Leucine-rich alpha-2-glycoprotein precursor	-0.6061
IPI00029751.1	Splice Isoform 1 of Contactin 1 precursor	-0.5999
IPI00022445.1	Platelet basic protein precursor	-0.5950
IPI00041065.3	HGF activator like protein	-0.5811
IPI00011261.1	Complement component C8 gamma chain precursor	-0.5084
IPI00291867.3	Complement factor I precursor	-0.4991
IPI00032258.4	Complement C4 precursor	-0.4465
IPI00553177.1	Alpha-1-antitrypsin precursor	-0.4460
IPI00294395.1	Complement component C8 beta chain precursor	-0.4404
IPI00022429.3	Alpha-1-acid glycoprotein 1 precursor	-0.4359
IPI00027848.1	Macrophage mannose receptor precursor	-0.4007
IPI00013954.3	PREDICTED: thioredoxin reductase 3	-0.3957
IPI00022895.5	Alpha-1B-glycoprotein precursor	-0.3820
IPI00000875.5	Elongation factor 1-gamma	-0.3777
IPI00022640.1	Neurogranin	-0.3721
IPI00294193.3	Splice Isoform 1 of Inter-alpha-trypsin inhibitor heavy chain H4 precursor	-0.3720
IPI00022371.1	Histidine-rich glycoprotein precursor	-0.3700
IPI00059940.2	APEM9336	-0.3684
IPI00032179.2	Antithrombin III variant	-0.3506
IPI00164623.3	Complement C3 precursor	-0.3465
IPI00022488.1	Hemopexin precursor	-0.3450
IPI00018305.1	Insulin-like growth factor binding protein 3 precursor	-0.3403
IPI00218732.2	Serum paraoxonase/arylesterase 1	-0.3401
IPI00022431.1	Alpha-2-HS-glycoprotein precursor	-0.3303
IPI00294004.1	Vitamin K-dependent protein S precursor	-0.3168
IPI00292950.3	Heparin cofactor II precursor	-0.3107

IPI^a	Protein Name	Fold Change NS:S
IPI00550991.1	Alpha-1-antichymotrypsin precursor	-0.3032
IPI00032291.1	Complement C5 precursor	-0.2979
IPI00414037.1	Myosin tail domain-containing protein	-0.2909
IPI00159652.10	PREDICTED: KIAA0826 protein	-0.2885
IPI00019568.1	Prothrombin precursor	-0.2879
IPI00009244.3	Adapter-related protein complex 1 sigma 1B subunit	-0.2858
IPI00298853.5	Vitamin D-binding protein precursor	-0.2847
IPI00402142.2	PREDICTED: similar to Hypothetical protein CBG23155	-0.2840
IPI00550315.1	Ig kappa chain C region	-0.2805
IPI00029863.3	Alpha-2-antiplasmin precursor	-0.2765
IPI00021727.1	C4b-binding protein alpha chain precursor	-0.2729
IPI00022389.1	Splice Isoform 1 of C-reactive protein precursor	-0.2690
IPI00019591.1	Splice Isoform 1 of Complement factor B precursor	-0.2577
IPI00019576.1	Coagulation factor X precursor	-0.2546
IPI00009793.1	Complement C1r-like proteinase	-0.2394
IPI00021304.1	Keratin, type II cytoskeletal 2 epidermal	-0.2349
IPI00298497.3	Fibrinogen beta chain precursor	-0.2334
IPI00021439.1	Actin, cytoplasmic 1	-0.2253
IPI00296176.1	Coagulation factor IX precursor	-0.2130
IPI00025276.1	Splice Isoform XB of Tenascin-X precursor	-0.2125
IPI00021891.5	Splice Isoform Gamma-B of Fibrinogen gamma chain precursor	-0.2077
IPI00239405.3	Splice Isoform 1 of Nesprin 2	-0.2063
IPI00043201.2	OTTHUMP00000042330	-0.2061
IPI00026199.1	Plasma glutathione peroxidase precursor	-0.2026
IPI00303602.1	Hypothetical protein FLJ12806	-0.1977
IPI00009920.1	Complement component C6 precursor	-0.1915
IPI00292530.1	Inter-alpha-trypsin inhibitor heavy chain H1 precursor	-0.1912
IPI00299026.3	Tissue alpha-L-fucosidase precursor	-0.1891
IPI00218746.1	Complement Component 1, q subcomponent, beta polypeptide precursor	-0.1842
IPI00220644.6	Pyruvate kinase 3 isoform 2	-0.1829
IPI00292946.1	Thyroxine-binding globulin precursor	-0.1827
IPI00029168.1	Apolipoprotein	-0.1815
IPI00007118.1	Plasminogen activator inhibitor-1 precursor	-0.1733
IPI00303963.1	Complement C2 precursor	-0.1716
IPI00032220.3	Angiotensinogen precursor	-0.1711
IPI00011694.1	Trypsin I precursor	-0.1622
IPI00242956.2	Fc fragment of IgG binding protein	-0.1614
IPI00456831.1	PREDICTED: similar to hemcentin	-0.1613
IPI00166930.4	62 kDa protein	-0.1523
IPI00000494.4	60S ribosomal protein L5	-0.1500
IPI00023014.1	Von Willebrand factor precursor	-0.1451
IPI00216691.4	Profilin-1	-0.1441

IPI^a	Protein Name	Fold Change NS:S
IPI00010295.1	Carboxypeptidase N catalytic chain precursor	-0.1422
IPI00028413.3	Inter-alpha (globulin) Inhibitor H3	-0.1403
IPI00032311.3	Lipopolysaccharide-binding protein precursor	-0.1245
IPI00009028.1	Tetranectin precursor	-0.1228
IPI00022391.1	Serum amyloid P-component precursor	-0.1145
IPI00020996.3	Insulin-like growth factor binding protein complex acid labile chain precursor	-0.1123
IPI00329775.4	PCPB protein	-0.1070
IPI00455197.1	PREDICTED: similar to RIKEN cDNA 2610034N24	-0.1043
IPI00291262.3	Clusterin precursor	-0.1015
IPI00006662.1	Apolipoprotein D precursor	-0.0996
IPI00289334.1	Splice Isoform 1 of Filamin B	-0.0917
IPI00007199.3	Protein Z-dependent protease inhibitor precursor	-0.0910
IPI00017601.1	Ceruloplasmin precursor	-0.0896
IPI00022331.1	Phosphatidylcholine-sterol acyltransferase precursor	-0.0879
IPI00292043.3	Sidekick 2	-0.0833
IPI00027230.3	Endoplasmin precursor	-0.0817
IPI00026314.1	Gelsolin precursor	-0.0813
IPI00296099.3	Thrombospondin-1 precursor	-0.0773
IPI00165421.3	SERPINC1 protein	-0.0760
IPI00296053.3	Fumarate hydratase, mitochondrial precursor	-0.0756
IPI00007425.1	Desmocollin 1b	-0.0750
IPI00219217.2	L-lactate dehydrogenase B chain	-0.0690
IPI00304273.1	Apolipoprotein A-IV precursor	-0.0655
IPI00293925.1	Splice Isoform 1 of Ficolin 3 precursor	-0.0650
IPI00011876.1	S-methyl-5-thioadenosine phosphorylase	-0.0645
IPI00017696.1	Complement C1s subcomponent precursor	-0.0619
IPI00290903.5	Hypothetical protein LOC130951	-0.0549
IPI00216699.1	Splice Isoform 2 of Unc-112 related protein 2	-0.0543
IPI00291866.3	Plasma protease C1 inhibitor precursor	-0.0482
IPI00234095.3	PREDICTED: similar to alanyl tRNA synthetase	-0.0482
IPI00219018.4	Glyceraldehyde-3-phosphate dehydrogenase, liver	-0.0476
IPI00021857.1	Apolipoprotein C-III precursor	-0.0431
IPI00022937.2	Coagulation factor V	-0.0421
IPI00291175.3	Vinculin isoform VCL	-0.0418
IPI00021263.3	14-3-3 protein zeta/delta	-0.0377
IPI00029739.3	Splice Isoform 1 of Complement factor H precursor	-0.0362
IPI00396348.1	Serine (or cysteine) proteinase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 4	-0.0263
IPI00010402.2	Hypothetical protein	-0.0263
IPI00011062.1	Splice Isoform 1 of Carbamoyl-phosphate synthase [ammonia], mitochondrial precursor	-0.0229
IPI00465439.4	Fructose-bisphosphate aldolase A	-0.0148
IPI00018903.1	Ventral anterior homeobox 2	-0.0136

IPI^a	Protein Name	Fold Change NS:S
IPI00297160.2	Hypothetical protein DKFZp451K1918	-0.0125
IPI00008558.1	Plasma kallikrein precursor	-0.0099
IPI00022434.1	Serum albumin precursor	-0.0026
IPI00298971.1	Vitronectin precursor	-0.0025
IPI00305461.2	Inter-alpha-trypsin inhibitor heavy chain H2 precursor	-0.0023
IPI00021817.1	Vitamin K-dependent protein C precursor	0.0006
IPI00470919.1	Hypothetical protein DKFZp686K08164	0.0062
IPI00029061.1	Selenoprotein P precursor	0.0120
IPI00001701.2	Doublecortin domain containing protein	0.0222
IPI00395488.2	Vasorin	0.0238
IPI00550349.1	Fetuin B	0.0302
IPI00180240.2	Thymosin-like 3	0.0328
IPI00022426.1	AMBP protein precursor	0.0333
IPI00010970.3	Scm-like with four mbt domains 2	0.0365
IPI00182739.3	Hypothetical protein FLJ37870	0.0409
IPI00020091.1	Alpha-1-acid glycoprotein 2 precursor	0.0410
IPI00171473.2	Spondin-1 precursor	0.0456
IPI00023728.1	Gamma-glutamyl hydrolase precursor	0.0502
IPI00022229.1	Apolipoprotein B-100 precursor	0.0530
IPI00010779.3	Tropomyosin 4	0.0547
IPI00296165.5	Complement C1r subcomponent precursor	0.0585
IPI00004373.1	Mannose-binding protein C precursor	0.0594
IPI00008603.1	Actin, aortic smooth muscle	0.0638
IPI00019943.1	Afamin precursor	0.0709
IPI00024057.2	Transgelin 2	0.0811
IPI00019581.1	Coagulation factor XII precursor	0.0821
IPI00163207.1	Splice Isoform 1 of N-acetylmuramoyl-L-alanine amidase precursor	0.0834
IPI00298828.1	Beta-2-glycoprotein I precursor	0.0848
IPI00333541.3	Filamin A	0.0854
IPI00027487.3	Creatine kinase, M chain	0.0856
IPI00064667.2	Glutamate carboxypeptidase-like protein 2 precursor	0.0882
IPI00017672.2	Hypothetical protein FLJ25678	0.0925
IPI00073772.4	Fructose-1,6-bisphosphatase	0.0966
IPI00002547.1	Calpain 6	0.0985
IPI00027235.1	Splice Isoform 1 of Attractin precursor	0.1013
IPI00220327.2	Keratin, type II cytoskeletal 1	0.1080
IPI00298994.3	Talin 1	0.1211
IPI00006114.4	Pigment epithelium-derived factor precursor	0.1235
IPI00013508.3	Alpha-actinin 1	0.1277
IPI00297550.7	Coagulation factor XIII A chain precursor	0.1313
IPI00419453.1	Ig kappa chain V-III region VG precursor	0.1322
IPI 00021885.1	Splice Isoform Alpha-E of Fibrinogen alpha/alpha-E chain precursor	0.1376

IPI^a	Protein Name	Fold Change NS:S
IPI00012011.3	Cofilin, non-muscle isoform	0.1469
IPI00025252.1	Protein disulfide-isomerase A3 precursor	0.1566
IPI00025864.1	Cholinesterase precursor	0.1617
IPI00003351.2	Extracellular matrix protein 1 precursor	0.1694
IPI00006146.3	Serum amyloid A2	0.1727
IPI00306311.7	Pleckstrin	0.1801
IPI00027223.2	Isocitrate dehydrogenase [NADP] cytoplasmic	0.1803
IPI00010180.3	Liver carboxylesterase 1 precursor	0.1886
IPI00023673.1	Galectin-3 binding protein precursor	0.1916
IPI00007257.4	Alcadein alpha-1	0.1928
IPI00022418.1	Splice Isoform 1 of Fibronectin precursor	0.1965
IPI00465436.3	Catalase	0.2045
IPI00296608.6	Complement component C7 precursor	0.2053
IPI00027462.1	Calgranulin B	0.2090
IPI00294542.1	WUGSC:H_2G3A.1 protein	0.2366
IPI00022394.2	Complement C1q subcomponent, C chain precursor	0.2374
IPI00218413.1	Biotinidase precursor	0.2507
IPI00216298.5	Thioredoxin	0.2646
IPI00216589.1	Splice Isoform 2 of ATP-binding cassette, sub-family B, member 9 precursor	0.2771
IPI00011651.1	Receptor-type tyrosine-protein phosphatase gamma precursor	0.2858
IPI00478493.1	Haptoglobin precursor	0.2885
IPI00003590.1	Quiescin Q6	0.2937
IPI00007739.1	HemK protein homolog	0.3321
IPI00398997.2	PREDICTED: similar to ribosomal protein L31	0.3609
IPI00018136.1	Splice Isoform 1 of Vascular cell adhesion protein 1 precursor	0.3809
IPI00029260.2	Monocyte differentiation antigen CD14 precursor	0.3941
IPI00020986.2	Lumican precursor	0.4339
IPI00019579.1	Complement factor D precursor	0.4561
IPI00235407.1	Serine/threonine kinase 36	0.5271
IPI00218816.5	Hemoglobin beta chain	0.5327
IPI00022420.3	Plasma retinol-binding protein precursor	0.5611
IPI00021842.1	Apolipoprotein E precursor	0.5732
IPI00298281.3	Laminin gamma-1 chain precursor	0.5847
IPI00410714.1	Hemoglobin alpha-1 globin chain	0.6605
IPI00478003.1	Alpha-2-macroglobulin precursor	0.7246
IPI00168178.1	Hypothetical protein FLJ34521	0.7644
IPI00004656.1	Beta-2-microglobulin precursor	0.9139

^aInternational Protein Index ID number