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Proteomics in sepsis: a pilot study

Proteômica na sepse: estudo piloto

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ABSTRACT

Gene expression is disrupted by sepsis. Genetic markers can only reveal a patient's genotype, and they are not affected by environmental biological processes. These processes are expressed by proteins.

This study was aimed to advance the insight into the molecular foundations of sepsis. It employed proteomic techniques to identify and analyze differential serum protein expressions taken from a patient throughout the stages of sepsis (sepsis, severe sepsis and septic shock).

Serum samples were collected at each stage of sepsis and submitted to one-dimensional electrophoresis, on gradient strips of immobilized pH, followed by two-dimensional 12.5% polyacrylamide gel electrophoresis. The gels obtained were stained, scanned and analyzed by the ImageMasterPlatinum program. Proteins that were differentially expressed in the gels were excised, digested with trypsin and identified through mass spectrometry.

Fourteen differentially expressed proteins were identified throughout the stages of sepsis, as well as a protein that was not expressed in all stages, suggesting the potential existence of a biomarker. The differentially expressed proteins identified were: serum amyloid A, apolipoprotein A-1 (2 isoforms), zinc finger protein 222, human albumin, PRO 2619, immunoglobulin kappa light chain VLJ region, monoclonal immunoglobulin M cold agglutinin, 7 proteinase inhibitors – alpha-1 antitrypsin.

The findings of this pilot study demonstrate the involvement of the complement and coagulation pathways, of the lipid metabolism and of genetic information in sepsis. The vast majority of proteins identified are involved in the immune system and the proteinase inhibitor proteins are predominant.

Keywords: Shock, septic; Human genome; Mass spectrometry; Electrophoresis, polyacrylamide gel; Proteomics; Sepsis; Case reports

INTRODUCTION

Sepsis is the main cause of death in non-coronary intensive care units. It may occur at any age (although it is more frequent in the elderly), gender (5% more common in men) and may be associated to any infectious process. It is responsible for more than 200,000 deaths a year in the United States (US).[1]

In sepsis, an acute inflammatory response is associated to the infectious insult. Some released proteins, like cytokines, may present an autocrine effect (when the target-cell is the cytokine secreting cell itself), paracrine effects (when the target-cell is a neighboring cell) or endocrine effects, that lead to reactions beyond local inflammatory limits and result in systemic manifestations.[2] The complex interaction between cytokines and cytokine-neutralizing molecules is likely to determine the clinical presentation and the course of sepsis.

In sepsis, success and survival depend on early and appropriate therapy. Protein expression analysis may provide a means of achieving faster sepsis diagnosis and prediction of therapeutic effects.

In the search for a sepsis biomarker, several molecules were investigated, such as procalcitonin (PCT);[3] several interleukins, including interleukin (IL)-6, IL-8, TNF;[4] and C-reactive protein (CRP).[5] High levels of macrophage migration inhibiting factor (MIF) are found both in sepsis and septic shock and, thus, have been considered mortality predictors in intensive care unit (ICU) infections.[6] As a patient's condition progresses from sepsis, or severe sepsis, to septic shock, a significant increase of sTREM-1 (soluble triggering receptor expressed on myeloid cells-1) [7] occurs. The HMGB-1 (High Mobility Group B-1) protein seems to be a late mediator in sepsis, and has been considered a severity marker.[8] To date, an ideal sepsis marker has yet to be found; the best way to foresee and monitor sepsis is probably through a combination of biomarkers.

Recent advances in genome sequencing have significantly impacted on scientific research, however very little has been learned in relation to the gene products, proteins. Protein analysis is needed, since a genomic study would not reflect the proteins' dynamic structure, which is where disease processes initiate.[9] Among the proteomic tools, the classic strategy consists of separating and quantifying the proteins of a sample (a cell, tissue, or fluid) by means of two-dimensional (2D) electrophoresis and, subsequently, identifying each of the proteins through mass spectrometry (MS).[10] Proteomics reveals itself promising in promoting a more comprehensive understanding of complex biological systems as well as leading towards the development of new biomarkers and therapeutic targets.[11]

This study objective was to employ proteomic techniques to analyze the variations in expression of serum proteins throughout the different stages of septic development – sepsis, severe sepsis and septic shock – in a single patient, by: identifying the proteins expressed in each stage of sepsis, evaluating the change in expression of these proteins from one stage to the next and searching for a protein or proteins that may serve as a marker/markers of severity or protection (biomarkers).

This original study contributes to medical science and raises issues for further investigation.

CASE REPORT

A prospective observational longitudinal study of a septic patient admitted to the Intensive Care Unit (ICU) of the Hospital Central do Exército (HCE), Rio de Janeiro, Brazil, after an Informed Consent Form (ICF) was obtained from his legal guardian and approved by the Ethics Committee (EC). This ICU is comprised of 20 beds. It was necessary for us to collect serum samples from 11 different patients over three consecutive months before encountering a patient that evolved sequentially through the three described stages. This study was performed in the Protein Chemistry Lab of the Rio de Janeiro Federal University's (UFRJ) Chemistry Institute. The institution's previously installed infrastructure and existing resources were sufficient for the research. The patent's treatment was conducted in compliance with the routine established by the ICU. No change or intervention was made by the researchers.

Study Design

Sepsis was defined in three stages according to the following:

– Sepsis – Systemic Inflammatory Response Syndrome – (SIRS) associated with a identified or suspected infective focus. This was defined as Stage **A.**

– Severe Sepsis – Sepsis associated with manifestations of tissue hypoperfusion and organ dysfunction, characterized by lactic acidosis, oliguria or change in level of consciousness, or, also, arterial hypotension with systolic blood pressure below 90 mmHg, however not requiring vasopressor agents. This phase was defined as Stage **B.**

– Septic Shock – Hypotension or hypoperfusion induced by sepsis refractory to appropriate volemic resuscitation, and followed by the need for vasopressor agents. This phase was defined as Stage **C.**

The patient progressed, respectively, from Stage A to B, B to C and then on to death. Blood samples were collected sequentially as the patient changed from one stage to the next, totaling three samples. The patient was his own comparator. There was no control case. Cultures were prepared from patient samples (blood, urine, and tracheal secretion), proteins were dosed, and the Acute Physiology and Chronic Health Evaluation (APACHE II) score[12] was calculated within the first 24 hours from admission, and the Sequential Organ Failure Assessment (SOFA)[13] score, was calculated daily.

Samples were submitted to one-dimensional and two-dimensional electrophoresis, producing nine gels, three for each stage of sepsis (triplicate). The gels were stained with Coomassie Blue revealing several proteins that were analyzed. Following analysis, the proteins that presented significant differences in expression between stages were excised from the gel, digested with trypsin and identified by mass spectrometry.[14]

Preparation for the 1st dimension

After the sample proteins were dosed, about 1,000 µg of proteins (16.1 µL of the sample A serum and 16.7 µL of the B and C samples) were diluted in a re-hydration buffer – 2.7 g urea 9.0M, 0.1g 2% 3-cholamidopropyl dimethyl-ammonio-1-propane (CHAPS), 3.5 mg 1% dithiothreitol (DTT), 1.75 µL immobilized pH gradients buffer (IPG buffer), 0.5% 3-10 non-linear, 10 µL 1% bromophenol blue/ Tris and 134 µL MilliQ water – yielding a final volume of 350 µL. Each sample was vortexed for 30 minutes, to shake and denature the proteins. Insoluble materials were removed by 5 minutes of 14,000 rpm centrifugation.

1st dimension

Immobilized pH gradient (IPG) isoelectric focusing (IEF) promotes protein separation according to the proteins' isoelectric points (pI). The EttanTM IPGPhor IITM (GE-Healthcare) system used for isoelectric focusing, with temperature control and programmable supply. Isoelectric focusing was performed in accordance with the following schedule: IEF 20ºC 50 µA/strip and several sequential cycles up to a total of 80,000Vh: 1st step, 30 V per 12:00 hours; 2nd step, 200 V for 1:00 hour, and 3rd step, 500 V for 1:00 hour; 4th step, 1,000 V for 1:00 hour; run: 8,000 V; 82,060 Vht in 25:00 hours. The IPG gel strips were sealed in plastic and stored at -80ºC.

2nd dimension

Polyacrylamide sodium dodecyl sulface gel (SDS-PAGE) 12.5% electrophoresis performed with Amersham Biosciences® equipment and reagents. Proteins were split according to their molecular weight (MW) using the Ettan DALT*six* system – a vertical electrophoresis system with water circulator for temperature control (Multitemp III®). Before proceeding to the second dimension, each strip was removed from the freezer at -80ºC, balanced in the presence of SDS and submitted to reduction with 100 mg dithiothreitol (DTT) for 20 minutes. Next, strips were alkylated with 250 mg IAA in equilibration solution (urea 6M, 30% glycerol, 2% SDS, 0.05 M Tris-HCL, pH 8.8) for 20 minutes. Following equilibration, the strips were immersed in running buffer (0.25 M Tris, 1.92 M glycine, 1% SDS and distilled water to complete 1,000 mL) for 10 seconds to remove excessive balance solution. Next they were placed on top of the second dimension gel and fixed with running buffer with 0.5% agar, and then submitted to the second dimension on the vertical SDS-PAGE Ettan DALT*six* (GE-HealthCare®) system, at 20ºC. The run was performed in two phases. During the first we used 1.5 W/gel for 30 minutes and during the second 16.6 W/gel for 03h30 minutes, totaling 299 V, 96 W, 312 mA.

At the conclusion of the runs, each gel was placed in an ethanol fixing solution: acetic acid: water (4 hours and 10 minutes: 50 v/v/v) for three hours, stained for 24 hours in 1% Coomassie Blue R-350 solution and discolored with methanol: acetic acid: water (40: 10: 50 v/v/v) in three 30 minute washes. Next, the gels were left in a 5% acetic acid water solution for 24 hours until the spots were revealed, where, potentially, each spot should correspond to a polypeptide chain species.

The nine gels were digitized with Amersham Biosciences Labscan v 3.0 software on a Umax scanner, with an integrated transparency system and stored in 5% acetic acid solution in sealed plastic. The images were digitized and analyzed to determine the molecular mass and isoelectric point (pIs) with reference to standards and an analysis of the pH buffer range of the immobilized pH gradients that were employed. The gels were analyzed with the ImageMaster 2D Platinum (Amersham Biosciences) software using a combination of automatic (performed by the program) and manual spot detection. Six hundred and thirty six spots were detected. Only proteins presenting a volume percent above 0.5 were considered for analysis. Proteins with increased or reduced intensity were selected for identification. Selection was based on the degree of variation seen between them. Those presenting a ratio of at least a twofold increase or reduction (in % of volume) were selected. The comparison between gels was performed automatically, after several gel reference points had been identified. Following digitization, the specific proteins in each gel were compared to the corresponding proteins in the reference gel. After editing and comparing the 9 gels (3 for each stage of sepsis), we proceeded to prepare a single synthetic gel to represent each phase. Each of the 3 Stage A gels generated a single synthetic gel to represent Stage A, the same occurring with the 3 from Stage B and from Stage C. This composition of three gels to refer to a single stage was performed as proof of the experiment's reproducibility. Again a comparison (differential analysis) between gels was conducted, this time, between each of the synthetic gels that represented a discrete stage (A, B or C).

The proteins of interest were manually excised from the two dimensional gels in 1 mm³ fragments with the aid of a scalpel and transferred to eppendorfs tubes e proteins were then discolored in a 50 mM NH4HCO3/ acetronile (1:1) solution in three 30 minute washes and dehydrated with acetronile (100%) for 5 minutes. The

remaining solvent was removed from the gel fragments by vacuum centrifugation (29). The proteins were rehydrated in ice for 15 minutes in a 15 µL solution of 25 mM ammonium bicarbonate containing 0.2 µg modified trypsin. Gel fragments were covered with a 20 µL buffer and digestion was performed in a 16 hour bath at 37ºC. The peptides were then extracted from the gel by a 50% acetonitrile solution and trifluoroacetic acid (5%) in water, and vacuum centrifuged until concentrated to a volume of approximately 5 µL.

Mass spectrometry

Following protein selection, excision and hydrolysis, the mixture containing the digested peptides was blended 1:1.5 with an α-cyano-4-hydroxycinnamic acid saturated solution in 50% acetronile/ 0.3% trifluoroacetic acid in water. One µL of the mixture was added to the MALDI plate, and matrix crystallization occurred at room temperature prior to mass spectrometer analysis.

The mass spectra were obtained using an ABI 4700 TOF/TOF (Applied Biosystems) device. The interactive mode was used, in which all samples were automatically analyzed in the MS reflector mode, after which the six most intensive peaks underwent further MS/MS analysis. Laser intensity was of 4800 in MS mode and 5200 for MS/MS, and the collision cell was set to 1 kV pressure 1 x 10-6 torr with atmospheric gas.

The combined analysis spectra (MS and MS/MS) were filtered by the peaks' signal to noise ratio, which was 20 for the MS data and 10 for MS/MS, and submitted to peptide mass homology and amino acid sequencing comparison searches in the non-redundant NCBI (National Center for Biotechnology Information – www.ncbi.nlm.nih.gov/NCBInr) protein database. The search was performed with the MASCOT (Matrix Science Ltd.) software interface, that employs algorithms to test the identifications statistical significance. A minimum ion score of 30 and minimum protein score of 70 were considered as statistical values for identification. The MS-BLAST software was also used for searches in case of doubtful identification redoing the sequence.[15]

DISCUSSION

The pathogen identified in the blood cultures drawn at each of the protocol defined stages; the SOFA score, calculated by the sampling times; the protein contents and other patient data, are described in chart 1.

The 15 proteins that presented a range ratio above 2 in this analysis were selected for identification. The differentially expressed proteins are identified in figure 1.
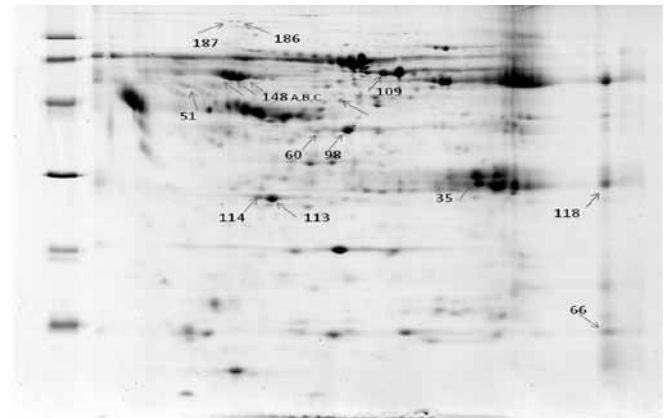

Figure 1 – Phase B 2D Gel with identified excised spots.

The proteins with differential expressions that were considered significant were: serum amyloid A, two apolipoprotein A1 isoforms, seven serpin family members, zinc finger protein 222, serum albumin, PRO 2619 (albuminoid super-family), immunoglobulin kappa light chain VLJ region, immunoglobin M monoclonal of cold aglutination. The identified serpin family proteins were, respectively, three alpha-1-antitrypsin isoforms, serpin, peptidase inhibitor, class A (antiproteinase alpha-1-antitrypsin), member 1, two serine proteinase inhibitor isoforms, class A (antiproteinase alpha-1, antitrypsin), member 1 and crystalized structure of serpin-protease complex, chain A.

Chart 2 shows proteins identified with the spot number, isoelectric point, and theoretical and experimental molecular weight, protein score (minimum 70), ion score (minimum 30), best Peptide Sequencing, NCBI database

Chart 1 – Studied patient demographic and clinical characteristics

Gender	Age	APACHE II	Samples	Pathogen	Phase	SOFA	Serum protein (g/dL)
Male	57	18	D1	*E. coli*	A	2	6.2
			D11	*E. coli*	B	4	6.0
			D15	*E. coli*	C	6	6.0

APACHE - Acute Physiology and Chronic Health Evaluation; SOFA - Sequential Organ Failure Assessment; g/dL- grams per deciliter; D1 – Day 1; E. coli - Escherichia coli.

Chart 2 – Differentially expressed proteins identification and description

Identification group	Proteins score/ Ions score	Peptides sequencing	Access number	Protein description	Function	Expression variation
66	196/174	GPGGAWAAEVISNAR SFFSFLGEAFDGAR	gi|337743	Serum amyloid A	Scavenger	C<B 0A
113	449/370	EQLGPVTQEFWDNLEK THLAPYSDELR WQEEMELYR DEPPQSPWDR	gi|90108664	Apolipoprotein A-1	Innate immunity	A>B>C
114	448/378	EQLGPVTQEFWDNLEK THLAPYSDELR WQEEMELYR	gi|90108664	Apolipoprotein A-1	Innate immunity	A>B>C
98	342/291	ITPNLAEFAFSLYR LYHSEAFTVNFGDTEEAKK VFSNGADLSGVTEEAPLK	gi|11514321	Serpin-Proteinase complex crystallized structure, chain A	Proteinase inhibitor	C<B>A
133	168/100	VFDEFKPLVEEPQNLIK FQNALLVR	gi|23307793	Serum albumin	Amino acid metabolism	A>B>C
109	481/342	HPYFYAPELLFFAK HPDYSVVLLLR VFDEFKPLVEEPQNLIK RPCFSALEVDETYVPK	gi|11493459	PRO 2619 (albuminoid super-family)	Amino acid metabolism	A>B>C
60	100/-	EKPFQGENCK SFCLRSSLNR	gi|20988840	Zinc finger 222 protein	Transcription factor	C<B>A
118	200/191	SGTASVVCLLNNFYPR VYACEVTHQGLSSPVTK TVAAPSVFIFPPSDEQLK	gi|21669353	Immunoglobulin kappa light chain VLJ region	Host defense	C>B>A
186	209/142	VFSNGADLSGVTEEAPLK DTEEEDFHVDQVTTVK	gi|50363217	Serin proteinase inhibitor, class A (alpha 1 anti-proteinase, antitrypsin), member 1	Proteinase inhibitor	C>B>A
187	191/143	ITPNLAEFAFSLYR LYHSEAFTVNFGDTEEAKK	gi|50363217	Serin proteinase inhibitor, class A (alpha-1 anti-proteinase, antitrypsin), member 1	Proteinase inhibitor	C>B>A
35	150/140	SGTASVVCLLNNFYPR VYACEVTHQGLSSPVTK TVAAPSVFIFPPSDEQLK	gi|10835792	Cold agglutination immunoglobulin M monoclonal	Host defense	C>B>A
51	172/143	ITPNLAEFAFSLYR VFSNGADLSGVTEEAPLK	gi|15080499	Serpin, peptidase inhibitor, class A (anti-proteinase alpha-1, antitrypsin), member 1	Proteinase inhibitor	C>B>A
148 A	229/181	LYHSEAFTVNFGDTEEAKK LYHSEAFTVNFGDTEEAK	gi|177831	Alpha-1- antitrypsin	Proteinase inhibitor	C>B>A
148 B	376/318	LYHSEAFTVNFGDTEEAKK LQHLENELTHDIITK VFSNGADLSGVTEEAPLK	gi|6137432	Alpha-1- antitrypsin	Proteinase inhibitor	C>B>A
148 C	396/355	LQHLENELTHDIITK LYHSEAFTVNFGDTEEAKK LQHLENELTHDIITK TDTSHHDQDHPTFNK GKWERPFEVK	gi|224224	Alpha-1- antitrypsin	Proteinase Inhibitor	C>B>A

entry number, protein function and differential expression in sepsis stages.

Each identified protein was categorized into one of the established functional categories: metabolism, genetic information processing, environmental information processing, cell processes and human diseases. The Kyoto Encyclopedia of Genes and Genomes (KEGG - www.genome.jp/kegg/) classification was adopted. Proteins were classified as follows: cell processes/immune system (66.6%), cell processes/endocrine system (13.3%), metabolism (13.3) and genetic information/transcription factor (6.6%), as can be seen in figure 2.
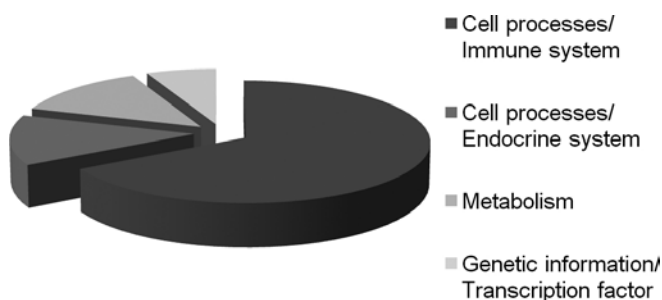

Figure 2 – Identified proteins relative functional distribution. The reported categories were previously reported by the Kyoto Encyclopedia of Genes and Genomes.

The relative abundance of proteins involved in immune system cell processes may be related to the body's reaction against the infectious process. These proteins are found in the coagulation cascade pathways and complement system. The results indicate a significant focus on these pathways, known for their significant role in sepsis.[16] Other systems involved were the endocrine, with its signaling pathways PPAR (13.3%), the metabolism (13.3%) and genetic information processing/transcription factor (6.6%). The PPAR signaling pathway is involved in the lipid mechanism (KEGG).

Identified proteins

Apolipoprotein A1 (APO A-1) is the main protein component of HDL (high density lipoprotein). APO A-1 binds to lipopolysaccharides or endotoxins, with a relevant role in HDL anti-endotoxin function.[17] It interacts with LBP, the LPS-binding protein, and circulates in plasma modulating LPS binding to HDL and acting as a cofactor for LPS neutralization. Lipoproteins could have a role in innate immunity, as they reduce cytokine response in animal sepsis models.[18] APO A-1 expression decreased with the progression of sepsis severity. This may indicate a reduction of innate immu-

nity action and anti-endotoxin effects.

Serum amyloid A (SAA) is an HDL complex apolipoprotein. SAA acts by redirecting LPS neutralized HDL from the CD14 macrophage activation pathway to the liver, with fast clearance and, consequently, reduced HDL levels. Therefore, during the acute phase LPS neutralizing ability is reduced, perpetuating systemic inflammatory response and contributing to organ failure and death.[19] In this study, SAA was not detected during the initial sepsis stage, and was most expressed during Stage B (severe sepsis), with decreased expression in the stage C of the study. These data may reflect a lower expression due to Stage C's decreased need for APO A-1 displacement or a reduction of the liver's ability to secrete it.

Also identified in the study within the immune system category, and a participant in the coagulation and complement cascade, is immunoglobulin kappa light chain VLJ region. Immunoglobulins integrate the humoral immune system. Their contribution to immunity may occur in three different forms: by binding to a pathogen to prevent its entry to the cell; by covering a pathogen to stimulate its removal by macrophages; and they may also trigger direct pathogen destruction by stimulating other immune responses such as complement pathway activation.[20] Another immunoglobulin identified in this study was immunoglobin M monoclonal of cold aglutination. Cold agglutinins are auto-immunoglobulin M antibodies, characterized by their ability to agglutinate at low temperatures with erythrocytes in vitro (4-22ºC).[21] Both of the different immunoglobins identified in the study presented greater expression in stage C, decreasing towards stage A, thus exhibiting greater expression in the more advanced stages of the infectious process.

The zinc finger 222 protein (ZNF 222) is a transcription factor that belongs to the Krueppel type C2H2 zinc finger protein.[22] A transcription factor is a protein that binds DNA to specific sites where it can regulate transcription. According to data obtained from the NCBI, the zinc finger 222 transcription factor would, supposedly, have a transcription repression role, regulating transcription, cell division and chromosome partition. Its expression increased from stage A to B and, then, was reduced in stage C. Its behavior in sepsis progression may reflect the body's attempt to block the production of proteins whose effects may be harmful to the body (e.g., cytokines). With its reduced concentration in stage C, less of these proteins would be repressed, leading to the onset of shock. The same

would apply to tissue regeneration, which is also among its functions.

Albumin is the most abundant plasma protein, comprising 50% of the human serum proteins.[23] This study identified a reduction of albumin expression as sepsis worsened. One of albumin's important functions is to maintain the volume of circulating plasma, due to its relatively low molecular weight and high concentration. Albumin's role also comprises: the maintenance of the acid-base balance; the transportation of a series of physiological substances; the distribution and metabolism of several different substances, both endogenous and exogenous; and, additionally, acting as an amino acid reserve.[24]

The PRO 2619 protein belongs to the albuminoid family, and is a serum albumin precursor. Therefore, its expression was also decreased as the sepsis worsened.

Several proteins related to the serpin family were also identified. "Serpins" is a name given to the proteinase inhibitors family. Serpins share a complex and well preserved tertiary structure. The following serpin family proteins were found: serine proteinase inhibitor, class A (alpha 1 anti-proteinase, antitrypsin), member 1; alpha-1-antitrypsin and serpin A chain present in a crystalline serpin-proteinase complex. Alpha-1-antitrypsin is a glycoprotein known to be a trypsin inhibitor, and plays a central role in homeostasis by neutralizing the detrimental effects of elastase, a powerful enzyme found in white blood cells. As proteinase inhibitors, serpins have an array of functions including blood clotting regulation, cell remodeling and motility, blood pressure regulation and angiogenesis.[25] The serpin identified in the database, as part of a serpin-proteinase complex, displayed increasing expression from the early stage to severe sepsis, and then dropped. Since this complex involves proteinases, this may suggest that these enzymes were reduced in septic shock, or that the enzyme amount would remain the same having less expression of serpins on the other hand. The remaining serpin expression increased as the patient's infection worsened, i.e., a greater expression from stage A to B and B to C, respectively. This profile demonstrates the involvement of the immune system and of the coagulation and complement cascade in sepsis progression, encompassing tissue repair and lung protection mechanisms. This condition probably arose as a consequence of the Acute Respiratory Distress Syndrome (ARDS) episode developed by the patient with a concomitant increase in elastase concentration.

Alpha-1-antitrypsin isoforms were isolated. The usually low level of isoform concentration, increases up to a hundredfold under inflammatory conditions,[26] in agreement with this study results. The increase may have resulted from alpha-1-antitrypsin fragmentation, which has an important lung function role. The fragmentation of this protein may be a factor in the ARDS pathogenesis, indicating that its increase may be relevant and a useful biological marker for ARDS diagnosis.

Apolipoprotein A-1 isoforms were similarly identified. Variations in the percentage of different APO A-1 isoforms may occur as a result of an alteration in the fractional catabolic rate of this apolipoprotein.[27] The decreasing expression of these isoforms as sepsis worsened in this study, may indicate reduced innate immunity action, with regard to anti-endotoxin effects and cardiovascular protection.

The functional meaning of these proteins was considered. While many of them are inflammation and acute response biomarkers, the list of over- or underexpressed proteins in this study shows that sepsis has significant effects on coagulation, the immune system, the lipid metabolism, apoptosis and genetic information, confirming the findings of previous studies. Figure 3 shows the profile presented by the proteins identified in this study, according to their range of expression throughout the stages.
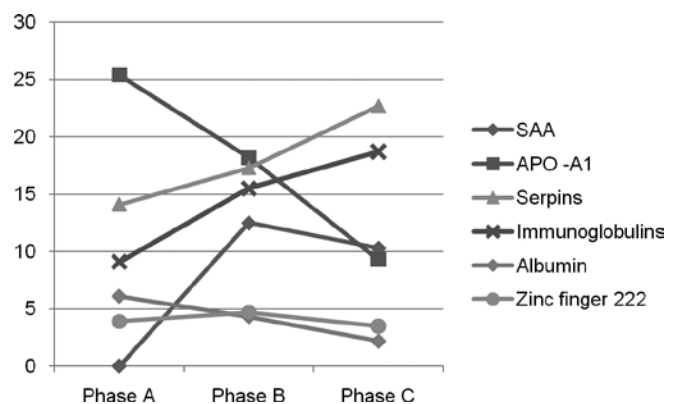

Figure 3 – Plot of the percent proteins expression in phases A, B and C.

Figure 3 clearly shows the variation of protein expression throughout the studied sepsis stages. Apolipoprotein A-1 and albumin expression decreased from Stage A to Stage C, while serpin and immunoglobulin expression increased. In this case, the rising proteinase inhibitor levels may represent the body's attempt to in-

hibit the increasing amounts of elastase expressed during the ARDS episode.

SAA was not seen in Stage A, it would only appear during Stage B and reduced expression during Stage C. This behavior could likely be explained by the reduced need of APO A-1 displacement during phase C, when its presence was lessened, or the liver's capacity of secreting it was diminished.

The zinc finger 222 protein increased its expression from Stage A to Stage B, then decreased in Stage C. Being a transcription repression factor, its reduced expression and, therefore, diminished repression of a harmful protein may have contributed to the onset of septic shock phase.

Stage A was characterized by a predominance of APO A-1 and albumins, representing the protective response and maintenance of homeostasis in sepsis. During Stage B, the zinc finger 222 transcription factor and SAA were predominant, demonstrating intense inflammatory activity by APO A-1 displacement and protein repression attempts. Stage C was characterized by increased expression of proteinase inhibitors and immunoglobulins, proteins that are functionally accountable for the body's defense.

The proteins identified in this study are related to those described in previous proteomic studies of sepsis. In this study, however, a longitudinal approach of the same patient was performed. While this study's analysis of a single patient may be considered a limitation, that does not allow results to be inferred, on the other hand, the study of a single patient, without a mixture of samples, presents greater value, due to the absence of confounding factors that would arise from mixing serums from patients of different genders, ages, ethnicity (African, Asian, European, etc), infecting organisms, immune responses towards insulting agents, nutritional habits – which are all factors that can influence protein expression. Several previous trials employed this mixed approach, potentially weakening their findings.

One of the major opportunities we envision through proteomic studies is the characterization of protein expression differences to identify potential therapeutic targets and disease biomarkers, thus widening our understanding of biological processes. A functional proteomic approach could provide data on sepsis' inflammatory signaling network, identifying critical regulation nodes. But how can proteomic data help us direct our therapy to a specific signaling pathway or critical crossroad between the connection pathways responsible for maintaining the septic state? The future challenge lies in translating what this research data can convey to clinical diagnosis, to greatly impact the decision process regarding patient therapy.

This investigation reveals the potential that proteomic techniques – two-dimensional electrophoresis and mass spectrometry – hold for advancing research into sepsis.

This is the first proteomic-based description, of sepsis' progressive changes in a single patient. Despite this study's limitations, it holds significant value as a starting point for other studies along this line that may validate the conclusions reached herein regarding protein expression patterns in sepsis.

RESUMO

Na sepse ocorre desregulação da expressão gênica. Os marcadores genéticos revelam apenas o genótipo do indivíduo, não sendo afetados pelos processos biológicos decorrentes da ação do ambiente, estes expressos nas proteínas. Este estudo teve como objetivo alcançar maior compreensão sobre as bases moleculares da sepse. Para tal realizou a identificação e análise da expressão diferencial de proteínas no soro de paciente séptico em diferentes estágios de gravidade (sepse, sepse grave e choque séptico) através de técnicas proteômicas. Amostras de soro referentes a cada estágio da sepse foram colhidas e submetidas à eletroforese unidimensional em fitas com gradiente de pH imobilizado seguida de eletroforese bidimensional em gel de poliacrilamida 12,5%. Os géis obtidos foram corados, escaneados e analisados através do programa ImageMasterPlatinum. As proteínas expressas diferencialmente nos géis foram excisadas, digeridas com tripsina e identificadas através de espectrometria de massa. Foram identificadas 14 proteínas expressas diferencialmente entre os estágios da sepse, assim como uma proteína não expressa em todos os estágios, sugerindo a existência de um possível biomarcador. Foram elas: amilóide sérico A, apolipoproteína A-1 (2 isoformas), proteína dedo de zinco 222, albumina humana, PRO 2619, imunoglobulina de cadeia leve kappa região VLJ, imunoglobulina M monoclonal de aglutinação a frio e 7 inibidoras de proteases - alfa-1 antitripsina. Os resultados obtidos neste estudo piloto demonstram a participação das vias do complemento e coagulação, do metabolismo lipídico e da informação genética na sepse. A grande maioria de proteínas identificadas está envolvida no sistema imune com predomínio das proteínas inibidoras de proteases.

Descritores: Choque séptico; Genoma humano; Eletroforese em gel de poliacrilamida; Espectrometria de massas; Proteômica; Sepse; Relatos de casos

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