Proteomic updates on sepsis

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Summary

The increased knowledge regarding proteomic analysis techniques has allowed for better understanding of the molecular bases related to the identification of cell signaling, modifying protein, and post-translational modification pathways, in addition to the characterization of specific biological markers. Thus, documenting certain proteins expressed in sepsis is a promising approach to elucidate pathophysiological, diagnostic, therapeutic, and prognostic aspects in this condition with a purpose of applying them to clinical practice. Although the studies are still preliminary, proteomics may offer good benefits for the better management of septic patients. Thus, this article aims to introduce a short review of the applications of proteomic studies to sepsis.

Keywords: Proteomics; sepsis; diagnosis; therapeutics; prognosis.

Study conducted at the Department of Medicine and Nursing and at the Department of Biochemistry and Molecular Biology, Universidade Federal de Viçosa (UFV), and Medical School, Centro Universitário Serra dos Órgãos (Unifeso) Teresópolis, RJ, Brazil

Submitted on: 10/30/2011
Approved on: 12/30/2011

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Conflicts of interest: None.

Introduction

Sepsis – a systemic inflammatory response syndrome (SIRS) triggered by infection (supposed or confirmed) – is an extremely important condition from a clinical care and public health perspective[1]. It is one of the most important infectious complications in contemporary medicine both for its incidence and severity, as well as for its great potential of progression to death (high lethality, depending on the stage presented when diagnosed)[2-5].

The different possibilities of interaction between *Homo sapiens sapiens* and different etiological agents[6] make different clinical manifestations possible, making it important to distinguish situations such as infection, SIRS, sepsis, severe sepsis, septic shock, and multisystem organ dysfunction (MSOD)[7,8].

In addition to the scientific issue – comparability across case studies – the terminological definition has aimed at early bedside detection. In this domain, instituting appropriate strategies to approach the patient could lead to a more favorable outcome and consequent reduced mortality. Diagnostic and therapeutic breakthroughs are the focus of scientific investigation, leading to an expansion of knowledge in the field, and stressing the recent role that proteomic techniques (identification of all proteins encoded by the genome[9]) have gained in the study of sepsis in terms of pathophysiology, diagnosis, therapeutics, and prognosis. To that effect, this article introduces a short review of the applications of proteomic studies in sepsis, considering their future incorporation into clinical practice.

Methods

The article was elaborated from a literature review with a definite search strategy. The articles were searched for in the U.S. National Library of Medicine (PubMed) and in the Scientific Electronic Library Online (SciELO), comprising the period from January 1, 2000 to September 1, 2011, with only studies performed in humans being selected. The terms used were:

Strategy 1 – sepsis + proteomics
Strategy 2 – sepsis + proteome
Strategy 3 – sepsis + proteomics + diagnosis
Strategy 4 – sepsis + proteomics + treatment
Strategy 5 – sepsis + proteomics + outcome
Strategy 6 – sepsis + proteomics + prognostic

In addition to articles, textbooks on internal medicine, infectology, and critical care were consulted as part of the bibliographical survey. The search retrieved the citations distributed according to Table 1. Out of the total articles retrieved, 25 were selected – resulting from empirical investigations and literature reviews –, mainly focusing on sepsis proteomic study and pathophysiological and clinical-therapeutic aspects, which formed the basis of the current investigation.

The articles were read and information was organized into different sections: (1) proteome concept; (2) proteome and sepsis pathophysiology; (3) proteome and sepsis diagnosis; (4) proteome and sepsis treatment; (5) proteome and sepsis prognosis; and (6) concluding comments.

The proteome concept

The proteome reflects the functional expression of the genome, that is, the current functioning status of a certain biological system in specific physiological conditions. This characteristic makes the study of the proteome an important challenge, as cell gene expression is quite dynamic, depending on the development status, the presence of activators or inhibitors and the environment conditions. Despite this, proteomics is now considered the most appropriate tool to understand gene functioning, as it analyzes the genome's end product[9]. Although identifying all of the proteins encoded into an organism genome appears to be a very difficult task, even in simpler organisms, the information from proteomic studies is increasingly complete[10]. These new findings are related to cell signaling pathways, regulatory protein sets, post-translational modifications as well as cell and organism states in health or sickness[11].

Since Wasinger et al.[12] proposed the proteome concept in 1995, investigations through proteomic analysis – involving systematic screening of great numbers of peptides contained in the cells, tissues, and biological fluids (e.g., cerebrospinal fluid, blood, urine, pancreatic fluid, amniotic fluid) – have rapidly advanced, characterizing the research field termed proteomics. These studies may lead to three basic aspects directly implicated in various biology, biotechnology and medical science fields: (1) the discovery of metabolic pathways in various cell steps, generating unprecedented knowledge in molecular biology and biochemistry; (2) identification of new bioactive molecules in natural biological extracts, leading to the development of new drugs; and (3) characterization of biological markers, that is, specific endogenous and exogenous molecules in a determined nosological entity. The ability to identify these molecules can become exceedingly useful in the early diagnosis of diseases and in the follow-up of treatment progress[11]. Currently, the main techniques used in proteomics are two-dimensional (2D) electrophoresis and mass spectrometry.

Proteomic analysis can be seen as a peptide screening aiming to document the overall distribution of peptides in cells, tissues, organs, and other specimens by identifying and characterizing individual proteins of interest and finally elucidating their interactions and roles in cell biology, in physiological and pathological contexts. Compared with the genome microarray technique, the

Table 1 – Number of articles obtained from the bibliographic survey

Search strategy	Database consulted	
	PubMed*	SciELO
Stragegy 1 (sepsis + proteomics)	69	1
Strategy 2 (sepsis + proteome)	40	0
Strategy 3 (sepsis + proteomics + diagnosis)	34	0
Strategy 4 (sepsis + proteomics + treatment)	26	0
Strategy 5 (sepsis + proteomics + outcome)	5	0
Strategy 6 (sepsis + proteomics + prognostic)	0	0

*To search PubMed database by employing English words, the following limits were used: articles on adult (> 19 years) humans published between January 1, 2000 and September 1, 2011.

proteomic approach has the advantage of detecting peptides previously, while microarrays only allow for the measurement of already defined genes. In parallel with proteomic advances, efforts to apply proteomic analysis to discover new biomarkers for pathophysiology description have been reported for a wide range of diseases, including sepsis. This point will be further discussed.

The proteome in sepsis pathophysiology

The pathophysiology of sepsis depends on the relationships established between the etiological agent and the host[8,13,14]. Many aspects concerning the triggering of this morbid condition are still unclear, likely because there is not a more appropriate understanding of the immune response biochemical aspects and of the inflammatory process[6]. Some hypotheses to explain sepsis genesis have been proposed, being considered in terms of (1) pathogen/innate immune system, (2) immune and adaptive inflammation/mediation, and (3) coagulation system, as discussed in a previous research[8].

The interaction between the microbial agent and the host is initiated by recognizing not-self substances (the host's non-particular substances) from the microorganism, the pathogen-associated molecular patterns (PAMPs) – non-variable molecules expressed by groups of etiologic agents, which are usually crucial for the microorganism's virulence and/or survival – identified by the pattern recognition receptors (PRRs), which are cell structures encoded by the germlines and expressed by innate immune system cells[15]. The most potent and best-studied PAMPs are the endotoxins of Gram-negative bacteria, derived from their cell walls and mainly formed by lipopolysaccharides (LPS). Regarding PRRs, the significant Toll-like family, whose molecules are identified in the surface of monocytes, macrophages, dendritic cells, and neutrophils, should be highlighted[16]. Polymorphisms in these receptors seem to decisively implicate in the possibility – or not – of a progression into severe sepsis and septic shock[17]. Continuing the recognition phase, various cell activation and cytokine production events succeed, resulting in SIRS.

Following the binding between PAMPs and Toll-like receptors, there is an intracellular domain activation in the latter, culminating in the activation of MyD88 protein (myeloid differentiation protein)[18]. The interaction of MyD88 with the IRAK (interleukin-1 receptor-associated kinase, a serine-threonine kinase) enzyme leads to the activation of kinases IkKa and IkKB, which form the KkK dimer, which, in turn, "disconnects" the protein IkB (NF-κB inhibitor), linked to the nuclear transcription factor NF-κB (nuclear factor κB), responsible for the activation of transcription genes in numerous cytokines which are part of SIRS (whether or not they are associated with infection)[19,20].

The intracellular events described, especially NF-κB release, determine the production and secretion of many proinflammatory cytokines, such as interleukins 1 (IL-1), 2 (IL-2), 6 (IL-6), 8 (IL-8), 12 (IL-12), tumor necrosis factor-alpha (TNF-α) and tumor necrosis factor-beta (TNF-β); this event is considered crucial to sepsis development. Of note, a number of patients progress to early death resulting from severe systemic inflammatory response. Nevertheless, anti-inflammatory cytokines, such as interleukins 4 (IL-4), 5 (IL-5), 10 (IL-10), 11 (IL-11), and 13 (IL-13) are equally produced – especially in settings wherein the patient survives systemic inflammation-associated disorders – making the development of anergy possible and slowing of the response to etiologic agents in a typical immunosuppression context[5], which, in sepsis, is differently named: immunoparalysis, immunodeficiency window, or compensatory anti-inflammatory response syndrome (CARS)[21]. This pro/anti-inflammatory balance regulation is complex, and the role of monocytes/macrophages as adaptive immune response activators must be emphasized. As macrophages phagocytize necrotic cells or bacteria, they induce Th1 lymphocyte phenotype, leading to the release of proinflammatory substances, such as alpha-interferon (a-IFN), delta-interferon (d-IFN), and IL-2; if they phagocytize apoptotic cells, Th2 lymphocyte phenotype is activated,

leading to IL-4 and IL-10 production, which "brakes" the proinflammatory response[22]. Indeed, apoptosis is one of the significant events triggering immunosuppressor processes[23]. The balance between proinflammatory and anti-inflammatory mediators is increasingly recognized as the key to explain the morbid condition progression either to resolution or death[8], as they can lead to a deep "immunological dissonance" termed mixed antagonist response syndrome (MARS), wherein both SIRS and CARS are simultaneously found in the same patient[24].

Proteomic studies have added important elements to the understanding of this complex "physiopathogenic web". In a pilot study performed by Paiva et al.[25] in order to better understand sepsis molecular bases, the differential expression of serum proteins in septic patients in different severity stages (sepsis, severe sepsis, and septic shock) were identified and analyzed through proteomic techniques. Fourteen differentially expressed proteins were identified across sepsis stages, as well as a protein not expressed in all stages, suggesting the possibility of the existence of a biomarker. The proteins were: serum amyloid A, apolipoprotein A-1 (two isoforms), zinc finger protein 222, human albumin, PRO 2619, immunoglobulin kappa light chain VLJ region, monoclonal immunoglobulin M with cold agglutinin activity, and seven alpha-1 antitrypsin protease inhibitors[25]. The results achieved from this pilot study demonstrated the participation of the complement and coagulation pathways of the lipid metabolism and of the genetic information in sepsis. The majority of peptides identified are involved in the immune system, and protease inhibitor peptides predominate[25].

Proteome and sepsis diagnosis

Despite the extensive knowledge production regarding pathophysiology and treatment, sepsis remains a difficult entity for clinical management[2,26]. Several studies have suggested the presence of specific genetic polymorphisms during sepsis[27]. Other investigations have used a microarray technology to compare gene expression levels after the administration of endotoxin[28]. However, gene expression studies cannot accurately predict the structure or the dynamics of the respective characteristic proteins in sepsis. RNA patterns do not appropriately reflect the proteomic pattern – that is, proteins expressed –, as in the analysis of many proteomic patterns of regulatory processes, such as post-translational modifications[29].

A great number of biological substances have been investigated as biochemical mediators and/or candidate biomarkers for sepsis laboratory investigation. C-reactive protein (CRP)[30], procalcitonin[30,31], and IL-6 are considered useful in the diagnosis and in the severity rating of sepsis, despite some limitations. More recently, attempts to show clinical usefulness as sepsis biomarkers were documented for a wide range of molecules, including the high mobility group box 1 protein (HBGB-1) and the triggering receptors expressed on myeloid cells (TREM-1)[8]. Some sepsis biomarkers, such as the cytokines, are also considered important disease mediators, so that the modulation of these substances may have therapeutic importance[32]. In addition, the combined use of multiple molecular markers or the use of more accurate prognosis scores for the severity allows for rating and predicting the sepsis outcome[8]. Finding new mediators involved in sepsis physiopathology, as well as new biomarkers allowing a more accurate sepsis diagnosis and prognosis is, thus, urgently needed.

Proteomic analysis methods can be used to investigate protein profiles in patients with sepsis and septic shock, thus revealing differences in protein electrophoresis mapping among patients who survive and those progressing to death. These studies indicate two important results. First, proteomic analysis can become a feasible tool to exclude early changes in peptide expression in patients with septic shock. Second, there are specific protein changes among survivors and non-survivors on day 28 in an initial stage of septic shock. This can be found in samples obtained over the first 12 hours after septic shock diagnosis.

Early sepsis diagnosis based only on clinical elements is known to be very difficult, although it is an essential aspect to approach patients, allowing the immediate initiation of an appropriate antibiotic therapy, which could greatly impact the patients' survival[33]. Paugam-Burtz et al.[34], by using a proteomic approach termed surface enhanced laser desorption/ionization – time-of-flight mass spectrometry (SELDI-TOF MS) for patients' serum evaluation within five days of liver transplantation, obtained a profile containing five peptides identifying sepsis. The comparison of protein profiles obtained in the sepsis group (n = 31) showed a total of 29 differentially expressed protein peaks, compared with the non-septic group (n = 30). Fourteen peptides profiles had their expression enhanced in the septic group, whereas 15 were restrained. As this is a preliminary study, the proteins are still being identified by those authors[34].

Proteome and sepsis treatment

The literature search proceeded with the terms sepsis + prognosis + proteomics not resulted in obtaining of citations in two databases consulted. However, information was gathered from articles selected for review.

Most proteomic studies involving sepsis focus on the disease physiopathology and on the detection of proteins that could serve as diagnostic biomarkers, proposing comparisons between sera from both septic and non-septic patients, and comparison across proteomic data from

patients with sepsis, severe sepsis, and septic shock, to identify protides specifically expressed either in this morbid condition or in one of its stages. Studies on sepsis treatment using proteomic technology are still rare.

Techniques of continuous renal replacement therapy (CRRT) have been occupying an important position in intensive care units (ICUs), employed in severe sepsis treatment when acute renal failure has already supervened[7,35]. Many water-soluble proteins with pro and anti-inflammatory activity play important roles in the severe sepsis pathophysiological process and are inflammatory response mediators. The clearance of these soluble proteins may account for a number of CRRT beneficial effects[36]. Changes occurring in serum proteome of patients undergoing CRRT remain unclear. As there is not a perfect understanding of CRRT, and there is no specific biomarker describing treatment progress, Gong et al.[37] investigated the proteome changes in patients with severe sepsis on CRRT. Ten proteins were identified as differentially expressed during CRRT. They include syntaxin-1B1 (an antithrombin III variant), CD5 antigen-like precursor, apolipoprotein A-IV precursor, apolipoprotein B-100 precursor, gamma-A isoform of fibrinogen gamma chain precursor, isoform 2 of ubiquitin E1-like activation enzyme, 36-kDa protein, MYH2 protein, and SPTAN1 protein (fragment). Among them, seven proteins were reduced in serum and three were increased during CRRT[37]. Western blot was performed to validate the study, evidencing the expression of CD5 antigen-like precursor and gamma-A isoform of fibrinogen gamma chain precursor in serum samples obtained from both patients on CRRT and controls (septic patients with no organ dysfunction and not treated by CRRT). The investigators detected both proteins in the serum of patients on CRRT but not in control patients[37].

CD5 antigen-like precursor was reduced in serum during CRRT. This protein: (1) plays an important role in regulating innate and adaptive immune systems[38], (2) induces aggregation of Gram-positive and Gram-negative bacteria, and (3) inhibits TNF-a secretion, a mediator playing a pivotal role in severe sepsis. Many of the innate immune response components normally involved in the *Homo sapiens sapiens* response to infection may occasionally damage cells and tissues, leading to multisystem organ failure[39]. CD5 antigen-like precursor was significantly high in patients' serum before CRRT and was reduced on CRRT[37].

An increase in gamma-A isoform of the fibrinogen gamma chain precursor in serum was observed during CRRT. Fibrinogen is a part of homeostasis events, being an acute phase reactant that responds to stress[40]. Different cells can produce cytokines, inducing an acute phase reaction and therefore increasing fibrinogen plasma levels[41]. The increased detection of serum gamma-A isoform of fibrinogen gamma chain precursor during CRRT suggests that the patients' immune system functioning has been partially restored[37].

By using differential gel electrophoresis (DIGE) – a proteomic technique in 2D gel using up to three different protide samples labeled by fluorescent dyes – Holly et al.[35] identified changes in the number of rat urinary proteins, including albumin, kidney brush-border enzymes (e.g., meprin-1-alpha), and serine protease inhibitors. Meprin is a brush-border enzyme playing a role in injuries related to ischemia and renal reperfusion. Meprin inhibition prevents in vitro hypoxic injury and in vivo ischemia/reperfusion injury[42]. This enzyme increase reflects kidney brush-border loss following sepsis-induced acute renal failure (chiefly septic shock). Treatment with actinonin, a meprin inhibitor, prevented acute renal failures in animal experiments[35]. This demonstrates the potential use of meprin as a sepsis biomarker and drug target in sepsis treatment.

Proteome and sepsis prognosis

In the prognosis evaluation of a patient with sepsis, the acute physiologic chronic health evaluation (APACHE II) score can be used, although the best strategy for this purpose is the sequential organ failure assessment (SOFA) score, which comprises respiratory, hematological, hepatic, cardiovascular, neurological, and renal variables[43]. The multiple organ dysfunction score (MODS) is also available, selecting six organ systems (respiratory, renal, hepatic, cardiovascular, hematological, and neurological) and easily scoring each observed dysfunction, allowing for an objective measurement of organic dysfunction severity at admission and follow-up by evaluating the dysfunction throughout hospitalization[44]. Recently, the association of inflammation biomarkers with these scores is considered to enhance the prognostic evaluation in patients with sepsis[45].

Another important reference to assess patients with sepsis is the PIRO concept, which is substantiated on multivariate elements, including predisposing conditions, insult quality and range, type and magnitude of the host response (deleterious response), and resulting or preexisting organic dysfunction (organic failure)[46]. The PIRO concept is interesting to rate septic patients, aiming at the development of studies to understand the physiopathology and improve therapeutics[47].

In addition to searching for biomarkers to identify sepsis and its variations, investigators seek markers to determine the disease prognosis by trying to identify the disease course and appropriate treatment on the basis of immune information and the patient's inflammatory

status. This information can be studied through proteomics technology, which, along with APACHE II, SOFA, and PIRO, will allow advances in sepsis treatment, prognosis, and outcome.

Sepsis prognosis studies using the above concepts and molecular studies, such as proteomics, are few[31,32,48]. In this field, recent investigation has revealed that, in early sepsis, there are significant differences in protein expression in patients surviving the condition versus those who do not. Patients surviving sepsis exhibited a strong activation of proteins involved in antibody-independent monocyte-mediated cytotoxicity, macrophage spread, plasminogen activation, and B lymphocyte proliferation. Survivors are thought to have a more efficient immune response. A study in 124 sepsis patients – with and without septic shock – was conducted by Oberholzer et al.[32] and evaluated not only the APACHE II and MODS scores, but also the proinflammatory and anti-inflammatory cytokine concentrations, as well as the procalcitonin and CRP levels. Correlations of these parameters with protein levels were established, and protein plasma concentrations of all cytokines and humoral mediators were high. IL-6 and sTNFR I concentrations, were significantly higher in patients surviving after 28 days, but not TNF-a, IL-8, IL-10, procalcitonin, and CRP concentrations. IL-6 concentration alone or in combination with APACHE-II or MODS scores is a strong candidate to predict clinical outcome in patients with severe sepsis[32].

Conclusion

Sepsis, despite being a very frequent condition in clinical practice, still remains enigmatic from different points of view. Indeed, there are very unclear points regarding physiopathology, diagnostic accuracy, therapy, and prognosis – which are related to the lack of information about many immune system aspects –, for which new investigations can bring light to in the near future.

In this field, proteomic studies stand out – being employed to understand different infectious conditions – and although the results are still quite preliminary in investigating sepsis, they have already shown great potential to become useful tools in the patient management, thus contributing to the much needed full care of patient.

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