Serum proteome alteration of severe sepsis in the treatment of continuous renal replacement therapy

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Abstract

Background. Continuous renal replacement therapy (CRRT) techniques have occupied an important position in the intensive care units (ICU). Serum proteome alteration and protein removal in this process are not clear. Since it has a poor understanding of mechanism of the treatment, there is a specific need of proteomics research for CRRT. The aim of this research was to study the serum proteome alterations of severe sepsis patients in the treatment of continuous veno-venous haemofiltration (CVVH). Improved knowledge of proteome alteration could lead to the development of more efficient treatment strategies.

Methods. In this study, 20 severe sepsis patients were enrolled. A proteomic approach with two-dimensional gel electrophoresis (2-DE) coupled with mass spectrometry and bioinformatics methods was utilized to identify proteins with altered expression at different times in the treatment of continuous veno-venous haemofiltration (CVVH). All proteins were identified on the appearance of the 2-DE gel at the appropriate molecular size and pH and score from peptide mass fingerprinting. Protein identifications were confirmed by sequencing of the tryptic peptides and an independent database search based on the sequence. A further validation study was performed by western blot.

Results. Thirty-four protein spots expressed differentially were separated. Ten proteins were identified to be the commonly differentially expressed proteins in the treatment. Seven proteins decreased in the serum and three increased.

Conclusions. This study gives a novel overview of serum proteome alteration of severe sepsis patient in the treatment of CVVH. Potentially interesting proteins have been revealed that are different from those identified by method of traditional biology.

Keywords: continuous renal replacement therapy (CRRT); continuous veno-venous haemofiltration (CVVH); proteome; proteomics; severe sepsis

Introduction

Continuous renal replacement therapy (CRRT) techniques have occupied an important position in the intensive care units (ICU). It was used in the treatment of severe sepsis. Severe sepsis refers to sepsis complicated by organ dysfunction including acute renal failure [1]. It is a major healthcare problem and is associated with high mortality. More than 200 000 severe sepsis patients die annually in the United States [2]. Many water-soluble proteins with pro- and anti-inflammatory action play important roles in the patho-physiological process of severe sepsis. These proteins are mediators of the inflammatory response. Blocking any one mediator has not led to a measurable outcome improvement. Removal of these soluble proteins may be responsible for some of the beneficial effects of CRRT. CRRT is a continuously acting therapy, which removes pro- and anti-inflammatory mediators in a non-selective way [3]. Broad areas of consensus exist for use of CRRT, and guideline development appears feasible. Equally broad areas of disagreement also exist, and additional basic and applied research is needed [4]. Serum proteome alteration in this process is not clear. Since there is a poor understanding of the mechanism of CRRT and there are no specific biomarkers to describe the progress of the treatment, there is a specific need of proteomics research for CRRT. Although cytokines and inflammatory proteins in the patho-physiological process of severe sepsis have been studied in recent years [5–8], all these studies used methods of traditional biology that identified only one protein at a time. These studies have many limitations and could not find some low-abundance inflammatory proteins.

In this study, a method of systems biology was used. We performed a proteomic approach with two-dimensional gel electrophoresis (2-DE) coupled with matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) and bioinformatic methods, as previously described [9], to study the proteome alterations and identify proteins in the serum of severe sepsis patients in the treatment of CRRT.

Proteomics is the large-scale study of proteins in a cell, tissue or biological fluids. It offers a high-throughput...
analysis of the expression of proteins in biological samples. Proteomics can be applied to provide a better understanding of disease pathogenesis and for treatment. It has been considered a powerful tool for the discovery of biomarkers. Particularly promising areas of proteomic research include delineation of altered protein expression in biological fluids, the development of novel biomarkers for diagnosis and early detection of disease [10]. The rapid development of proteomics technologies has provided new technology platforms to study the mechanism of CRRT.

Subjects and methods

Patients and samples

In this study, 20 patients with severe sepsis were included. Fourteen were men and six were women. They were all induced by sepsis. All patients were with acute renal failure and acute respiratory failure. The mean ages (±SD) were 57.6 ± 6.5 years. Informed consent was obtained from each subject. The protocol for this research project has been approved by the Ethics Committee of the Affiliated Renmin Hospital of Jiangsu University. It conforms to the provisions of the Declaration of Helsinki in 1995 (as revised in Edinburgh 2000). The diagnosis of severe sepsis was performed according to the criteria that were accepted by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) Consensus Conference 1992 Committee [11] and 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference [1]. Acute renal failure was defined as a rise of the serum creatinine >176.8 µmol/l (2 mg/dl) and a urine output <20 ml/h despite volume correction and intensive diuretic therapy. The mean of serum creatinine of these patients was 735.3 µmol/l at the beginning of CRRT. Their serum creatinine varied from 567.5 µmol/l to 937.9 µmol/l. The patient was submitted to a 72-h session of CRRT. The Acute Physiology and Chronic Health Evaluation II (APACHE II) score of each patient was recorded. The Sequential Organ Failure Assessment (SOFA) score of each patient was calculated daily. The exclusion criteria included patients receiving corticosteroids in the past 4 weeks, patients with diabetes mellitus and patients with acquired immune deficiency syndrome. The characteristics of severe sepsis patients are shown in Table 2.

CRRT was performed on the PRISMA machine, and the modality of CRRT was continuous veno-venous haemofiltration (CVVH). Vascular access for CRRT was obtained via the internal jugular vein catheterization with a double-lumen catheter. The blood flow rate was 120 ml/min and the ultrafiltration rate was 30 ml/kg/h. Unfractionated heparin was used as an anticoagulant. The dialyser was a Prisma M60, AN69 hollow fibre haemofilter/dialyser and was replaced every 24 h. The method of fluid replacement was predilutional and the type of replacement fluid used contained a bicarbonate buffer. The blood samples for the proteomics analysis were taken from a vein of the patients.

Serum samples of patients before CRRT (0 h) and serum samples at 24 h, 48 h and 72 h during the treatment of CRRT were obtained for the study. Serum samples of 20 controls (sepsis patients without organ dysfunction) were also collected. Their mean ages (±SD) were 51.3 ± 5.7 years. Ten were men and 10 women. The details of the control group are listed in Table 2. The blood samples were immediately centrifuged. A total of 20 µl of each patient sample was pooled to form four distinct samples (0 h group, 24 h group, 48 h group and 72 h group). All serum samples were stored at −80°C until analysis.

Table 1. An overview of the common differentially expressed proteins identified in the serum of severe sepsis patients treated by continuous renal replacement therapy

<table>
<thead>
<tr>
<th>Spot no.</th>
<th>Spot ID</th>
<th>IPI accession number</th>
<th>Protein name</th>
<th>Overall trend in the treatment of CRRT</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>133</td>
<td>IPI00025204</td>
<td>CD5 antigen-like precursor</td>
<td>Decreased detection in the serum</td>
<td>38 088 Da</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
<td>IPI00018067</td>
<td>Syntaxin-1B1</td>
<td>Decreased detection in the serum</td>
<td>33 245 Da</td>
</tr>
<tr>
<td>3</td>
<td>94</td>
<td>IPI00743092</td>
<td>36 kDa protein</td>
<td>Decreased detection in the serum</td>
<td>36 504 Da</td>
</tr>
<tr>
<td>4</td>
<td>181</td>
<td>IPI00032179</td>
<td>Antithrombin III variant</td>
<td>Decreased detection in the serum</td>
<td>52 602 Da</td>
</tr>
<tr>
<td>5</td>
<td>199</td>
<td>IPI00555997</td>
<td>MYH2 protein</td>
<td>Decreased detection in the serum</td>
<td>223 044 Da</td>
</tr>
<tr>
<td>6</td>
<td>163</td>
<td>IPI00746615</td>
<td>SPTAN1 protein (fragment)</td>
<td>Decreased detection in the serum</td>
<td>18 152 Da</td>
</tr>
<tr>
<td>7</td>
<td>1175</td>
<td>IPI00022229</td>
<td>Apolipoprotein B-100 precursor</td>
<td>Decreased detection in the serum</td>
<td>515 563 Da</td>
</tr>
<tr>
<td>8</td>
<td>917</td>
<td>IPI00847179</td>
<td>Apolipoprotein A-IV precursor</td>
<td>Increased detection in the serum</td>
<td>45 399 Da</td>
</tr>
<tr>
<td>9</td>
<td>1401</td>
<td>IPI00827491</td>
<td>Ubiquitin-activating enzyme E1-like protein 2</td>
<td>Increased detection in the serum</td>
<td>77 960 Da</td>
</tr>
<tr>
<td>10</td>
<td>961</td>
<td>IPI00219713</td>
<td>Isoform gamma-A of fibrinogen gamma chain precursor</td>
<td>Increased detection in the serum</td>
<td>51 512 Da</td>
</tr>
</tbody>
</table>

Table 2. The characteristics of severe sepsis patients and the control group (septic patients without organ dysfunction)

<table>
<thead>
<tr>
<th>Characteristics of severe sepsis patients and the control group (septic patients without organ dysfunction)</th>
<th>Number of severe sepsis patients</th>
<th>Number of the control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male patients</td>
<td>14</td>
<td>10</td>
</tr>
<tr>
<td>Female patients</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>The mean ages (±SD)</td>
<td>57.6 ± 6.5 years</td>
<td>51.3 ± 5.7 years</td>
</tr>
<tr>
<td>The APACHE II score (mean ± SD)</td>
<td>21.3 ± 6.7</td>
<td>15.6 ± 4.1</td>
</tr>
<tr>
<td>The outcome of patients (nonsurvival) (n %)</td>
<td>11(55%)</td>
<td>14(70%)</td>
</tr>
<tr>
<td>The outcome of patients (survival) (n %)</td>
<td>9(45%)</td>
<td>6(30%)</td>
</tr>
<tr>
<td>Source of infection (n %)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung</td>
<td>14(70%)</td>
<td>11(55%)</td>
</tr>
<tr>
<td>Wound</td>
<td>3 (15%)</td>
<td>4 (20%)</td>
</tr>
<tr>
<td>Blood</td>
<td>2 (10%)</td>
<td>2 (10%)</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>1 (5%)</td>
<td>3 (15%)</td>
</tr>
</tbody>
</table>

SD, standard deviation; APACHE II, acute physiology and chronic health evaluation II.
2-DE and image analysis

A large number of low-abundance proteins are often masked by a few high-abundance proteins because the intrinsic large dynamic range of serum proteins. To remove high-abundance proteins from serum samples, 50 μl of each of the four groups of pooled serum samples was processed with the ProteoExtract™ Albumin/IgG Removal Kit (Calbiochem, San Diego, CA, USA), which selectively removes albumin and IgG from the serum sample. After albumin and IgG depletion, excess salts followed by precipitation of proteins using the ProteoExtract™ Protein Precipitation Kit (Calbiochem) were removed in each of the four groups’ pooled samples. The samples were processed according to the manufacturer’s instructions. The protein concentrations of serum samples were determined by using the Bradford assay. Then, proteins were repeated three times. Protein concentrations in sera for the validation in this study was as previously described [12]. Each blot of the controls were described before. The aim of the western blot was without organ dysfunction who were not treated by CRRT. The controls were septic patients with severe sepsis who were treated by CRRT. The controls were septic patients.

The commonly differential expressed protein spots were picked from the 2-DE gel and digested with trypsin. The spots were dehydrated with 50 μl ACN, rehydrated in 5 μl porcine trypsin for 30 min, and then 10 μl 25 mM ammonium bicarbonate was added. Proteolysis was allowed to continue overnight at 37°C and stopped by adding 10 μl 2% formic acid, desalted using C18 zip tips. The resulting peptides were concentrated, and mixed with α-cyano-4-hydroxycinnamic acid (α-HCCA, Sigma, St Louis, MO, USA), deposited on a 384-well MALDI target and air-dried. Analyses were performed using a Biflex IV (Bruker Daltonics, Germany), as previously described [9]. By using the MASCOT search engine (http://www.matrixscience.com) based on the Swiss-Prot protein database, the peptide mass fingerprinting was performed for protein identification from trypic fragment sizes using the following assumption that peptides are monoisotopic. Though most matches did not contain any missed cleavages, one missed trypsin cleavage was allowed. Mass tolerance of 100 p.p.m (parts per million) was the window of error to be allowed for matching the peptide mass values.

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry and protein identification

The commonly differential expressed protein spots were picked from the 2-DE gel and digested with trypsin. The spots were dehydrated with 50 μl ACN, rehydrated in 5 μl porcine trypsin for 30 min, and then 10 μl 25 mM ammonium bicarbonate was added. Proteolysis was allowed to continue overnight at 37°C and stopped by adding 10 μl 2% formic acid, desalted using C18 zip tips. The resulting peptides were concentrated, and mixed with α-cyano-4-hydroxycinnamic acid (α-HCCA, Sigma, St Louis, MO, USA), deposited on a 384-well MALDI target and air-dried. Analyses were performed using a Biflex IV (Bruker Daltonics, Germany), as previously described [9]. By using the MASCOT search engine (http://www.matrixscience.com) based on the Swiss-Prot protein database, the peptide mass fingerprinting was performed for protein identification from trypic fragment sizes using the following assumption that peptides are monoisotopic. Though most matches did not contain any missed cleavages, one missed trypsin cleavage was allowed. Mass tolerance of 100 p.p.m (parts per million) was the window of error to be allowed for matching the peptide mass values.

Western blot validation

Since the CD5 antigen-like precursor and isoform gamma-A of the fibrinogen gamma chain precursor mouse monoclonal antibody (Abcam, Cambridge, UK), or an anti-human fibrinogen gamma chain rabbit polyclonal antibody (Abcam). After washing, the blots were incubated in 1:2000-diluted horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG, or anti-rabbit IgG (KPL Gaithersburg, MD, USA) for 1 h at 37°C. Proteins were detected with a Phototope-HRP Western Blot Detection Kit (Cell Signaling Technology, Danvers, MA, USA).

Results

In this study, a triplicate 2-DE gel image of each group was constructed and the ImageMaster software was used to make an average gel representing the medial protein expression level of each group. The 2-DE gel image of 0 h group was compared with that of 24 h group, 48 h group and 72 h group, respectively. Using the ImageMaster software, we identified protein spots with significant differences in expression levels that were 2-fold increased or decreased in the serum during the treatment of CRRT. Thirty-four protein spots expressed differentially were separated. Ten protein spots were identified to be the commonly differentially expressed protein spots during the treatment of CRRT. They were excised and digested with trypsin. Peptide masses were obtained by MALDI-TOF MS, and MASCOT searches allowed identification of ten different proteins. These commonly differentially expressed proteins include syntaxin-1B1, antithrombin III variant, CD5 antigen-like precursor, apolipoprotein A-IV precursor, apolipoprotein B-100 precursor, isoform gamma-A of the fibrinogen gamma chain precursor, isoform 2 of ubiquitin-activating enzyme E1-like protein 2, 36 kDa protein, MYH2 protein, SPTAN1 protein (fragment), as shown in Figure 1. Among them, seven proteins were decreased in the serum and three were increased in the serum during the treatment of CRRT. An overview of these proteins is presented in Table 1, which includes information of protein IPI accession numbers, protein names, overall trend (decreased or increased in the serum) in the
treatment of CRRT and the molecular weight of each protein. Quantitative comparison of the 10 protein spots at 0 h, 24 h, 48 h and 72 h groups is presented in Figure 2. The volume of spots was normalized and quantified as relative intensity by ImageMaster™ 2D Platinum software. The spot numbers are the same as those shown in Table 1. An example of identification of the CD5 antigen-like precursor from spot number 1 by peptide mass fingerprinting is seen in Figure 3. The top panel shows the spectrum obtained by MALDI-TOF MS of the tryptic fragments.

Using the method of western blot, we further studied the expression of the CD5 antigen-like precursor and isoform gamma-A of the fibrinogen gamma chain precursor in a new set of serum samples and the controls (septic patients without organ dysfunction who were not treated by CRRT) for validation. The results showed the detection of these two proteins in the new samples but in none of the controls, as shown in Figure 4.

Discussion

It has been considered that circulating cytokines and proteins cause universal endothelium injury in organs in the early phase of severe sepsis. In the later phase of severe sepsis, over-expression of inflammatory mediators in the interstitial space of various organs is considered a main mechanism of parenchyma injury [14]. De Vriese AS et al. studied the cytokine removal during continuous haemofiltration in septic patients and found that CVVH with an AN69 membrane in septic patients removes cytokines from the circulation, mainly by membrane adsorption [15]. But they used methods of traditional biology such as a solid-phase enzyme-linked immunosorbent assay that identified only one protein at a time. Lefler DM et al. investigated proteins present in ultrafiltrate of CRRT and identified several proteins by using proteomics technologies [16]. For some pro-inflammatory proteins, reduction in serum levels may be largely mediated by binding of the protein to the membrane. Research on the identification of proteins in ultrafiltrate obtained during CRRT is not complete. The results could not reflect the overall alteration trend of these identified proteins in the treatment of CRRT. In this study, a serum proteomics-based approach was used. All proteins were identified on the appearance of the 2-DE gel at the appropriate molecular size and pI and score from peptide mass fingerprinting. Protein identifications were confirmed by sequencing of the tryptic peptides and an independent database search based on the sequence. A further validation study was performed by western blot. Since the process of CRRT is dynamic, the research on the serum alteration of patients during CRRT is of great value. Identification of these proteins may provide more information about the mechanism of CRRT. It is interesting that one of the proteins in the ultrafiltrate of CRRT identified by Lefler DM et al. was apolipoprotein A-IV. It was also identified in the serum of severe sepsis patients during CRRT in our study.

All identified proteins in this study have a relatively high molecular weight that is more than 18 000 Da. They are poorly cleared by dialysis (diffusion). However, the elimination of these proteins might be enhanced by an epuration modality based on convection that depends on the extracorporeal flow rates of CVVH [17]. Brunet and coworkers confirmed that convection is more efficient than diffusion in removing middle molecular weight solutes in the treatment of CVVH (AN69 membrane, 0.9 m², substitution rate of 2 l/h) [18]. In our study, the ultrafiltration rate of CVVH was 30 ml/kg/h. The dialyser was an AN69 hollow fibre haemofilter/dialyser. Therefore, some proteins with relatively high molecular weights can be filtered through the haemofilter.

The decreased detection of the identified proteins in this study revealed the overall alteration trends of these proteins in the treatment of CRRT that included two parts. The first part is the filtration of CVVH including the binding of the proteins to the membrane of dialyser and the second part is the natural course of the proteins during sepsis. Data suggested that the combination of a relatively high ultrafiltration rate and frequent filter changes results in clinically measurable decreases in systemic cytokine concentrations [17]. For larger molecules during CRRT, the solute removal mechanisms of convection and adsorption have been emphasized. However, the overall alteration trends of these proteins during the treatment of CRRT are more important in clinical practice. We discuss potentially therapeutic and detrimental effects of the alteration of these proteins below.

Syntaxin-1B is one of the two isoforms of syntaxin 1, which plays an essential role in synaptic vesicle exocytosis. Mobilization of human neutrophil granules is critical for the innate immune response against infection and for the outburst of inflammation. Innate immune provides a first line of host defence that occurs rapidly and involves the coordinated action of diverse cells and proteins [19]. Human neutrophil–specific and tertiary granules are readily exocytosed upon cell activation. Research showed that syntaxin underlies the differential mobilization of granules in human neutrophils [20].

The CD5 antigen-like precursor was found decreased in the serum during CRRT. An alternative name is Sp alpha. It
is expressed by macrophages present in lymphoid tissues, and it binds to myelomonocytic and lymphoid cells, which suggests that it may play an important role in the regulation of the innate and adaptive immune systems [21]. It can induce aggregation of gram-positive and gram-negative bacteria strains and inhibit TNF-alpha secretion, which plays a strategic role in severe sepsis. Many of the components of the innate immune response that are normally concerned with host defence against infection can, under some circumstances, cause cell and tissue damage and hence multiple organ failure [19]. In this study, the CD5 antigen-like precursor showed significantly high abundance in the serum of patients before CRRT and was decreased in the treatment of CRRT.

In this study, the apolipoprotein A-IV precursor was found to be increased in the serum during CRRT.
The antiatherogenic properties suggest that this protein is an endogenous anti-inflammatory protein. The anti-inflammatory effect likely involves the inhibition of P-selectin-mediated leukocyte and platelet adhesive interactions [22]. The shifts of serum levels of the apolipoprotein A-IV precursor parallel changes in inflammatory parameters such as serum interleukin-6 (IL-6) activity [23] and the down-regulation of apolipoprotein A-IV messages may be mediated by IL-6 and TNF-alpha [24]. IL-6 induces a broad array of acute-phase proteins that limit inflammation, such as the α-1-acid-glycoprotein or C-reactive protein [25]. Apolipoprotein A is a highly polymorphic glycoprotein that forms a covalent complex with apolipoprotein B-100. Researchers have found that serum apolipoprotein A-IV levels are especially sensitive to the interruption of enteral feeding and rapidly fall to very low values during prolonged fasting [26]. Therefore, apolipoprotein A-IV has a close relationship with the nutritional conditions of patients. The loss of nutritional substrates must be considered in the treatment of CRRT. This is one of the side effects and may be harmful to patients treated with CRRT. However, the elimination of endotoxin and/or mediators might be at least potentially beneficial [27].

Isoform gamma-A of the fibrinogen gamma chain precursor was found to be increased in the serum during CRRT. Fibrinogen is a 340 000 kD trinodular dimeric protein consisting of three pairs of non-identical polypeptide chains, namely, (Aα/Bβ/γ)2 [28]. Fibrinogen participates in events other than haemostasis, and it is an acute phase reactant that responds to injury and stress [29]. It interacts with the binding of plasmin with its receptor, and finally it represents a major acute phase protein [30]. All cells are able to produce cytokines that induce an acute phase reaction, and thus increase fibrinogen plasma levels [30]. Researchers found that the depletion of fibrinogen may alter host susceptibility to the consequences of infection in mice [31]. Fibrinogen is necessary for immune inflammatory responses via stimulation of leukocyte adhesion and migration across endothelial cell layers [32]. Leukocyte activation causes production of inflammatory mediators, an increase in migration and infiltration, phagocytosis and degranulation, as well as receptor phosphorylation and signal transduction. The increased detection in the serum of isoform gamma-A of the fibrinogen gamma chain precursor during CRRT suggests that the immune functions of the patients were partially restored.

Since hypercoagulability was closely related to severe sepsis and endothelial cell activation/endothelial cell injury (ECA/ECI), treatment for reducing hypercoagulability and ECA/ECI was thought to be justified as one of the therapies for acutely ill septic patients [33]. Researchers found that the thrombin-antithrombin III complex was positively correlated with the multiple organ failure score and blood thrombomodulin levels in patients with severe sepsis [33]. The decreased detection in the serum of antithrombin III variant in this study shows that the conditions of the severe sepsis patients were improved with the treatment of CRRT. Isoform 2 of ubiquitin-activating enzyme E1-like protein 2 is a new protein that was found to be increased in the serum during CRRT in the study. It activates ubiquitin [34]. Ubiquitination is catalyzed by a cascade of enzymes consisting of E1, E2 and E3. Several studies have addressed the importance of various ubiquitin-like (UBL) post-translational modifiers. These UBLs are covalently linked to most target proteins through an enzymatic cascade analogous to ubiquitination, consisting of E1 (activating), E2 (conjugating) and E3 (ligating) enzymes [35]. Its increased detection in the serum during CRRT suggests that the immune functions of the patients have been improved with the treatment of CRRT.

In this study, we did not identify cytokines such as IL-1β, TNF-α, IL-6 and IL-8 that were considered to be inflammatory mediators in the patho-physiology process of severe sepsis. This is because previous studies used methods of traditional biology. They are antibody-based assays such as ELISA to identify inflammatory cytokines in the serum. These assays have sensitivity several orders of magnitude greater than the protein stains used in this study that used proteomic approaches.

Proteomics offers a high-throughput analysis of the expression of proteins in biological samples. 2-DE and mass spectrometry (MS) have become a key technology in proteome research. It is well known that each method has its own advantages and disadvantages, and no single method will be optimal in all applications. The method of 2-DE also has some limitations. For example, the dynamic range of detection possible in gels can be exceeded by the dynamic range of protein abundance in samples, making the detection of low-abundance proteins difficult. This limitation is being improved by using 2-DE together with other technologies to take advantage of the complementary strengths of each [36]. There are different ranges of pH of NL IPG strips. The broad range of NL IPG strips is pH 3–10 and the narrow range is pH 3–6, pH 5–8, pH 7–10 and pH 4–7. Wide gradients are applied for detecting and analysing the entire protein spectrum of various samples. In this study, we used the 24 cm, pH 3–10 range, NL IPG strips that are the broad-range NL IPG strips.

This study used a method of systems biology. It gives a novel overview of serum proteome alterations of severe sepsis patients in the treatment of CRRT. Potentially, interesting proteins have been revealed that are different from those identified by the method of traditional biology. Ten proteins were identified to be the commonly differentially expressed proteins in the serum of severe sepsis patients during the treatment of CRRT. These results would give novel insights into the mechanism of CRRT.
Supplementary data

Supplementary data are available online at http://ndt.oxfordjournals.org.

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Conflict of interest statement. None declared.

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