Review Article

Mass Spectrometry-based Proteomics in Acute Respiratory Distress Syndrome: A Powerful Modality for Pulmonary Precision Medicine

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Abstract

Objective: Acute respiratory distress syndrome (ARDS) is an acute and lethal clinical syndrome that is characterized by hypoxemic respiratory failure and diffuse alveolar inflammatory damage. This review aimed to search and discuss the mass spectrometry (MS)-based proteomic studies on different subsets of ARDS patients.
Data Sources: Original research articles were collected from the PubMed database published in English up to December 2015.
Study Selection: The literature search was done using the term "(acute lung injury OR acute respiratory distress syndrome) AND (proteomics OR proteome OR mass spectrum OR differential in-gel electrophoresis OR two-dimensional polyacrylamide gel electrophoresis)". Related original research articles were included and were carefully analyzed.
Results: Eight original proteomic researches on ARDS patients were found. The common proteomic modalities were two-dimensional (2D) high-performance liquid chromatography-based electronic spray ion-MS/MS and 2D-polyacrylamide gel electrophoresis/differential in-gel electrophoresis-based matrix-assisted laser desorption ionization-time of flight/MS. They compared the proteome between ARDS patients and normal controls and analyzed the dynamic changes of proteome at different ARDS stages or severity. The disturbed proteome in ARDS patients includes plasma acute-phase proteins, inflammatory/immune-associated proteins, and coagulation proteins.
Conclusions: Although several previous studies have provided some useful information about the lung proteome in ARDS patients and gained several interesting disease-associated biomarkers, clinical proteomic studies in ARDS patients are still in the initial stage. An increased cooperation is still needed to establish a global and faithful database containing disease-specific proteome from the largest ARDS subsets.

Key words: Acute Respiratory Distress Syndrome; Biomarkers; Mass Spectrometry; Proteomics

Introduction

The acute respiratory distress syndrome (ARDS) is manifested by acute and severe hypoxemia, bilateral diffuse infiltration in the lung, and a remarkable reduction in pulmonary compliance.[1] The most common trigger for clinical ARDS is severe sepsis, either pulmonary or nonpulmonary origin, which accounts for 79% of the cases.[2] Other etiologies of ARDS include aspiration, acute pancreatitis, multiple trauma, major surgery, massive transfusion, and burn injury.[3,4] Despite decades of extensive studies, the mortality of ARDS has not significantly improved and remains high. A most recent large observational study from fifty countries across five continents showed that hospital mortality was 34.9% for mild, 40.3% for moderate, and 46.1% for severe ARDS patients.[5] The mainstay of treatment for ARDS is lung-protective mechanical ventilation together with other assistant strategies aiming at decreasing ventilation-associated lung injury.[6-8]

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Lacking of effective drug therapy and a high mortality rate highlights the need for identifying new disease-specific biomarkers for ARDS. As with genomics, proteomic methods can simultaneously detect a much more comprehensive proteome as opposed to single or several proteins.[9,10] Therefore, by proteomic studies, we can better understand the molecular pathogenesis of disease and can identify the potential biomarkers that associate with disease diagnosis, prognosis, stratification, and treatment. Although the clinical proteomic studies on ARDS are still in the immature stage, some preliminary clinical studies have started to define the distinct proteome of ARDS patients using mass spectrometry (MS)-based proteomic technology. Here, we will discuss the clinical proteomic studies on the biological specimens, including bronchoalveolar lavage fluid (BALF), plasma, and lung cells, which are derived from ARDS patients.

Overview of Current Proteomic Technologies

The major objective of proteome research is to identify and characterize the comprehensive responses of a cell or biological system to different stimuli.[9] The emerging proteomic studies largely depend on the advances in protein separation, identification technology, and bioinformatics. A typical workflow for the proteomics studies should include several processes including sample acquisition, storage, preparation and separation/fractionation, protein/peptide quantification, and identification and bioinformatics for gene ontology (GO) and pathway analysis.[10] The two most popular proteomic modalities combine proteins/polypeptides separation by two-dimensional gel electrophoresis (2-DE) or by 2D liquid chromatography (strong cation exchange [SCX] followed by a reversed-phase column) with their identification/quantification by matrix-assisted laser-desorption ionization-time of flight-MS (MALDI-TOF-MS) or electrospray ionization (ESI)–tandem MS (MS/MS).[11-14]

Proteomic separation technology

Two main techniques are currently available to separate the protein/polypeptide cocktails. The first is gel-based separation technology named as 2-DE.[11] In the first separation phase (isoelectric focusing electrophoresis), proteins will migrate according to their isoelectric point in a gel with a consecutive pH gradient (usually from 3 to 10). They will stop moving at the place where they are neutrally charged. The second dimension of separation is dependent on the polyacrylamide gel electrophoresis (PAGE) according to different molecular weight. Then, the resulting 2D protein maps are stained and compared for the differential display proteomics.[11] The molecular members existing in the distinct proteome can be the potential disease-specific biomarkers.

The second and more popular approach for peptides separation is a gel-independent method called column-based liquid chromatography (LC). Typically, proteins in a complex biological sample are first digested with trypsin. Then, the labeled or unlabeled peptides are separated by two-dimensional high-performance liquid chromatography (2D-HPLC). The peptides are first separated on an SCX support followed by a reversed-phase column chromatography. Overall, higher or lower molecular weights proteins (with the molecular weight outside 10–150,000 range), insoluble hydrophobic proteins, and proteins with extreme pH which are difficult to separate on a gel can be well separated with 2D-HPLC.

Proteomic identification and quantification technology

John Fenn and Koichi Tanaka won the Nobel Prize for Chemistry in 2002 for the development of the two soft ionization techniques that greatly revolutionized biological MS named by MALDI and ESI, respectively.[9] Generally speaking, when proteins are separated by 2-DE, the interested protein spots are isolated and digested. Then, the peptides are spotted onto an MALDI target plate and are introduced into the TOF-MS where they are separated and recorded according to their mass. Alternatively, when the complex protein mixtures are free of gel-based separation, they will be first enzymatically digested into peptides, separated by 2D-HPLC, and ionized directly into a tandem MS to identify proteins. The protocol of 2D-HPLC coupled with MS/MS is also referred to as "shotgun" proteomics.[15,16]

Bioinformatics

Using database searching, the observed mass of peptides originated from a trypsinized parent protein is compared with theoretical digests of all proteins in the database (e.g. Sequest, Mascot, Comet, and X!tandem).[17] This process is named by peptide mass mapping (PMM) and peptide sequencing. Following the database searching, a long list of possible protein candidates is obtained, and a score is also ranked to indicate the level of significance.[10] For further data exploration, some mathematical methods are used including hierarchical clustering, self-organizing maps, and artificial neural networks.[10] By bioinformatics analysis, the biological process and molecular function of differentially expressed proteins can be well characterized. Furthermore, by incorporating unidentified proteins using proteomics technology, the network analysis can easily predict the potential central hubs that may be implicated in the pathogenesis of a specific disease.

Taken together, current MS-based proteomics is mainly performed by 2-DE-MALDI-TOF/MS (gel based) and 2DLC-ESI-MS/MS (shotgun) methods. There are some other alternatives MS-based proteomic technologies such as surface enhanced laser desorption ionization (SELDI)-TOF/MS, difference gel electrophoresis (DIGE)-TOF/MS, and isobaric tag for relative and absolute quantitation (iTRAQ)-2DLC-MS/MS. The detailed description and comparison of these technologies are out of the focus here and can be found in the related reviews elsewhere.[18-20]

Clinical Proteomic Studies Based on Biological Mass Spectrometry of Acute Respiratory Distress Syndrome Patients

The lung is a highly orchestrated organ which is composed by many cells types including epithelial cells, endothelial cells, immune cells, and resident lung fibroblasts. Furthermore, because the lung is directly exposed to the air particulate, its proteome may be multifaceted and vary with environmental compounds.[9] Currently, the majority of the lung proteomic studies are descriptive using various biological specimens including plasma/serum, epithelial lining fluid (ELF), lung tissue biopsies, and lung cell culture.[9,10,21] In the following, we will discuss the existing literature describing ARDS lung proteomes.

Plasma proteome in acute respiratory distress syndrome

Human plasma is a protein-rich informational reservoir that contains proteome originating from a variety of tissues and blood cells by a form of active secretion or passive leakage.[22] Plasma can be easily acquired by less invasive method compared with other human body fluid. Hence, plasma proteomics is an attractive area for disease investigation. Furthermore, biomedical studies have demonstrated that protein profiles from blood may largely reflect human physiological or pathological status and can be used as the potential biomarkers for disease diagnosis, prognosis, and treatment.[23] Plasma proteome contains a dynamic range of protein concentrations of at least 9–10 orders of magnitude, indicating a multifaceted manner. How to identify and quantify all of the plasma proteins remains a critical challenge.

Bowler *et al*.[24] first compared the plasma proteome between 16 acute lung injury (ALI)/ARDS patients and 12 normal volunteers in 2004. They found several elevated proteins in the plasma of ALI patients including albumin, serum amyloid protein, hemopexin, IgG heavy chain, complement component 3, α2 or β-hemoglobin, α2 or β2-glycoprotein1, and α2-Heremans-Schmid-glycoprotein. By contrast, the protein expression level of α1-antitrypsin, haptoglobin, and transthyretin was remarkably reduced in ALI plasma. The differentially expressed proteins in ALI plasma compared with normal counterparts indicate an acute-phase response and a serious diseased milieu.

More recently, Chen *et al*.[25] conducted a study aiming at elucidating novel biomarkers for disease diagnosis/pathophysiology and identifying the potential ARDS treatment targets using iTRAQ-labeled proteomic analysis (IEF-MALDI-TOF-MS). In this study, volunteers were divided into three groups including direct acute lung injury (AD, n = 6) group, indirect acute lung injury (AI, n = 5) group, and control group (n = 15). They found 16 differentially expressed proteins in ARDS patients, of which 11 proteins were in both the AI and AD group, while 5 proteins were AI specific. GO annotation of the differential proteins showed that they fell into different categories such as metabolic process, immune system process, transport/cellular process, and response to stimulus. Furthermore, pathway analysis showed that distinct pathways may be activated in ARDS such as acute phase response signaling and inflammation signaling in macrophages.[25]

Taken together, current limited and preliminary plasma proteome studies focusing on ARDS may provide novel biomarker candidates and may shed new light on the pathogenesis of ARDS. Nevertheless, we must bear in mind that biomarkers derived from the plasma proteome may not be lung specific and require further validation.

Lung tissue proteome in acute respiratory distress syndrome

To obtain the lung tissues, more invasive sampling techniques are required such as open lung biopsy (OLB) and transbronchial lung biopsy. Hence, the majority of studies have focused on the comparison between normal and cancer lung tissue proteome using 2-DE-based MALDI-TOF/MS and shotgun proteomic approaches.[26,27] There are also lung tissue proteome studies on other lung diseases which usually need pathological diagnosis such as different forms of interstitial lung diseases, especially idiopathic pulmonary fibrosis (IPF).[28] Furthermore, some studies used 2-DE-based proteomic technique to study the features of the lung tissue protein profiles obtained from patients with chronic obstructive lung disease.[29] However, as far as we known, there is still a lack of proteomic studies focusing on identifying and quantifying lung tissue proteins obtained from ARDS patients. This is largely due to the difficulty to access enough lung tissues in the critically ill patients. Nevertheless, some previous studies have shown that bedside OLB is a helpful and acceptably safe diagnostic- and therapeutical-guiding technique for patients with ARDS.[30-32] Thus, the lung tissue proteome studies could be simultaneously manipulated in these ARDS patients who received OLB performance. This is vital important because lung tissue-specific proteomics has more power to find the faithful and valuable biomarkers for ALI/ARDS diagnosis, prognosis, pathogenesis, and treatment than plasma proteomics do.

Lung cell proteome in acute respiratory distress syndrome

Proteomic approach that targets specific lung cells has also been performed. It was previously shown that alveolar macrophages (AMs) have the unique proteomic cocktails in terms of their physiologic role in proteolysis, actin reorganization, and cellular adaptation compared with their counterparts of blood mononuclear cells.[33,34] Using 2-DE-based MS technology, Silva *et al*.[35] conducted a study aiming at finding the proteomic alterations of AMs in pulmonary sarcoidosis as compared with healthy volunteers. Recently, Dong *et al*.[36] performed a comparative analysis of the alveolar macrophage proteome in ALI/ARDS patients

between the exudative and recovery phase. Fourteen patients with ALI/ARDS were enrolled and received bronchoalveolar lavage (BAL) to obtain BALF AMs on days 1 (exudative phase) and 5 (recovery phase) upon diagnosis, respectively. Seventeen proteins were found more abundant at the recovery phase (e.g., protein S100-A8/A9, interleukin-1 receptor antagonist protein, tumor necrosis factor alpha, and leukocyte elastase inhibitor), while ten proteins were remarkably upregulated at the exudative phase (e.g., cathepsin B and heat shock protein 27). They concluded that AMs underwent a functional switch during ALI/ARDS onset and resolution, in which AMs in the exudative phase can initiate, augment, and perpetuate acute inflammation, while AMs in the recovery phase have a distinct role in anti-inflammatory response to prevent further lung injury. Unfortunately, we do not find any proteomic study that compares the proteome of AMs derived from ALI/ARDS patients and normal controls in different disease stages.

Bronchoalveolar lavage fluid proteome in acute respiratory distress syndrome

Physiologically, the airways, as well as alveoli, are covered with a thin layer of ELF.[10] ELF proteins can be originated from the release of the lung resident cells and from the passive diffusion or active transport of plasma proteins through the alveolar-capillary membrane.[37] ELF gained by BAL (BALF) may faithfully reflect the comprehensive proteome from the airspaces and small airways.

The first proteome analysis of BALF was done in 1979 by Bell and Hook.[38] They used 2D-PAGE technology and compared the differential BALF proteome between normal volunteers and patients with pulmonary alveolar proteinosis.[38] Then, Lenz *et al*.[39,40] compared the BALF proteomes among IPF, sarcoidosis, asbestosis, and normal controls using 2D-PAGE technology.

Our focus was on the first proteomic report aiming at studying BALF and edema fluid (EF) proteomes in 12 normal volunteers and 16 ALI patients conducted by Bowler *et al*.[24] Using 2-DE-MALDI-TOF/MS, they found that albumin, transferrin, IgG, clusterin, serum amyloid protein, α2 or β-hemoglobin, α2 or β2-glycoprotein1, α1-antitrypsin, and α2-Heremans-Schmid-glycoprotein were increased in the EF of all ALI patients. The enrichment of acute-phase plasma proteins in EF indicates an increased permeability of the alveolar-capillary barrier in ALI patients. On the contrary, SP-A was significantly decreased in the EF of ALI patients, indicating that alveolar type II cell function may be remarkably impaired. Furthermore, they found different isoforms of the same protein (such as haptoglobin and complement component 3) were only evident in the EF of ALI patients, but not in the normal volunteers, suggesting that enhanced proteolytic activity or other posttranslational modifications exist in the affected lung.

Soon after, de Torre *et al*.[41] identified several inflammatory biomarkers, including apolipoprotein A1, and S100 calcium-binding proteins A8 and A9, in 11 patients with ARDS using SELDI-TOF/MS and 2-DE-MALDI-TOF/MS proteomics methods. Simultaneously, Schnapp *et al*.[42] used a shotgun proteomic approach (2D-HPLC-MS/MS) to analyze BALF proteomics from three ARDS patients. They showed that BALF proteins in ARDS patients had extensive coverage of abundant plasma proteins, including albumin, ceruloplasmin, fibrinogen α chain, and other acute-phase reactant proteins, such as α1 chymotrypsin, α2-Heremans-Schmid-glycoprotein, and antitrypsin inhibitor. This further indicates the remarkable leakage properties of alveolar barrier in ARDS patients. Moreover, they identified insulin-like growth factor-binding protein-3 (IGFBP-3) as a novel mediator of apoptotic pathways in ARDS. This was validated by the following ELISA assay which showed a marked increase of IGFBP-3 in patients at risk for ARDS and in those with established ARDS.

The studies listed above-studied BALF proteomics in ARDS patients at only a single time, so the dynamic changes of BALF proteins that occur during the evolution of ARDS remain elusive. To address this problem, Chang *et al*. used DIGE-MALDI-TOF/MS proteomics approach to characterize BALF proteins of ARDS patients at days 1, 3, and 7 after disease onset and compared the results with normal BALF protein profiles.[43] An average of 991 protein spots was detected in 2D gels, of which 80 protein spots (representing 37 unique proteins) were identified by TOF/MS method. Overall, differentially expressed BALF proteins in ARDS patients and normal volunteers represented diverse protein families including immune response, antioxidants, basement membrane proteins, coagulation proteins, and plasma acute-phase proteins. Furthermore, the principal component analysis suggested that the perturbed BALF protein profiles were comparable over the subsequent course (days 3 and 7) of ALI/ARDS.

In addition to these studies aiming at better understanding of ARDS pathogenesis, some other BALF proteomic studies aimed to find new potential biomarkers that may provide faithful diagnostic and prognostic information. Recently, Bhargava *et al*.[44] launched a study of the BALF proteome to identify proteins that may discriminate ARDS survivors from nonsurvivors using high-resolution MS-based proteomics (iTRAQ-labeled 2D-LC-MS/MS). In this study, ARDS patients were divided into early phase (1–7 days after initiation of mechanical ventilation) survivors (n = 7) and nonsurvivors (n = 8) and late phase (8–35 days after initiation of mechanical ventilation) survivors (n = 7). They found that the upregulated proteins in early phase survivors included the coagulation and fibrinolysis factors (such as coagulation factor II/XII, plasminogen, and antithrombin III), immune responsive proteins (such as complement C5/C1r), and proteins maintaining the cation and iron homeostasis (such as hemopexin, ferritin, and ceruloplasmin). However, early phase nonsurvivors had more abundant proteome which

participated in carbohydrate catabolism (such as enolase 1 and glyceraldehyde-3-phosphate dehydrogenase-like 6) and collagen metabolic process (such as Type I/III/V collagen and matrix metallopeptidase 9). In contrast, cell migration- and actin organization-associated proteins were more significant in late phase survivors, indicating the dynamic changes in the BALF proteome. The authors concluded that differentially expressed BALF proteins in the early phase of ARDS can be used as the faithful biomarkers to differentiate nonsurvivors from survivors for disease prognostication.

Another study released by Nguyen *et al*.[45] aimed to discriminate the ventilator-associated pneumonia (VAP) in patients with ALI using shotgun proteomic analysis based on tandem MS (2D-LC-MS/MS). In this study, BALF was obtained from five normal controls and thirty ALI patients with VAP (VAP^+, $n = 14$) and without VAP (VAP^-, $n = 16$). One hundred and sixty-six differentially expressed proteins were found between normal and ALI volunteers, of which 47 proteins were more abundant in normal BALF, whereas 119 were enriched in the BALF of ALI patients. GO analysis showed the upregulated BALF proteins in ALI patients involved in the defense/inflammatory/immune response and wound healing process. Conversely, decreased BALF proteins in ALI patients were more closely related with endopeptidase inhibitor activity and metabolic processes. Furthermore, they identified 76 differentially expressed BALF proteins between VAP^+ and VAP^- ALI patients, of which 60 proteins were more abundant in VAP^+ subset. Proteins enriched in VAP^+ group involved in defense, immune response, and leukocyte migration, whereas proteins more abundant in VAP^- group were largely related with fibrinogen complex, cell surface binding, wound healing, and developmental processes. Finally, the authors identified and validated a triad of limited proteomic biomarkers that can separate VAP^+ from VAP^- patients: S100A8, lactotransferrin, and actinin 1.

Collectively, these "differential-display proteomic" studies compared the distinct proteomes in various lung milieus (i.e., patients with different subsets of ALI/ARDS and healthy controls) and obtained a comprehensive view on the characteristics of lung proteome. By analyzing the previous proteomic data, we can better understand the pathogenetic factors, signals, and events underlying ARDS and can identify the potential biomarkers with the ability to define disease status, to get earlier diagnosis, and to guide clinical management for ARDS. The proteomic studies on ARDS patients are summarized in Table 1.

Future Directions of Clinical Proteomics in Acute Respiratory Distress Syndrome

The preliminary studies discussed in this review provided the feasibility that ARDS proteome can be manipulated using proteomic technologies with the clinical samples such as plasma, cells, and BALF. However, clinical proteomics in defining ARDS patients is still in its infancy. There are some limitations that need to overcome in the future. First, although proteomics is defined to achieve the goal of detecting the whole set of proteins expressed by a cell or organism, it seems impossible with current proteomic technologies because of the complexity of a biological specimen and the limitations in protein separation and MS methods. 2-DE-MS is a genuine "top-down" analytical method; however, certain groups of proteins, including low abundant proteins, membrane, and hydrophobic proteins, and proteins with very high or low molecular mass or extreme pH cannot be well separated by 2-DE, thus cannot be detected by subsequent MS.[11,46] Although the coupling of LC with MS significantly improved the separation, identification, and quantification of small, minor, or hydrophobic proteins, it is still unable to detect the relatively low abundant proteins in the multifaceted biological cocktails, such as chemokines/cytokines/growth factors, intracellular signaling proteins, or transcription factors. Thus, the current proteomic technology only defines a selected subset not a global set of proteome in ARDS patients. Other proteins that may closely connect with disease warning, diagnosis, stratification, and treatment would be unintentionally ignored. A popular method that aims at increasing the identification of low abundance proteins is immunodepletion strategy.[24] With this method, high-abundance plasma proteins may be depleted, such as albumin, transferrin, haptoglobin, antitrypsin, IgG, and IgA.[43] However, many of the highly expressed plasma proteins are associated with the pathogenesis of ARDS, so alternative technological advances should be anticipated to explore a more comprehensive lung proteome.

Second, the current clinical studies focusing on ARDS proteomics are dependent on limited human volunteers, which may not fully depict the whole proteomic nature of ARDS patients. Because human beings show a great individual difference in protein expression, a large human cohort may be recruited to identify the valid, disease-specific biomarkers. Furthermore, ARDS is a multifaceted, seriously disorganized disease with diverse etiology and evolution process, so well designed, ARDS subset based (i.e., direct or indirect, infective or noninfective, etc.), while large cohort, prospective clinical studies are needed. Meanwhile, proteomic studies on ARDS should not only base on BALF but also on plasma/serum and diseased lung tissues and lung cells. Only by this way, we can realize the ultimate goal of the clinical proteomics studies that is to identify useful disease-associated indicators.

Third, some proteomic studies lack validations for the potential biomarkers using other more accurate molecular biological techniques, such as antibody-based Western blot, and ELISA. Most proteomic experiments typically identify hundreds of differentially expressed proteins, yet few of them are the real or helpful biomarkers for disease. We believe that the expression levels of interesting biomarker candidates should be evaluated in a larger sample size. Moreover, their functional role in the pathophysiology of ARDS should be

Table 1: Representative proteomic studies on ARDS

References	Samples	Study design	Proteomic technology	Major findings	Validation assay
Bowler *et al*.[24]	EF, BALF, plasma	To detail the protein profiles from 16 ALI patients and 12 normal volunteers	2D-PAGE; MALDI-TOF/MS	a: 158 proteins were identified b: Transferrin, IgG, clusterin, serum amyloid protein, hemopexin, IgG heavy chain, complement component 3, α2/β-hemoglobin, α2/β2-glycoprotein1, α2- Heremans-Schmid glycoprotein↑ c: SP-A, α1-antitrypsin↓ d: Truncation or other posttranslational modifications↑	None
Chen *et al*.[25]	Plasma	To elucidate novel biomarkers for disease diagnosis/ pathophysiology and identify the potential ARDS treatment targets by analyzing 11 ARDS patients and 15 normal controls	iTRAQ; IEF; MALDI-TOF-MS	a: 132 plasma proteins were confirmed b: Alpha-1-antitrypsin, complement component C9, alpha-1-acid glycoprotein 1, alpha-2-glycoprotein, alpha-1-antichymotrypsin, isoform 1 of C-reactive protein and serum amyloid A protein↑ c: Complement factor H, apolipoprotein A-I, serotransferrin↓	None
de Torre *et al*.[41]	BALF	To assess markers of lung inflammation by enrolling 33 volunteers challenged with endotoxin or saline and 11 patients with ARDS	SELDI-TOF/ MS; 2D-PAGE; MALDI-TOF/MS	a: Apolipoprotein A1, S100 calcium-binding proteinsA8 and A9↑ b: ATIII, transthyretin, hemoglobin A chain b↑	Western blots
Schnapp *et al*.[42]	BALF	To obtain a more complete protein profile of 3 ARDS patients	2D-HPLC; ESI-MS/ MS (Shotgun)	a: 870 proteins were identified b: Albumin, ceruloplasmin, fibrinogen α chain, α1 chymotrypsin, α2-HS-glycoprotein and antitrypsin inhibitor were abundant c: IGFBP-3 were identified	Western blots; ELISA
Chang *et al*.[43]	BALF	To examine the changes in protein expression of ARDS patients on days 1 (*n*=7), 3 (*n*=8), and 7 (*n*=5)	DIGE; MALDI-TOF-MS	a: 991 protein spots were detected b: Immune response (complement C3 precursor, S100 calgranulin A9)↑ c: Fibrinogen alpha chain↑ d: α1-antitrypsin, apolipoprotein A1, hemopexin precursor↑	None for identified proteins; cytometric bead-based immunoassay system and ELISA kit of incorporated proteins by pathway analysis that were not identified from the proteomics experiments, for example, TNF-α, IL-1β, and IL-6
Bhargava *et al*.[44]	BALF	To define the proteomic profiles in 24 ARDS patients that differentiate survivors from nonsurvivors	iTRAQ; 2D-HPLC; ESI-MS/MS	a: 724 proteins were identified b: Coagulation factor II/XII, plasminogen, antithrombin III, Complement C5/C1r, hemopexin, ferritin, and ceruloplasmin were more abundant in early survivors c: Type I/III/V collagen, matrix metallopeptidase nine were more abundant in early nonsurvivors	ELISA

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Table 1: Contd...

References	Samples	Study design	Proteomic technology	Major findings	Validation assay
Nguyen *et al*.[45]	BALF	To discriminate VAP in thirty ALI patients	2D-HPLC; ESI-MS/MS	a: 119 proteins (defense/inflammatory/immune response and wound healing process) in ALI patients↑ b: 47 proteins (endopeptidase inhibitor activity and metabolic processes) in ALI patients↓ c: S100A8, lactotransferrin, and actinin 1 were more abundant in VAP^{+}than VAP^{-}ALI patients	ELISA
Dong *et al*.[36]	Aveolar macrophage	Comparative analysis of the alveolar macrophage proteome in 14 ALI/ARDS patients between the exudative phase and recovery phase	2D-PAGE; MALDI-TOF-MS	a: 27 informative proteins were identified b: 17 proteins were more abundant at the recovery phase (e.g., protein S100-A8/A9, IL-1 receptor antagonist protein, TNF-α, leukocyte elastase inhibitor) c: 10 proteins were remarkably upregualated at the exudative phase (e.g., cathepsin B, heat shock protein 27)	Western blots

ALI: Acute lung injury; ARDS: Acute respiratory distress syndrome; BALF: Bronchoalveolar lavage fluid; DIGE: Difference in-gel electrophoresis; EF: Edema fluid; ESI: Electrospray ionization; IEF: Isoelectric focusing; iTRAQ: Isobaric tags for relative and absolute quantitation; MALDI-TOF/MS: Matrix-assisted laser desorption/ionization-time of flight-mass spectrometry; SELDI: Surface-enhanced laser desorption ionization; 2D-HPLC: Two-dimensional high-performance liquid chromatography; 2D-PAGE: Two-dimensional polyacrylamide gel electrophoresis; IL-6: Interleukin-6; TNF-α: Tumor necrosis factor-alpha; VAP: Ventilatorassociated pneumonia; ↑: Up-regulated; ↓: Down-regulated.

further validated by both *in vitro* and *in vivo* experimental studies.

Conclusions

The aim of proteomic studies on ARDS is to search for clinically relevant disease biomarkers. If the results of proteomic studies can be easily translated into clinical practice, they will shed new light on the understanding of the mechanisms of ARDS and be beneficial to the discovery of new therapeutic candidates for disease prevention and treatment. To realize this goal, an increased international cooperation is needed to establish a global and faithful database containing ARDS-specific proteome derived from plasma/serum, BALF, as well as lung cells/tissues with the largest ARDS subsets.

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Conflicts of interest

There are no conflicts of interest.

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