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Invited critical review

New markers in pneumonia

Chao-Wen Cheng [a], Ming-Hsien Chien [a,b], Shih-Chi Su [c], Shun-Fa Yang [d,e,*]

[a] Graduate Institute of Clinical Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan
[b] Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan
[c] Department of Urology, the University of Texas MD Anderson Cancer Center, Houston, TX, USA
[d] Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
[e] Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan

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ABSTRACT

Pneumonia is one of the most common causes of death from infectious diseases worldwide, and the most common fatal infection acquired in hospitals. Despite advances in prevention strategies, such as antibiotic therapies and intensive care, significant improvement in the mortality rate is still lacking. This high mortality is largely due to the limitations in current clinical practices and laboratory tests, which delay the timing of adequate antibiotic therapy. In recent years, many indicators (biomarkers) are present in scenarios where infectious pathogens invade into the body. These biomarkers, as reflected in specific biological responses to infections, have been reported to demonstrate the ability to facilitate the diagnosis, risk stratification, and management of pneumonia. This review provides a schematic overview of these new potential biomarkers based on the categories of (1) microorganisms and their derivatives, (2) inflammation mediators, (3) inflammation response proteins, and (4) stress-sensing proteins. In addition, approaches to identifying new biomarkers are also briefly introduced. Although no current biomarker can solely achieve a definitive diagnosis, many of them can be complemented, rather than replaced outright, in routine clinical practices to improve decision-making processes regarding pneumonia.

Contents

1. Introduction to pneumonia

Pneumonia, an acute infection in the lung parenchyma leading to an abnormal pulmonary function, is the most common infection during hospitalization [1]. The classifications of pneumonia include community-acquired pneumonia (CAP), hospital-acquired pneumonia (HAP), ventilator-associated pneumonia (VAP), and nursing home-associated pneumonia (NHAP) [2]. CAP refers to pneumonia

* Corresponding author at: Institute of Medicine, Chung Shan Medical University, 110 Chien-Kuo N. Road, Section 1, Taichung 402, Taiwan. Tel.: +886 4 24739595x34253; fax: +886 4 24723229.
E-mail address: ysf@csmu.edu.tw (S.-F. Yang).

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acquired outside of hospitals; HAP describes infections acquired in the hospital setting; VAP is a subtype of HAP that occurs when receiving mechanical ventilation; NHAP is defined as infection acquired in an extended-care facility. In the United States, an estimated 5.6 million patients are diagnosed with CAP annually, with 1.1 million requiring hospital admission. The mortality rate of CAP outpatients is in the range of 1%–5%, with 12% of patients requiring hospitalization, and as much as 40% of patients requiring admission to intensive care units (ICUs) [3,4]. Although the clinical presentations are often similar, pneumonia can also be classified into typical or atypical types according to the differences in pathogens. The typical type of pneumonia is usually caused by bacteria such as streptococcus and staphylococcus, while the atypical type is caused by the influenza virus, mycoplasma, chlamydia, legionella, adenovirus, or other unidentified microorganisms [5]. Ideally, as an early and definitive diagnosis can be achieved by verifying the specific pathogens involved, subsequent treatment with specific antibiotics can improve the prognosis of pneumonia. However, a rapid and accurate diagnosis of pneumonia remains challenging.

The common clinical symptoms of pneumonia patients include cough, fever, chills, fatigue, dyspnea, rigors, and pleuritic chest pain [6]. Depending on the various types of pathogens involved, pneumonia may be accompanied with different features. According to the clinical guidelines, the gold standard for diagnosing pneumonia involves the use of chest radiography to detect the presence of pulmonary infiltrates. However, chest radiography is not sensitive enough for detecting specific pulmonary infiltrates and the early course of the disease. Up to one-third of possible pneumonia diagnoses may be missed when using chest radiography [7]. In addition, certain chronic diseases, such as congestive heart failure, chronic obstructive pulmonary disease, and malignancy, can also interfere with the results. Laboratory tests, including leukocyte count, sputum Gram stain, 2 sets of blood cultures, and urine antigens, can also help provide a definitive diagnosis and guide therapeutic decision making. However, the results of these tests on patients with suspected CAP vary because of the uncertain quality of specimens and the use of antimicrobial drugs. A positive blood culture may have no correlation with the severity of the illness or outcome [6]. Such uncertainty and time-consuming limitation may obscure accurate diagnoses and delay the beginning of treatment. Therefore, innovative approaches for identifying the indicators of biological state of pneumonia are eager to be achieved.

2. Biomarkers in pneumonia

The term “biomarker” is defined as a biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition [8]. According to this definition, a qualified biomarker of pneumonia should be used to accomplish at least one or more of the following tasks: perform a rapid and accurate diagnosis and less affected by other diseases, identify the types of pathogenic microorganisms for specific antibiotics treatment, and reflect the status of successful prognosis therapy or remain elevated with the infectious stimulus [9,10]. Consequently, the potential biomarkers in pneumonia exist in the specific biological responses to infections and the scenario where the infectious pathogens invade in the host body. Noteworthily, most of the potential markers in pneumonia are also described in the sepsis. Therefore, biomarkers in pneumonia can fall into four categories: (1) microorganisms and their derivatives; (2) inflammation mediators; (3) inflammation response proteins; and (4) stress-sensing proteins (Table 1).

Table 1
Potential biomarkers presented the biological state of pneumonia.

Category	Biomarker
Microorganisms and their derivatives	*Conserved bacterial genomic sequence (PCR)*
	Endotoxin
Inflammation mediators	*IL-1β*
	IL-6
	IL-8
	IL-10
Inflammation response proteins	*C-reactive protein*
	Long pentraxin 3
	Procalcitonin
	Pro-adrenomedullin
	Soluble form of Triggering receptor expressed in myeloid cell-1
Stress-sensing proteins	*Copeptin*
	Cortisol

2.1. Microorganisms and their derivatives

In the progress of pneumonia, pathogenic bacteria that undergo host respiratory colonization and growth can be detected and characterized by performing conventional laboratory tests. Although these methods are highly specific, the result is affected by the usage of antimicrobial drugs and the sensitivity is poor. The application of polymerase chain reaction (PCR) in species-specific genes and conserved bacterial DNA sequence-based diagnostic tests has been developed [11,12]. In contrast to negative sputum cultures, most samples from patients infected with *Streptococcus pneumoniae* and receiving antibiotics can still be accurately evaluated by applying PCR [13]. Examination by using a real-time (RT) PCR technique can reduce the time consumption associated with culture methods and permit early diagnosis. In a prospective study in Denmark, a quantitative multiplex PCR method was evaluated by detecting *S. pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis* in bronchoalveolar lavage fluids (BALF) and cerebrospinal fluids (CSF). Both the sensitivities and specificities for *S. pneumoniae* and *H. influenzae* can reach 80%–90% in a cut-off of 10^5 genomic copies/mL for clinical positivity. In a previous study where patients had taken antibiotics prior to sampling, 36% of *S. pneumoniae* infections and 53% of *H. influenza* infections could be detected using PCR, whereas only 6% and 20%, respectively, could be detected in culture. In addition, identifying the *S. pneumoniae* and *N. meningitidis* in CSF can achieve 100% sensitivity and specificity [14]. Pathogens detected by PCR are not found by using the culture methods, and vice versa, suggesting that PCR can provide additional information compared to current culture methods [14,15]. PCR results may show the existence of specific infectious pathogens, which, however, is not considered the “illness” of pneumonia.

VAP is the main infection acquired in an ICU and with the use of antibiotics. The microbiologic flora associated with VAP encompasses multidrug-resistant bacteria. Up to 80% of VAP cases are caused by Gram-negative bacteria, which contain abundant endotoxins in the outer membrane. The concentration of endotoxins in BALF has exhibited a relationship with the quantity of Gram negative bacteria in VAP. An amount greater than or equal to 6 EU/mL can be used to distinguish patients with Gram-negative or -positive bacterial pneumonia (sensitivity, 81%; specificity, 87%; positive predictive value, 67%; negative predictive value, 95%). Determining the concentration of endotoxins in BALF can facilitate the diagnosis of Gram-negative pneumonia for further antibiotic therapies, although a Gram stain of BALF can provide the same information [16,17].

2.2. Inflammation mediators

Once the infectious pathogen invades and begins growing, the host rapidly releases a cohort of inflammatory cytokines, such as interleukin (IL)-1β, IL-6, Tumor necrosis factor (TNF)-α, and IL-8, in response to the infection. However, these proinflammatory cytokines have not met the criteria for clinical applications because of their short half-life and low specificity, as well as the existence of natural inhibitory factors in the serum [9,18]. In a study of 24 patients with CAP, IL-6 and TNF-α were detected in most of the patients on

admission and decreased significantly thereafter, and IL-6 presented as a prognostic marker in correlation with the Acute Physiology and Chronic Health Evaluation II (APACHE II) scores on admission. Based on an evaluation, high levels of both IL-6 and IL-10 increase the risk of mortality in CAP [19,20].

Conway-Morris et al. found that BALF IL-1β, IL-8, Granulocyte-macrophage colony-stimulating factor (GM-CSF), and Macrophage inflammatory protein (MIP)-1α were significantly higher in the VAP patients. In patients with BALF IL-1β lower than 10 pg/mL, the post-test probability of VAP was only 2.8%, whereas a level of IL-8 greater than 2 ng/mL corresponded to a 61% probability of VAP being present. In addition, the cytokines could be detected within 4 h and showed a significant impact on clinical decision making [21,22].

2.3. Inflammation response proteins

2.3.1. Pentraxins

The innate immunity system is the first line of host defense against pathogens and comprises both cellular and soluble compartments. The major components in soluble compartments are pattern recognition proteins, demonstrating the abilities to recognize pathogen-associated molecular patterns (PAMP), such as collectins, ficolins, and pentraxins, in initiating an immune response. Pentraxins are a family of acute-phase reactants that are highly conserved in biological classes from arachnids to mammals. Based on the primary structure, pentraxins are divided into two groups: short and long pentraxins. C-reactive protein (CRP) and the serum amyloid P component (SAP) are prototypic short pentraxins, and long pentraxin 3 (PTX3) is the prototypic long pentraxin [23,24]. Plasma pentraxins, which are barely detectable under normal conditions but rapidly increasing while counteracting with the infections, represent invaluable diagnostic indicators in pneumonia.

2.3.1.1. C-reactive protein. CRP was first identified in 1930 from the serum of pneumonia patients. CRP has been observed to disappear in recovering patients and not detectable in healthy people [25,26]. In the presence of calcium, CRP can recognize and bind to several PAMPs present in bacteria, fungi, and parasites, and become involved in the clearance of microorganisms. CRP is synthesized in hepatocytes through the induction of IL-6, although other cytokines, such as TNF-α and IL-1β, are also involved. In healthy human adults, the concentration of CRP is lower than 3 mg/L [27], and the secretion of CRP starts from between 4 and 6 h after stimulation, doubling every 8 h, and reaching the maximum value after 36–50 h. In the existence of stimuli, CRP production can still be evaluated at even more than 1000-fold of the reference value. Once the stimulus is removed, the CRP value falls rapidly with a half-life of 19 h [25,28].

Serum CRP has been reported in some studies regarding its role in identifying patients with pneumonia. The elevation of CRP levels is both sensitive and specific for predicting pneumonia, and its diagnostic performance is superior to white blood cell counts or erythrocyte sedimentation rates [29]. In a prospective hospital-based CAP study, serum CRP levels were elevated above 50 mg/L in all of the patients and above 100 mg/L in 75% of the patients on the day of hospital admission. Patients who had received antibiotics prior to hospital admission exhibited significantly lower CRP levels [30]. In radiologically confirmed pneumonia, the specificity of CRP at a cut-off value of 100 mg/L in predicting pneumonia can reach 91.2% [31]. Serum CRP values are significantly higher in CAP patients, compared to healthy controls and suspected CAP patients, and high CRP values are suggestive of severity in determining the appropriateness of inpatient care. In addition, serum CRP levels have been demonstrated to be greatly increased by most severe bacterial infections, but only modestly increased by viral respiratory tract infections [29,33]. High CRP values are especially present in patients with pneumonia caused by *S. pneumonia* or *Legionella pneumophila* [32]. The single parameter of CRP concentration greater than 150 mg/L appears to be highly specific for a bacterial infection. Combination with other markers did not improve the diagnostic accuracy in differentiating patients with a bacterial or viral lower respiratory tract infection (LRTI), compared with CRP alone [34]. By contrast, some previous studies have inferred that CRP lacks specificity for bacterial infection, and a high CRP level could be due to other inflammatory conditions [9]. The serum level of CRP may largely depend on the status of systemic inflammatory responses rather than the direct outcomes of the disease.

The general characteristics of patients, such as age and sex, may also strongly influence serum CRP values. Alternatively, assessment results of relative variations may provide more information about the course of infection than that of absolute variations can. The serial measurements of CRP in a patient may represent the progression of the clinical course and also help assess the response to antibiotic therapy. In a cohort study of 64 HAP patients, the CRP ratios of Day 10 to the admission day could separate the "good" and "poor" responses at 53% and 20% mortality rates, respectively [35]. In a multicenter prospective observational study of community-acquired sepsis, Póvoa et al. indicated that, as early as Day 3 onward, the CRP concentrations of survivors decrease to a level that was significantly lower than that of non-survivors [36]. Póvoa et al. also suggested that, after 72–96 h of antibiotic therapy, the CRP value of severe CAP survivors should decrease by 30%–50% from the admission day. Patients with consistent decreasing CRP ratios usually have a more favorable response to antibiotic therapy [37].

2.3.1.2. Long pentraxin 3. PTX3, similar to the short pentraxins, is an acute-phase reactant that plays a crucial role as a soluble innate immune pattern recognition receptor in the activation of innate immunity. However, PTX3 exhibited different features from short pentraxins in its structure, gene location, and cellular source, inducing stimuli and ligand recognition. The structure of PTX3 is related to CRP and SAP, but contains an unrelated long amino-terminal domain coupled to the carboxyl-terminal pentraxin domain [23]. The genes of short pentraxins are located on chromosome 1, whereas PTX3 is located on chromosome 3q25. Unlike CRP that is produced in the liver by the stimulation with the secondary cytokine, IL-6, PTX3 is induced by the Toll-like receptor engagement and primary cytokines, TNF-α and IL-1β, in various cell types, particularly mononuclear phagocytes, dendritic cells, fibroblasts, endothelial cells, and epithelial cells [38,39]. IL-6 does not induce PTX3 production. Taken together, these findings imply that the serum PTX3 level may directly reflect the severity of infections and is less affected by other inflammatory conditions.

The levels of PTX3, which are low under normal conditions (<2 ng/mL in human), have been examined in several clinical settings for infectious diseases, thus revealing a correlation with severity and mortality. A cohort study consecutive critically ill patients admitted to the ICU with systemic inflammatory response syndrome, sepsis, or septic shock showed that PTX3 was elevated with a gradient from systemic inflammatory response syndrome to septic shock. PTX3 levels not only correlated with clinical scores but also reflected the severity of disease, and higher levels of PTX3 were associated with an unfavorable outcome [40]. In severe leptospirosis patients, the plasma PTX3 levels were also associated with the severity and mortality of diseases, while CRP levels could not be used to differentiate the severe from the severest cases [41]. In studies on patients with meningococcal disease, high plasma concentrations of PTX3 were seen at admission and correlated to patients with shock, whereas CRP level at admission was negatively correlated. PTX3 level was not correlated with mortality or days spent in the ICU [42]. In bacteremia patients, PTX3 values on Days 1–4 were markedly higher in non-survivors compared to survivors and showed 72% sensitivity and 81% specificity for fatal disease at a cut-off level of 15 ng/mL. PTX3 showed a higher prognostic value compared to CRP in the prognostic stratification of bacteremia patients [43,44]. In our recent data on CAP, PTX3 and CRP levels were both decreased after antibiotic treatment. The plasma

concentration of PTX3 was correlated with the severity of CAP and the length of hospital stay.

2.3.2. Calcitonin gene peptide superfamily

Calcitonin gene peptides, including procalcitonin (PCT), CT gene-related peptides, and adrenomedullin (AM), are a superfamily of endocrine cell-produced hormones with systemic activities. The classical production of calcitonin gene peptides is mainly carried out by the neuroendocrine cells. In addition, calcitonin gene peptides can also be expressed in the various parenchymal cells and are released by the microbial toxins or pro-inflammatory cytokine stimulation. The systemic release of calcitonin gene peptides occurs in a cytokine-like manner and is considered a prototype of hormokines. The serum levels of calcitonin gene peptides may be applied as indicators for the status of infections.

2.3.2.1. Procalcitonin. Procalcitonin (PCT), a thyroidal parafollicular cell-produced prohormone (13 kDa), is low in serum (<0.1 ng/mL) of healthy people, whereas circulating PCT levels, primarily derived from non-thyroidal tissues, can increase by several 1000-fold in patients with sepsis [45]. In the process of microbial infection, lipopolysaccharide (LPS), IL-1β, IL-6, and TNF-α can promote CALC-I gene expression and increase the release of PCT into circulation from parenchymal tissues, such as the liver and peripheral blood mononuclear cells. After a septic stimulus, PCT protein production of parenchymal cells can be detected after 10 h with a half-life of approximately 22–35 h [28,46]. In addition, the production of PCT may also be markedly increased under noninfectious conditions such as trauma, surgery, burns, heat stroke, and rejection after transplantation [28,47]. PCT levels are attenuated by the cytokines released in response to a viral infection. The prohormone requires further post-translational processing to form a mature type of hormone, and may demonstrate distinct biological activities. In a hamster septic model, the concentration of PCT was rapidly evaluated and peaked at 12 h after induction of sepsis. Because the exogenous PCT significantly increased the mortality rate, a prophylactic blockade of PCT attenuated the lethal effects of sepsis. The increase in circulation of PCT levels may exacerbate the mortality rate, and diminishing the production of PCT increases the survival [47]. It inferred that PCT may play a detrimental role in systemic inflammation. In addition, the changes in circulating PCT levels may represent the severity of infections and may be used as a prognostic marker. Therefore, the circulating PCT level has been examined to monitor the progression of severe sepsis, septic shock, and the response to antimicrobial therapy [48].

Two commercial PCT detection assays that demonstrate high sensitivity, low detection limitations, and a fast readout are currently available. Several studies have reported the value of using PCT levels in managing CAP. It has been suggested that rising PCT can facilitate early identification of the risk of mortality in critically ill patients [49]. From the results of two randomized prospective studies, in 373 CAP patients from a total of 545 patients with suspected lower respiratory tract infections, PCT presented a higher diagnostic accuracy in differentiating CAP from other diagnoses than did CRP or the total leukocyte count. The PCT levels also demonstrated higher accuracy in predicting bacteremia and the severity of CAP [50]. In a VAP cohort study with sequential measurement of PCT and CRP levels, both PCT and CRP levels were significantly higher in patients with confirmed VAP, and PCT demonstrated higher accuracy. However, the PCT and CRP levels in BALF cannot help differentiate between confirmed and non-confirmed VAP [51]. In addition to discriminating the accurate diagnosis of pneumonia, PCT levels demonstrated their prognostic value in CAP patients [52,53]. In a study where the PCT, CRP, WBC, and CRB-65 scores in 1671 CAP patients were all determined on admission and followed-up for 28 days to ascertain mortality, a similar prognostic accuracy of the CRB-65 score showed that PCT levels on admission can predict the severity and outcome of CAP. PCT levels show a higher prognostic accuracy than do those of CRP or leukocyte count, and provide independent identification of patients at low risk of death [53]. By using PCT levels, Christ-Crain et al. reduced antibiotic exposure in patients with CAP from a median of 12 to 5 days in a randomized trial investigating the guidance of antibiotic treatment duration in CAP [54]. The dynamic levels of PCT may present a guide for the choice and duration of the antibiotic therapies.

2.3.2.2. Pro-adrenomedullin. The adrenomedullin (AM) gene encodes a 185-amino acid preprohormone, which can be generated into a 164-amino acid peptide pro-AM with removal of the signal peptide and further processed to form the active peptides AM and PAMP (define PAMP) [55]. AM is expressed in many tissues including the adrenal medulla, atrium, lung, pancreas, and small intestine [56]. In addition to its potent vasodilating activities, AM can modulate the complement activation to perform bactericidal activities [57]. In cultured vascular smooth-muscle and endothelial cells, AM production can be largely induced by LPS, TNF, and IL-1 stimulation. By contrast, AM also suppresses IL-1β-induced TNF-α secretion and gene transcription in Swiss 3T3 fibroblasts [56,58]. The regulation of AM production may be involved in a feedback loop during inflammatory responses. The serum AM levels might reflect the severity of infections. However, AM is rapidly cleared from circulation. Determining the level of Pro-AM can alternatively represent the levels of the rapidly degraded active peptide AM [44].

Pro-AM levels are helpful for individual risk assessment and outcome prediction in sepsis. A previous study demonstrated that, the median Pro-AM level was 0.4 nM (0.21–0.97) in healthy people, 1.8 nM (0.4–5.8) in those with sepsis, 2.3 nM (1.0–17.6) in those with severe sepsis, and 4.5 nM (0.9–21) in patients with septic shock. On admission, it was also significantly higher in non-survivors than in survivors [59]. In a prospective observational study, Pro-AM levels were correlated with the severity of CAP and could predict the mortality on admission. Combining the PSI and Pro-AM levels increased the prognostic accuracy to predict the risk stratification of CAP patients [60]. In pediatric CAP patients, the Pro-AM level was also related to the development of complications during hospitalization [61]. Although Pro-AM level can help distinguish the severity and risk of CAP, it shows a high coverage of the concentration ranges, and the cut-off values in pneumonia still need to be evaluated.

2.3.3. S-TREM

The triggering receptor expressed in myeloid cell-1 (TREM) is a member of immunoglobulin superfamily receptors expressed at high levels in neutrophils and monocytes that infiltrate human tissues infected with bacteria. In microbial sepsis patients and an animal model of LPS-induced shock, TREM was upregulated in the peritoneal neutrophils [62,63]. Silencing of TREM in vivo in a fecal peritonitis mouse model blunted the inflammatory response and increased mortality. These effects occur with an impairment of bacterial clearance that was related to marked inhibition of the neutrophil oxidative burst [64]. Raising TREM expression levels may promote neutrophil activities in eliminating microorganisms. Once the TREM binds to its ligand and is activated through the association with the adapter protein, DAP12, it can further induce the secretion of inflammatory cytokines, such as IL-8, monocyte chemotactic protein-1, and TNF-α to induce neutrophil degranulation in response to infections [65]. TREM is highly expressed in neutrophils caused by *Staphylococcus aureus*, *Bartonella henselae*, and *Aspergillus fumigatus* infections. This phenomenon has not been observed in samples from patients under non-infectious inflammatory conditions [62]. This indicates that the upregulation of the membrane-bound and the soluble form (S-TREM) of TREM may be specifically involved in infectious diseases.

Because the increased expression of TREM is found in the infiltrated neutrophils and monocytes, the S-TREM levels in BALF may be of diagnostic value in pneumonia. In a prospective study of suspected CAP and VAP patients, the presence of BALF S-TREM by itself was

more accurate than any clinical findings or laboratory values in identifying the presence of bacterial or fungal pneumonia, suggesting that the BALF S-TREM may serve as an independent predictor of pneumonia [66]. By contrast, in a study that was conducted to evaluate the usefulness of S-TREM in BALF of ICU patients as a rapid diagnostic test for VAP, the S-TREM levels in BALF could not discriminate for VAP, although the confirmed VAP patients exhibited higher concentrations of S-TREM in BALF than did the unconfirmed VAP patients [67]. In another prospective cohort study, the mean S-TREM concentration was higher in confirmed VAP patients, but this finding lacks statistical significance. Patients with alveolar hemorrhage had the greatest values for S-TREM concentration, while a cut-off value for S-TREM greater than 200 pg/mL yielded a diagnostic sensitivity of 42.1% and a specificity of 75.6% for definite VAP [68]. In addition to being measured in BALF, the serum levels of S-TREM were also reported with significant elevation in pneumonia, COPD, and asthma exacerbations [69]. The levels of S-TREM may correlate with an inflammation condition but appear less accurate as a sole marker for pneumonia. Therefore, S-TREM levels must be further combined with other markers for accurate evaluation.

2.4. Stress-sensing proteins

In sepsis, the innate immune system releases multiple inflammation mediators and related proteins in response to the pathogens. In this period, the uncontrolled secretion of mediators, outgrowth of pathogens, and the release of endotoxin can all cause detrimental physiological stresses such as tissue edema, organ failure, and shock, to affect the vital signs. The neuroendocrine system hypothalamo-pituitary-adrenal (HPA) axis regulates many of the physical processes to appropriately react to stress through the release of corticotropin-releasing hormones (CRHs). In the co-stimulation of CRH, another stressor, arginine vasopressin (AVP), induces adrenocorticotropic hormone (ACTH) secretion and further controls the adrenal cortex to produce cortisol. In this cascaded process, the circulation levels of AVP and cortisol may be used as indicators for the severity of a particular illness [70,71].

2.4.1. Copeptin

The level of circulating AVP is difficult to measure because it is released in a pulsatile manner, unstable in plasma, and bound to platelets [70]. Active AVP is derived from a larger precursor peptide, the 164-amino-acid preprovasopressin, which consists of a signal peptide, AVP, neurophysin II, and copeptin [72]. Copeptin is a glycosylated peptide with a leucine-rich core segment that is simultaneously released with AVP during precursor processing, and considered as an alternative indicator for AVP concentration. In addition, copeptin is stable for days after blood withdrawal and can be quickly and easily measured [28,73].

The increase in copeptin levels has shown the positive association of copeptin with the severity and outcome of illness, such as sepsis, hemorrhagic shock, and stroke [74]. In a study on lower respiratory tract infection (LRTI) patients, copeptin levels were significantly increased in LRTI patients, and the highest levels of copeptin were observed in patients with CAP [75]. The copeptin level was also associated with the severity of CAP, and the non-survivors exhibited significantly higher copeptin levels on admission. Copeptin levels are a powerful predictor of short-term and long-term risk stratification of patients with CAP [75,76]. In addition to CAP, copeptin levels are also an indicator for the severity and mortality of VAP [77,78]. The circulating copeptin level represents an accurate marker for early risk stratification and can be applied to management of pneumonia patients.

2.4.2. Cortisol

The activation of the HPA axis is characterized by an increase in cortisol levels, thus indicating that cortisol, as copeptin, is essential for survival and parallels the degree of stress. The majority of circulating cortisol in human serum is bound to proteins; however, the free cortisol, rather than the protein-bound fraction, is responsible for the physiologic functions. Although the total cortisol level more accurately reflects the intensity of activation in the HPA axis, the free cortisol concentrations can still represent an indicator of critically ill patients [79,80]. Both total and free forms of cortisol have been reported to increase with the increasing severity of CAP and have been observed to be significantly higher in non-survivors [81,82]. Cortisol shows the potential for predicting the mortality and critical disease in CAP patients, independently from clinical scores and inflammatory biomarkers.

3. Approaches in identifying new markers

As mentioned, the potential biomarkers in pneumonia may exist in the scenario where infectious pathogens invade into the body, as reflected in specific biological responses to infections. In addition to the pneumonia markers that have been discussed, other biomarkers, such as pro-atrial natriuretic peptide [83,84], B-type natriuretic peptide [85–87], and red cell distribution width [88], have been examined and demonstrated to have the ability to improve the diagnosis and prognosis of pneumonia.

With advances in proteomic research on developing 2D gel electrophoresis, mass spectrometry, and bioinformatic analysis, global protein analysis can be applied as an inventory for identifying potential pneumonia markers. Because of the deduction of cost, higher resolution, and fast performance, the proteomic technology can be used at the stage of large clinical intervention trials [89]. Yip et al. applied protein chip array profiling analysis to identify biomarkers that might be useful in monitoring the clinical course of severe acute respiratory syndrome patients. They identified the concentration of serum amyloid A protein, a short pentraxin, as a biomarker potentially useful in monitoring the extent of pneumonia [90]. A research group in Taiwan applied 2D gel electrophoresis and mass spectrometry to identify the differential levels of haptoglobin, immunoglobulin kappa chain, apolipoprotein A-I, and transthyretin in the plasma protein profiles of children with different severities of pneumococcal pneumonia [91].

In addition to proteomic studies, metabolomics is a rapidly expanding field of identifying and quantifying the changes in the levels of all metabolites within a biofluid, such as blood, saliva, and urine. In a study aiming to compare *S. pneumonia* with other microbe-mediated pneumonia, the urinary metabolomic profile of pneumococcal pneumonia patients was significantly different from that of patients with viral and other bacterial forms of pneumonia [92]. The results from a metabolomic analysis of severe childhood pneumonia in Gambia also clearly distinguished severe pneumonia patients from community controls. Those identified metabolites are related to the host response to infection through antioxidant, inflammatory, and antimicrobial pathways, as well as the energy metabolism [93]. These findings indicate that NMR-based analysis of metabolites in urine may provide new information for the diagnosis and etiology of pneumonia.

4. Conclusion

Pneumonia is the leading cause of severe sepsis, which is present with a cohort of processes from the entry of pathogens to host defense responses. These potential biomarkers may provide different clinical values in detecting pneumonia according to their original biological features. First, identifying microorganisms and their derivatives can help ascertain the etiology of pneumonia. Second, evaluating the inflammation mediators and response proteins can provide information on the degree of immunity to combat pulmonary pathogens that cause the disease, and can facilitate pneumonia management. Finally, the circulating stress-sensing proteins that reflect the condition of illness can be useful in stratifying risks and predicting prognosis. However, due to the heterogeneity of pneumonia patients, who vary in age, sex, comorbid conditions, and the development of

complications, the clinical cut-off value of these potential biomarkers must be determined further. In conclusion, the implication of these biomarkers should be considered complementary to routine clinical and laboratory features to improve decision making regarding pneumonia.

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