

REVIEW

Biomarkers in community-acquired pneumonia: A state-of-the-art review

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Community-acquired pneumonia (CAP) exhibits mortality rates, between 20% and 50% in severe cases. Biomarkers are useful tools for searching for antibiotic therapy modifications and for CAP diagnosis, prognosis and follow-up treatment. This non-systematic state-of-the-art review presents the biological and clinical features of biomarkers in CAP patients, including procalcitonin, C-reactive protein, copeptin, pro-ANP (atrial natriuretic peptide), adrenomedullin, cortisol and D-dimers.

KEYWORDS: Pneumonia; Biological Markers; Community-Acquired Infections.

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INTRODUCTION

Approximately 4 million adults develop community-acquired pneumonia (CAP) in the United States (U.S.) annually; CAP is also the eighth leading cause of death in the U.S. (1). Severe CAP is responsible for 6.6% to 16.7% of pneumonia hospitalizations in Europe and the U.S. (2,3). The highest mortality rates, between 20% and 50%, are observed in severe CAP infections in Spanish and British intensive care units (ICUs) (4,5).

Hospitalized CAP patients undergo clinical, radiological and laboratory tests to determine the disease severity, need for ICU hospitalization and possible complications. Hemograms, urea, creatinine, glucose, hepatic function tests, pulse oximetry, arterial blood gasometry and blood and sputum cultures are critically important (6,7). Identifying the etiological agent has no relevant effect on the hospitalization time or mortality in the first 30 days or between the comparisons of focused therapy and the identified agent or empirical therapy across a large spectrum (8).

Severity scores, such as the *Pneumonia Severity Index* (PSI) and CURB-65 (confusion, urea, respiratory rate, arterial blood pressure and age) scores, have been developed and validated. These scores can aid the decision-making process of hospitalization and ICU referral (9).

Biomarkers are useful tools in the diagnosis, prognostics and follow-up treatment of CAP and for investigating

antibiotic modifications. This article presents a non-systematic, state-of-the-art review of the biological and clinical features of CAP biomarkers.

Procalcitonin

Procalcitonin (PCT) is a protein that is encoded by the CALC-I gene on chromosome 11, which produces calcitonin and several additional free peptides after several post-translational modifications (10).

PCT concentrations in the serum of healthy subjects are undetectable or low, generally <0.1 ng/mL (11). PCT is detected in other tissues in healthy subjects, but the transcription of the extra-thyroid CALC-I gene is poor in the absence of infection. PCT mRNA is up-regulated in sepsis, which increases the expression and secretion of this peptide in tissue (10).

Inflammatory and infectious injuries stimulate the increase in serum PCT (11). The synthesis of this peptide is particularly induced during severe bacterial infection, sepsis, septic shock and multiple organ dysfunction syndrome (12).

PCT supports a CAP diagnosis, and this protein is a predictor of complications and mortality. PCT and C-reactive protein (CRP) enhance the diagnostic accuracy of the clinical signs and symptoms that are routinely used for screening and diagnosing CAP (13). The standard clinical model exhibited a diagnosis accuracy of 0.79 (IC 95% 0.75–0.83) in this study, and including these biomarkers increased the accuracy to 0.92 (IC 95% 0.89–0.94), which was significantly better than the association of one of these biomarkers alone ($p < 0.001$ for both comparisons).

Boussekey et al. (11) have also evaluated the prognostic value of PCT for CAP and demonstrated that PCT >2 ng/mL was associated with an increased incidence of bacteremia,

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septic shock, multi-organ failure and mortality. No association for CRP was observed. Antibiotic administration must be based on the PCT cutoff ranges (14). Antibiotic treatment is intensified when the infection is severe and the PCT levels remain elevated (>0.25 or 0.5 ng/L). Antibiotics may be discontinued when the PCT levels decrease rapidly.

Christ-Crain et al. (15) demonstrated that using PCT for therapeutic guidance substantially reduced total antibiotic exposure and decreased the treatment duration by 55% compared to the standard therapeutic treatment (median 12 days *vs.* 5 days, $p<0.001$). Reduced adverse effects and microbiological resistance rates and shortened antibiotic therapy courses improve resource allocations, which is an important factor in public healthcare.

C-Reactive Protein

C-reactive protein (CRP) was the first "acute phase" protein to be described (16). CRP was discovered in the serum of patients with pneumococcal pneumonia; the CRP precipitated at the C-polysaccharides from the bacterial membrane. Combining CRP with the phosphocholine molecule responded to C-polysaccharide and other bacterial and host cell membrane constituents. Other ligands have also been described.

CRP activates the classical complement pathway, stimulates phagocytosis, binds to the immunoglobulin receptors, and interacts with several molecules (17). CRP values <3 mg/L are normal, and values >10 mg/L indicate significant inflammation (18). CRP is a sensitive inflammatory biomarker, but it exhibits low specificity. CRP values between 3 mg/L and 10 mg/L may reflect numerous conditions, such as obesity, smoking, diabetes mellitus, uremia, hypertension, low physical activity, oral hormone replacement therapy, sleep disturbances, chronic fatigue, alcohol consumption, depression, aging and other states that do not necessarily include inflammation (19).

A cut-off point of 11 mg/L serum CPR demonstrated a 94% sensitivity and 95% specificity in healthy individuals and CRP patients, respectively. These data suggest that CPR values below this point may exclude a confirmed CAP diagnosis. With an 83% sensitivity and 44% specificity, a cut-off point of 33 mg/L CRP distinguished the patients with a confirmed CAP diagnosis from the patients with similar clinical symptoms but different clinical conditions (20).

Chalmers et al. (21) concluded that CRP values <100 mg/L in CAP patients on the day of admission and four days later were independently associated with a low 30-day mortality rate, low probability for mechanical ventilation and/or inotropic support and low rates of complicated pneumonia. The risks of 30-day mortality, need for mechanical ventilation and/or inotropic support and complicated pneumonia increased when the CPR levels did not drop by at least 50% until the fourth day of admission.

A cohort of 53 subjects (22) demonstrated that daily measurements of serum CRP in the patients with severe CAP are useful for identifying the patients with a poor prognosis, and this biomarker is a better predictor than the commonly used markers of infection, such as body temperature and leukocyte count. This study also demonstrated that shorter antibiotic therapy might exhibit the same efficacy with less toxicity in patients with a rapid drop in CRP levels, thereby avoiding the emergencies that are

associated with resistant strains and reducing hospitalization costs.

Copeptin

Arginine-vasopressin (AVP) is a hormone that is produced in the paraventricular nuclei of the hypothalamus and stored in the posterior part of the pituitary gland. Several stimuli, such as hypotension, hypoxia, hyperosmolarity, acidosis and infections, stimulate the release of AVP (23). AVP is released into the circulatory system by osmotic and hemodynamic stimuli. AVP exerts antidiuretic and vasopressor effects, which may restore the vascular tonus in vasodilatation hypotension (24).

Copeptin is a 39-amino acid glycopeptide, and its physiological function is unknown. AVP and neurophysin II comprise the terminal portion of the pre-pro-vasopressin molecule (25). Copeptin may play an important role in the correct structural formation of the AVP precursor, which is required for its proteolytic maturation efficiency (26).

Serum AVP levels have limitations because of the short half-life of AVP and its molecular instability. However, copeptin is highly stable *ex vivo* even for several days at room temperature. *Ex vivo* copeptin may be an indirect parameter to estimate the AVP plasma concentrations in critical patients, including the patients with sepsis and septic shock, for whom the levels of these biomarkers are high (27,28).

The presence of copeptin indicates the need for follow-up treatment for different types of pneumonia. Copeptin may be an independent predictor of mortality in CAP. CAP was an independent predictor of mortality in ventilation-associated pneumonia, and mortality rates increased with the severity of the sepsis (29).

Pro-ANP

Members of the family of natriuretic peptides are established biomarkers for congestive heart failure (30). These proteins defend the body against hypertension and salt and water retention by antagonizing the renin-angiotensin-aldosterone system. Natriuretic proteins alter renal sodium reabsorption, vascular tonus and cell growth. The smooth muscles of the blood vessels and kidneys are the primary targets of atrial natriuretic peptide (ANP). ANP distends the smooth muscles of the vessels, and increases the permeability of capillaries, which facilitates the removal of water and sodium. This hormone also inhibits the function of several other hormones, such as endothelin and vasopressin (31).

ANP is predominantly produced in the atrium of the heart, and this peptide comprises 98% of the natriuretic peptides in circulation. The pre-pro-ANP hormone is composed of 151 amino acids. The amino acid chain is called pro-ANP after removing a 25-amino acids signal sequence. The pro-ANP is likely cleaved by the membrane proteins in a functional ANP chain to a 28-amino acid peptide and an amino-terminal fragment of 98 amino acids (the NT-pro-ANP) prior to exocytosis (32).

Distended atrial walls signal the ANP release. High cardiac output, sympathetic stimulation and metabolic factors influence the ANP release. It is also suspected that hypoxia influences the ANP release. The half-life of ANP is 2 to 5 minutes, and its degradation rate is approximately 14 to 25 mL/min/kg (33).

ANP is a marker for the prevention and differential diagnosis of several diseases. The use of this peptide in diagnosing dyspnea caused by heart failure is more efficient than traditional methods (34). ANP and pro-ANP are interesting new sepsis and pneumonia markers (35,36). Morgenthaler et al. (37) have compared the pro-ANP levels to the APACHE II score (Acute Physiology and Chronic Health Evaluation) as an outcome predictor in septic patients.

Adrenomedullin

Human adrenomedullin (ADM) is a 52-amino acid peptide that is synthesized as part of pre-pro-adrenomedullin, a larger precursor molecule (38). The ADM gene is expressed in a wide range of tissues, but initial studies on the distribution of this gene have suggested that the highest levels of expression are observed in the adrenal medulla, ventricular chambers, kidneys and lungs (39). The ADM gene is more highly expressed in the endothelial cells than the adrenal medulla, and this peptide is a secretory product of the vascular endothelium, which also includes nitric oxide (NO) and endothelin (40).

The plasma half-life of ADM is approximately 22 minutes (41). The normal plasma concentrations of ADM range from 1 to 10 ng/mL, and most values are between 2 and 3.5 ng/mL (42). However, obtaining reliable measurements of ADM release in blood circulation is difficult because ADM immediately binds to receptors near the site of its production. The short half-life of ADM and technical difficulties also complicate the plasma measurements (43).

The plasma ADM levels are elevated in a wide range of disease states, usually as a compensatory response to cardiovascular disturbances (42). ADM likely participates in the pathophysiology of septic shock because this is the only pathological condition in which the plasma levels of this protein approach the levels that are required for receptor activation. The ADM plasma levels in sepsis patients are directly responsible for hypotension during septic shock (44).

Christ-Crain et al. (45) have noted that the levels of MR-pro-ADM on admission increased according to the CAP severity (based on the PSI score). MR-pro-ADM is a stable, functionally irrelevant fragment of ADM degradation that is used in some studies because of its better technical viability. This progressive increase was also observed in procalcitonin ($p < 0.0001$). However, no statistical significance was observed for the C-reactive protein, total leukocyte count, and body temperature.

The ADM levels upon admission were significantly higher in the patients who died during the follow-up compared to the patients who survived: 2.1 (1.5–3.0 nmol/L) vs. 1.0 (0.6–1.6 nmol/L) ($p < 0.001$). An analysis of the “treatment failure” and “death” outcomes demonstrated that the prognostic accuracy of ADM was similar to the PSI score but higher than other parameters (44).

Krüger et al. have demonstrated that the MR-proANP (mid-regional pro-atrial natriuretic peptide), copeptin, CT-proET-1 (proendothelin-1), and MR-proADM (mid-regional proadrenomedullin) biomarkers are strong predictors of the 28- and 180-day CAP mortality, and MR-proADM exhibited the best performance. The combination of CRB-65 and MR-proADM was the best predictor for short- and long-term mortality (46).

Cortisol

The hypothalamic-pituitary-adrenal circuit is activated by central stress control circuits to produce and secrete the corticotropin-releasing hormone (CRH). CRH stimulates the anterior portion of the pituitary gland to synthesize and release proopiomelanocortin (POMC), an adrenocorticotropic (ACTH) precursor. In the systemic circulation, ACTH activates the transcription of steroids, particularly cortisol, in the adrenal gland (47).

Cortisol secretion increases in amplitude but not frequency after three to five hours of sleep, and secretion peaks a few hours before waking until one hour after waking. Cortisol amplitude decreases in the morning and reaches a minimum level at dawn (48). The half-life of cortisol is approximately 80 minutes, which is longer than the 8-minute half-life of ACTH (49). The plasma cortisol levels are higher in cases of severe trauma, burns, major surgery, hypoglycemia, fever, blood pressure changes, exercise and exposure to intense cold (50-53).

Salluh et al. have demonstrated that treatment with supraphysiological doses of hydrocortisone increases the survival rates of severe CAP patients who develop adrenal failure during septic shock (54). A study of 72 CAP patients demonstrated that the baseline level of total cortisol was significantly higher in non-survivors. These results confirm the interference of infection in adrenal functions and support the value of cortisol as a better predictor of mortality compared to severity-related scores (APACHE II, CURB-65, SOFA) and laboratory markers (CRP, leukocyte count, and d-dimers) (55).

D-dimers

D-dimers are released into the blood during the dissolution process of fibrin emboli in the fibrinolytic system. D-dimers are the smallest fragments of the fibrin degradation, and these proteins are detectable in blood plasma. The half-life of this protein is approximately 8 hours, and it is cleared from the plasma via urinary excretion and the action of the reticuloendothelial system (56).

High d-dimers levels have been detected in patients with disseminated intravascular coagulation (DIC), severe sepsis, thrombotic events, hepatic diseases, surgery and trauma (57-59). The most important application of d-dimers is related to thrombotic events. D-dimers have been studied extensively as a diagnostic method for deep vein thrombosis (DVT) and pulmonary embolism (PE). A negative result has diagnostic utility that is comparable to normal lung scans or negative duplex ultrasound findings (60).

The application of the d-dimers analysis to CAP is a novel approach. In a cohort study of 68 CAP patients, Shilon et al. have demonstrated a positive correlation between d-dimers and PSI, APACHE II, hospitalization time, organ failure, fever duration, and hospital mortality (61). Another study of 302 CAP patients (62) investigated the relationships between plasma d-dimers levels and the prognostic variables that are included in the PSI. High d-dimers levels were associated with radiological pneumonia extension findings.

Using biomarkers may aid in the diagnosis, treatment and prognosis of CAP. Table 1 summarizes the reviewed biomarkers and triggers. The PCT serum levels may provide valuable support to the clinical diagnosis of CAP and aid in the differential diagnosis of bacterial and viral pneumonia. PCT is particularly useful because the results are obtained several days prior to the culture tests. These biomarkers also

Table 1 - Summary of the studied biomarkers and triggers.

Biomarker	Trigger
Procalcitonin	Inflammatory and infectious injuries
C-Reactive Protein	Inflammatory and infectious injuries
Copeptin	Hypotension, hypoxia, hyperosmolarity, acidosis, infections
Pro-ANP	Distension of the atria walls, high cardiac output, sympathetic stimulation
Adrenomedullin	Cardiovascular disturbances, sepsis
Cortisol	Septic shock, severe trauma, burns, major surgery, hypoglycemia, blood pressure changes
D-dimers	Disseminated intravascular coagulation, severe sepsis, thrombotic events, hepatic diseases, surgery, trauma

aid in identifying the low-risk patients who can be treated in outpatient environments. Reducing unnecessary hospitalizations decreases treatment costs and patient discomfort.

Protocols based on PCT levels can substantially reduce the use of antibiotics and treatment times. Antibiotic prescriptions can be encouraged or discouraged via the use of PCT serum levels. The clinical course of pneumonia is reflected in the serum levels of PCT and CRP. CRP is already a widely used biomarker during the follow-up of infectious processes, and it is included in the clinical protocols of several hospitals. Decreasing the levels of these biomarkers is critical to predicting patient survival, and increased biomarker levels indicate the progression to septic shock, multiple organ failure and death. New biomarkers, such as pro-ANP and copeptin, are under investigation, and these markers demonstrate effective prognostic powers.

Finally, PCT is currently the most appropriate biomarker. PCT distinguishes cases according to their severity, and the PCT levels may direct the treatment of complicated cases. PCT levels rise in proportion to the severity of the bacterial infection, but the levels do not increase in viral infections. Therefore, low PCT levels preclude the need for antibiotics. Elevated PCT levels are associated with an increased rate of bacteremia, septic shock, multi-organ failure and mortality. Decreasing PCT levels during antimicrobial treatment indicate a favorable outcome with a lowered risk of death.

The behavior of infections remains unclear. Biomarkers may assist clinicians in determining the severity of the patient symptoms in these diseases.

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AUTHOR CONTRIBUTIONS

All of the authors were equally involved in the bibliographic revision, data compilation and manuscript writing and revision.

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