The current status of biomarkers for the diagnosis of nosocomial pneumonias

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Purpose of review
Nosocomial pneumonia is a frequent and severe nosocomial infection divided in two distinct groups: hospital-acquired pneumonia and ventilator-associated pneumonia (VAP). In this context, the VAP is notoriously difficult to diagnose clinically, resulting from the lack of a 'gold standard' method of diagnosis.

Recent findings
The use of biomarkers may potentially improve the early diagnosis of infections allowing earlier and better identification and treatment. An exhausting list of biomarkers has been studied and although far from perfect, procalcitonin (PCT) and C-reactive protein (CRP) are the most studied biomarkers used in clinical practice. Data coming from literature suggests the use of PCT for VAP prognosis and as a based algorithm tool for the reduction of duration of pneumonia therapy, as well as, the use of the CRP dynamics to the early prediction of VAP and the response to the antibiotics.

Summary
The evidence for the use of biomarkers to diagnose nosocomial pneumonia as a stand-alone tool is low to moderate. Improved performance for both PCT and CRP can be obtained by using them in association with clinical features or scoring systems but prospective studies are still needed to validate this hypothesis.

Keywords
biomarkers, nosocomial pneumonia, ventilator-associated pneumonia

INTRODUCTION

The use of biomarkers in clinical practice has increased substantially specially because enthusiasts claim that biomarkers may improve the early diagnosis of infections and be available as a point-of-care tool. This allows earlier and better identification and treatment of patients with severe life-threatening infections. Nosocomial pneumonia is divided in two distinct groups: hospital-acquired pneumonia denotes an episode of pneumonia that occurs more than 48 h after hospital admission and was not in incubation at the time of admission, and ventilator-associated pneumonia (VAP) is defined as pneumonia that occurs more than 48 h after the initiation of invasive mechanical ventilation. In the context of VAP, one of the most challenging problems is the correct identification, resulting from the lack of a 'gold standard' method of diagnosis [1▪]. The commonly used criteria are based in clinical variables lacking specificity [2]; as a result, up to 50% of patients diagnosed with VAP do not have the condition [3]. Thus, there is a need for a clinical tool to improve the diagnosis and the decision-making process to achieve a balance between overdiagnosis (and thus antimicrobial overuse) and underdiagnosis (or late diagnosis), both of which could result in worse outcomes. A perfect biomarker (not expensive, not invasive, timely, that helps to avoid excess antibiotic use and assist in the conduct of clinical investigation) is still lacking, but various biological mediators have been proposed, among which procalcitonin (PCT), C-reactive protein (CRP), and soluble triggering expressed on myeloid cells type 1 (sTREM-1) are the most frequently studied.

The aim of the present article is to review the recent advances in the medical literature to support

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KEY POINTS

- Nosocomial pneumonias and VAP are amongst the most common infections contributing significantly to the antibiotic consumption burden namely in ICU.
- The use of biomarkers may potentially improve the early diagnosis of infections allowing earlier and better identification and treatment of patients with severe life-threatening infections, but clinical evidence is lacking. The performance of biomarkers to diagnose nosocomial pneumonia may be potentially improved by using them in association with clinical features or scoring systems and hopefully by using novel approaches that incorporate its dynamic assessment either to predict or to re-evaluate the diagnosis of pneumonia. A translational approach, with application of genomics, proteomics and metabolomics methodologies is required to better understand the disease, however, it remains unclear if some of these new technologies will be incorporated in the clinical routine for the diagnosis of nosocomial pneumonia.

the use of biomarkers for the diagnosis of nosocomial pneumonia.

BIOMARKERS IN CURRENT PRACTICE, WHERE ARE WE NOW?

Classically, VAP diagnosis is based on the presence of a pulmonary and systemic inflammatory response in patients who are under mechanical ventilation [4]. The clinical criteria for diagnosis of VAP have high sensitivity but rather low specificity [2,3]. They are therefore useful to raise suspicion of pneumonia, but confirmation of the presence of VAP is a much more difficult task, being one of the most controversial and challenging issues in critically ill patients. There are other complications of critical illness and mechanical ventilation that can mimic nosocomial pneumonia or VAP namely atelectasis, pulmonary edema, pulmonary embolism, and pulmonary traumatic contusion. The use of biomarkers is proposed to render VAP diagnosis more specific. An exhausting list of biomarkers has been studied as potential adjunctive tools for diagnosis, prognosis, assessment of response to antibiotics, and lately antibiotic stewardship (Table 1). Although far from being perfect, PCT is one of the most extensively studied biomarkers used in everyday clinical practice.

Procalcitonin is a pro-hormone, precursor of calcitonin and it is synthesised in virtually all organs and in macrophages in response to inflammatory stimuli [16]. To our knowledge, only a few studies have evaluated the potential usefulness of PCT for diagnosing VAP in the intensive care unit (ICU) [15▪▪,17–19], and demonstrate that PCT is a poor marker for VAP diagnosis. Procalcitonin is not a good biomarker for the diagnosis of VAP or nosocomial pneumonia in ICU patients as compared with those with sepsis admitted from the community, mainly because most of the former have already developed systemic inflammation response syndromes, multiple organ failure, and/or a previous infection, conditions known to raise the PCT levels and thus making the usual cut-offs proposed for the diagnosis of infection less useful [20▪]. In addition, other VAP patients present very low or even undetectable PCT levels at the day of diagnosis, possibly as a result of VAP being hypothetically a compartmentalized infection.

The value of PCT in evaluating the outcomes of VAP has also been studied. A recent systematic review concluded that high baseline PCT levels were associated with decreased survival for VAP patients [21]. Additionally, Li *et al.* [22] enrolled 115 critically ill patients with VAP and found that high serum PCT level was an independent prognostic biomarker of mortality risk (OR = 2.32; 95% CI 1.25–4.31; $P = 0.008$).

Studies have attempted to correlate the clinical pulmonary infection score (CPIS) and PCT. As the CPIS is a commonly used score, it was proposed and demonstrated that the incorporation of PCT in the CPIS may improve the prediction accuracy and the prognostic assessment [5,23]. In a single-center study evaluating 92 patients, Su *et al.* [5] explored the value of PCT and the CPIS in the diagnosis and prognostic assessment of VAP. On the day of diagnosis, patients who had pneumonia had higher PCT levels and its association with the CPIS were the most reliable tool for diagnosis and prognostic assessment (AROC 0.848; 95% CI 0.67–1.02).

Zagli *et al.* [24] evaluated 221 patients using their modified score that associated PCT and lung echography to the CPIS. In this pilot study, the modified score showed a significantly higher diagnostic value as compared with a CPIS more than six alone (AROC 0.829 versus 0.616, respectively; $P < 0.0001$).

The diagnosis and the outcome predictions for VAP are not the only fields of application of PCT. Studies propose that it could guide the duration of antibiotic therapy, allowing a well tolerated individualised strategy. However, the efficacy and safety of PCT-guided antibiotic is not fully established as studies have yielded conflicting results [20▪].

Recommendation for or against initiation or discontinuation of antibiotic therapy was based on initial PCT levels and the kinetics of PCT over time [20▪,25]. As expected, different PCT cut-offs triggered stronger or weaker recommendations for or against antibiotic therapy. Procalcitonin

Table 1. Characteristics of recent studies: association of biomarkers and VAP

Author	Year	Biomarker	Endpoint	Sample size (*n*)	Main finding	Reference
Su	2012	sTREM-1 PCT	Diagnosis and prognosis	92	BAL sTREM-1 + CPIS for diagnosis PCT for prognosis	[5]
Jiao	2014	PCT	Diagnosis	92	Association PCT and SOFA Cardiac surgery patients	[6]
Shi	2014	PCT kinetics	Treatment failure	60	Patients at least 65 years old In association with CPIS was a marker of clinical efficacy	[7]
Tanriverd	2015	PCT CRP	Mortality	45	CRP was superior Day 3 PCT level was the strongest predictor of mortality	[8]
Kiaei	2015	PCT CRP	Effect of antibiotic	50	Determined the efficacy 't' of antibiotic therapy	[9]
Liu	2015	TLR4	Development of postoperative pneumonia	17	Cardiac surgery with cardiopulmonary bypass Early-onset VAP	[10]
Shelhamer	2015	IL-8	Mortality risk of VAP	62	Burn patients	[11]
Li	2015	PCT	Mortality	115	Independent prognostic biomarker of mortality risk	[12]
Muzlovic	2016	CD64 index	Mortality	32	Recognized VAP-induced sepsis With positive cultures predict survival	[13]
Hellyer	2016	IL-1β IL-8	Exclusion of VAP	210 (study protocol)	BAL Rapid discontinuation of antibiotics	[14▪]
Póvoa	2016	CRP kinetics	Prediction and diagnosis	105	Daily CRP monitoring	[15▪▪]

BAL, bronchoalveolar lavage; CD64, cluster of differentiation 64; CPIS, clinical pulmonary infection score; CRP, C-reactive protein; IL, interleukin; PCT, procalcitonin; SOFA, sequential organ failure assessment; sTREM-1, soluble triggering expressed on myeloid cells type 1; TLR4, toll-like receptor 4; VAP, ventilator-associated pneumonia.

guidance resulted in a relative reduction in the duration of antibiotics by 27% in VAP [26]. However, it should be clear that control groups seldom followed the treatment duration recommended by guidelines (less than 8 days) and courses of 14 days or more were frequent. It also remains unclear if in those patients with very early responses and shorter courses of antibiotics (less than 3 days) PCT demonstrates response to therapy or simply identify patients that did not have VAP at all and allowed clinicians to safely interrupt antibiotics.

It has been reported that the diagnostic accuracy of PCT in patients with acute kidney injury (AKI) is lower than those without AKI because PCT is thought to be eliminated through the kidneys and/or liver, and it has been suggested that PCT may not be a reliable indicator of sepsis in patients with severe AKI or renal replacement therapy [27]. However, Nakamura *et al.* [28] reported that the accuracy of PCT was not significantly different between groups with or without renal dysfunction, but that the optimal cut-off value was significantly different making it hard to use in a homogeneous and protocolized way in critically ill patients.

C-reactive protein was the first acute-phase protein to be described. The synthesis of CRP is rapidly upregulated in hepatocytes, under the control of cytokines originating at the site of pathology, in particular IL6. The use of a single measurement of CRP in the diagnosis of VAP has not consistently shown positive results. A single elevated plasma CRP concentration is not very informative, thus making CRP not specific enough to diagnose nosocomial pneumonia or VAP [29]. However, the monitoring of CRP dynamics is very useful in the early prediction of VAP and the response to the antibiotics [15▪▪,30,31].

TREM-1 is a glycoprotein member of the immunoglobulin family. TREM-1 expression is

up-regulated in the presence of extracellular bacteria and fungi and some (noninfectious) inflammatory conditions. In response to infection, sTREM-1 can then be measured in body fluids, whereassTREM-1 levels are not detectable at baseline in normal individuals. Grover *et al.* [32] found that sTREM-1 may be a good predictor of VAP, but other studies have contradicted these findings, showing instead that sTREM-1 is elevated in the bronchoalveolar lavage (BAL) fluid of patients with and without confirmed VAP [33]. Further studies are required to fully determine the diagnostic utility of sTREM-1 for VAP.

The systematic reviews performed by the 2016 HAP/VAP guideline panel concluded that biomarkers and clinical scores are not recommended for the diagnosis of HAP/VAP based on current evidence [1▪]. Although it can be claimed that the ability of biomarkers alone to diagnose VAP was no better than that of existing clinical scores, however, in some studies it has improved the predictive capability whenever used together. Thus, the use of a biomarker should always be complementary to the clinical diagnosis and a thorough cost-effectiveness evaluation is required to understand if this approach should be widely implemented in all cases of suspected VAP, especially whenever PCT and other novel (and expensive) biomarkers are considered. A proposed model of potential uses is provided on Fig. 1 [34].

NEW APPROACHES TO OLD BIOMARKERS

Several studies have assessed the diagnostic performance of nosocomial pneumonia of a single measurement of a biomarker [30,35–38]. However, looking at a single measurement of a variable is like looking at a snapshot of a movie, it is impossible to catch the entire story! That single measurement ignores the dynamics that is essential to understand if biomarker levels are increasing or decreasing, as a result, repeated measurements, can be much more informative than a single measurement.

The statistical assessment of the value of repeated measurements over time of a biomarker is more complex and requires a more sophisticated mathematical approach. Though, the analysis of such data cannot be done by repeated testing, like repeated Student's *t* tests, at different time points. In these structures, there are typically strong hierarchies because there is much more variation between individuals than between measurements done at different time points in the same individual. There are several ways to overcome these problems and to correctly perform analysis of variance whenever the same variable is measured several times on different occasions on the same individual, namely two-way analysis of variance, multilevel statistical analysis for repeated measures data, General Linear Model (GLM) repeated measures, GLM univariate repeated measures or also performing a linear regression of the repeated measurements creating two new variables for each patient, that are the intercept and the slope [30,31,39,40]. With these statistical models for repeated measures data, we can study how the dependent variable, is influenced by the explanatory variables, correcting for the between and within patient variation.

Our original work, with this innovative method of analysis, demonstrated that daily CRP determinations could be useful as a marker of infection prediction in ICU patients as well as in the assessment of response to antibiotic therapy [30,31,40,41▪▪].

Our group has recently conducted the BioVAP study designed to evaluate the additional information biomarkers can bring in the management of VAP. We assessed 138 mechanically ventilated patients without an infection at ICU admission [15▪▪]. A total of 35 patients (25.4%) developed a microbiologically documented VAP. Among the studied biomarkers, CRP and CRP ratio (relative changes in CRP concentration in relation to initial level) showed the best performance in VAP prediction. The time-dependent analysis of CRP and CRP ratio was significantly different between noninfected controls and patients that went on to develop a VAP ($P < 0.001$ and $P < 0.001$, respectively). The time-dependent analysis of PCT was not significantly different between groups ($P = 0.685$). But more interestingly, it was also clear that CRP began to present significant differences around 48 h before VAP diagnosis, as we had already identified in a previous study [31].

As already pointed out, the biomarker slope describes the average rate of change per day of a particular variable in each patient from the beginning of mechanical ventilation that is day 1, till day 6. We studied the slopes of the biomarkers to assess how the magnitude of concentration change over time, that is the kinetics of the biomarker, was associated with the VAP prediction [41▪▪]. The slope of CRP change over time (OR = 1.62; 95% CI 1.21–2.19; $P = 0.001$) during the first 6 days of mechanical ventilation, was markedly associated with VAP development. A patient with an average increase in CRP concentration of 1 mg/dl/day from day 1 till day 6 of mechanical ventilation has 62% greater chance of having VAP when compared with a patient with no CRP increase. The same was shown with the CRP-slope calibration plot showing that the higher the slope, the higher the probability of developing a VAP.

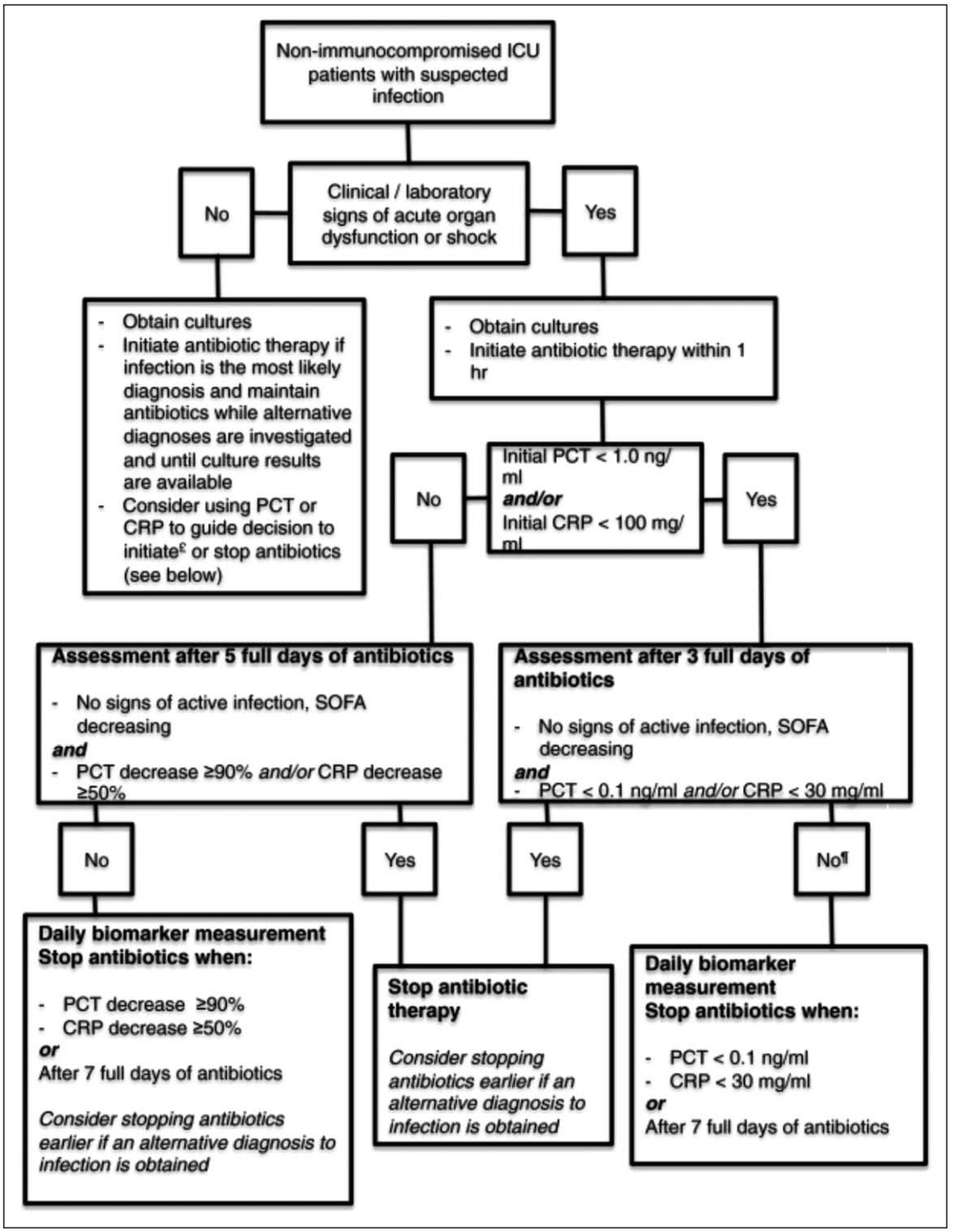

FIGURE 1. Use of C-reactive protein and procalcitonin to guide antimicrobial therapy in critically ill patients. C-reactive protein (CRP) was tested only in a single-center trial with predominantly medical ICU patients. This flowchart does not apply to immune-compromised patients (for example, febrile neutropenia) or to patients with infections requiring long-term antibiotic therapy (for example, infectious endocarditis, cerebral abscess, bacteremia due to *Staphylococcus aureus*). £Most trials investigating procalcitonin (PCT)-guided protocols tested the role of this marker in guiding the decision of antibiotic interruption. Initiating antibiotics for all critically ill patients with suspected infection is probably the safest decision, regardless the levels of laboratory biomarkers. However, this decision must be reassessed daily. PCT and CRP are proposed as additional tools to diagnose infection, and different cut-off levels have been proposed in the literature. Consider stopping antibiotics before day 7 in patients with no proven infection (for example, negative cultures) regardless the levels of CRP or PCT. SOFA, sequential organ failure assessment. Reproduced with permission (open access) from [34].

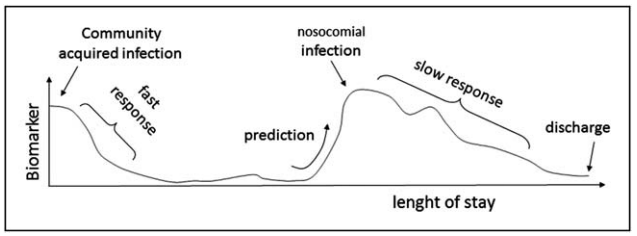

FIGURE 2. Biomarkers single vs. serial determinations. **Transverse** – useful along all ICU stay, either in the *presence* as well as in the *absence* of **infection**, and together with a complete clinical and laboratory evaluation, serial **biomarker** determinations could be very valuable in daily clinical practice.

This is particulary relevant, as monitoring the course of a single biomarker overtime we could have pertinent clinical information in relation to VAP prediction that is much easier to interpret that calculating a score. A proposed model is summarized in Fig. 2.

NOVEL BIOMARKERS FOR THE DIAGNOSIS OF NOSOCOMIAL PNEUMONIA

Genome-wide transcriptional studies have recently emerged as a powerful investigational tool. Kothari *et al.* [42] reported that overexpression of the gene of the tumor necrosis factor (TNF) α is associated with increase in the incidence of severe sepsis and septic shock from all causes, including pneumonia. Another study in critically ill patients demonstrated that the gene *PIK3R3*, that encodes the protein of phosphoinositide 3 kinase regulatory subunit gamma, expressed in immune cells, and involved in chemoattractant-induced cell migration, contributed to sepsis and organ damage [43]. None of these genes alone, however, is sufficient enough to answer the fundamental question why certain patients develop VAP, whereas others do not.

Rapid detection of infectious disease can be achieved by the exhaled breath that contains volatile organic compounds (VOCs) that result from bacterial metabolism and/or host response to the environment [44]. Capture of VOCs in an exhaled breath analysis has shown to be safe and reliable in mechanically ventilated critically ill patients [45] and the presence of bacteria may be detected based on a small panel of VOCs [46].

A number of individual proteins have been proposed as biomarkers for the presence of VAP, but single biochemical measurements are not consistent predictors of either onset or severity. In the recent years, it is being recognized that a multibiomarker model could be more promising than a single biomarker for risk assessment of disease [14▪,32,47]. This approach has progressed by recent advances in clinical proteomics. Proteomics applies the techniques of molecular biology, biochemistry, and genetics to analyse the structure, function, and interactions of the proteins produced by the genes of a particular cell or tissue [48]. In 2008, Lu *et al.* [49] published the first description of the BAL proteome from patients with VAP. They identified 206 proteins, defining a proteome map. Four selected proteins (gelsolin, serum amyloid P-component, vitamin D-binding protein and pyruvate kinase) were significantly higher in BAL from patients with VAP ($P < 0.05$). On the other hand, Nguyen *et al.* [50] applying a modern proteomic approach, identified a BAL protein 'signature' that discriminated VAP patients with acute lung injury, that is constituted by: S100A8, lactotransferrin, and actinin-1.

Although our insight has significantly increased over the past years, a translational approach, with application of genomics, proteomics, and metabolomics methodologies is required to better understand the disease. We should search for deeper interactions among basic science researchers and clinicians to push on translational approaches in the newest fields, which could provide new insights and possibilities on VAP diagnosis. Despite promising there is no current role and it remains unclear if and when, at least some of these technologies will be incorporated in the clinical routine for the diagnosis of nosocomial pneumonia.

CONCLUSION

There is no clear evidence in favour of the use of biomarkers to diagnose nosocomial pneumonia as a stand-alone tool despite being widely employed in clinical practice. Improved performance for both PCT and CRP may be obtained by using them in association with clinical features or scoring systems but prospective studies are still needed to validate this hypothesis. Hopefully by using these biomarkers with novel approaches that incorporate its dynamic assessment either to predict or to re-evaluate the diagnosis of pneumonia will bring further progress to the field.

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Conflicts of interest

There are no conflicts of interest.

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