

# Usefulness of Various Inflammatory Markers to Differentiate Pulmonary Edema from Pneumonia

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**ABSTRACT:** **Background:** Community-acquired pneumonia requiring hospitalization is a severe illness with high mortality, especially if the appropriate treatment is delayed. Sometimes diagnosis is difficult due to an equivocal clinical picture or chest film, or to accompanying diseases that mask or simulate pneumonia. **Objectives:** To assess the usefulness of certain inflammatory markers in differentiating pulmonary edema from pneumonia throughout the hospital stay in patients admitted for pneumonia or pulmonary edema of non-infectious origin and to monitor the response to treatment. **Methods:** The study group comprised 50 patients admitted for pneumonia, 50 admitted for pulmonary edema and 30 healthy individuals. Blood samples for determination of leukocyte count, erythrocyte sedimentation rate (ESR), fibrinogen, C-reactive protein (CRP), albumin, sCD14 and oxidized fibrinogen were drawn upon admission, at 48 and 72 hours after admission, and at discharge from the intensive care unit. **Results:** The levels of sCD14 were similar in both patient groups but higher than control levels during the first 48 hours ( $P < 0.03$ ). They decreased gradually with hospital stay. The concentration of oxidized fibrinogen was similar in both patient groups and significantly lower than that of the healthy control group throughout the hospitalization period. **Conclusions:** Oxidized fibrinogen and sCD14 are not reliable markers for the diagnosis of pneumonia, for its differential diagnosis from pulmonary edema, and for patient follow-up throughout hospitalization. The finding of elevated levels of oxidized fibrinogen in the group of healthy controls warrants further study to identify the factors responsible for altering fibrinogen oxidation. The other markers are more indicative.

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solidation, especially with co-morbidities such as pulmonary embolism or pulmonary fibrosis, other markers that could assist the differential diagnosis are required. Indeed, many studies have focused on the ability of acute-phase proteins [4-6], particularly C-reactive protein and cytokines, to serve as such diagnostic and predictive markers.

CRP increases dramatically soon after bacterial infection, and decreases rapidly after effective therapy [5-7]. CRP is sensitive enough to discern pneumonia from other non-inflammatory pulmonary processes [8], but it is not specific enough to allow differentiation of community-acquired pneumonia from other lung infections.

Fibrinogen is also a positive acute-phase protein whose level reportedly increases two- to fourfold upon infection or tissue damage [9]. However, in infectious processes, the changes are not specific and contribute little to the differential diagnosis or to the assessment of therapeutic efficacy [10]. Nonetheless, recent works have demonstrated that an oxidized form of fibrinogen is associated with smoking, lung cancer [11] and renal disease requiring hemodialysis [12].

Another acute-phase protein is the polysaccharide-binding protein that mediates the binding of lipopolysaccharides to the CD14 glycoprotein. Rapid increase in plasma levels of soluble CD14 is indicative of a bacterial infection, and in gram-negative sepsis sCD14 was directly correlated with mortality [13]. However, this protein was also found elevated in a variety of other diseases, such as asymptomatic human immunodeficiency virus, active sarcoidosis, rheumatoid arthritis, and multiple organ failure, correlating with CRP level [14-17].

The objective of the present study was to investigate whether plasma levels of fibrinogen, oxidized fibrinogen, sCD14 and CRP can be correlated with the clinical and laboratory parameters of patients hospitalized for pneumonia or for pulmonary edema of non-infectious origin. We also wished to examine the suitability of these markers for differentiating between pneumonia and pulmonary edema, to evaluate the extent of fibrinogen oxidation, and to determine whether it is more diagnostically advantageous than other indicators of inflammation.

CRP = C-reactive protein  
sCD14 = soluble sCD14

**P**neumonia is the sixth leading cause of death in general, and the first among infectious diseases [1-3]. Yet the diagnosis of pneumonia, especially in its early stages, is difficult to reach. When the clinical presentation is unclear and chest radiographs do not demonstrate infiltration or con-

## PATIENTS AND METHODS

Two groups, each of 50 patients admitted to the pulmonary intensive care unit and internal medicine department during a 30-month period, and a similar group of healthy individuals were included in this prospective study. Their demographic and clinical admission data are detailed in Table 1. All participants signed an informed-consent agreement for blood sampling approved by the institutional committee in accordance with the Helsinki Declaration.

Members of the first group were diagnosed based on the distinctive clinical presentation, together with fever, left-shift leukocytosis purulent sputum and typical radiographs, and whether a specialist radiographer interpreted the chest radiographs. Pulmonary edema presented as hypopnea, vasocongestion and symptoms of hypoxia, as well as the radiographic findings. Acute coronary syndrome and myocardial infarction were excluded by measuring cardiac troponin, enzymes and serial electrocardiogram. Echocardiography showed valvulopathy in most of the patients, some with rapid atrial fibrillation, some with uncontrolled hypertension and a minority with unknown cause. Exclusion criteria were malignancy, renal disease requiring hemodialysis, immunocompromised status, and in-hospital death.

Blood samples were obtained at admission, at 48 hours, at 72 hours and at discharge. Part of the serum was frozen and stored at  $-70^{\circ}\text{C}$  for determination of fibrinogen by an immunoturbidometric assay (Cobas Mira analyzer, Hoffman-La Roche Ltd, Basel, Switzerland). Oxidized fibrinogen was quantified by derivatization with 2,4-dinitrophenylhydrazine, separation on gel electrophoresis and subsequent western blot analysis, as detailed elsewhere [12]. sCD14 was quantified with a commercial kit based on a sandwich enzyme immunoassay (Quantikine, R&D Systems, Minneapolis, MN, USA).

## FIBRINOGEN AND CRP

Fibrinogen levels were measured in plasma using the K-Assay<sup>®</sup> kit (Kamiya Biomedical Company, USA) by chemical analyzer (Cobas Mira, Roche Diagnostics, Germany).

Blood CRP levels were measured using a commercial kit (Biokit, Spain) and the Hitachi 917 Automatic analyzer (Roche Diagnostics, Germany).

## STATISTICAL ANALYSIS

Values obtained were analyzed by ANOVA with repeated measurements, and when homogeneity of variance was violated non-parametric tests such as Mann-Whitney and Kruskal Wallis were applied. Values are presented as mean  $\pm$  SD, and the significance level was set at  $P < 0.05$ . Either the two groups or the different time interval within each group was compared by Student's *t*-test, using Prism version 3.0 statistical software (GraphPad software, San Diego, CA, USA). Correlations between different study parameters were performed using Pearson correlation coefficients.  $P < 0.05$  was considered significant.

## RESULTS

In admitted pneumonia patients, fever was a distinct diagnostic feature differentiating them for the first 48 hours from pulmonary edema patients and healthy controls [Figure 1A], with values of  $38 \pm 0.9^{\circ}\text{C}$  and  $37.2 \pm 0.8^{\circ}\text{C}$  in these two groups, compared to  $36.4 \pm 0.4^{\circ}\text{C}$  and  $36.5 \pm 0.3^{\circ}\text{C}$  in edema patients ( $P < 0.001$  and  $P = 0.015$ , respectively). As for leukocyte count, both patient groups had high and statistically similar counts relative to the control group, and this level (from  $12.8 \pm 5.4 \times 10^6$  cell/ml to  $10.5 \pm 5.6 \times 10^6$  cell/ml upon discharge, versus  $6.8 \pm 1.6 \times 10^6$  cell/ml in the control group) remained significantly high throughout the entire hospital stay ( $P < 0.01$ ).

The erythrocyte sedimentation rate was also elevated in both patient groups on admission [Table 1] and decreased only slightly during the study period. The ESR of the pneumonia group was significantly higher than that of patients with pulmonary edema on admission and at 72 hours ( $P = 0.04$  and  $P = 0.02$ , respectively; data not shown). Similarity between the two experimental groups was also observed in serum albumin levels, which did not change throughout the study period. Compared to the healthy individuals, the albumin in both patient groups was significantly decreased [Table 1] ( $P < 0.001$  between healthy controls and either group).

CRP levels in the pneumonia and pulmonary edema patients were significantly different ( $P < 0.001$ ) from those of the healthy control group [Figure 1B], and a significant difference between the pneumonia and pulmonary edema cases was noted at admission and at 72 hours ( $P = 0.007$  and  $0.03$ , respectively). With the decrease in CRP towards discharge, the groups became statistically similar.

The behavior of fibrinogen and oxidized fibrinogen is depicted in Figure 1C. Fibrinogen levels in pneumonia and

**Table 1.** Demographic and patient admission data (mean  $\pm$  SD)

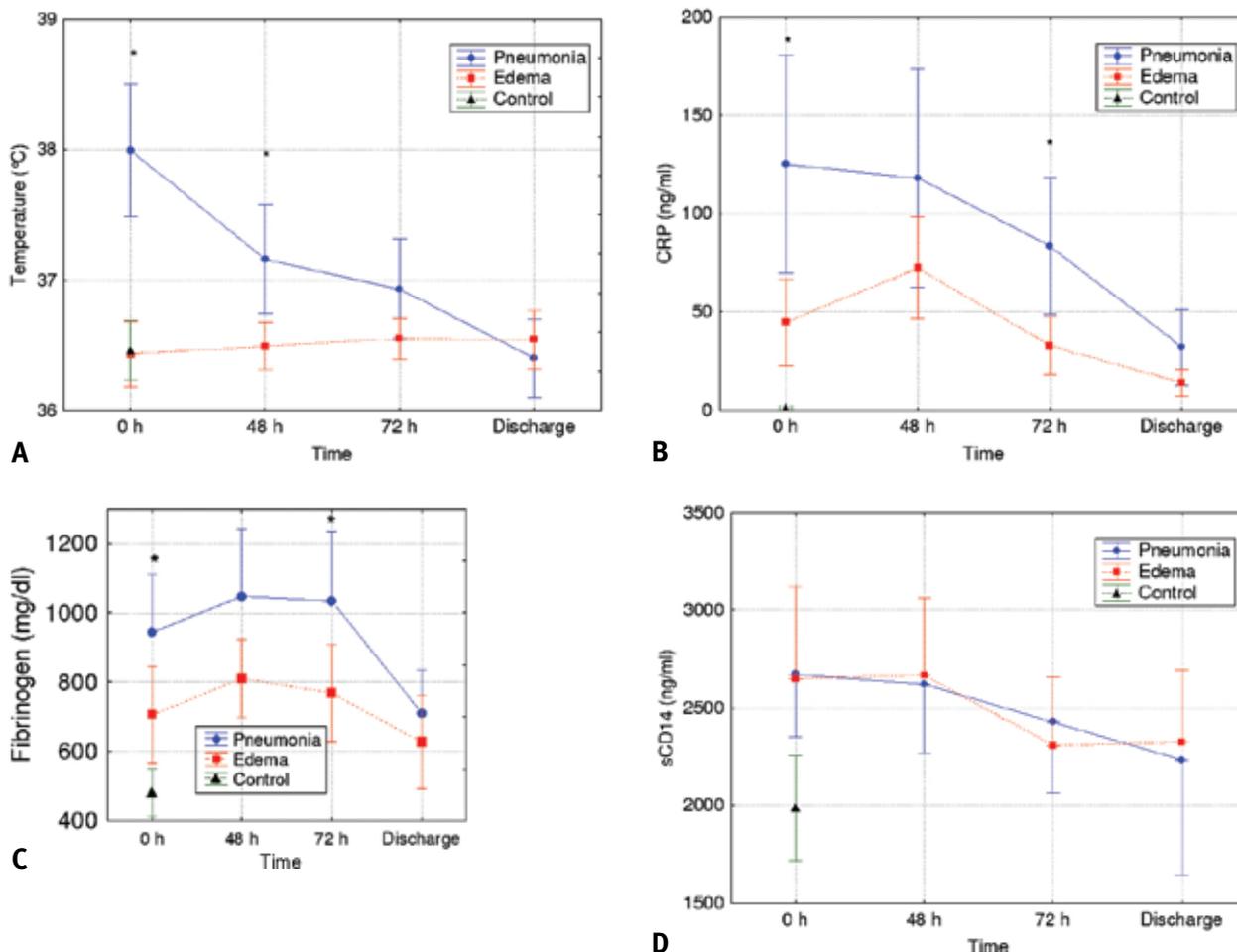
	Normal values	Pneumonia	Edema
Patients		50	50
Age (yrs) (range)		67.5 $\pm$ 17.9 (35–89)	69.5 $\pm$ 6.2 (54–79)
Fever ( $^{\circ}\text{C}$ )	36.0–37.0	38.0 $\pm$ 0.9	36.4 $\pm$ 0.4
WBC ( $\times 10^3/\text{ml}$ )	3200–11,000	15,787 $\pm$ 6309	17,007 $\pm$ 5000
ESR (mm)	0–30	77 $\pm$ 32	48 $\pm$ 30
Albumin (mg/dl)	3.4–4.8	3.6 $\pm$ 0.1	3.9 $\pm$ 0.1

WBC = white blood cell

ESR = erythrocyte sedimentation rate

**Figure 1.** Time course in the study groups of **[A]** fever (bars denote 95% confidence interval); \* $P < 0.05$  between pneumonia and pulmonary edema. **[B]** CRP (bars denote 95% confidence interval); \* $P < 0.05$  between pneumonia and pulmonary edema. **[C]** fibrinogen (top) and oxidized fibrinogen (bottom) (bars denote

95% confidence interval); \* $P < 0.05$  between pneumonia and pulmonary edema and # $P < 0.05$  between control and patient groups. **[D]** sCD14 (bars denote 95% confidence interval); \* $P < 0.05$  between pneumonia and pulmonary edema.



pulmonary edema were significantly elevated compared to those of the healthy controls. In pneumonia, fibrinogen levels were significantly higher than in pulmonary edema patients at admission and at 72 hours ( $P = 0.04$  and  $P = 0.02$ , respectively). However, the level of oxidized fibrinogen was significantly lower in the two hospitalized groups than in the control individuals (pneumonia  $P = 0.01, 0.007, 0.01$  and  $0.03$  for the respective time points, and correspondingly for edema  $P = 0.003, 0.007, 0.04$  and  $0.1$ ) [Figure 1C]. The degree of fibrinogen oxidation increased with time in the edema group but not in the pneumonia group, yet statistically those two patient groups were similar.

sCD14 levels were similar in both patient groups and higher than levels in the control individuals during the first 48 hours

( $P < 0.01$  for pneumonia and  $P < 0.03$  for edema); the sCD14 values decreased gradually with hospital stay [Figure 1D].

## DISCUSSION

The findings of this study support the common notion that body temperature is an effective primary marker for the initial diagnosis and for treatment follow-up of infectious diseases, pneumonia in particular, and it can differentiate them from non-infectious pulmonary processes. Nevertheless, many studies have demonstrated that fever is unrelated to infection in as many as 50% of patients in intensive care. Likewise, the significant rise in leukocyte count in admitted pneumonia patients and its decrease during hospital stay concur with the

accepted notion that this variable contributes to the diagnosis of infections, especially pneumonia [8]. Yet leukocytosis was also observed in the studied patients with non-infectious pulmonary edema, precluding application of the leukocyte count for differentiation of lung infection, in agreement with the conclusions of two studies [18,19].

ESR is also a well-established tool for the assessment of inflammation, since it is based primarily on the plasma concentration of fibrinogen. ESR values, however, change slowly and correlate poorly with variations in patients' clinical status, as observed in this cohort of patients. Although an ESR > 60 mm supports the initial diagnosis of an infectious disease, particularly pneumonia [8,19], later the ESR becomes valueless and cannot be used for assessing clinical status and response to treatment.

The application of CRP for diagnosing infection and monitoring inflammatory response in systemic diseases has been increasing in recent years. Several studies on CRP in pneumonia illustrated the sensitivity of this marker for diagnosis and for follow-up of response to antibiotics [5,6]. Others, however, have emphasized the low specificity of CRP for the differential diagnosis of pneumonia caused by different agents, but still appreciated its good correlation with clinical and laboratory variables [4]. In the present study, CRP increased in patients admitted with pneumonia by up to 25 times the normal value, and decreased gradually with hospital stay, corresponding to the decrease in body temperature, white cell count and clinical presentation. Thus, our findings support CRP as a reliable marker during follow-up in pneumonia patients. In pulmonary edema, CRP also increased, but to a much lesser degree than in pneumonia, and continued to increase for at least 2 days after admission. This is similar to the reported behavior of CRP in patients admitted and treated for acute cardiac insufficiency [20]. However, we did not observe a rapid and substantial decrease in CRP in pulmonary edema patients even after the clinical signs indicated improvement. Therefore, we concur with researchers who found CRP not to be a reliable diagnostic marker of infection during intensive care [21].

Albumin, despite its classification as a negative acute-phase protein, also had no diagnostic and follow-up value in the studied patients.

sCD14 is related to the immune response, and its activation results in aggregation of leukocytes and their binding to endothelial cells. Indeed, elevated sCD14 was demonstrated in numerous diseases, such as sarcoidosis, psoriasis, atopic dermatitis, lupus, asymptomatic HIV, and multisystem failure. It was also associated with increased risk of mortality in gram-negative sepsis, but others noted that in sepsis such increased levels could also have beneficial effects [13,22,23]. We observed a gradual decrease of sCD14 during the hospital

stay in both patient groups, but it was not specific enough and therefore is not a suitable diagnostic or follow-up marker. A decreasing level of sCD14 was described recently in congestive heart failure [24].

The serum levels of fibrinogen, another positive acute-phase protein, increase during infection in the elderly, in smokers, and in patients with diabetes or hypertension. In our study the level of fibrinogen in the control group was higher than the norm, which could be attributed to the older age of these individuals (67 years). Still, on admission, both patient groups presented with elevated fibrinogen, up to twice that of the control group. The increase of fibrinogen peaked between 48 and 72 hours and did not level off to normal values by discharge. This marker was significantly higher in the pneumonia group, at least in the early stage of admission, and could serve as a differentiating factor of pneumonia from pulmonary edema. However, it was a poor indicator of clinical improvement.

Oxidized fibrinogen, which is reportedly increased among smokers and some patients [11,12], reflects the severity of the oxidative stress of existing inflammatory processes. Surprisingly, in our study oxidized fibrinogen was elevated in the control individuals and very low in pneumonia patients, and to a lesser degree in the pulmonary edema patients. Since normal values of oxidized fibrinogen were established in individuals aged about 50 years [12], these values probably do not reflect the normal range of older individuals.

Hence, the surprising finding in this study was the relatively decreased levels of oxidized fibrinogen among patients with pulmonary disease. It is unlikely that the oxidative stress among these patients is lower than among healthy controls, and therefore we speculate that because oxidized fibrinogen was measured relative to fibrinogen and because the level of fibrinogen was elevated by 150% to 200% compared to healthy individuals, the increase in total fibrinogen artificially decreased the oxidized fibrinogen values. Indeed, when normalizing by total fibrinogen, no significant difference was found among the three study groups. We demonstrated these findings regarding fibrinogen in a previous study but with a small number of patients; in the previous study oxidized fibrinogen and sCD14 were not assessed [25]. Therefore, for assessing the effects of oxidative stress other factors should be followed such as peroxidation of fatty acids. Since this was not performed in the present study, the degree of oxidative stress that the patients had suffered remains unknown. It is also possible that the prolonged exposure of oxidized proteins to a highly oxidative environment might cause further protein modification, in which case the quantity of carbonyl residues does not reflect the real extent of protein oxidation.

In conclusion, the findings of the present study indicate that in the acute phase fibrinogen could serve as a diagnostic marker for pneumonia and could differentiate it from pulmonary edema.

HIV = human immunodeficiency virus

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