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Plasma calprotectin in the emergency department: a potential clinical biomarker for patients with infectious diseases

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ABSTRACT
Increased levels of plasma calprotectin are reported in patients with infectious diseases. However, the clinical usefulness of calprotectin as a biomarker to identify patients with infectious diseases in the emergency department (ED) setting has not been investigated. To study the ability of calprotectin to discriminate patients with acute infectious diseases and dyspnea from patients with other causes of acute dyspnea in the ED setting. Patients aged ≥18 years seeking ED during daytime on weekdays between March 2013 and July 2018, with acute dyspnea, were included. Participants (n = 1287) were triaged according to Medical Emergency Triage and Treatment System-Adult score (METTS-A) or Rapid Emergency Triage and Treatment System (RETTs), and blood samples were collected. The association between calprotectin and other markers of infectious diseases, i.e. biomarkers (CRP, leukocytes) and body temperature, was studied. The predictive value of calprotectin for the outcome of acute infection was evaluated with receiver operating characteristic (ROC) analysis. Univariate cross-sectional regression showed significant associations between calprotectin and leukocytes, CRP and body temperature. Patients with severe infections including pneumonia (n = 119) had significantly higher concentrations of calprotectin compared to patients with heart failure (n = 162) or chronic obstructive pulmonary disease (n = 183). When tested for the outcome of acute infection (n = 109), the area under the ROC curve (AUROC) was for CRP 0.83 and for calprotectin 0.78. Plasma calprotectin identifies infectious diseases in ED patients with acute dyspnea, and the clinical usefulness of Calprotectin in the ED has to be further studied.

Introduction
Calprotectin, a heterodimer of S100A8 and S100A9, constitutes approximately 45% of the cytosolic protein in neutrophils [1]. Calprotectin is released upon neutrophil activation [2], and plays an important but not fully understood role in the inflammatory responses. Fecal calprotectin is a valuable biomarker for inflammatory bowel disease [3-5], and previous studies have shown increased levels of calprotectin in several other inflammatory diseases [6-8]. In recent years, elevated levels of circulating calprotectin have been reported in patients with infectious diseases and/or sepsis [9,10], raising the question if calprotectin could prove a useful biomarker for these conditions. White blood cell and neutrophil counts are widely used as infectious markers. The problem with cell counts is that when the cells are activated they will adhere to the vascular endothelium and extravasate [11]. Thus, a neutrophil activation marker should therefore in theory be superior to the neutrophil counts for this reason.

Acute dyspnea is a symptom of a variety of underlying conditions including life-threatening ones such as heart failure and infectious diseases and/or sepsis. It is a common chief complaint in patients presenting to the emergency department and acute dyspnea itself is a predictor of increased mortality and rapid and correct diagnosis and treatment is of high priority [12]. Clinical tools available for diagnostic evaluation of patients with acute dyspnea in the ED usually consist of clinical examination, vital parameters and routine laboratory parameters.

The aim of this study was to investigate the ability of circulating calprotectin to discriminate between patients with acute infectious diseases and dyspnea from patients with other causes of acute dyspnea in the ED setting.

Methods
Study population

The study was conducted at the ED of Ska’ne University Hospital in Malmö (SUS Malmö), which is responsible for a catchment area of nearly 400,000 people with up to 85,000
visits per year. Patients 18 years of age and older who presented to the ED during daytime on weekdays, 8:00 am to 5:00 pm, with acute dyspnea as their main complaint between 6 March 2013 and 1 July 2018 were approached by a research nurse and were offered to take part in the study. Participants were triaged according to Medical Emergency Triage and Treatment System-Adult score (METTS-A) or Rapid Emergency Triage and Treatment System (RETTS) where after blood samples were collected [13,14]. In total, 1710 patients were included, and of these 423 patients were excluded due to missing data (leucocytes, body temperature, CRP, Calprotectin-analyses, creatinine and BMI), leaving 1287 patients eligible for further analyses.

Clinical parameters

Patients with acute dyspnea were included with the help of special trained research nurses. Further care at the ED, such as patient acute care, patient primary diagnosis and administration of pharmaceutics was carefully followed and documented by the nurses. The research nurses reviewed the patient journals in order to confirm all details involving the acute care of the patient as well as the medical history of the patient and current medication, with the support of senior emergency medicine physicians. The patients’ primary ED-diagnosis was set at the ED by the ED physician in charge of the patient. Only patients with serious infection including pneumonia, heart failure or COPD were included in this study. Other primary ED diagnoses were not included. Originally, METTS-A and RETTS use a five clinical priority levels with increasing clinical priority: blue (lowest clinical priority - not life-threatening), green, yellow, orange and red (highest clinical priority – life-threatening). The lowest clinical priority level blue was not used in the clinical triage of the patients included here due to the local triage routines, and the lowest clinical triage priority here was green. Blood pressure, oxygen saturation and heart rate were recorded by an automated oscillometer (CARESCAPE Monitor B850 or B650, General Electric Healthcare) and consciousness was determined according to the Reaction Level Scale [15]. The respiratory rate was calculated manually, and no dyspnea scale was used.

Blood analyses

High sensitivity plasma CRP was analyzed by a particle enhanced turbidimetric assay (PETIA), and creatinine was analyzed by an IDMS calibrated enzymatic creatinine assay [16]. The respiratory rate was calculated manually by ED nurses and blood pressure, oxygen saturation and heart rate were measured with a fully automated oscillometric device (CARESCAPE Monitor B850 or B650, General Electric Healthcare) [17,18]. Plasma concentration of CRP was measured using a Radiometer ABL800 Flexmachine [19], or Afinion AS100 Analyzer System [20]. Lactate and creatine were analyzed according to local clinical routines at the University hospital laboratory. Within an hour of presentation at ED, blood was sampled, serum and plasma separated and subsequently put away for storage at −80°C for future analysis. Blood samples were taken before administration of antibiotics. Calprotectin was analyzed in EDTA plasma samples on a Mindray BS380 chemistry analyzer (Mindray, Shenzhen, China) using calprotectin reagents from Gentian AS (Moss, Norway).

Statistical analysis

Baseline values given as number or percentages, mean values with standard deviation (SD) or median values (for Calprotectin). Univariate linear regression analyses were used to study the association between calprotectin, with patient clinical characteristics at baseline such as age, BMI, blood pressure, respiratory rate, CRP, lactate and creatinine. Logarithmic calprotectin and CRP were used in the regression analyses. The association between calprotectin and clinical endpoints as final diagnosis was analyzed using logistic regression, for one SD increase in calprotectin. A p-value of < .05 was considered statistically significant. We used three models, i.e. Model A adjusted for age and sex, Model B as Model A but also adjusted for BMI and creatinine and Model C as Model B but also adjusted for CRP. Receiver operating characteristics (ROC) analysis was calculated for calprotectin alone as well as for other markers of infection. Area under the ROC curve (AUROC) was measured to determine the accuracy of the model. A p-value of < .05 was considered statistically significant. Dataset was handled and regression models, ROC curves and AROC were all computed with IBM SPSS statistics 25 (SPSS Inc., Chicago, IL).

Results

Baseline data of included patients (n = 1287) are shown in Table 1. Median age was 74 years.

Association between baseline characteristics and calprotectin is shown in Table 2, with statistically significant findings between calprotectin and respiratory rate, CRP, body temperature and white blood cell count (p < .001), and to some extent between calprotectin and lactate and creatinine (p < .05).

Table 3 shows values of calprotectin, CRP and white blood cell count among patients with severe infectious diseases including pneumonia, COPD and heart failure, respectively, with statistically significant differences for calprotectin and CRP between patients with severe infectious diseases including pneumonia compared to the other two patient groups (p < .001), and for white blood cell count between patients with serious infectious diseases including pneumonia compared to patients with heart failure (p < .001).

Table 4 shows the results of the ROC analysis for the risk of being diagnosed with a severe infection including pneumonia at the ED (n = 119), with highest AUROC for CRP (0.83), where addition of body temperature, white blood cell count and calprotectin did not improve this value.
Associations were analyzed with linear regression models. Logarithmic calprotectin and CRP were used only for regression analyses.

| Table 1. Baseline characteristics for patients. |
|-----------------------------------------------|
| Variable                                      | Patients (Median (IQR) unless otherwise specified) |
| Number of participants                        | 1287 |
| Female                                        | 54% |
| Age at survey (years)                        | 74 (63–84) |
| Body mass index (kg/m²)                      | 26 (23–30) |
| Admitted to hospital care                    | 60% |
| Vital parameters                              |
| Systolic blood pressure (mmHg)                | 141 (126–164) |
| Diastolic blood pressure (mmHg)               | 80 (70–91) |
| Heart rate (frequency)                       | 89 (77–104) |
| Respiratory rate (frequency)                 | 24 (20–28) |
| Blood oxygen saturation (%)                  | 95 (90–97) |
| Body temperature (degree Celsius)            | 36.9 (36.5–37.3) |
| METTS-A (%)                                   |
| Red                                           | 14% |
| Orange                                        | 33% |
| Yellow                                        | 48% |
| Green                                         | 5% |
| Laboratory parameters                         |
| Calprotectin (mg/L)                           | 0.5 (0.25–1.0) |
| C-reactive protein (mg/L)                    | 11 (3.4–36) |
| White blood cell count (10⁹/L)               | 9 (7.1–11.6) |
| Lactate (mmol/L)                              | 1.5 (1.2–2.1) |
| Creatinine (µmol/L)                           | 81 (65–107) |
| Comorbidities (%)                             |
| Diabetes                                      | 19% |
| Cancer                                        | 20% |
| Hypertension                                  | 42% |
| Chronic heart failure                        | 35% |
| Coronary artery disease                      | 31% |
| COPD                                          | 31% |
| Asthma                                        | 10% |
| Current smoker                                | 18% |
| Previous smoker                               | 51% |

Median values (with IQR). Calprotectin, CRP, lactate and creatinine were analyzed in plasma.

| Table 2. Association between calprotectin and baseline characteristics. |
|---------------------------------------------------------------|
| Variable                                      | Calprotectin (B (95% CI)) |
| Sex                                           | 0.038 (–0.72,0.15) |
| Age at survey (years)                            | 0.002 (–0.001,0.005) |
| Body mass index (kg/m²)                         | –0.008 (–0.02,0.001) |
| Systolic blood pressure (mmHg)                  | –0.001 (–0.003,0.000) |
| Diastolic blood pressure (mmHg)                 | –0.003 (–0.006,0.001) |
| Respiratory rate (frequency)                    | 0.015 (0.008,0.022)** |
| C-reactive protein (CRP, mg/L)                  | 0.26 (0.23,0.29)** |
| Body temperature (centigrade)                  | 0.19 (0.11,0.28)** |
| White blood cell count (10⁹/L)                  | 0.18 (0.01,0.02)** |
| Lactate (mmol/L)                               | 0.06 (0.005,0.11)* |
| Creatinine (µmol/L)                            | 0.001 (0.000,0.002)* |

*p < .05, **p < .01.
Logarithmic calprotectin and CRP were used only for regression analyses. Associations were analyzed with linear regression models.

**Comparison with the literature**

Previous studies have shown a high performance of calprotectin to identify bacterial infectious diseases and a possibility to distinguish between viral and bacterial infectious diseases.

A Swedish study of critically ill patients found a slightly higher AUROC value for calprotectin compared to CRP, 0.78 vs. 0.71, but significantly higher than WBC and procalcitonin in the early diagnosis of bacterial infectious diseases [21]. In contrast, in the present study, AUROC for calprotectin was slightly lower than that of CRP, i.e. 0.77 vs. 0.83. In a study among patients with sepsis, a higher AUROC value for calprotectin was found compared to procalcitonin in distinguishing between patients with sepsis from trauma patients 0.78 vs. 0.49 [9]. A study among surgical patients with bacterial sepsis also found a high AUROC for calprotectin, 0.889 for the prediction of septic AKI and in-hospital mortality [22]. Interestingly, two recent studies showed high performance of calprotectin in identifying bacterial infections and distinguishing these from viral infectious diseases.

In one study with patients with respiratory infectious diseases, calprotectin was compared to heparin-binding protein (HBP) and procalcitonin (PCT) showing greater performance in distinguishing bacterial and mycoplasma respiratory infectious diseases from viral infectious diseases [23].
Moreover, when comparing patients diagnosed with bacterial sepsis to patients with viral infectious diseases or healthy controls, calprotectin performed better than other conventional biomarkers in distinction between bacterial and viral infectious diseases with an AUROC value of 0.91 which was higher than a value for white blood cell count (0.84) and procalcitonin (0.88), with however no value for CRP [10].

**Potential mechanisms**

Neutrophil granulocytes are one of the first responders to bacterial infectious diseases. Calprotectin, an antibacterial protein and neutrophil activator and one of the most abundant cytosolic proteins in the neutrophils, is released from activated neutrophils and increases in the blood within hours in response to bacteria or endotoxin [24], signaling the presence of an acute infection and/or inflammation. Calprotectin works in the body against bacterial pathogens through a mechanism termed ‘nutritional immunity’, by binding to Mn(2+) and Zn(2+) and thus starving the bacteria to these essential nutrients [25,26].

**Clinical implications**

In the acute situation at ED, identifying patients with potential life-threatening infectious diseases is essential in order to determine and initiate proper medical therapy and reduce the risk of mortality. It is also of great importance to avoid improper treatment with antibiotics and contribution to the increasing development of antibiotic resistance and detrimental effects on the microbiota. In the present study of an unsorted group of patients admitted to the ED with acute dyspnea, calprotectin successfully identified patients with severe infections including pneumonia and calprotectin might be a promising biomarker in this clinical situation.

**Limitations and strengths**

There are some limitations of the study. The study is performed in a special group and is limited to patients with acute dyspnea seeking care at a hospital ED. However, the study is similar to other studies with acute or even critically ill patients seeking care [10,21,22,27]. As the outcomes were based on the ED physician’s primary assessment, it is likely that some of the diagnoses were misclassified. Moreover, the fact that the clinical infection diagnosis included consideration of patients CRP levels and white blood cell count could lead to increase of the diagnostic performance estimates for these clinical biomarkers and thus preclude an unbiased comparison with plasma calprotectin, especially as CRP and calprotectin are shown to be highly correlated [28]. Nevertheless, a potential overestimation of the number of infectious states should only account for a small number of patients. We cannot generalize to other age or ethnic groups or to other clinical settings. Only one blood sample was drawn, and comparisons between the state before and after the admittance to the ED cannot be explored. Among strengths of the study are the characterizations of study participants. The database only includes validated diagnoses, practically, this means that individual patient records have been thoroughly studied by the study personal to confirm the correctness of the documented care of the patient, including the patients’ diagnoses. The final ED-diagnosis was set at patient discharge from the ED, either to further in-hospital care or if the patient was dismissed from the ED. Diagnosis was set after careful examination of the ED-physician, blood sampling of the patient as well as suitable adapted procedures such as radiologic imagining or ECG.

**Conclusions**

Calprotectin might be a promising biomarker of infectious diseases in the ED setting.

**Ethical approval**

This study has an ethical approval from ‘Regionala Etikprövningsnämnden EPN’, Lund, Sweden. Dnr 2014/82.

**Informed consent**

All patients left their written informed consent to take part in the study.

**Author contributions**

TR, MW, OM, TW designed the study, TR analyzed data, and all authors participated in the interpretation of data; MW and PW drafted the manuscript and all other authors revised it critically for important intellectual content.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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