The role of acute phase proteins for predicting SARS-CoV-2 positivity upon emergency department admission

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ABSTRACT

Background: due to the important abnormalities observed in the concentration of many inflammation/infection biomarkers in patients with coronavirus disease 2019 (COVID-19), this study was aimed to evaluate whether the assessment of C-Reactive Protein (CRP), interleukin 6 (IL-6) and procalcitonin (PCT) could help predicting SARS-CoV-2 positivity at emergency department (ED) presentation in patients with suspected infection.
Methods: the study population consisted of patients consecutively admitted to the ED of the University Hospital of Verona, with clinical suspicion of SARS-CoV-2 infection over a 2-week period. Blood samples as well as oropharyngeal and nasopharyngeal swabs were collected upon ED admission.
Results: the final study population consisted of 92 patients, 48 with negative and 44 with positive SARS-CoV-2 swabs. No significant differences were observed in concentrations of CRP, IL-6, or PCT between patients with or without acute SARS-CoV-2 infection. A significant correlation was found between CRP and IL-6 in both negative (r=0.77) and positive (r=0.74) SARS-CoV-2 cases, between CRP and PCT in SARS-CoV-2 negative (r=0.38) and positive (r=0.44) cases, and between IL-6 and PCT in SARS-CoV-2 negative (r=0.37) and positive (r=0.40) cases. The area under the curve (AUC) of none of the biomarkers could efficiently discriminate patients with negative or positive swabs (CRP: 0.52; IL-6: 0.51; PCT: 0.53).
Conclusions: routine measurement of CRP and IL-6, together with PCT, does not seem a useful pre-test strategy in ED patients with clinical suspicion of COVID-19.

INTRODUCTION

The continuous spread of severe acute respiratory coronavirus 2 (SARS-CoV-2), which is responsible for the ongoing coronavirus disease 2019 (COVID-19) pandemic outbreak, is causing a devastating impact on healthcare services, society and economy across the world (1). With the number of COVID-19 cases exponentially growing, the volume of molecular tests, which are still considered the gold standard for diagnosing SARS-CoV-2 infection, has increased to such a large extent that most clinical laboratories are no longer capable of meeting testing needs. According to a recent survey of the American Association of Clinical Chemistry (AACC) carried out in September 2020, more than half of respondent laboratories declared to be unable to obtain the materials needed to perform all the necessary tests (2). Specifically, over 60% of all laboratories which participated in the survey reported that they were unable to secure test kits and supplies, whilst more than half reported being plagued by a shortage of staff required to run the tests.

Overall, the data clearly demonstrates that the current testing strategy seems to have failed in many countries (3,4), and that different approaches are urgently need for counteracting the ongoing emergency

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of providing tests to all who will need them. Some strategies have been suggested for enhancing the efficacy and the likelihood of obtaining positive results of molecular testing, including pre-test probability calculation of nucleic acid amplification test (NAAT) positivity, the use of rapid molecular tests which would not require RNA extraction and purification, pooling together and then measuring a variable number of clinical samples, and rapid (antigen) testing (5,6). With respect to pre-test probability calculation, a number of models have been proposed and validated, with the aim of quickly and accurately assessing the individual risk of SARS-CoV-2 infection, especially during the triage in the emergency room (7-11). Although the cumulative diagnostic performance of these algorithms is rather heterogeneous, some of these - such as the so-called "corona-score" - display acceptable accuracy (i.e., between 70-90%) (7,8).

One essential and common element of all these pre-test scoring systems is the incorporation of laboratory tests within the algorithm, especially inflammatory biomarkers, such as C-Reactive Protein (CRP) and ferritin, as well as pro-inflammatory cytokines, such as interleukin-6 (IL-6). This is mostly due to the fact that the pathogenesis of COVID-19 is characterized by a systemic immune hyperactivation, which can be accompanied by a so called "cytokine storm syndrome" (frequently abbreviated to "cytokine storm"), especially in patients with severe and/or critical disease. This syndrome is typically characterized by considerable release of pro-inflammatory mediators, imbalance of the renin-angiotensin-aldosterone system, as well as by a paradigmatic form of vascular disease, called COVID-19-associated coagulopathy (CC) (12). Importantly, the adverse clinical progression of COVID-19 seems also influenced by development of secondary (especially bacterial) co-infections (13,14), so that the measurement of procalcitonin (PCT) seems an important variable in patient evaluation and monitoring (15).

Due to the important role played by inflammatory and infection biomarkers in COVID-19 (16), the aim of this article was to evaluate whether the assessment of some of these molecules could help predicting the positivity of SARS-CoV-2 molecular testing upon patient presentation to the emergency department (ED).

METHODS

The study population consisted of a series of patients consecutively admitted to the ED of the University Hospital of Verona, with clinical suspicion of SARS-CoV-2 infection (i.e., fever, dyspnea, cough, gastrointestinal and other pathognomonic symptoms) over a 2 weeks period. Blood samples were collected upon ED presentation along with oral- and nasopharyngeal swabs, according to standard procedures and in accordance with the World Health Organization (WHO) recommendations (17). Molecular analysis for detecting SARS-CoV-2 RNA was carried out using Seegene AllplexTM2019-nCoV Assay (Seegene, Seoul, South Korea), in which viral RNA identification is based on multiplex Real time polymerase chain reaction (PCR) targeting three SARS-CoV-2 genes (*E, RdRP* and *N*), and thus fulfilling validated international testing protocols. The serum concentration of CRP, PCT and IL-6 was measured on MAGLUMI 800 (SNIBE, Shenzhen, China), according to manufacturer's instructions. All these immunoassays are based on chemiluminescent detection. The performance of the tests, as declared by the manufacturer, are as follows: CRP: functional sensitivity 0.00015 mg/L, linearity up to 100 mg/L, total imprecision ≤6%; IL-6: limit of detection 1.5 pg/mL, linearity up to 5000 pg/mL, and total imprecision ≤7%; PCT: limit of detection 0.04 ng/mL, linearity up to 10 000 ng/mL (with dilution) and total imprecision ≤10%.

Results were finally expressed as median with interquartile range (IQR). The comparison of biomarker values between SARS-CoV-2 negative and positive patients was carried out with Mann-Whitney U or Chi-Squared tests when appropriate, the potential associations were assessed with Spearman's correlation (and relative 95% confidence interval; 95% CI), whilst their diagnostic performance was assessed with receiver operating characteristics (ROC) curve analysis. Statistical significance was set at $p<0.05$. The statistical analysis was carried out using Analyse-it (Analyse-it Software Ltd, Leeds, UK). The general study design has been cleared by the local Ethical Committee (University Hospital of Verona; SOPAV-2; protocol n. 35747).

RESULTS

The final study population included 92 patients, 48 with negative swab [mean (SD), 54 (20) years; 58% women] and 44 with positive swab for SARS-CoV-2 [57 (23) years; 50% women]. No additional information on the final diagnosis could be obtained for patients testing negative for SARS-CoV-2 infection. The concentration of none of the three biomarkers appeared significantly different between patients testing positive or negative for SARS-CoV-2. Specifically, the concentration of the three biomarkers was CRP: 20 mg/L (IQR, 3-75 mg/L) *versus* 17 mg/L (IQR, 5-66 mg/L; p=0.198); IL-6: 27 pg/mL (IQR, 12-65 pg/ml) *versus* 24 pg/mL (IQR, 12-59 pg/mL; p=0.146) and PCT: 0.06 ng/mL (IQR, 0.01-0.11 ng/mL) *versus* 0.05 ng/mL (IQR, 0.01-0.11 ng/mL; p=0.279) (Figure 1). Although a significant number of patients displayed biomarker values above the relative upper reference limit (URL), such percentages for CRP (URL, 0.7 mg/L; 86% *versus* 90%; p=0.634), IL-6 (URL, 7 pg/mL; 98% *versus* 98%; p=0.950) and PCT (URL, 0.05 ng/mL; 50% *versus* 52%; p=0.842) were not found to be significantly different in patients testing positive or negative for SARS-CoV-2 infection.

A significant correlation was found between CRP and IL-6 in both negative (r=0.77; 95% CI, 0.61-0.87; p<0.001) and positive (r=0.74; 95% CI, 0.57-0.84; p<0.001) SARS-CoV-2 cases, between CRP and PCT in SARS-CoV-2 negative (r=0.38; 95% CI, 0.10-0.61;

p=0.010) and positive (r=0.44; 95% CI, 0.18-0.65; p=0.002) cases, as well as between IL-6 and PCT in SARS-Cov-2 negative (r=0.37; 95% CI, 0.08-0.60; p=0.013) and positive (r=0.40; 95% CI, 0.13-0.61; p=0.005) cases.

The analysis of the area under the curve (AUC) revealed that none of these three biomarkers could efficiently discriminate patients with negative or positive swab for SARS-CoV-2 (Figure 2). More specifically, the serum concentration of neither CRP (AUC, 0.52; 95% CI, 0.40-0.64), IL-6 (AUC, 0.51; 95% CI, 0.39-0.63), or PCT (AUC, 0.53; 95% CI, 0.41-0.65) was found to have a statistically significant accuracy for predicting swab positivity for SARS-CoV-2.

DISCUSSION

Although a pro-inflammatory state seems commonplace in patients with SARS-CoV-2 infection, especially in those with unfavorable progression (18), our evidence on the values of CRP, IL-6 and PCT in a cohort of patients acutely presenting to the ED with clinical suspicion of SARS-CoV-2 infection clearly attests that these biomarkers are not predictive enough of swab positivity to be used as a valid pre-molecular test strategy.

These results are not really surprising according to recent literature data. Sinha et al. studied 39 patients with SARS-CoV-2 (19), and compared their clinical and laboratory data with those of 39 patients with acute respiratory distress syndrome (ARDS) caused by

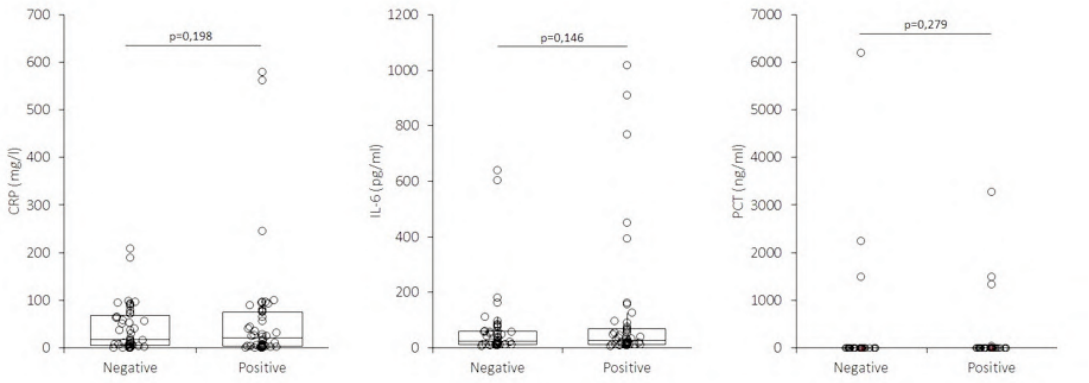

Figure 1
Serum concentration of C-Reactive Protein (CRP), interleukin-6 (IL-6) and procalcitonin (PCT) upon emergency department presentation in a cohort of patients who tested negative or positive for suspected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

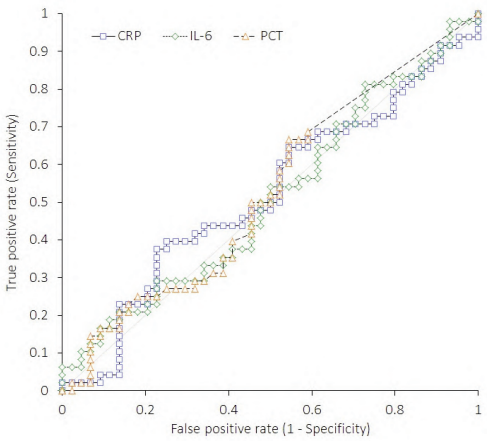

Figure 2
Diagnostic performance in receiver operating characteristics (ROC) curve analysis of C-Reactive Protein (CRP), interleukin-6 (IL-6) and procalcitonin (PCT) upon emergency department admission in a cohort of patients who tested negative or positive for suspected severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection.

diseases other than COVID-19. Notably, not only was the rate of hyperinflammatory syndrome found to be slightly lower in patients with COVID-19 ARDS than in those with respiratory failure due to other reasons (i.e., 21% *versus* 28%), but also the concentration of some pro-inflammatory biomarkers, especially IL-6 (p=0.35), was found to be similar between the two patient cohorts. In an ensuing article, Leisman et al. carried out a meta-analysis of all COVID-19 studies reporting cytokine concentrations in patients with severe/critical COVID-19 compared to those with sepsis, cytokine release syndrome or ARDS unrelated to COVID-19 (20). In keeping with our evidence, the "cytokine storm" in patients with COVID-19 was found be of lesser extent compared to that of the other conditions. In particular, the increase of several pro-inflammatory biomarkers such as CRP, IL-6 and ferritin was found to be similar or even globally lower in COVID-19 patients with severe and critical illness than in patients with sepsis, cytokine release syndrome or ARDS unrelated to COVID-19. The serum concentration of PCT was also found to be lower in patients with COVID-19 that in those with sepsis, whilst D-dimer values were found to be consistently higher. However, interferon response, seemed to be substantially suppressed in COVID-19 compared to the other diseases. This would hence confirm that COVID-19 may be characterized by a tendency of developing a viral-induced immunosuppression/anti-inflammatory cytokine response, i.e. an "immunoparalysis" (21), leading to a dysregulated, or even paradoxically inadequate, inflammatory response to SARS-CoV-2 infection, whilst direct cytopathic injury in several organs and tissues, along with the profuse endothelial damage, would place immunothrombosis at the very core of the pathogenesis of COVID-19 (1,22).

Although we could not collect information on the final diagnosis of patients who tested negative for SARS-CoV-2, which is the major limitation of this study, we can however conclude that CRP, IL-6 and PCT cannot efficiently discriminate COVID-19 from other acute diseases leading patient presentation to the ED. There is significant heterogeneity with respect to clinical indications and patient needs for presentation in various EDs, that may often be impacted by geographical variability, as well as seasonal trends (e.g., influenza), which further complicates the use of these biomarkers even in well-validated SARS-CoV-2 pre-test probability scoring systems (23). Therefore, the routine measurement of CRP, IL-6, and PCT, does not appear a useful pre-test strategy in patients presenting to the ED with clinical suspicion of COVID-19. Their measurement should be reserved for disease monitoring and prognostication, rather than for specific diagnostic purposes.

CONFLICT OF INTEREST

None.

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