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Searching for a role of procalcitonin determination in COVID-19: a study on a selected cohort of hospitalized patients

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Abstract

Objectives: Procalcitonin (PCT) has been proposed for differentiating viral vs. bacterial infections. In COVID-19, some preliminary results have shown that PCT testing could act as a predictor of bacterial co-infection and be a useful marker for assessment of disease severity.

Methods: We studied 83 COVID-19 hospitalized patients in whom PCT was specifically ordered by attending physicians. PCT results were evaluated according to the ability to accurately predict bacterial co-infections and death in comparison with other known biomarkers of infection and with major laboratory predictors of COVID-19 severity.

Results: Thirty-three (39.8%) patients suffered an in-hospital bacterial co-infection and 44 (53.0%) patients died. In predicting bacterial co-infection, PCT showed a relatively low accuracy (area under receiver-operating characteristic [ROC] curve [AUC]: 0.757; 95% confidence interval [CI]: 0.651–0.845), with a strength for detecting the outcome not significantly different from that of white blood cell count and C-reactive protein (CRP). In predicting patient death, PCT showed an AUC of 0.815 (CI: 0.714–0.892), not better than those of other more common laboratory tests, such as blood lymphocyte percentage (AUC: 0.874, p=0.19), serum lactate dehydrogenase (AUC: 0.860, p=0.47), blood neutrophil count (AUC: 0.845, p=0.59), and serum albumin (AUC: 0.839, p=0.73).

Conclusions: Procalcitonin (PCT) testing, even when appropriately ordered, did not provide a significant added value in COVID-19 patients when compared with more consolidated biomarkers of infection and poor clinical outcome. The major application of PCT in COVID-19 is its ability, associated with a negative predictive value >90%, to exclude a bacterial co-infection when a rule-out cut-off (<0.25 μg/L) is applied.

Keywords: bacterial infection; COVID-19; procalcitonin; severe acute respiratory syndrome coronavirus 2.

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Introduction

The role of serum procalcitonin (PCT) measurement in laboratory practice has been largely debated by discussing the availability of robust scientific evidence and some critical issues still affecting its clinical use [1, 2]. Among others, some evidence supports the PCT role in differentiating viral vs. bacterial infections in patients with acute respiratory symptoms [3]. PCT concentrations in serum are markedly elevated in severe pulmonary bacterial infections but found normal or slightly elevated in viral infections and non-specific inflammatory processes. The release of PCT from C cells of different tissues in response to bacterial infections is directly induced by cytokines, such as interleukins 1β and 6, and tumor necrosis factor-α (TNF-α), while infection of viral origin does not generally affect PCT values, due to virus-stimulated production of interferon-γ, which inhibits TNF-α [4, 5]. As previously described [1, 6], to warrant the cost-effectiveness of PCT test and to increase its appropriateness, in our institution the test ordering should fulfill a restricted policy. Particularly, PCT can be freely ordered only by intensive care units (ICU), whereas for all other clinical wards, the PCT request has to be preventively approved by laboratory specialists, who should be contacted by physicians to discuss about the clinical suspicion supporting the PCT request in addition to other already openly available tests (e.g., C-reactive protein [CRP]).

At the end of 2019, an outbreak of atypical pneumonia of unknown cause was detected in Wuhan, China.

The etiological agent of this disease was later identified to be a novel coronavirus, named 'severe acute respiratory syndrome coronavirus 2' (SARS-CoV-2), phylogenetically similar but distinct from other coronaviruses known to cause disease in humans, such as human severe acute respiratory syndrome and Middle East respiratory syndrome [7]. The disease caused by SARS-CoV-2, named COVID-19, can relatively frequently evolve towards the development of a severe respiratory disease, characterized by interstitial pneumonia and progressively worsening respiratory impairment requiring ventilatory assistance. These patients are also at risk of developing sepsis, septic shock and multiorgan failure [8]. PCT testing has been suggested in these patients because raised marker concentrations may reflect the establishment of a bacterial co-infection, which in turn increases disease severity and possibly drives systemic sepsis, also allowing a more targeted use of antimicrobials and promoting antibiotic stewardship even in COVID-19 [9, 10]. A preliminary meta-analysis of the available data has shown a cumulative odds ratio (OR) of 4.76 (95% confidence interval [CI], 2.7–8.3) for increased PCT for predicting severe COVID-19 [11]. A further meta-analysis of 16 studies has highlighted the association of elevated serum PCT with mortality and severe COVID-19 [12]. However, no studies so far have specifically focused on the role of PCT determination in COVID-19 patients. Here, we investigated the PCT value in a selected cohort of adult COVID-19 patients admitted to our reference hospital for infectious disease for (a) detecting patients with bacterial co-infections and (b) predicting poor outcome. Given the implemented standard gating policy mentioned above, in our setting PCT was ordered only for COVID-19 patients with high clinical suspicion of bacterial co-infection or sepsis, and this helped to increase the appropriateness and the positive predictive value (PPV) of the test. PCT diagnostic performance was compared with that of other classical biochemical and hematological blood markers of infection [CRP, white blood cell (WBC), neutrophil (NE) and immature granulocyte (IG) counts] and with established laboratory markers of COVID-19 severity [lactate dehydrogenase (LDH), serum albumin, CRP, WBC, NE, and absolute and percentage values of lymphocytes (LY)] [13–15].

Materials and methods

Subjects

We performed a retrospective, observational study on a selected cohort of adult COVID-19 patients, enrolled among those admitted between February and March 2020 to the 'Luigi Sacco' academic hospital in Milan, one of the two national reference centers for infectious diseases. Particularly, in the indicated period, our laboratory received blood samples from 427 patients with confirmed diagnosis of COVID-19, 83 (19%) of whom had at least one PCT request and were therefore included in the study. SARS-CoV-2 infection was confirmed by detection of viral RNA in nasopharyngeal swab specimens, using a real-time reverse transcription polymerase chain reaction method. The Institutional Review Board approved the study.

Methods

Patients' data were extracted from the hospital information system. In particular, we scrutinized the clinical records of recruited patients by searching for the presence of a bacterial co-infection confirmed by microbiological analyses, i.e., positivity of blood cultures and/or of cultures of lower respiratory tract specimens (bronchoalveolar lavage fluid or bronchial aspirate).

As more than one test result was often available for each patient, the highest result of the whole hospitalization period was initially considered for analysis, except for blood cell count parameters that were recruited on the same day of the worst PCT value. As the clinical course of COVID-19 could be variable, to avoid some bias due to the timing of sample collection, we also repeated the analysis of biomarkers evaluated for detecting bacterial co-infection using the initial PCT value during hospitalization and those of other markers obtained in the same day. Serum PCT was measured by the Brahms proprietary immunoassay on a Cobas e601 platform (Roche Diagnostics) applying the electrochemiluminescence technique, with a limit of detection of 0.1 μg/L [16]. The mean CV in the study period was 2.2% at a mean PCT concentration of 0.6 μg/L. According to the PRORATA trial [17], in ICU patients with lower respiratory tract infections, PCT values <0.25 μg/L indicate bacterial etiology as very unlikely, while values ≥1.0 μg/L indicate a high probability for this etiology. CRP, serum albumin, and LDH were determined on the Alinity *c* platform (Abbott Diagnostics) by using immunoturbidimetry (CRP and albumin) and enzymatic (LDH) assays [18–20]. The mean CVs in the study period were 3.1% for CRP, 1.8% for albumin, and 1.2% for LDH, at a mean analyte concentration of 12.0 mg/L (CRP), 39.6 g/L (albumin), and 395 U/L (LDH), respectively. Adult reference limits, derived from previously performed ad hoc local studies, are: CRP, up to 10 mg/L; albumin, 35–50 g/L; LDH, 125–220 U/L. The complete blood cell count was performed on a Sysmex XN-9000 hematology system [21]. Mean CVs were: 1.6% for WBC, 2.1% for NE count, 1.3% for NE percent, 3.0% for LY count, 2.5% for LY percent, 5.5% for IG count, and 4.8% for IG percent, respectively. Adult reference limits are: WBC, 4.19–9.35 10^9/L; NE, 1.91–6.23 10^9/L and 40.1–72.5%; LY, 1.13–3.37 10^9/L and 19.6–46.5%; IG, up to 0.07 10^9/L and up to 1%.

Statistical analysis

Biomarkers were evaluated according to the following outcomes: (1) presence/absence of bacterial co-infection during hospitalization, and (2) in-hospital death (non-survivors) vs. hospital discharge after clinical recovery (survivors). Data were reported as percentages for categorical variables and median with interquartile range (IQR) for quantitative variables. Differences between variables in different categories were assessed by applying chi-squared test (categorical) and Mann–Whitney rank-sum test (quantitative). To evaluate the

efficacy of the tests in predicting outcomes, the receiver operating characteristic (ROC) curve analysis was performed and areas under ROC curve (AUC) estimated and compared. Test cut-offs were selected by maximizing specificity, i.e. rule-in ability, and positive likelihood ratio (LR+) and PPV associated with these cut-offs were then derived. LR+ expresses the quotient between the probability that a value of the test overlapping the indicated cut-off is associated with the defined outcome and the probability that it does not associate with such outcome. The strength of the indication for the presence of the selected outcome provided by the positive result of the test is relevant when LR+ ≥10, modest when 5≤ LR+ <10, and poor when 2≤ LR+ <5 [22]. Reported PPV indicate the number of bacterial co-infected or deceased COVID-19 patients that a test accurately identifies out of the total number of co-infected or dead patients. Univariate logistic regression was used to estimate variables' odds ratios (OR) and their 95% confidence intervals (CI) in relation to the selected outcome. A multivariate logistic regression model was then applied to variables significant at the univariate analysis. Final selection of variables included in the multivariate model was done by applying a stepwise approach. A p-value <0.05 denoted statistical significance. All statistical analyses were carried out using MedCalc software.

Results

The median age for the 83 COVID-19 patients of the studied cohort was 64 years (IQR: 53.3–72.0), 82% being males. Out of these, 44 (53%) patients died during hospitalization, while 39 were discharged after clinical recovery. Analysis of clinical records showed that 33 (39.8%) patients had a confirmed bacterial co-infection during hospitalization, 31 (93.9%) of them being admitted to ICU. Their mortality rate (28 out of 33, 84.8%) was significantly higher (p<0.001) than in patients without a bacterial co-infection (16 deaths out of 50 patients, 32%).

Values of selected laboratory tests were significantly different between groups for both the examined outcomes (Table 1). Figure 1 displays the peak PCT values in the enrolled patients. The median PCT (IQR) was 0.8 μg/L (0.2–2.5). By applying the cut-offs derived from the PRORATA study (that was however a sepsis trial with bacterial infection patients and thus quite different from the presented COVID-19 cohort), 24 (28.9%) patients had PCT values <0.25 μg/L suggesting no bacterial co-infection, while 39 (47%) had values ≥1.0 μg/L indicating bacterial superinfection. By comparing PCT results with the clinical history, our data confirmed that a biomarker peak value <0.25 μg/L held a sensitivity of 93.4% (CI: 79.8–99.3) and high negative predictive value (91.7%; CI: 73.4–97.8) to exclude the presence of a bacterial infection. Conversely, the best PCT cut-off for ruling in a bacterial co-infection in COVID-19 should be settled much higher, at a concentration of 2.5 μg/L (Table 2). For comparison, the PRORATA rule-in cut-off (≥1.0 μg/L) gave lower specificity (72.0%; CI: 57.5–83.8) and a similar positive predictive value (64.1%; CI: 52.4–74.4). However, neither PCT nor the other evaluated tests of infection exhibited acceptable LR+ and PPV, able to predict the presence of bacterial co-infection (Table 2). The ROC curve analysis confirmed the poor ability of laboratory tests in identifying bacterial co-infection in COVID-19 patients, with AUC ranging between 0.757 for PCT to 0.631 for IG percentage (Figure 2A). Pairwise comparison of PCT AUC with other biomarker

Table 1: Laboratory findings in COVID-19 patients included in the study. The highest results for procalcitonin (PCT) and C-reactive protein of the whole hospitalization period were considered, while for blood cell count parameters data were from the same day of the highest PCT value.

	With bacterial co-infection (n=33)	No bacterial co-infection (n=50)	p-Value
	Median (IQR)	Median (IQR)	
Procalcitonin, μg/L	1.9 (1.2–6.1)	0.3 (0.2–1.4)	0.0001
CRP, mg/L	319.9 (235.8–394.8)	218.6 (142.2–303.4)	0.0019
WBC, $\times10^9$/L	11.35 (8.85–15.58)	8.02 (5.90–10.07)	0.0008
Neutrophils, $\times10^9$/L	9.93 (6.67–14.24)	6.80 (3.99–9.27)	0.001
Neutrophils, %	87.7 (81.7–91.1)	82.5 (70.9–88.6)	0.012
IG, $\times10^9$/L	0.16 (0.07–0.57)	0.08 (0.03–0.13)	0.0054
IG, %	1.2 (0.8–3.9)	0.9 (0.6–1.5)	0.044
	Non-survivors (n=44)	**Survivors (n=39)**	**p-Value**
	Median (IQR)	**Median (IQR)**	
Procalcitonin, μg/L	1.8 (0.7–7.5)	0.2 (0.1–0.8)	<0.0001
LDH, U/L	709 (588–918)	448 (362–558)	<0.0001
Albumin, g/L	18 (16–20)	24 (21–27)	<0.0001
CRP, mg/L	321.8 (227.6–389.6)	193.8 (131.2–278.3)	<0.0001
WBC, $\times10^9$/L	11.97 (8.62–17.57)	6.52 (5.43–9.12)	<0.0001
Neutrophils, $\times10^9$/L	10.74 (7.27–15.87)	4.79 (3.73–7.27)	<0.0001
Neutrophils, %	88.7 (84.6–91.6)	77.4 (68.2–83.5)	<0.0001
Lymphocytes, $\times10^9$/L	0.77 (0.46–1.09)	0.96 (0.68–1.36)	0.028
Lymphocytes, %	5.2 (3.9–9.3)	14.3 (8.7–22.2)	<0.0001

IQR, interquartile range; CRP, C-reactive protein; WBC, white blood cells; IG, immature granulocytes; LDH, lactate dehydrogenase.

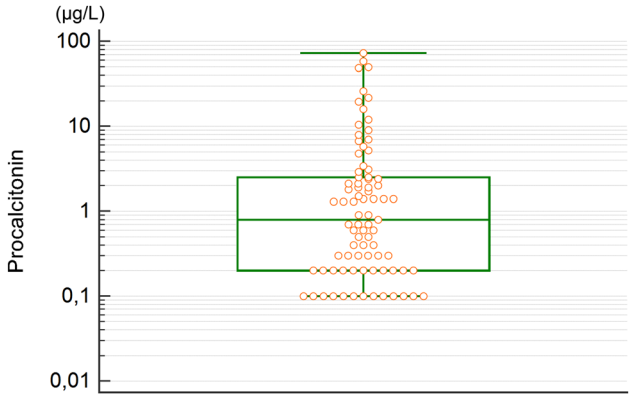

Figure 1: Box and whisker plots of serum procalcitonin peak concentrations (in µg/L) measured in the 83 studied COVID-19 patients.

AUC always showed a not statistical significance (p ≥0.10). The reanalysis using initial PCT data did not improve the performance of evaluated tests in detecting bacterial co-infection (Table 3).

In terms of predicting in-hospital death of COVID-19 patients, among the evaluated tests blood LY percentage determination reached the best accuracy, with an AUC of 0.874, an LR+ of 22.2 and a PPV of 96.2% when a cut-off ≤5.5% was employed (Table 4). Other tests providing a relevant LR+ were NE count (20.4), NE% (14.2), and serum albumin (13.3). PCT displayed an AUC of 0.815, lower but not statistically different from that of LY% (p=0.19) and other laboratory tests (always p≥0.47) (Figure 2B). The LR+ of 10.6 for >6.7 µg/L concentrations showed a moderate strength of serum PCT values for predicting the risk of in-hospital death in COVID-19 patients.

At univariate analysis, ORs for detecting bacterial co-infection during COVID-19 hospitalization were significantly higher for patients with concentrations of all evaluated tests, except PCT (Table 5). On the other hand, all the evaluated laboratory tests, except LY count, together with age and the presence of bacterial co-infection, were predictor of death (Table 5). At the multivariate analysis, WBC and CRP were still minimally associated with high OR for predicting bacterial co-infection, while age, high blood concentrations of LDH and WBC, and low concentrations of albumin remained significantly associated with high OR of death (Table 5).

Discussion

Sepsis is a common complication in COVID-19 [8]. In that context, PCT may potentially fit the profile of the biomarker that medical laboratories should offer to target high-risk patients, allowing an improvement of clinical management of COVID-19 [14, 23]. Previous papers, all evaluating Chinese populations of COVID-19 patients, gave however partial and heterogeneous information about the role of PCT. In a small cohort of nine patients with mild to moderate COVID-19 and without any demonstrated bacterial co-infection, no PCTs results >0.5 µg/L were obtained [24]. In larger but clinically heterogeneous cohorts, including COVID-19 patients covering the whole spectrum of disease severity, the number of patients with PCT results >0.5 µg/L spanned from 8 to 35% [25, 26]. Finally, in the largest cohort from China, PCT resulted >0.5 µg/L in only 5.5% of patients [7]. However, the increase was significantly associated with a severe disease (OR: 4.14, p<0.001) and with other primary composite endpoints, i.e., admission to ICU, use of mechanical ventilation, or death (OR: 7.69; p<0.001). The mentioned studies were promptly meta-analyzed by Lippi and Plebani [11] showing that increased PCT values were

Table 2: ROC curve analysis and diagnostic ability of procalcitonin (PCT) and other biomarkers of infection to predict bacterial co-infection in studied COVID-19 patients using the best cut-off maximizing the predictive positive value of tests, when the highest results for PCT and C-reactive protein of the whole hospitalization period were considered. Blood cell count parameters data were from the same day of the highest PCT value.

Test	AUC (95% CI)	Selected cut-off	Sensitivity (95% CI)	Specificity (95% CI)	LR+[a] (95% CI)	PPV (95% CI)
Procalcitonin	0.757 (0.651–0.845)	>2.5 µg/L	39.4% (22.9–57.9)	86.0% (73.3–94.2)	2.8 (1.3–6.3)	65.0% (45.4–80.7)
CRP	0.702 (0.592–0.798)	>312.5 mg/L	51.5% (33.5–69.2)	82.0% (68.6–91.4)	2.9 (1.5–5.6)	65.4% (49.0–78.8)
WBC	0.719 (0.609–0.812)	>12.85 10^9/L	42.4% (25.5–60.8)	90.0% (78.2–96.7)	4.2 (1.7–10.7)	73.7% (52.7–87.6)
Neutrophils, count	0.715 (0.606–0.809)	>10.58 10^9/L	48.5% (30.8–66.5)	88.0% (75.7–95.5)	4.0 (1.8–9.3)	72.1% (64.6–78.5)
Neutrophils, %	0.664 (0.552–0.764)	>90.9%	30.3% (15.6–48.7)	88.0% (75.7–95.5)	2.5 (1.0–6.3)	62.5% (40.2–80.6)
IG, count	0.681 (0.569–0.779)	>0.29 10^9/L	36.4% (20.4–54.9)	92.0% (80.8–97.8)	4.6 (1.6–12.9)	75.0% (51.4–89.5)
IG, %	0.631 (0.518–0.734)	>3.4%	27.3% (13.3–45.5)	94.0% (83.5–98.7)	4.6 (1.3–15.6)	75.0% (46.8–91.1)

[a]The strength of the indication for the presence of the selected outcome provided by the positive result of the test is relevant when LR+≥10, modest when 5≤LR+<10, and poor when 2≤LR+<5 [22]. AUC, area under the ROC curve; CI, confidence interval; LR+, positive likelihood ratio; PPV, positive predictive value; CRP, C-reactive protein; WBC, white blood cells; IG, immature granulocytes.

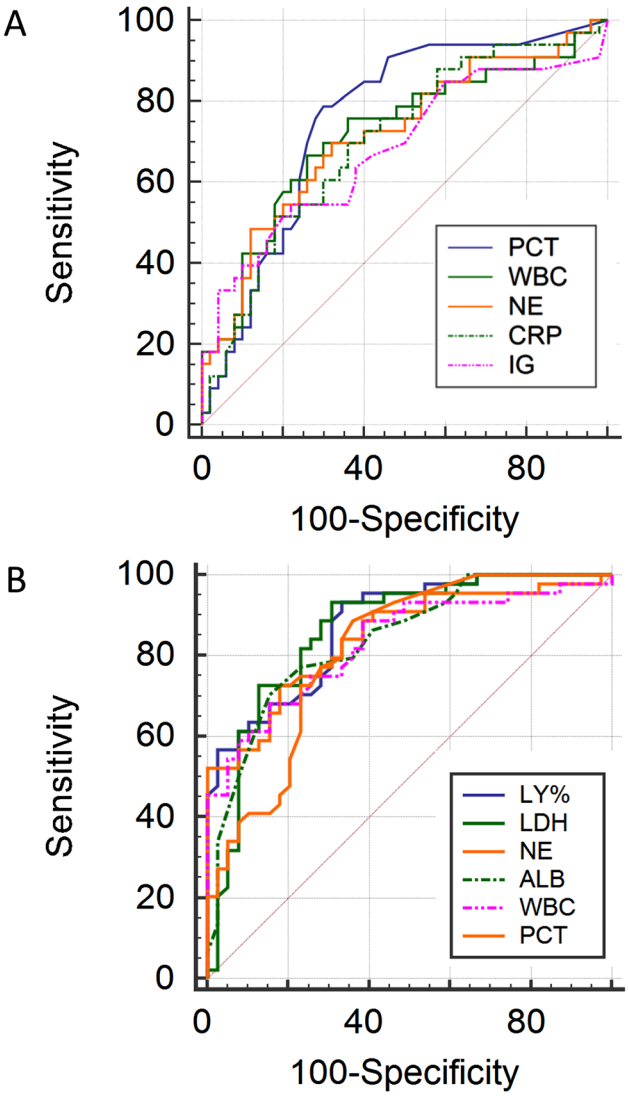

Figure 2: Receiver operating characteristic curve of evaluated markers for identification of bacterial co-infection (A) and prediction of COVID-19 fatality (B).
PCT, procalcitonin; WBC, white blood cells; NE, neutrophil count; CRP, C-reactive protein; IG, immature granulocytes; LY, lymphocytes; LDH, lactate dehydrogenase; ALB, serum albumin.

associated with a nearly 5-fold higher risk of severe COVID-19. The authors argued that substantial increase of PCT would reflect bacterial co-infection in COVID-19 patients, thus contributing to more severe forms of disease. More recently, the same authors further expanded the meta-analysis to seven studies, concerning more than 1,000 patients, substantially attenuating the overall PCT discriminative ability to detect COVID-19 severity, and highlighting the need of more categorical data [14]. This uncertain situation did not however prevent the authors to suggest that "PCT should be regularly measured to serve as a marker of secondary bacterial infection", which is frequently found in COVID-19 non-survivors [14]. In a parallel meta-analysis of 16 *Chinese* studies by an independent group, an elevated PCT value in COVID-19 was associated with an increased composite poor outcome [risk ratio (RR), 3.92 (CI: 2.4–6.4), p<0.001], comprising mortality, severe COVID-19, acute respiratory distress syndrome, and the need for ICU care [12]. Subgroup analysis showed that an elevated PCT was associated with increased mortality (5 studies; RR: 6.26 [CI: 1.8–22.4], p=0.005) and severe COVID-19 (8 studies; RR: 2.90 [CI: 1.8–4.8], p<0.001). A publication bias and small-study effects were however detected, and further studies encouraged. It must be also underlined that no information about the assays used for PCT testing in patients of the studies made in China was available. The lack of standardization of PCT measurements can make results obtained with different assays not comparable, although this is fully ignored in clinical papers, including those published on valued journals [27].

By analyzing the data from a multicentre study exploring risk factors for in-hospital death of COVID-19 patients, interesting information on bacterial co-infection and PCT can be gathered [8]. First, the PCT association with death appears to be undisputable. Second, half of patients who died experienced a secondary infection, diagnosed with the same criteria adopted in our study, i.e., a positive culture of a new pathogen from lower respiratory tract specimens or blood samples, vs. only 0.7% of survivors, making the occurrence of a co-infection itself a strong risk factor, complicating the clinical course and worsening the outcome of COVID-19 patients. Despite a direct relationship between PCT concentrations and secondary infection in COVID-19 was not demonstrated, PCT could help in early identifying these high-risk patients. Thirdly and more relevant, all non-survivors developed sepsis, but only 37% of them showed an increase of PCT values, while sepsis occurred also in 42% of survivors, with a 16% PCT positivity. Zhou et al. [8] speculated that, in some COVID-19 patients, sepsis, the most common complication in the evaluated cohort, might be directly caused by SARS-CoV-2 infection and not necessarily related to a bacterial co-infection, which is usually considered as the leading cause of this syndrome. Therefore, we cannot exclude that in a subgroup of patients with severe COVID-19, those manifesting the so-called 'cytokine storm syndrome', a sepsis caused by hyperinflammation driven by SARS-CoV-2 infection itself may occur [28]. By checking the clinical records of our cohort, we also found three patients to whom diagnosis of sepsis was made without any positive microbiological test. Two of them died, but no one displayed a positive PCT. On the other hand, although the diagnostic standard for bacterial sepsis is still considered the blood

Table 3: ROC curve analysis and diagnostic ability of procalcitonin (PCT) and other biomarkers of infection to predict bacterial co-infection in studied COVID-19 patients using the best cut-off maximizing the predictive positive value of tests, when the initial result of the whole hospitalization period for PCT was considered. C-reactive protein and blood cell count parameters data were from the same day of the initial PCT value.

Test	AUC (95% CI)	Selected cut-off	Sensitivity (95% CI)	Specificity (95% CI)	LR+[a] (95% CI)	PPV (95% CI)
Procalcitonin	0.668 (0.556–0.767)	>0.8 µg/L	60.6% (42.1–77.1)	72.0% (57.5–83.8)	2.2 (1.3–3.7)	58.8% (45.9–70.7)
CRP	0.542 (0.428–0.652)	>164.8 mg/L	36.4% (20.4–54.9)	79.6% (65.7–89.8)	1.8 (0.9–3.6)	54.5% (37.0–71.0)
WBC	0.625 (0.512–0.729)	>10.75 10^9/L	33.3% (18.0–51.8)	86.0% (73.3–94.2)	2.4 (1.0–5.5)	61.1% (40.4–78.4)
Neutrophils, count	0.630 (0.517–0.733)	>9.88 10^9/L	33.3% (18.0–51.8)	86.0% (73.3–94.2)	2.4 (1.0–5.5)	61.1% (40.4–78.4)
Neutrophils, %	0.650 (0.537–0.751)	>91.4%	18.2% (7.0–35.5)	90.0% (78.2–96.7)	1.8 (0.6–5.5)	54.5% (28.5–78.3)
IG, count	0.544 (0.431–0.654)	>0.29 10^9/L	18.2% (7.0–35.5)	96.0% (86.3–99.5)	4.6 (1.0–21.2)	75.0% (39.2–93.3)
IG, %	0.513 (0.401–0.624)	NA	NA	NA	NA	NA

[a]The strength of the indication for the presence of the selected outcome provided by the positive result of the test is relevant when LR+≥10, modest when 5≤LR+<10, and poor when 2≤LR+<5 [22]. AUC, area under the ROC curve; CI, confidence interval; LR+, positive likelihood ratio; PPV, positive predictive value; CRP, C-reactive protein; WBC, white blood cells; IG, immature granulocytes; NA, not applicable because AUC not significantly different from 0.5.

culture, it is well known that it may be inconclusive in up to 40% of cases [29].

Our study possesses specific characteristics that should be highlighted. First, PCT was requested in less than 20% of COVID-19 patients admitted to our hospital during the study period. Given the gating policy implemented from many years in our academic hospital [1, 6, 16], all PCT requests in our COVID-19 patients were dictated by a strong suspicion of bacterial co-infection or sepsis. This makes the patient cohort enrolled for the study very selected and clinically characterized. Nevertheless, our data suggest that the major application of PCT determination in COVID-19, if any, is its ability, associated with a negative predictive value >90%, to exclude the presence of a bacterial co-infection when a rule-out cut-off (<0.25 µg/L) is applied. Conversely, none of the evaluated tests of infection, including PCT, exhibited a good accuracy to predict the presence of bacterial co-infection in COVID-19. The AUCs <0.76 and the poor LR+ and PPV (always <5 and ≤75%, respectively) do not permit to support the confident use of any laboratory biomarker for ruling in bacterial co-infection in COVID-19 even in very selected individuals. The use of high cut-offs for every test did not seem to change this conclusion.

Table 4: ROC curve analysis and diagnostic ability of procalcitonin (PCT) and other evaluated tests to predict in-hospital death in studied COVID-19 patients using the best cut-off maximizing the predictive positive value of the tests. The highest results for PCT and C-reactive protein of the whole hospitalization period were considered, while for blood cell count parameters data were from the same day of the highest PCT value.

Test	AUC (95% CI)	Selected cut-off	Sensitivity (95% CI)	Specificity (95% CI)	LR+[a] (95% CI)	PPV (95% CI)
Procalcitonin	0.815 (0.714–0.892)	>6.7 µg/L	27.3% (15.0–42.8)	97.4% (86.5–99.9)	10.6 (1.4–78.1)	92.3% (62.0–98.9)
LDH	0.860 (0.767–0.927)	>660 U/L	61.4% (45.5–75.6)	92.3% (79.1–98.4)	8.0 (2.6–24.3)	90.0% (74.7–96.5)
Albumin	0.839 (0.742–0.911)	≤16 g/L	34.1% (20.5–49.9)	97.4% (86.5–99.9)	13.3 (1.8–96.1)	93.7% (67.5–99.1)
CRP	0.766 (0.660–0.852)	>335.5 mg/L	43.2% (28.3–59.0)	94.9% (82.7–99.4)	8.4 (2.1–33.9)	90.5% (70.2–97.4)
WBC	0.832 (0.734–0.905)	>11.28 × 10^9/L	54.6% (38.8–69.6)	94.9% (82.7–99.4)	10.6 (2.7–42.1)	92.3% (75.2–97.9)
Neutrophils, count	0.845 (0.749–0.915)	>10.17 × 10^9/L	52.3% (36.7–67.5)	97.4% (86.5–99.9)	20.4 (2.9–144.0)	95.8% (76.5–99.4)
Neutrophils, %	0.815 (0.714–0.892)	>90.8%	36.4% (22.4–52.2)	97.4% (86.5–99.9)	14.2 (2.0–102.1)	94.1% (69.0–99.1)
Lymphocytes, count	0.641 (0.528–0.743)	≤0.39 × 10^9/L	20.5% (9.8–35.3)	97.4% (86.5–99.9)	8.0 (1.1–60.2)	90.0% (54.4–98.5)
Lymphocytes, %	0.874 (0.783–0.937)	≤5.5%	56.8% (41.0–71.7)	97.4% (86.5–99.9)	22.2 (3.1–156.0)	96.2% (78.0–99.4)

[a]The strength of the indication for the presence of the selected outcome provided by the positive result of the test is relevant when LR+≥10, modest when 5≤LR+<10, and poor when 2≤LR+<5 [22]. AUC, area under the ROC curve; CI, confidence interval; LR+, positive likelihood ratio; PPV, positive predictive value; LDH, lactate dehydrogenase; CRP, C-reactive protein; WBC, white blood cells.

Table 5: Univariate and multivariate logistic regression analyses for predictors of bacterial co-infection and death during hospitalization of COVID-19 patients.

	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	p-Value	Odds ratio (95% CI)	p-Value
Biomarkers as predictor of bacterial co-infection				
Procalcitonin	1.04 (0.99–1.08)	0.09	–	–
C-reactive protein	1.01 (1.00–1.01)	0.003	1.005 (1.000–1.010)	0.041
WBC	1.13 (1.04–1.23)	0.004	1.099 (1.011–1.195)	0.027
Neutrophils, %[a]	1.07 (1.01–1.12)	0.016	–	–
IG, %[a]	1.37 (1.04–1.79)	0.024	–	–
Biomarkers as predictor of death				
Age	1.07 (1.03–1.11)	0.0006	1.115 (1.023–1.215)	0.013
Bacterial co-infection	11.9 (3.9–36.5)	<0.0001	–	–
Procalcitonin	1.32 (1.04–1.68)	0.022	–	–
LDH	1.01 (1.00–1.01)	<0.0001	1.009 (1.003–1.014)	0.001
Albumin	0.71 (0.61–0.82)	<0.0001	0.813 (0.664–0.995)	0.044
C-reactive protein	1.01 (1.01–1.02)	0.0001	–	–
WBC	1.43 (1.19–1.71)	0.0001	1.345 (1.041–1.738)	0.023
Neutrophils, %[a]	1.14 (1.07–1.22)	0.0001	–	–
Lymphocytes, %[a]	0.76 (0.67–0.86)	<0.0001	–	–

CI, confidence interval; WBC, white blood cells; IG, immature granulocytes. [a] The additional inclusion of the absolute values of these parameters did not add significance to the analysis.

In our selected cohort of patients, PCT demonstrated a relatively good ability in predicting the worst outcome (AUC: 0.815), which, however, was not better than those of other more common laboratory tests, such as blood lymphocyte percentage and neutrophil count, or serum LDH and albumin concentrations, already previously validated by our and other groups [15, 19, 30, 31]. Interestingly, the PCT AUC value reported above is not far from those previously reported both in primary studies and in meta-analyses. Liu et al. found an AUC of 0.812 for PCT in detecting clinical severity in a retrospective cohort of COVID-19 patients, concluding that the validity of PCT testing needed to be further investigated [32]. Huang et al. [12] in their meta-analysis showed a summary AUC of 0.88 (CI: 0.84–0.90) when PCT was associated to a poor outcome. The same authors however highlighted the limitations of their work, which included a number of studies published on preprints servers and that were not yet peer-reviewed, requiring caution in interpreting results. As for other scientific papers related to the COVID-19, even some reports on PCT clinical utility in this disease are oddly affected by the approach of addressing conclusions that overcome the study experimental findings, presenting positive remarks largely beyond the evidence of the data.

In conclusion, despite the early emphasis on the potential role for PCT in predicting bacterial co-infection or sepsis in COVID-19, our data suggest that PCT cannot be recommended for this use. Even when appropriately ordered, PCT testing did not provide a significant added value when compared with more common markers of infection. The possible application of PCT in COVID-19 lies in its ability to exclude a bacterial co-infection when a cut-off for ruling out is applied. However, this needs further validation in *ad hoc* structured studies.

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