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Letter to the Editor

Serum calprotectin is not an independent predictor of severe COVID-19 in ambulatory adult patients

Dear editor,

Whilst COVID-19 represents a self-limiting disease in most patients infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), a small proportion of individuals may develop life threatening respiratory and multi-organ failure associated with prolonged hospital admission and intensive care stay[1].

In the United Kingdom (UK), same-day emergency care (SDEC) units operating an ambulatory-care-by-default model review a high volume of patients at risk of developing severe disease where rapid decisions must be made regarding treatment and the setting of care delivery, predominantly based on prediction of risk of deterioration[1]. To support attending clinicians, models incorporating age, comorbidities, clinical metrics and blood test results (particularly C-reactive protein) have been established that estimate the risk of adverse clinical outcomes, encompassing the need for intensive care unit (ICU) admission, non-invasive ventilation (NIV), or death[2,3].

Calprotectin is a cytosolic protein with pro-inflammatory signalling properties released by activated neutrophils. Both an elevated circulating calprotectin concentration and excessive neutrophil degranulation have been mechanistically associated with severe COVID-19 disease[4,5]. Recent studies, including two published in this journal, have subsequently proposed serum calprotectin as an early sensitive biomarker for predicting deterioration from COVID-19 in Emergency Departments (ED)[6–8]. Whether the utility of serum calprotectin extends to adult patients attending SDEC, and whether its addition to existing deterioration models improves prediction of deterioration is unknown.

To determine this, from February to April 2021 we prospectively asked SDEC clinicians in Oxford University NHS Foundation Trust, UK, to order a pre-specified panel of biochemical blood tests through an electronic 'Careset' (EPR, Cerner Millenium) for all patients suspected of having COVID-19 based on symptomology or epidemiology. Selection of the Careset triggered automatic analysis of serum calprotectin using a particle enhanced turbidimetric assay (Gentian AS, Moss, Norway). Clinicians were blinded to calprotectin results, and laboratory staff blinded to COVID-19 status.

We subsequently employed the Infections in Oxfordshire Research Database (IORD) to identify individuals and hospital episodes with serum calprotectin available on the Laboratory Information Management System, then extracted demographic, clinical, biochemical and outcome information stored on the electronic healthcare records in a structured and anonymised way for downstream analysis. IORD has Research Ethics Committee and Confidentiality Advisory Group approval (19/SC/0403, 19/CAG/0144) for use as a de-identified electronic research database[9]. COVID-19 diagnosis was retrospectively defined as a composite of select internationally standardised diagnostic codes (using ICD10 codes Z208, Z861, J128, M358, Z115 and/or a positive PCR test for SARS-CoV2 during the patient encounter. We determined that a patient had deteriorated if they required application of NIV, were admitted onto ICU or died within four weeks of admission. Any remaining patients were censored at that point. All data analyses were undertaken in R (version 4.0.2).

Out of 3280 patients reviewed in acute ambulatory care over the study period, 771 (23.5%) were suspected of having COVID-19, with serum calprotectin levels available within 48 h of presentation to hospital (Table 1). Of the 771, 222 (28.8%; 6.8% of all those reviewed) had a final diagnosis of COVID-19 and 25 (11.3%) of these patients reached the composite endpoint (18 died, 6 commenced on NIV and 2 admitted onto ICU, where one individual died after commencing on NIV).

Univariate associations with risk of deterioration were observed for several variables including Charlson comorbidity index ($P = 8.1 \times 10^{-5}$), oxygen saturation ($P = 7.6 \times 10^{-3}$), and C-reactive protein ($P = 4.5 \times 10^{-5}$), but not neutrophil count or calprotectin (Table 1). Calprotectin had a univariate area under the receiver operator characteristic curve (AUROC) estimate of 0.61 (95% confidence interval 0.48–0.74) compared to 0.76 (0.67–0.84) for CRP in patients with COVID-19 (Fig. 1A) with a similar pattern observed for all 771 individuals tested (AUROC 0.62 and 0.79 respectively). Use of CRP had a statistically greater capability of discriminating risk of deterioration compared to calprotectin in both analyses ($P = 0.01$).

Testing correlation between measured biomarkers in the full dataset of 771 individuals, the highest correlation was observed between neutrophil count and calprotectin (rho=0.61) with a similar estimate observed between calprotectin and CRP (rho=0.58; Fig. 1B). In multivariate models, when used alongside CRP with age and sex, calprotectin led to only a marginal improvement in predicative capability. There was no incremental benefit when using all available data as part of a gold standard predictive model (Supplementary Table 1).

We conclude that in this large cohort attending SDEC during a surge of COVID-19 in the UK, whilst serum calprotectin was found to be a potential predictor of deterioration, there was little evidence that it offered benefit over and above existing biomarkers including peripheral cell counts or CRP.

Other studies investigating calprotectin as a potential predictor of adverse outcome include a German ED study that enroled 66 individuals with suspected COVID-19 where calprotectin was found to have a AUROC of 0.87, compared to 0.70 for CRP, for predicting multi-organ failure within 72 h[6]. Another Spanish ED study found estimates of 0.80 and 0.79 respectively for predicting death during admission[7]. Thus, these reported estimates for CRP are similar to ours whereas our estimate for calprotectin is significantly lower. This discrepancy could be due to the timing of sampling in

Table 1

Characteristics of patients with calprotectin levels available at presentation to ambulatory care during the months of February to April 2021. Univariate *P*-values were calculated using the Mann-Whitney-U test for continuous variables and Fisher's Exact Test for nominal variables.

| Characteristic	All(n = 771)	COVID-19 diagnosis(n = 222)	COVID-19 with no outcome(n = 197)	COVID-19 with outcome(n = 25)	Evidence of difference between COVID-19 groups (P)
Demographics					
Age at presentation in years	64 (45–78)	69 (48–81)	68 (47–80)	77 (58–89)	*
Female sex	422 (55)	111 (50)	101 (51)	10 (40)	NS
BMI	27 (24–32)	27 (24–32)	28 (24–32)	25 (23–31)	NS
Charlson comorbidity index	3 (0–10)	3 (0–10)	3 (0–8)	13 (4–19)	***
Index of multiple deprivation	11 (7–17)	11 (7–17)	11 (7–17)	8 (6–15)	NS
Clinical measures at presentation					
Supplemental oxygen	33 (4)	18 (8)	15 (8.0)	3 (12.0)	NS
Respiratory rate, breaths/min	18 (18–19)	18 (17–19)	18 (17–19)	18 (18–19)	NS
Oxygen saturation,%	97 (95–98)	97 (95–98)	97 (95–98)	95 (93–96)	***
Glasgow coma scale	15 (15–15)	15 (15–15)	15 (15–15)	15 (14–15)	*
Outcomes					
Death	47 (6)	18 (8)	–	18 (72)	NA
Requiring NIV	6 (1)	6 (3)	–	6 (24)	NA
ICU Admission	2 (1)	2 (1)	–	2 (8)	NA
Composite outcome	54 (7)	25 (11)	–	25 (100)	NA
Biomarkers					
Neutrophil count, x10×9/L	5.3 (3.9–7.5)	5.8 (4.4–8.5)	5.7 (4.4–8.4)	6.9 (4.9–9.6)	NS
Lymphocytes count, x10×9/L	1.5 (1.0–1.6)	1.4 (0.9–2.0)	1.4 (1.0–1.9)	1.0 (0.7–1.5)	*
Urea, mmol/L	5.1 (3.9–7.1)	5.7 (4.2–7.7)	5.5 (4.2–7.2)	7.7 (5.2–13.9)	**
C-reactive protein, mg/L	6.0 (1.6–28.7)	12.4 (3.5–72.8)	10.4 (3.0–64.1)	70.5 (38.8–165.6)	***
Calprotectin, mg/L	1.9 (1.1–3.3)	2.2 (1.3–4.1)	2.1 (1.3–3.8)	3.7 (1.7–5.7)	NS

Continuous variables are represented with median and IQR, nominal variables with frequency and column percentage (of valid cases).
NS: non-significant,.
* *P*<0.05.
** *P*<0.01.
*** *P*<0.001; NA: not applicable.
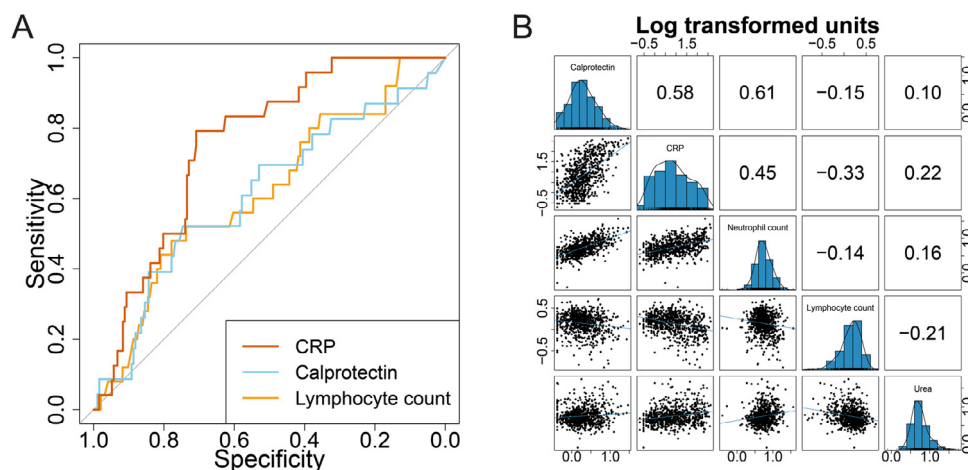
Fig. 1. Biomarkers to predict the risk of deterioration of patients presenting to ambulatory care with COVID-19. A) The receiver operating curve (ROC) distributions for three biomarkers associated with deterioration measured in 222 individuals with confirmed COVID-19. ROC analyses were undertaken using the pROC package and the comparison of the paired ROC statistics was calculated using DeLong's test[10]. B) The Spearman rank rho correlation between the $\log_{10}$-transformed distributions of the predominant biomarkers associated with disease severity and deterioration in COVID-19 studies to date as measured in 771 individuals presenting to ambulatory care with symptoms consistent with COVID-19.

relationship to clinical deterioration[4]. Equally, as neither calprotectin nor neutrophil count were associated with risk of deterioration in our SDEC cohort there is the possibility that calprotectin may be more helpful in patients presenting more unwell to ED.

Although we were unable to collect some important variables such as duration of symptoms and standardised radiology results owing to the priority of anonymization, our double-blind prospective design using standardised clinical data available through electronic healthcare records with multivariable analyses reduces the risk of selection bias and offers an effective, pragmatic opportunity for the rapid assessment of other such biomarkers in the future.

Our findings do not support the widespread uptake of serum calprotectin use in ambulatory adult patients to predict COVID-19 deterioration, but does support the ongoing use of biomarkers such as CRP alongside standard clinical assessment in this setting as well as for predicting deterioration from other respiratory disease in SDEC.

Supporting information

Supplementary Table 1

Declaration of Competing Interest

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