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## A novel near-patient host-protein score predicts COVID-19 deterioration at presentation to secondary care

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# **A novel near-patient host-protein score predicts COVID-19 deterioration at presentation to secondary care**

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## **A novel near-patient host-protein score predicts COVID-19 deterioration at presentation to secondary care**

COVID-19 remains a significant driver of healthcare utilisation. Despite widespread immunity from natural infection and vaccination, severe disease and adverse outcomes are still observed, albeit at a reduced frequency. Identifying individuals 'at risk' of deterioration at the point of presentation is challenging, however vital to inform decision making around the setting, intensity and escalation of care.

The COVID-19 Severity™ assay (C19-SA) (MeMed, Israel) is a novel near-patient in vitro diagnostic designed for integration into acute care pathways. Using 0.1mL serum the assay quantifies the concentration of three host-released immune proteins (tumour necrosis factor-related apoptosis inducing-ligand, TRAIL; C-reactive protein, CRP; and interferon gamma-induced protein 10, IP-10) and integrates them via a proprietary algorithm (1,2) in 15 minutes. A numerical score from 0-100 is released to the clinician: 0 representing a low likelihood and 100 a high likelihood of severe outcome.

To date, the C19-SA score has been evaluated in only one unpublished study of 394 patients with COVID-19 (3). Conducted in Germany, Israel and the USA during the first (wild-type) wave of SARS-CoV-2, the score was predictive of severe disease (area under receiver operator curve, AUROC 0.86, 95%CI 0.81-0.91), displaying superior performance to IL-6 (AUROC 0.77) and CRP (AUROC 0.78) alone. The utility of the assay in the context of modern variants and a population with established immunity is unknown.

Using a similar approach to our prior analysis of serum calprotectin (4), we sought to determine the ability of the C19-SA to independently predict a future adverse clinical outcome defined as requirement of non-invasive ventilation (NIV), admission to an intensive care unit (ICU), or death within 4 weeks of presentation to secondary care in patients diagnosed with COVID-19, including assessment of the value of individual components of the composite score (TRAIL, IP-10, CRP).

Between 10<sup>th</sup> January to 3<sup>rd</sup> March 2022, we collected remnant serum from the initial blood draw of all patients presenting to the John Radcliffe Hospital, Oxford University NHS Foundation Trust, via the emergency department (ED), ambulatory assessment unit (AAU) or emergency assessment unit (EAU). Samples were then retrospectively analysed on the C19-SA if a positive SARS-CoV-2 PCR was obtained and their presentation was consistent with acute, symptomatic COVID-19 infection (via independent clinical review, see Supplementary Figure 1). Clinicians were blinded to the C19-SA results as well as the individual biomarker concentrations. The Infections in Oxford Research Database (IORD) (Research Ethics Committee and Confidentiality Group Approval: 19/SC/0403,

19/CAG/0144) was used to retrospectively identify individuals and hospital episodes with a C19-SA score to extract relevant demographic, clinical, biochemical and outcome information in an anonymised manner. All analyses were performed in R (version 4.0.2).

In total 52 patients were included. Of these, 6 individuals reached the composite endpoint (3 required NIV, 2 died and 1 individual was admitted onto ICU and subsequently died).

Univariate associations with risk of deterioration were observed for a limited number of variables (Table 1). The median value of C19-SA score at presentation in those who deteriorated was 54.5 (IQR 36.8-73), significantly higher than in those who did not (23, IQR 8-42). Oxygen saturation and IP-10 at time of presentation, but not TRAIL, CRP, Glasgow Coma Scale, Body Mass Index, or Charlson comorbidity index were associated with risk of deterioration. The C19-SA score had an AUROC estimate of 0.80 (95%CI 0.63-0.96) compared to 0.71 (0.46-0.96) for CRP (Figure 1A). Correlation between the C19-SA score and commonly used biomarkers of disease severity (CRP  $\rho=0.41$ ; lymphocyte count  $\rho=-0.08$ ) was low (Figure 1B). TRAIL exhibited the strongest correlation with the overall C19-SA score ( $\rho=-0.92$ ).

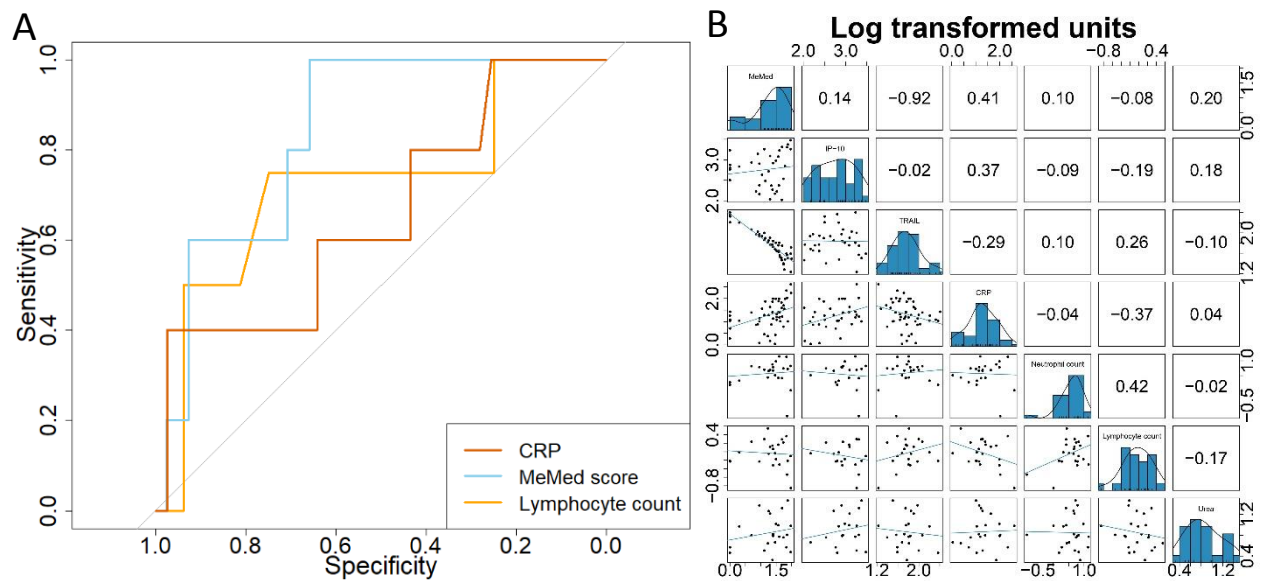


Figure 1: Biomarkers to predict the risk of deterioration of patients presenting to ambulatory care and A&E with COVID-19. A) The receiver operating curve (ROC) distributions for two biomarkers and the MeMed COVID-19 Severity™ assay measured in 52 patients with confirmed COVID-19. ROC analyses were undertaken using the pROC package and the comparison of the paired ROC statistics was calculated using DeLong's test (5). B) The Spearman rank rho correlation between the log<sub>10</sub>-transformed distributions of the predominant biomarkers associated with deterioration in COVID-19, measured in 52 patients with confirmed COVID-19.

Table 1: Characteristics of patients with a MeMed COVID-19 Severity™ assay score available at presentation to AAU, EAU or ED during January to March 2022. Univariate P-values were calculated using the Mann-Whitney-U test for continuous variables and Fisher's Exact Test for nominal variables.

Characteristic	COVID-19 diagnosis (n=52)	COVID-19 with no outcome (n=46)	COVID-19 with outcome (n=6)	Evidence of difference between outcome groups (P)
<b>Demographics</b>				
Age at presentation in years	66 (50-80)	64 (48-80)	68 (64-85)	NS
Female sex	23 (44)	19 (41)	4 (67)	NS
BMI	28 (24-32)	28 (24-31)	35 (26-36)	NS
Charlson comorbidity index	3 (0-13)	0 (0-12)	6 (4-15)	NS
Index of multiple deprivation	16 (8-21)	16 (8-22)	17 (15-18)	NS
<b>Clinical measures at presentation</b>				
Supplemental oxygen number	14 (27)	11 (24)	3 (50)	NS
Respiratory rate	19 (18-22)	19 (18-21)	21 (18-24)	NS
Oxygen saturation	97 (96-99)	98 (96-99)	95 (93-95)	**
Glasgow coma scale	15 (15-15)	15 (15-15)	15 (15-15)	NS
<b>Outcomes</b>				
Death	3 (6)	-	3 (50)	NA
Requiring NIV	3 (6)	-	3 (50)	NA
ICU Admission	1 (2)	-	1 (17)	NA
<b>Biomarkers</b>				
Neutrophil count (x10 <sup>9</sup> /L)	4.9 (2.6-6.4)	4.8 (2.6-6.4)	4.0 (2.0-7.5)	NS
Lymphocytes count (x10 <sup>9</sup> /L)	0.9 (0.6-1.6)	1.1 (0.6-1.7)	0.6 (0.4-0.8)	NS
Urea (mmol/L)	6.3 (4.1-10.1)	6.0 (3.7-10.2)	6.4 (6.1-9.8)	NS
C-reactive protein (mg/L)	18.7 (10.3-48.4)	17.4 (7.8-40.0)	61.7 (17.9-102.9)	NS
MeMed score, (units)	26.0 (9.5-46.3)	23.0 (8.0-42.0)	54.5 (36.8-73.0)	*

IP-10, (pg/ml)	656.5 (235.0-1273.8)	522.0 (235.0-908.0)	1696.0 (1199.0-2028.0)	*
TRAIL, (mg/L)	57.0 (39.0-84.5)	58.0 (39.0-86.5)	54.5 (40.8-62.3)	NS

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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.

NA: not applicable.

In multivariate analysis, the addition of the C19-SA score to a basic risk of deterioration model (age+sex) led to an incremental increase in predictive value, however this could be replicated via substitution with the lymphocyte count (Supplementary Table 1). Employing the C19-SA score in the full risk model, as opposed to CRP alone or the lymphocyte count, again afforded only limited improvement (C-statistic 0.96 (95% confidence interval 0.96-1) vs 0.91 [0.89-1] and 0.92 [0.88-1] respectively).

We conclude that in this cohort of patients presenting to a tertiary hospital during the Omicron surge of COVID-19 in the UK, the MeMed C19-SA score alone could predict deterioration, as defined by a composite endpoint. In multivariate risk models it however demonstrated limited additive predictive value over traditional and routinely available biomarkers including CRP and the lymphocyte count.

Our findings agree with a previous evaluation of the C19-SA (3), although suggest a lower overall predictive ability (AUROC 0.8 vs 0.86). This may reflect our smaller sample size, more diverse cohort including those not admitted to hospital, or the reduced frequency in adverse outcomes with modern variants and prevalent immunity. The data is also in accordance with studies investigating the C19-SA's component biomarkers. Elevated IP-10 has been reported in patients with COVID-19 and is associated with ICU admission and mortality, whereas TRAIL exhibits an inverse correlation between length of hospital and ICU stay (6, 7). Alternative studies have demonstrated the predictive capability of IP-10 and TRAIL at day 3 of hospitalisation, however not on presentation (8). Whilst we observed significant elevation in IP-10 in patients experiencing an adverse outcome (1696 pg/mL vs 522 pg/mL) but no difference in TRAIL (54.4 mg/L vs 58 mg/L), the high correlation between TRAIL and the overall C19-SA suggests a meaningful contribution.

Given its size, our study is at risk of type II error and further work evaluating the C19-SA is required to clarify its place and value in different care settings. Whilst the double-blind design minimises risk of selection bias and has previously demonstrated the ability to observe statistically significant associations between biomarkers and clinical outcomes (4), serum may not have been available from all eligible patients (no sample, insufficient volume). We believe this was a random effect and does not introduce systematic bias. Finally, it should be noted that the data relates to patients infected with currently circulating variants, includes those attending lower acuity services (e.g. same day emergency care) and patients not requiring hospitalisation.



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**Declaration of Competing Interest.** This was an investigator-led study and MeMed had no control over the study design, data analysis or interpretation. MeMed reviewed the study design, result and interpretation and made no changes prior to submission. Reagents for MeMed COVID-19 Severity™ measures were provided by MeMed, no financial support was received aside from this. All authors declare no conflicts of interest.

## Supplementary Figure 1

Positive COVID-19 PCR from ED / EAU / AAU

**Inclusion**

**Exclusion**

Primary team suspects COVID-19 infection within differential AND symptoms consistent with COVID-19 infection\* in last 14 days

OR

Symptom onset <14 days and consistent with COVID-19 infection\* AND the absence of a clear alternative diagnosis precipitating admission

Age of patient at admission <18 years

OR

Known pregnancy (self-reported or tested positive)

OR

Hospitalised due to reasons other than symptomatic SARS-CoV-2 infection

OR

Currently intubated with mechanical ventilation

OR

Under current renal replacement therapy

\*Symptoms:

- Fever
- Cough
- Dyspnoea
- Sore throat
- Runny nose
- Sneezing
- Malaise
- Headache
- Myalgia
- Nausea & vomiting
- Diarrhoea
- Anosmia
- Ageusia

**Supplementary Table 1: Multivariate analysis**

<b>Model</b>	<b>C-statistic (95% CI)</b>
<b><i>Incremental full model</i></b>	
Age	0.64 (0.47-0.89)
Age+sex	0.70 (0.53-0.95)
Age+sex+Charlson	0.70 (0.56-0.95)
Age+sex+Charlson+supplemental oxygen	0.76 (0.66-0.99)
Age+sex+Charlson+supplemental oxygen+oxygen saturation	0.85 (0.73-1.00)
Age+sex+Charlson+supplemental oxygen+oxygen saturation+lymphocyte count	0.92 (0.88-1.00)
Age+sex+Charlson+supplemental oxygen+oxygen saturation+lymphocyte count+CRP	0.91 (0.89-1.00)
Age+sex+Charlson+supplemental oxygen+oxygen saturation+lymphocyte count+MeMed	0.96 (0.96-1.00)
<b><i>Biomarker specific – CRP</i></b>	
Age+sex+CRP	0.72 (0.58-1.00)
Age+sex+MeMed	0.79 (0.64-0.99)
Age+sex+CRP+MeMed	0.84 (0.73-1.00)
<b><i>Biomarker specific – lymphocyte count</i></b>	
Age+sex+lymphocyte count	0.81 (0.64-1.00)
Age+sex+MeMed	0.79 (0.64-0.99)
Age+sex+lymphocyte count+MeMed	0.82 (0.69-1.00)

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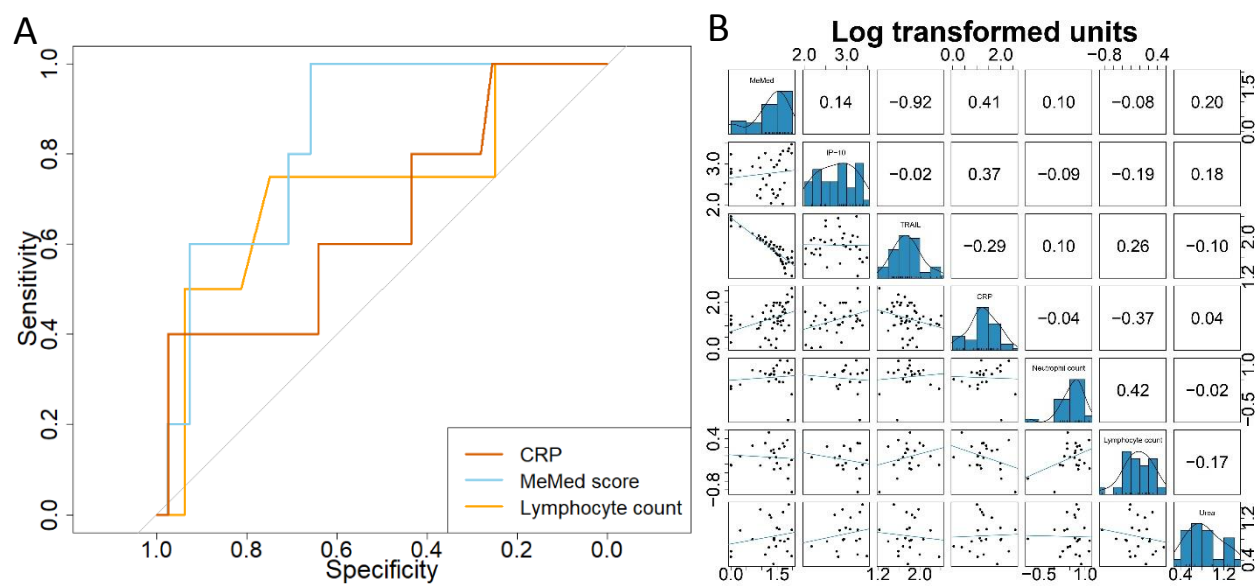


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**Supplementary file**

Supplementary Figure 1.docx



