
Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

visibly damaged mask materials or elastic straps. Remaining masks were disinfected with immersion in 35% VHP using a Bioquell® BQ-50 machine (Bioquell UK Ltd, Andover, UK). Control biological indicator (BI) release of 6-log *Geobacillus stearothermophilus* spores was performed with every load of masks disinfected and incubated for 7 days. Processed masks were held in quarantine until final culture and BI results were obtained. No mask loads demonstrated any pathogen or BI growth. These standard tests only verified the sterility of a mask and did not verify that the masks would protect a healthcare worker from a viral challenge, such as SARS-CoV-2.

We tested the widely available 3M model 1860 N95 FFR. Our protocol was modelled after testing used for certification of all new N95 mask designs, but built on certification protocols by using the more robust quantitative NIOSH fit testing in accordance with 29 Code of Federal Regulations 1910.134, utilising a TSI PortaCount® Pro (TSI Incorporated, 500 Cardigan Road, Shoreview, Minnesota, 55126, USA) with an 8026 aerosol generator, to produce 40–70 nm NaCl aerosol challenge particles (approximately the size of SARS-CoV-2). The independent fit testing was performed using a single-volunteer repeated-measures design to remove the variability of fit attributable to various head sizes and shapes. We evaluated 30 masks: one cohort of 10 randomly selected new masks and two cohorts of 10 randomly selected masks taken from our mask reprocessing programme, where each cohort was subjected to either five or 10 repeated cycles of disinfection using immersion in VHP. Figure 1 shows the means and 95% confidence interval of the mask cohorts on the overall fit factor and each activity, as analysed using R Core Team (2019). All masks (both new and recycled) passed testing with the raw data and pictures of fit testing supplied in Supplementary Table 1 and Supplementary Figure 1.

These results are the first to demonstrate that repeated vaporised hydrogen peroxide processing does not degrade the fit and function of this N95 mask. Based on these results, our institution has reprocessed and stockpiled approximately 200 000 3M 1860 N95 FFRs. Further analysis and testing with repeated wear cycles and on various other types of masks from other manufacturers are ongoing.

## Declarations of interest

The authors declare that they have no conflicts of interest.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2020.12.021.

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# Association between inflammation, angiopoietins, and disease severity in critically ill COVID-19 patients: a prospective study

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Editor—Current evidence on coronavirus disease 2019 (COVID-19) shows that the most severely affected patients tend to have higher cytokine concentrations. The so-called cytokine storm was proposed as a rationale to test therapies based on cytokine antagonists and more specifically to interleukin-6 (IL-6). However, further investigations revealed that cytokine levels in COVID-19 are not higher than in other virus-related infections.[1] In addition, some data support a potential role for endothelial injury in the pulmonary vascular shunts, extra-pulmonary manifestations, and thrombosis pathogenesis.[2] We analysed inflammation and endothelial injury biomarkers with regard to disease severity of critically ill patients with COVID-19 to investigate the potential mechanism of disease progression.

We conducted a single-centre prospective study at Amiens Hospital University (Amiens, France) as an ancillary study of a prospective database of critically ill patients with COVID-19 (registered on ClinicalTrials.gov NCT04354558 and Commission Nationale de l'Informatique et des Libertés number PI2020_843_0026). The population study comprised adult patients admitted to our ICU with a confirmed diagnosis of COVID-19 (RT–PCR diagnosed from nasopharyngeal swab). Severity was defined according to the WHO case definition.[3] The severe group included patients with respiratory distress syndrome (rate ≥30 bpm) or oxygen saturation ≤93% at rest or ratio of arterial partial pressure of oxygen to fractional concentration of oxygen in inspired air <300 mm Hg (X kPa), or >50% lesion progression over 24–48 h by pulmonary imaging. The critical group included patients with respiratory failure and requiring mechanical ventilation, or with shock or organ failure that requires ICU care.

Peripheral blood samples were collected on ICU admission in ethylenediamine tetra-acetic acid-containing tubes and centrifuged within 30 min of sampling for 10 min at 1000 × *g*. Plasma samples were collected and stored at −80°C until use. Cytokine levels were analysed in two-fold diluted plasma samples using ProteinSimple® (San Jose, CA, USA) microfluidic enzyme-linked immunosorbent assay (ELISA) technology, according to the manufacturer's instructions. The inter-assay and intra-assay coefficients of variation for tumour necrosis factor-α (TNF-α) and IL-6 were all <9%. Levels of angiopoietin (Ang)-1 and Ang-2 were determined in five-fold diluted plasma samples using commercially available ELISA kits according to the manufacturer's instructions (ELH-Angiopoietin1 and ELH-Angiopoietin2; RayBiotech, Norcross, GA, USA). Assay sensitivity was 30 pg $ml^{-1}$ for Ang-1 and 10 pg $ml^{-1}$ for Ang-2. The inter-assay and intra-assay coefficients of variation for Ang-1 and Ang-2 were all <12%.

From March to May 2020, 65 patients were included in the study: 17 patients in the severe group (26%) and 48 in the critical group (74%). Patient data are reported in Table 1.

At ICU admission, TNF-α and IL-6 were significantly higher in the critical group in comparison with the severe group ($P$=0.006 and $P$=0.038, respectively). Ang-1 did not significantly differ between groups ($P$=0.221), whereas Ang-2 and Ang-2/Ang-1 were significantly higher in the critical group ($P$=0.025 and $P$=0.028, respectively). Ang-2 was positively correlated to IL-6 and TNF-α ($P$=0.029 and $P$<0.0001); Ang-2/Ang-1 was correlated to TNF-α ($P$<0.0001) (Supplementary figure).

In univariate logistic regression analysis, TNF-α, IL-6, Ang-2, and Ang-2/Ang-1 were associated with vasopressor use ($P$<0.0001, $P$=0.038, $P$=0.013, and $P$=0.041, respectively). The TNF-α and Ang-2 levels were associated with renal replacement therapy (RRT) requirement ($P$=0.001 and $P$=0.046, respectively), but not IL-6 or Ang-2/Ang-1. Tumour necrosis factor-α was the only plasma marker associated with in-ICU death ($P$=0.025) (Supplementary table). After adjustment for clinical severity, only TNF-α was an independent factor associated with vasopressor use (odds ratio [OR]: 54.03; 95% confidence interval [CI]: 1.12–2611), RRT requirement (OR: 142.7; 95% CI: 4.3–4716.1), and in-ICU death (OR: 22.5; 95% CI: 1.1–440.6).

This study has three main findings. First, IL-6, TNF-α, and Ang-2 were increased in the critical group. Second, TNF-α was associated with organ failure and mortality. Third, TNF-α levels correlated with Ang-2. The first finding supports the so-called cytokine storm pathogenesis. Nevertheless, our findings on IL-6 are not in accordance with previous reports. It was suggested that IL-6 is a key factor in COVID-19-associated cytokine response, allowing therapies with IL-6 antagonists, such as tocilizumab. However, the first clinical trials with IL-6 antagonists were disappointing with negative results regarding mortality or disease progression.[4] Moreover, a recent meta-analysis confirmed that IL-6 elevation in COVID-19 is actually lower than in other respiratory virus-related diseases.[5]

In our report, TNF-α was a better correlate of initial severity and progression of ICU stay. Observations from large registries of patients with COVID-19 and with chronic rheumatism or inflammatory bowel diseases suggested that anti-TNF-α therapies may have prevented COVID-19 progression during the outbreak.[6] These results confirm the pro-inflammatory state associated with COVID-19 and emphasise the confirmed benefit of corticosteroid therapy on disease progression and mortality in critically ill patients.[7]

We also assessed endothelial injury with Ang-2 elevation. This result reinforces our previous hypothesis that COVID-19 is, at least partially, a vascular disease with endothelial damage, angiogenesis, and thrombosis.[8] Angiopoietin-2 expression can be promoted by different stimuli, including inflammation and hypoxaemia. We confirmed a positive correlation between Ang-2 and TNF-α levels, suggesting endothelial activation induced by inflammation, although an additive effect of tissue hypoxia on Ang-2 release was not excluded.[9] Critical patients had higher Ang-2 elevation than less severe patients. Angiopoietin-2, by promoting endothelial permeability, may increase pulmonary oedema, and hence disease severity. In accordance with our results, Smadja and colleagues[10] showed that Ang-2 level at hospital admission predicted COVID-19 severity.

The lack of a non-COVID-19 comparative group is a major limitation of this study. For example, Ang-2 levels in patients with COVID-19 might be similar to those of patients with other causes of acute respiratory distress syndrome. Nevertheless,

**Table 1** Patient and biological characteristics of COVID-19 patients according to the level of the severity. Ang, angiopoietin; COPD, chronic obstructive pulmonary disease; DVT, deep venous thrombosis; ECMO, extracorporeal membrane oxygenation; IL-6, interleukin-6; MV, mechanical ventilation; PE, pulmonary embolus; RRT, renal replacement therapy; SOFA, sequential organ failure assessment; TNF-α, tumour necrosis factor-alpha; WBC, white blood cell count.

| Variable | Overall population (*n*=65) | Severe group (*n*=17) | Critical group (*n*=48) | *P*-value |
|---|---|---|---|---|
| Age (yr) | 63 [56–69] | 61 [50–70] | 64 [58–69] | 0.207 |
| Male gender, *n* (%) | 45 (70) | 11 (65) | 34 (71) | 0.638 |
| BMI (kg $m^{-2}$) | 29.7 [26.8–33.5] | 28.5 [26.7–34.5] | 29.9 [27.0–33.1] | 0.726 |
| Comorbidities, *n* (%) | | | | |
| Hypertension | 34 (52) | 9 (53) | 25 (52) | 0.951 |
| Dyslipidaemia | 19 (29) | 6 (35) | 13 (27) | 0.547 |
| Severe obesity | 12 (19) | 3 (18) | 9 (19) | 1.000 |
| Smoking | 7 (11) | 1 (6) | 6 (13) | 0.664 |
| Diabetes mellitus | 17 (26) | 3 (18) | 14 (29) | 0.523 |
| Coronary artery disease | 7 (11) | 0 (0) | 7 (15) | 0.176 |
| COPD | 3 (5) | 0 (3) | 3 (6) | 0.559 |
| Days from symptoms onset to hospital admission (days) | 6 [3–8] | 7 [2–8] | 6 [4–8] | 0.938 |
| WBC ($mm^{-3}$) | 6800 [4700–8900] | 7650 [5350–9390] | 7300 [5200–9870] | 0.874 |
| Lymphocytes ($mm^{-3}$) | 700 [600–1200] | 710 [600–1100] | 700 [598–1107] | 0.409 |
| C-reactive protein (mg $L^{-1}$) | 109 [58–241] | 71 [51–137] | 145 [94–234] | 0.013 |
| Creatinine (μmol $L^{-1}$) | 70 [51–175] | 68 [52–77] | 85 [61–183] | 0.085 |
| D-dimers (ng $ml^{-1}$) | 1110 [770–2100] | 1065 [790–5915] | 1550 [770–5060] | 0.702 |
| $Paco_2$ (kPa) | 4.53 [4.26–5.33] | 4.93 [4.53–5.20] | 5.07 [4.40–5.87] | 0.410 |
| $Pao_2$ (kPa) | 10.13 [9.20–12.93] | 12.00 [10.00–15.87] | 10.67 [9.20–13.73] | 0.528 |
| Arterial lactate (mmol $L^{-1}$) | 1.7 [1.3–2.4] | 1.8 [1.2–2.4] | 1.8 [1.6–2.2] | 1.000 |
| SOFA score | 5 [2–9] | 2 [1–4] | 7 [4–12] | <0.0001 |
| Cytokines (pg $ml^{-1}$) | | | | |
| TNF-α | 20.0 [16.2–33.9] | 16.5 [15.6–19.8] | 26.7 [16.7–35.5] | 0.006 |
| IL-6 | 87.3 [31.1–173.0] | 36.4 [27.2–112.0] | 88.9 [42.1–293.0] | 0.038 |
| Angiopoietins (pg $ml^{-1}$) | | | | |
| Ang-1 | 9408 [6583–11783] | 9642 [7650–12243] | 9018 [6461–11598] | 0.221 |
| Ang-2 | 3992 [2188–6731] | 3015 [2054–3473] | 4484 [2408–7169] | 0.025 |
| Ang-2/Ang-1 | 0.49 [0.23–1.11] | 0.22 [0.18–0.57] | 0.57 [0.29–1.14] | 0.028 |
| Duration of MV (days) | NA | NA | 22 [16–32] | NA |
| ICU stay (days) | 18 [8–31] | 5 [3–7] | 25 [14–34] | <0.0001 |
| Hospital stay (days) | 27 [14–38] | 14 [11–21] | 32 [18–43] | <0.0001 |
| RRT, *n* (%) | 19 (29) | 0 (0) | 19 (40) | 0.001 |
| Vasopressor use, *n* (%) | 19 (29) | 0 (0) | 19 (40) | 0.003 |
| ECMO, *n* (%) | 4 (6) | 0 (0) | 4 (8) | 0.03 |
| DVT or PE | 5 (8) | 0 (0) | 5 (10) | 0.023 |
| Discharge from ICU | 53 (82) | 17 (100) | 36 (75) | <0.0001 |
| Discharge from hospital | 52 (80) | 17 (100) | 35 (73) | <0.0001 |
| ICU mortality (%) | 12 (18) | 1 (6) | 11 (23) | 0.088 |

we compared Ang-2 according to COVID-19 severity, showing that the most severe patients had higher markers of endothelial injury. Another major limitation is the single-time-point measurement.

Our main mechanistic hypothesis is that after the initial phase of viral infection, a pro-inflammatory state occurs, aggravated by local hypoxaemia, leading to progression of pulmonary vascular injuries.

## Declarations of interest

The authors declare that they have no conflicts of interest.

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## Appendix A. Supplementary data

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# Phenotypes of severe COVID-19 ARDS receiving extracorporeal membrane oxygenation

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Editor—Patients with acute respiratory distress syndrome (ARDS) caused by coronavirus 2019 (COVID-19) have heterogeneous clinical presentation, inflammatory status,[1] and respiratory mechanics.[2] Although venous-venous extracorporeal membrane oxygenation (ECMO) is utilised in patients with COVID-19, international data include varied outcomes.[3] Some of these differences may reflect variable initiation criteria and case-mix, and the possibility of differing phenotypes in this population has not been explored. Identification of latent phenotypes using readily available clinical data can help identify patients at greater risk of deterioration,[4,5] or patients who might benefit from a particular therapy.

We used an unsupervised clustering algorithm to assess for the existence of distinct phenotypes of COVID-19 patients on ECMO, utilising data available on Day 0 of ECMO commencement. We hypothesised that distinct phenotypes may inform risk of mortality and organ failure. This retrospective study incorporated all adult COVID-19 ECMO patients admitted to Guy's and St Thomas' Foundation Trust (GSTFT), a regional ECMO centre in the UK, up to July 1, 2020 ($n$=56) with institutional ethics approval (reference number 10796). We selected 15 variables, representing typical data available at ECMO initiation. These included patient characteristics, respiratory parameters at time of ECMO referral, and ECMO Day 0 laboratory values (see Supplementary material). Primary outcome was survival to hospital discharge, with secondary outcomes of organ support requirements. A k-means clustering algorithm (see Supplementary material), used previously in critical care datasets,[6] was used to group patients based on similarities across all variables. Clusters were validated internally, on stability and cohesion metrics, and externally, based on association to outcomes. Multivariable models were constructed to test the association of cluster membership with distal outcomes when adjusted for baseline characteristics.

Three clusters were identified, demonstrating distinct phenotypes with significant differences in characteristics and outcomes (Table 1, Supplementary Figs S1 and S2). There was a significant survival difference between phenotypes ($P$=0.0023), with phenotype 1 membership having 96% survival to ICU discharge, and a significant difference in renal replacement requirements ($P$=0.0052).

*Phenotype 1* ($n$=24 [42.8%], low mortality, hypoinflammatory, low organ support) included younger, mostly female patients, with low requirement for renal replacement characterised by lower pre-ECMO sequential organ failure assessment (SOFA) scores and markers of inflammation and thrombosis. More patients received steroids before ECMO (29.2% *vs* 5% and 16.7% in phenotypes 2 and 3, respectively, $P$=0.113). ICU mortality was 4.2%.

*Phenotype 2* ($n$=20 [35.7%], intermediate mortality, hyperinflammatory, high organ support) patients required the most renal replacement therapy. Patients had a significantly longer time (median 5 days [inter-quartile range 5–6]) between start of invasive mechanical ventilation (IMV) and ECMO, the