Physiological and biological heterogeneity in COVID-19-associated acute respiratory distress syndrome

One of the most common causes of hospital admission and death in patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is acute respiratory distress syndrome (ARDS), a clinical syndrome characterised by acute lung inflammation and increased-permeability pulmonary oedema due to injury to the alveolar capillary barrier. As clinicians care for a surge of patients with ARDS due to COVID-19, two questions arise. First, is COVID-19-associated ARDS intrinsically different from ARDS unrelated to COVID-19? The answer to this question has implications for the use of evidence-based therapies such as lung-protective mechanical ventilation, proning, and conservative fluid management in COVID-19-associated ARDS. Second, is COVID-19-associated ARDS a uniform syndrome, or can phenotypes be identified? Recent clinical studies in so-called classical ARDS (a term used here to refer to ARDS unrelated to COVID-19, the causes and characteristics of which are heterogeneous) using latent class analysis have shown distinct hyperinflammatory and hypoinflammatory biological phenotypes of ARDS,[1] and emerging evidence indicates that these phenotypes respond differently to some clinical interventions.[2,3] Identification of similar, or new, distinct phenotypes within the scope of COVID-19-associated ARDS could shed light on mechanisms of lung injury in COVID-19 and have implications for clinical trial design.

In *The Lancet Respiratory Medicine*, two Articles begin to answer these questions. To address the first question, Giacomo Grasselli and colleagues[4] studied clinical and laboratory characteristics of 301 adults with COVID-19-associated ARDS admitted to intensive care units (ICUs) in seven Italian hospitals over a 2-week period in March, 2020. Lung mechanics were assessed in the first 24 h of ICU admission and compared with findings in historical cohorts of patients with classical ARDS. Similar to classical ARDS, the distribution of values for static compliance of the respiratory system was broad. Although patients with COVID-19-associated ARDS had higher median static compliance (41 mL/cm H_2O [IQR 33–52]) than those with classical ARDS (32 mL/cm H_2O [25–43]), this difference diminished in multivariable models controlling for other clinical characteristics. Furthermore, almost all of those with COVID-19-associated ARDS (280 [94%] of 297 patients) had static compliance values below the 95th percentile of reported values for classical ARDS, and the extent of pulmonary oedema in patients with COVID-19, measured by calculation of total lung weights from lung CT scans, was similar to that of patients with classical ARDS. D-dimers in 261 patients with COVID-19 were associated with ventilatory ratio, which is a surrogate for dead-space ventilation. A subgroup of patients with D-dimer concentrations greater than the median and static compliance equal to or less than the median (high D-dimers, low compliance [HDLC]) had markedly worse 28-day mortality than the others subgroups of high D-dimers, high compliance (HDHC); low D-dimers, low compliance (LDLC); and low D-dimers, high compliance (LDHC). 28-day mortality was 56% (40 of 71 patients) in the HDLC group, 27% (18 of 67 patients) in the LDHC group, 22% (13 of 60 patients) in the LDLC group, and 35% (22 of 63 patients) in the HDHC group. This worse survival in the HDLC group suggests that the intersection of more severe dysregulation of coagulation and fibrinolysis with more severe lung injury in COVID-19-associated ARDS is highly deleterious, supporting a pathophysiological role for pulmonary microvascular thrombosis in COVID-19-associated ARDS, as has been reported in classical ARDS. Overall, the findings of this large, systematic, multicentre study provide new evidence that lung physiology in COVID-19-associated ARDS is heterogeneous and not fundamentally different from that of classical ARDS, in contrast to previous single-centre reports in small groups of patients that suggested otherwise.[5] As such, these findings support recent calls for the application of evidence-based ARDS care, such as lung-protective mechanical ventilation and proning, in COVID-19-associated ARDS.[6]

The Article from Pratik Sinha and colleagues[7] addresses the question of whether the previously described hyperinflammatory and hypoinflammatory phenotypes of classical ARDS are present in COVID-19-associated ARDS. Validated models for phenotype

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classification[8] were applied to 39 patients with COVID-19-associated ARDS, using point-of-care biomarker measurements at the bedside. Patients could be classified into the two phenotypes with a high degree of certainty, suggesting that the previously identified ARDS phenotypes are robust in this new patient population. Overall mortality in COVID-19-associated ARDS was higher (17 [44%] of 39 patients had died by day 28 of the study) than in a matched cohort of patients with classical ARDS from the HARP-2 study (132 [24%] of 539). Consistent with classical ARDS, mortality in the hyperinflammatory phenotype (five [63%] of eight patients) was substantially higher than in the hypoinflammatory phenotype (12 [39%] of 31). Yet, in COVID-19-associated ARDS, only four (10%) to eight (21%), depending on cutoffs applied, were classified as hyperinflammatory, which was considerably lower than the proportion with this phenotype in the HARP-2 matched cohort (186 [35%] of 539). These findings are surprising, given the prevalent speculation in the literature that severe COVID-19 is characterised by an excessive inflammatory response or so-called cytokine storm. However, a report comparing interleukin-6 (IL-6) levels in patients with COVID-19-associated ARDS to levels measured in classical ARDS showed that IL-6 levels, on average, were lower in the patients with COVID-19 than in those with classical ARDS.[9] Taken together, these findings suggest that the pathophysiology of COVID-19-associated ARDS is more complex than a simple overproduction of cytokines, and that there is heterogeneity within COVID-19 that is similar to that of classical ARDS, albeit with different distributions.

Although both of these studies provide new information about COVID-19-associated ARDS, there are some limitations. The study by Grasselli and colleagues[4] was done over a 2-week period during a rapid spike in COVID-19 cases, which might have affected clinical care; and it reflects cases in only one country. The study by Sinha and colleagues[7] is also geographically limited and had a very small sample size. Both studies assessed patients at a single timepoint, which might not be reflective of the protracted course of critical illness in many patients with COVID-19-associated ARDS. A report of deep immune profiling of patients with COVID-19 identified three immunophenotypes that were quite stable over 7 days, but some immunological signatures that were highly dynamic over time, underscoring the need for serial analyses.[10] Despite the limitations, the authors of both of these studies are to be commended for applying high-quality research methods during a surge of COVID-19 cases.

The ongoing COVID-19 pandemic reminds physicians daily of the importance of clinical investigation as a tool for improving the understanding and treatment of human disease. Like all good clinical investigations, the studies from Grasselli and colleagues[4] and Sinha and colleagues[7] provide answers that lead to new questions. The study by Grasselli and colleagues[4] prompts the question of whether identification of an HDLC group could be used to predictively enrich trials of empirical therapeutic anticoagulation to reduce sample size and improve the ratio of benefit to risk. The fact that only a small minority of the patients in the study by Sinha and colleagues[7] had a hyperinflammatory phenotype raises the question of whether dexamethasone treatment, which has been shown to be effective in patients with severe COVID-19 disease, will be uniformly beneficial across both phenotypes of COVID-19-associated ARDS. To answer these questions and the many others that arise during the daily care of patients with COVID-19-associated ARDS, it is imperative that high-quality clinical investigation proceeds, despite the inherent challenges of implementing research protocols in the uncertain and risky environment of a pandemic.

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Pathophysiology of COVID-19-associated acute respiratory distress syndrome: a multicentre prospective observational study

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Summary

Background Patients with COVID-19 can develop acute respiratory distress syndrome (ARDS), which is associated with high mortality. The aim of this study was to examine the functional and morphological features of COVID-19-associated ARDS and to compare these with the characteristics of ARDS unrelated to COVID-19.

Methods This prospective observational study was done at seven hospitals in Italy. We enrolled consecutive, mechanically ventilated patients with laboratory-confirmed COVID-19 and who met Berlin criteria for ARDS, who were admitted to the intensive care unit (ICU) between March 9 and March 22, 2020. All patients were sedated, paralysed, and ventilated in volume-control mode with standard ICU ventilators. Static respiratory system compliance, the ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air, ventilatory ratio (a surrogate of dead space), and D-dimer concentrations were measured within 24 h of ICU admission. Lung CT scans and CT angiograms were done when clinically indicated. A dataset for ARDS unrelated to COVID-19 was created from previous ARDS studies. Survival to day 28 was assessed.

Findings Between March 9 and March 22, 2020, 301 patients with COVID-19 met the Berlin criteria for ARDS at participating hospitals. Median static compliance was 41 mL/cm H_2O (33–52), which was 28% higher than in the cohort of patients with ARDS unrelated to COVID-19 (32 mL/cm H_2O [25–43]; p<0·0001). 17 (6%) of 297 patients with COVID-19-associated ARDS had compliances greater than the 95th percentile of the classical ARDS cohort. Total lung weight did not differ between the two cohorts. CT pulmonary angiograms (obtained in 23 [8%] patients with COVID-19-related ARDS) showed that 15 (94%) of 16 patients with D-dimer concentrations greater than the median had bilateral areas of hypoperfusion, consistent with thromboembolic disease. Patients with D-dimer concentrations equal to or less than the median had ventilatory ratios lower than those of patients with D-dimer concentrations greater than the median (1·66 [1·32–1·95] *vs* 1·90 [1·50–2·33]; p=0·0001). Patients with static compliance equal to or less than the median and D-dimer concentrations greater than the median had markedly increased 28-day mortality compared with other patient subgroups (40 [56%] of 71 with high D-dimers and low compliance *vs* 18 [27%] of 67 with low D-dimers and high compliance, 13 [22%] of 60 with low D-dimers and low compliance, and 22 [35%] of 63 with high D-dimers and high compliance, all p=0·0001).

Interpretation Patients with COVID-19-associated ARDS have a form of injury that, in many aspects, is similar to that of those with ARDS unrelated to COVID-19. Notably, patients with COVID-19-related ARDS who have a reduction in respiratory system compliance together with increased D-dimer concentrations have high mortality rates.

Funding None.

Introduction

The COVID-19 pandemic has affected millions of people and caused hundreds of thousands of deaths worldwide. Although most patients have a favourable prognosis, pneumonia and severe hypoxaemia associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection can lead to acute respiratory distress syndrome (ARDS), which is associated with a high mortality rate.[1]

The proportion of patients with COVID-19 who are diagnosed with ARDS on the basis of oxygenation criteria ranges between 20%[2] and 67%[1] in patients admitted to hospital and is 100% in mechanically ventilated patients.[3] However, few data are available that link the physiological, laboratory, and imaging features of these patients. This information is important because several studies have suggested that patients with COVID-19-associated ARDS have markedly higher lung compliances than do patients with ARDS unrelated to COVID-19 (so-called classical ARDS), so typical protective ventilatory settings might not be indicated in patients with COVID-19-related

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Research in context

Evidence before this study

We searched PubMed on July 8, 2020, with the search terms "COVID-19" and "ARDS", for research published in English between March 1 and July 1, 2020. Our search found 457 PubMed-indexed articles. Limitations of these studies included small sample size; retrospective design; and single-centre observation. Nevertheless, despite these limitations, highly cited studies have spread the knowledge that patients with COVID-19 that are diagnosed with ARDS might not have what we think of as classical ARDS, because they have significant hypoxaemia but relatively normal respiratory system compliance. These findings resulted in the clinical recommendation that suggested abandoning the previously proven best practices for lung protection.

Added value of this study

We completed a systematic analysis of clinical and laboratory features in patients with COVID-19-associated ARDS in a large (301 patients), unbiased (all consecutive patients prospectively enrolled in seven Italian hospitals) series, and compared the pathophysiology of COVID-19-related ARDS with that of classical ARDS using two large historical datasets. We present evidence that patients with COVID-19-associated ARDS have a form of injury that is similar to that of classical ARDS, characterised by decreased compliance and increased lung weight. In many patients, this injury is complicated by increased dead space, which is probably related to diffuse microthrombi or emboli of the pulmonary vascular bed. When pulmonary damage occured together with high D-dimer concentrations in our cohort, mortality was extremely high.

Implications of all the available evidence

The proposal that evidence-based lung-protective ventilatory strategies might not be recommended for some patients with COVID-19-associated ARDS is not backed up by our data, since the morphological hallmark of ARDS was essentially similar in COVID-19-related and classical ARDS. In view of these data, limitation of tidal volume to 6 mL/kg and plateau pressure to 30 cm H_2O is still recommended. The observation of higher values of dead space might suggest the use of lower levels of positive end-expiratory pressure, especially in patients in the higher range of compliance. Our results also have implications for the design of clinical trials, because patients with the phenotype characterised by low respiratory system compliance and high D-dimers have an extremely high 28-day mortality rate.

ARDS.[4–6] Additionally, patients with COVID-19-associated ARDS are thought to have substantial pulmonary thrombotic injury,[7] associated with increased D-dimer levels.[8] If confirmed, these findings could have major implications in terms of treatment strategies and prognosis.

The objective of this study was to examine the functional and morphological features of invasively ventilated patients with COVID-19-related ARDS and to assess whether the physiological and biological characteristics in patients with COVID-19 are similar to those previously described for classical ARDS.

Methods

Study design and participants

This prospective observational study was done at seven Italian hospitals (Policlinico di Sant'Orsola, Bologna; Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan; University Hospital of Modena, Modena; Grande Ospedale Metropolitano Niguarda, Milan; Ospedale San Gerardo, Monza; Humanitas Clinical and Research Center—IRCCS, Milan; and Fondazione Policlinico Universitario A Gemelli IRCCS, Rome).

Institutional review boards at each hospital approved the study protocol and decided that consent could be waived in the context of the COVID-19 pandemic. We enrolled all consecutive patients older than 18 years with confirmed COVID-19[9] who were admitted to intensive care units (ICUs) of participating hospitals between March 9 and March 22, 2020, with the following inclusion criteria in the first 24 h after admission: (1) presence of all Berlin definition criteria for ARDS;[5] and (2) receiving invasive mechanical ventilation.

Procedures

All patients were sedated, paralysed,[10,11] and ventilated in volume-control mode with standard ICU ventilators. Positive end-expiratory pressure (PEEP) selection was not protocolised. Tidal volume, respiratory rate, and airway pressures were recorded from the ventilator monitors. End-inspiratory and end-expiratory occlusions were performed using ventilator functions. End-inspiratory plateau pressure and total PEEP were measured as previously described.[12,13] The most representative set of measurements of ventilatory and physiological variables was collected within the first 24 h of ICU admission on the basis of the senior attending physician's assessment.

Static compliance of the respiratory system was calculated as tidal volume/(end-inspiratory plateau pressure–total PEEP), with a normal mean value being 67 mL/cm H_2O (SD 4).[12,14] Chest CT scans and CT-pulmonary angiograms were obtained when clinically indicated and technically feasible.[15] Total lung weight was estimated from standard non-contrast chest CT scans (done at clinical levels of PEEP) with a dedicated medical imaging software equipped with a semi-automated segmentation algorithm (3D Slicer).[16] Presence of pulmonary intravascular clots was assessed by analysing CT-pulmonary angiograms using software

installed on the IntelliSpace Portal release 11. The application uses an advanced automatic computer-aided design algorithm for detecting filling defects.[17,18] In addition, to estimate the hypoperfused areas of the lung parenchyma, the application provides a Hounsfield unit-based colour map of the lungs as an experimental feature.

Oxygenation was quantified as the ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air (PaO_2/FiO_2). Ventilatory ratio was calculated and used as a surrogate of dead space. Ventilatory ratio=measured minute ventilation×measured $PaCO_2$/(predicted minute ventilation×predicted $PaCO_2$), where minute ventilation=tidal volume×respiratory rate; predicted minute ventilation is calculated as predicted bodyweight in kg×100 (mL/min); predicted $PaCO_2$ is the expected $PaCO_2$ (37·5 mm Hg) if the patient is ventilated with the predicted minute ventilation. Ventilatory ratio is unitless; values greater than 1 suggest increased dead space.[19]

Clinical and physiological variables and D-dimer concentrations were collected within 24 h of study admission. Values of static compliance and results of pulmonary CT scans in patients with COVID-19-related ARDS were compared with a dataset of non-COVID-19-related classical ARDS obtained from the physiological database (n=269) used in the creation of the Berlin definition,[5] and the database of the LUNG-SAFE study (n=3022).[20] To minimise the potential effects of confounding variables in such comparisons, we first performed a stratified analysis for gender, body-mass index (BMI), ARDS severity (PaO_2/FiO_2 criteria[5]), and presence of pneumonia as the underlying disease causing ARDS and then built a multivariable linear model that used COVID-19 ARDS versus classical ARDS, gender, age, BMI, and PaO_2/FiO_2 as independent variables, and static compliance or lung weight as the dependent variable.

Statistical analysis

Continuous variables were expressed as medians and IQRs. Categorical variables were summarised as numbers and percentages. Comparison of continuous data between groups was done using Wilcoxon-Mann-Whitney or Kruskal Wallis test and comparison of categorical data was done using χ^2 or Fisher's exact test. We used the Kaplan-Meier method to estimate survival to day 28 from ICU admission and we assessed differences in survival curves using the log-rank test. A Cox proportional hazard model was used to estimate adjusted hazard ratios (HRs) with 95% CIs and to assess the influence of D-dimer and static compliance on survival. The relevant available clinical variables in the adjusted model were sequential organ failure assessment score at ICU admission, sex, age, and PaO_2/FiO_2 ratio.

All statistical tests were two sided. $p<0·05$ was considered statistically significant and analyses were done without any imputation for missing data. Analyses were done using SAS version 9.4, R version 3.4.0, and Graphpad Prism version 8.4.3 software packages.

Role of the funding source

There was no funding source for this study. ASS, APe, and VMR had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

During the study period of March 9–22, 2020, 301 patients fulfilled all Berlin criteria for ARDS and were recruited to the study.[5] Median time from hospital admission to intubation was 2 days (IQR 0–4). Median age was 63 years (55–70), 232 (77%) were men and 69 (23%) were women, and all were ventilated according to a conventional protective ventilatory strategy.[13] D-dimer concentrations in the first 24 h from ICU admission were available for 261 (87%) patients (appendix p 2).

See Online for appendix

Chest CT scans were obtained for 43 (14%) patients; median time from ICU admission to CT scan was 0·5 days (IQR 0–6). Quantitative analysis of lung CT scans was done in 20 (7%) patients. Analysis of pulmonary CT angiograms was done in 23 (8%) patients.

Baseline characteristics of COVID-19 ARDS compared with classical ARDS[5,20] were significantly different with regards to sex, BMI, incidence of mild and severe ARDS, and incidence of pneumonia (table 1).

Median static compliance of the respiratory system was 28% higher in patients with COVID-19 (n=297; 41 mL/cm H_2O [IQR 33–52]) than in those with classical ARDS (n=960; 32 mL/cm H_2O [25–43], $p<0·0001$). The distribution of static compliance was unimodal in the two groups, with a slight shift to the right (ie, towards higher values) in the COVID-19 group (appendix p 12). Only 17 (6%) of 297 of patients with COVID-19-related ARDS had compliances greater than the 95th percentile of the patients with classical ARDS. Static compliance decreased as PaO_2/FiO_2 decreased in patients with classical ARDS and in a pneumonia subset of patients with ARDS, while it remained unchanged in patients with COVID-19 ARDS (appendix p 3). Total lung weight did not differ between patients with COVID-19 ARDS and classical ARDS (figure 1).

The stratified analysis showed that differences in static compliance between COVID-19 ARDS and classical ARDS tended to become smaller (at least in some subgroups) after controlling for gender, BMI, severity of ARDS, and pneumonia (appendix p 4). Application of the multivariable linear model showed that static compliance was dependent on cause of ARDS, sex, and PaO_2/FiO_2 (appendix p 4), while lung weight was dependent on sex and PaO_2/FiO_2 but independent of cause of ARDS (appendix p 4).

Quartile analysis of D-dimer concentrations (n=261; normal range <500 ng/mL) and compliance (n=297) in

	COVID-19 ARDS	Classical ARDS	p value
Sex			
Men	232/301 (77·1%)	1580/2548 (62·0%)	<0·0001
Women	69/301 (22·9%)	968/2548 (38·0%)	..
Age, years*	63 (55–70)	63 (49–73)	0·943
Body-mass index, kg/m²†	27·8 (25·3–31·1)	26·0 (22·9–30·4)	<0·0001
ARDS severity			
Mild	33/300 (11·0%)	772/2634 (29·3%)	<0·0001
Moderate	163/300 (54·3%)	1263/2634 (47·9%)	0·2254
Severe	104/300 (34·7%)	599/2634 (22·7%)	0·0005
Underlying disease			
Pneumonia	301/301 (100·0%)	1523/2643 (57·6%)	<0·0001
Non-pneumonia	0	1120/2643 (42·4%)	..

Data are n/N (%) or median (IQR). ARDS=acute respiratory distress syndrome. *n=301 for COVID-19 ARDS and n=2643 for classical ARDS. †n=294 for COVID-19 ARDS and n=2186 for classical ARDS.

Table 1: **Baseline characteristics of patients with COVID-19 and classical ARDS**[5,20]

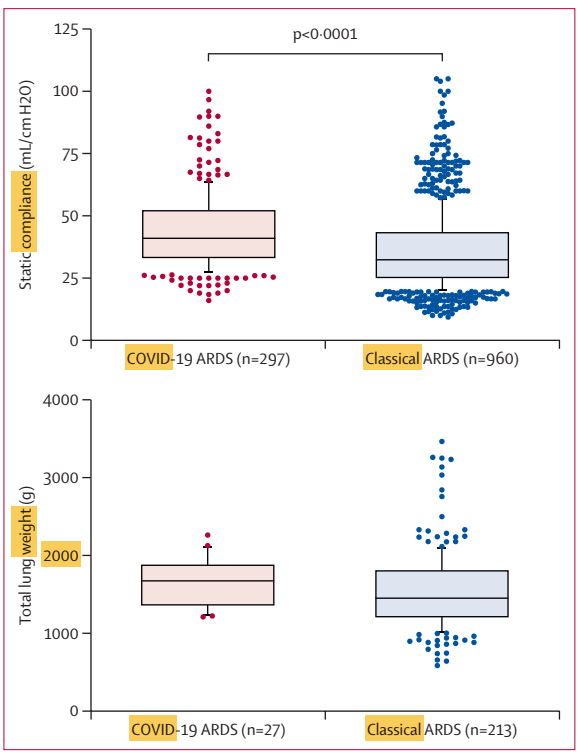

Figure 1: **Static compliance of the respiratory system and total lung weight of patients with COVID-19-associated ARDS or classical ARDS**[5,20]
Boxes show medians and IQRs; whiskers show the tenth to 90th percentiles. ARDS=acute respiratory distress syndrome.

patients with COVID-19 is shown in the appendix (pp 6–7). Patients with D-dimers equal to or less than the median (ie, 1880 ng/mL [IQR 820–6243]; n=131) had ventilatory ratios lower than those observed in patients with D-dimer concentrations greater than the median (n=130; 1·66 [1·32–1·95] *vs* 1·90 [1·50–2·33], p=0·0001; appendix p 6). Distributions of hyperinflated, normally inflated, poorly aerated, and non-aerated lung tissue in patients with static compliance either greater than or equal to or less than the median are shown in the appendix (p 14). Patients with static compliance greater than median (n=8) tended to have more hyperinflated and normally inflated lung tissue and less poorly aerated and non-aerated lung tissue than patients with static compliance equal to or less than the median (n=9), but none of these differences was statistically significant.

Based on quartiles of D-dimer concentrations and static compliance, patients were classified into four groups. The high D-dimers, low compliance (HDLC) group was patients with D-dimer concentrations greater than the median in COVID-19 ARDS (1880 ng/mL) and static compliance equal to or less than the median (41 mL/cm H_2O; 71 [27%] patients). The low D-dimers, high compliance (LDHC) group was patients with D-dimer concentrations equal to or less than the median and static compliance greater than the median (67 patients [26%]). The low D-dimers, low compliance (LDLC) group was patients with D-dimer concentrations and static compliance equal to or less than the medians (60 [23%] patients). The high D-dimers, high compliance (HDHC) group was patients with D-dimer concentrations and static compliance greater than the medians (63 [24%] patients; appendix p 8).

Patients with D-dimer concentrations equal to or less than median had normal perfusion scans regardless of compliance (figure 2). 15 (94%) of 16 patients with D-dimer concentrations greater than the median had bilateral, diffuse areas of hypoperfusion, consistent with the presence of thrombi or emboli (appendix p 9); this was the case in patients with both high and low static compliance.

28-day mortality was 36% (93 of 261 patients). The HDLC group had significantly higher 28-day mortality than the other three groups (40 [56%] of 71 in the HDLC group *vs* 18 [27%] of 67 in the LDHC group, 13 [22%] of 60 in the LDLC group, and 22 [35%] of 63 in the HDHC group, all p=0·0001). Kaplan-Meier analysis of survival for the four groups is shown in figure 3. In the Cox model with HDLC as the reference group, the adjusted HRs for 28-day mortality were 0·420 (95% CI 0·215–0·818) for the LDHC group, 0·386 (0·152–0·985) for the LDLC group, and 0·448 (0·230–0·873) for the HDHC group (table 2). Biological sex does not appear to be a risk factor for 28-day mortality.

Discussion

Our study provides two major findings. First, patients with COVID-19-related ARDS have lung morphology and respiratory mechanics that largely match those of classical ARDS. Second, there is a subgroup of patients with COVID-19-related ARDS who have disease characterised by low static compliance of the respiratory system and high D-dimer concentration and have a markedly increased mortality compared with other patients.

ARDS is a form of lung injury that occurs in response to various predisposing events and is characterised by inflammation, increased pulmonary vascular permeability, and loss of aerated lung tissue. The diagnosis of ARDS is based on severe hypoxaemia and bilateral radiographic opacities occurring within 7 days of exposure to known predisposing factors.[5] Central to the pathophysiology of ARDS is the presence of fibrin-rich exudates (hyaline membranes) due to activation of coagulation and inhibition of fibrinolysis.[21] Upregulation of procoagulant activity in the alveolar compartment has been proposed as the driving force for intra-alveolar fibrin deposition and has been implicated in the development of ARDS.[22] Concentrations of D-dimer, a proteic fragment present in the blood resulting from clot degradation commonly found in patients with suspected thrombotic disorders, are significantly increased in the oedema fluid of patients with ARDS.[23] Early studies proposed that widespread pulmonary vascular thrombosis was a consistent feature of ARDS,[24–26] and increased serum levels of D-dimers[7] and pulmonary vascular endothelialitis, thrombosis, and angiogenesis[27] have been observed in patients with COVID-19. Furthermore, dysregulation of other factors related to coagulation (eg, low vitamin K-dependent protein C and increased plasminogen activator inhibitor 1) has been associated with very high mortality in ARDS.[28]

Although unsupported by large studies, several authors have concluded that patients with COVID-19 who are diagnosed with ARDS might actually not have what we think of as classical ARDS because of the fact that they have significant hypoxaemia but quite compliant lungs.[6,29,30] Mean static compliance of 50·2 mL/cm H_2O (SD 14·3) was reported for 16 patients mechanically ventilated for COVID-19.[4]

To answer the question of whether patients with COVID-19-related ARDS have characteristics found in classical ARDS, we selected reference values from the dataset of 269 patients used to empirically assess the Berlin definition of ARDS[5] and from the 3022 patients included in the LUNG-SAFE database.[20] Patients with COVID-19-related ARDS have a median compliance 28% higher than the median in classical ARDS cohorts. Regardless, only 5·7% of patients with COVID-19 related ARDS had static compliance greater than the 95th percentile of those with classical ARDS. Notably, other published case series of critically ill patients with COVID-19 have reported median static compliance of 20–43 mL/cm H_2O,[31,32] similar to those in classical ARDS. In the three most recent and largest studies, median static compliance was 27 mL/cm H_2O (IQR 22–36; n=257), 28 mL/cm H_2O (IQR 23–38; n=267), and 35 mL/cm H_2O (IQR 27–45; n=296).[33–35] Furthermore, by quantitative analysis of lung CT scans, we found that total lung weight was similar to that in classical ARDS and was virtually identical to classical ARDS, when normalised to ARDS severity (appendix p 4). Together, these data strongly suggest that patients with COVID-19-related ARDS have values of static compliance that overlap those in classical ARDS.

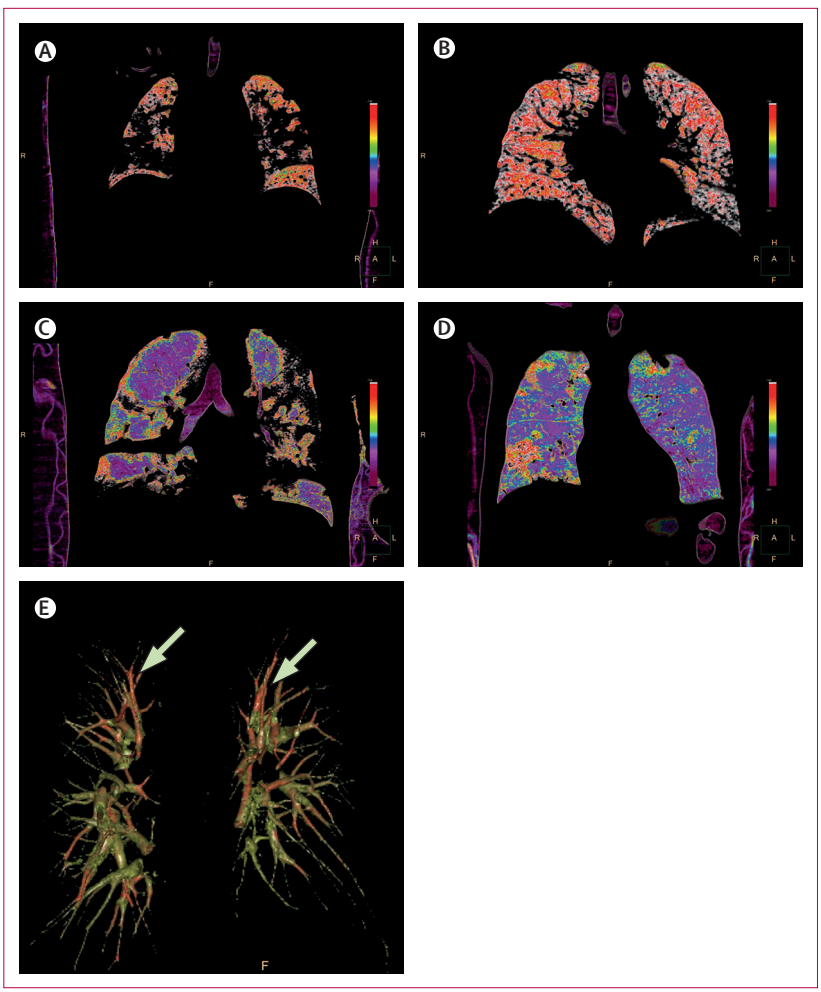

Figure 2: **Distribution of perfusion through CT angiogram coronal slices of patients representative of each D-dimer and compliance subgroup**
(A–D) CT angiogram in patients with COVID-19. (A) A 42-year-old man from the LDLC group (static compliance 38 mL/cm H_2O; D-dimer 1260 ng/mL; PaO_2/FiO_2 144). (B) A 70-year-old man from the LDHC group (static compliance 46 mL/cm H_2O; D-dimer 587 ng/mL; PaO_2/FiO_2 114). (C) A 62-year-old man from the HDLC group (static compliance 32 mL/cm H_2O; D-dimer 15 430 ng/mL; PaO_2/FiO_2 52). (D) A 75-year-old man from the HDHC group (static compliance 50 mL/cm H_2O; D-dimer 21 010 ng/mL; PaO_2/FiO_2 76). Purple-blue colouring indicates hypoperfusion. (E) Three-dimensional reconstruction of the pulmonary vascular arterial tree from the patient in panel D. Red (arrows) shows thromboembolic lesions. HDHC=high D-dimers, high compliance. HDLC=high D-dimers, low compliance. LDHC=low D-dimers, high compliance. LDLC=low D-dimers, low compliance. PaO_2/FiO_2=ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air.

Similarly to a previous study,[8] we found that most of our patients had markedly increased D-dimer concentrations (median 1880 ng/mL [IQR 820–6243]), a biomarker linked to increased inflammation, fibrin degradation, and possibly to vascular endothelial injury. Although we cannot demonstrate a direct link between D-dimer concentrations and thrombotic burden, we found that the ventilatory ratio, a marker of dead space, was higher in patients with COVID-19-related ARDS who had very high D-dimer concentrations irrespective of the patients' static compliance. Moreover, we showed

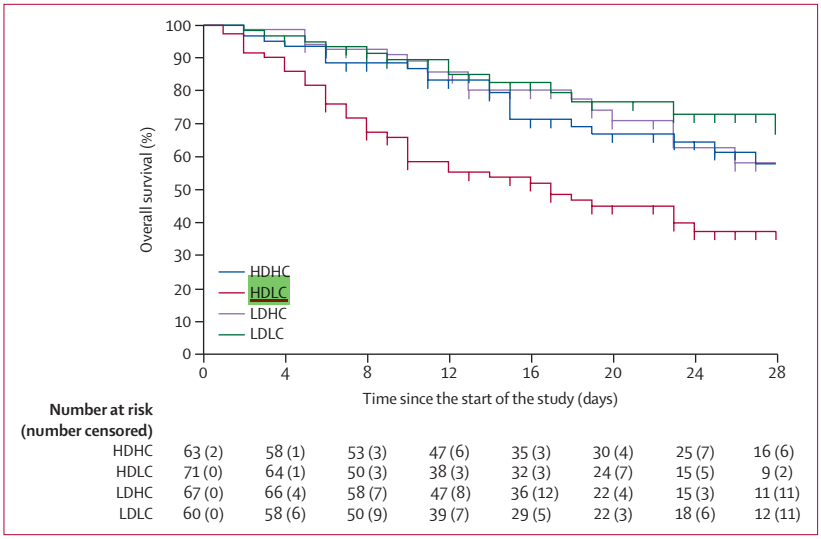

***Figure 3*: Kaplan-Meier analysis of 28-day survival in the four D-dimer and static compliance subgroups**
HDHC=high D-dimers, high compliance. HDLC=high D-dimers, low compliance. LDHC=low D-dimers, high compliance. LDLC=low D-dimers, low compliance.

	Hazard ratio (95% CI)
Class	
High D-dimers, low compliance	1 (ref)
High D-dimers, high compliance	0·448 (0·230–0·873)
Low D-dimers, high compliance	0·420 (0·215–0·818)
Low D-dimers, low compliance	0·386 (0·152–0·985)
Sex	
Female	1 (ref)
Male	1·803 (0·679–4·788)
Age	1·048 (1·002–1·095)*
PaO_2/FiO_2	0·996 (0·992–1·000)*

PaO_2/FiO_2=ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air. *Change in risk of death per one unit increase (years for age and mm Hg for PaO_2/FiO_2).

***Table 2*: Cox proportional risk analysis for mortality**

a dose–response association with higher values of ventilatory ratio at higher D-dimer concentrations (appendix p 6).

CT angiogram studies showed filling defects or occlusions of the pulmonary vasculature that were more prominent in patients with high D-dimer concentrations. Although limited by the experimental algorithm used to identify clots, this finding is similar to that observed in patients with H1N1-associated ARDS who had a significantly higher incidence of pulmonary embolism than patients with ARDS of different causes.[36] Although increased D-dimer concentrations might be driven by inflammatory mechanisms and dead-space ventilation might be due to mechanisms other than microclots, our study suggests that intravascular pathology plays a major role increasing dead space and causing hypoxaemia in COVID-19-related ARDS. This role could explain the observation that static compliance and PaO_2/FiO_2 were not correlated in COVID-19-related ARDS, but were correlated in classical ARDS (appendix p 3).

We also found a dramatic increase in mortality in a subgroup of patients that had a combination of very high D-dimer concentrations and low static compliance. The 28-day mortality in this group was more than two times higher than in patients who had increases of either D-dimer concentration or static compliance individually. These data suggest that patients have poor prognosis if SARS-CoV-2 attacks both the pulmonary cells and vascular system; although we cannot distinguish between injury in the pulmonary or systemic vasculature. Our findings are consistent with data showing that the lungs of patients with COVID-19 display distinctive vascular features, consisting of severe endothelial injury associated with the presence of intracellular virus and disrupted cell membranes.[25]

The observational nature of this study is its major weakness and affected several aspects of the study. First, the decision to use the physiological or ventilatory variables judged as most representative of the patient's status by the senior attending physician might have introduced inconsistencies because different selection criteria were used in the two historical comparators (ie, temporal criteria for the LUNG-SAFE[20] and protocol-driven criteria for the Berlin definition[5]). Second, since the number of CT scans and CT angiograms was limited by the risk of contagion[15] and since the angiograms might have been ordered in response to high D-dimer concentrations, we cannot exclude a selection bias in the subset of patients in whom CT scans were done, and they might not have been representative of the entire population. However, although quantitative CT scan analysis was done in a subset of patients with more severe ARDS (appendix p 11), stratified analysis showed that lung weight in severe COVID-19-related ARDS was essentially identical to the lung weight in severe classical ARDS (appendix p 4). Third, PEEP levels during CT scans in COVID-19-related ARDS (clinically set) and in classical ARDS (protocolised in an experimental settings)[5] were different, thus adding an element of heterogeneity in the comparisons; however, this should not have affected measurements of total lung weight. Fourth, although we did a stratified analysis and built a multivariable model to account for a number of potential confounding factors, the differences between COVID-19-related ARDS and classical ARDS could be influenced by many other factors not captured by our analysis—eg, comorbidities and onset of complications during ICU stay. Moreover, by definition, all patients with COVID-19-related ARDS had a viral origin for their ARDS, whereas classical ARDS can have various causes. However, our stratified analysis examining a subgroup of patients with classical ARDS caused by pneumonia yielded similar results. Fifth, physiological values obtained from previous studies were probably not taken at the same

timepoints as values obtained from our patients with COVID-19-related ARDS. In fact, this issue might partially explain the great heterogeneity in classical ARDS. Also, not all patients with COVID-19-related ARDS had all ventilatory and laboratory variables assessed.

The major strength of this study is the systematic analysis of physiological, laboratory, and clinical features obtained from a large, unbiased, multicentre series of patients. As such, it might have important implications for the clinical management of patients with COVID-19-related ARDS. The statement that classical protective ventilatory strategies[13] might not be recommended for some[4,36] patients with COVID-19-related ARDS is not backed up by our data. Under these circumstances, protective ventilatory strategies[13] are still recommended. The observation of higher values of ventilatory ratios (a marker of dead space) in patients with very high D-dimer concentrations might suggest that lower levels of PEEP should be used, especially in patients in the higher range of static compliance.[37] Furthermore, a meta-analysis of the use of PEEP in ARDS found that higher PEEP was associated with decreased mortality in patients with a PaO_2/FiO_2 less than 200, possibly related to lower static compliance.[38] The absence of correlation between PaO_2/FiO_2 and static compliance in patients with COVID-19 (appendix p 7) suggests that this conclusion will have to be reassessed in these patients.

Our results also have implications for the design of clinical trials. When SARS-CoV-2 affects both the pulmonary parenchyma and the coagulation or vasculature system, the 28-day mortality rate is extremely high. Identification of this phenotype is important for ongoing trials of anticoagulants or thrombolytics.

In conclusion, this study provides evidence confirming that patients with COVID-19-related ARDS have a form of injury similar to classical ARDS. When an easily identified phenotype of increased parenchymal damage (low static compliance) and increased D-dimer concentrations occurs together, mortality is extremely high.

Contributors

GG, TT, JL, CF, FL, MC, RF, SN, J-LV, MA, ASS, APe, and VMR were responsible for study design, data analysis, data interpretation, and preparing the first draft of the manuscript. All authors were responsible for data acquisition and data interpretation. CF did the statistical analysis. GG, TT, ASS, APe, and VMR finalised the manuscript. ASS, APe, and VMR are responsible for study data integrity. All authors reviewed the manuscript and approved the final submitted version.

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Declaration of interests

GG reports personal fees and non-financial support from Getinge and Biotest; personal fees from ThermoFisher Scientific; grants and personal fees from Fisher & Paykel; and personal fees from Draeger Medical, outside the submitted work. AZ has patent ES2732104 licensed to AW Technologies and patents US2017348472 and US2017224898 licensed to Fresenius. MC reports personal fees from Edwards Lifesciences, Directed Systems, and Cheetah Medical, outside the submitted work. ASS reports personal fees from Baxter and Novalung/Xenios. APe reports personal fees from Maquet, Novalung/Xenios, Baxter, and Boehringer Ingelheim, outside the submitted work. All other authors declare no competing interests.

Data sharing

Deidentified individual participant data that underlie results reported in this Article will be available. Applicants must provide (1) a methodologically sound approach to achieve scientific aims and (2) formal documents of approval from the ethics committee of the applicant's institution. Data will be made available pending authorisation of the Policlinico di Sant'Orsola ethics committee, which will review applicants' requests, and after signing an appropriate data sharing agreement. Proposals should be directed to the corresponding author. Data will be available immediately after publication with no end date.

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Prevalence of phenotypes of acute respiratory distress syndrome in critically ill patients with COVID-19: a prospective observational study

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Summary

Background In acute respiratory distress syndrome (ARDS) unrelated to COVID-19, two phenotypes, based on the severity of systemic inflammation (hyperinflammatory and hypoinflammatory), have been described. The hyperinflammatory phenotype is known to be associated with increased multiorgan failure and mortality. In this study, we aimed to identify these phenotypes in COVID-19-related ARDS.

Methods In this prospective observational study done at two UK intensive care units, we recruited patients with ARDS due to COVID-19. Demographic, clinical, and laboratory data were collected at baseline. Plasma samples were analysed for interleukin-6 (IL-6) and soluble tumour necrosis factor receptor superfamily member 1A (TNFR1) using a novel point-of-care assay. A parsimonious regression classifier model was used to calculate the probability for the hyperinflammatory phenotype in COVID-19 using IL-6, soluble TNFR1, and bicarbonate levels. Data from this cohort was compared with patients with ARDS due to causes other than COVID-19 recruited to a previous UK multicentre, randomised controlled trial of simvastatin (HARP-2).

Findings Between March 17 and April 25, 2020, 39 patients were recruited to the study. Median ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air (PaO_2/FiO_2) was 18 kpa (IQR 15–21) and acute physiology and chronic health evaluation II score was 12 (10–16). 17 (44%) of 39 patients had died by day 28 of the study. Compared with survivors, patients who died were older and had lower PaO_2/FiO_2. The median probability for the hyperinflammatory phenotype was 0·03 (IQR 0·01–0·2). Depending on the probability cutoff used to assign class, the prevalence of the hyperinflammatory phenotype was between four (10%) and eight (21%) of 39, which is lower than the proportion of patients with the hyperinflammatory phenotype in HARP-2 (186 [35%] of 539). Using the Youden index cutoff (0·274) to classify phenotype, five (63%) of eight patients with the hyperinflammatory phenotype and 12 (39%) of 31 with the hypoinflammatory phenotype died. Compared with matched patients recruited to HARP-2, levels of IL-6 were similar in our cohort, whereas soluble TNFR1 was significantly lower in patients with COVID-19-associated ARDS.

Interpretation In this exploratory analysis of 39 patients, ARDS due to COVID-19 was not associated with higher systemic inflammation and was associated with a lower prevalence of the hyperinflammatory phenotype than that observed in historical ARDS data. This finding suggests that the excess mortality observed in COVID-19-related ARDS is unlikely to be due to the upregulation of inflammatory pathways described by the parsimonious model.

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Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel virus leading to COVID-19 that has resulted in a global pandemic and is associated with high mortality and morbidity.[1–3] SARS-CoV-2 pneumonia in its most severe form can lead to profound hypoxia and acute respiratory distress syndrome (ARDS) requiring invasive mechanical ventilation.[1,3] Little is understood about the pathophysiology of COVID-19, though many have speculated that a central pathophysiological abnormality associated with severe COVID-19 is an exaggerated systemic inflammatory response or a so-called cytokine storm.[4–6] However, no objective data-driven evidence supports this theory.[7]

Considerable evidence does exist for the presence of subgroups of ARDS with exaggerated inflammation. In secondary analyses of five ARDS randomised controlled trials, two phenotypes, termed hyperinflammatory and hypoinflammatory, have been consistently identified using latent class analysis (LCA).[8–11] The hyperinflammatory phenotype is associated with exaggerated inflammation evidenced by greatly increased levels of circulating proinflammatory cytokines and increased incidence of shock. Mortality rates in the phenotype with

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Research in context

Evidence before this study

We searched PubMed and Google Scholar using the search terms "COVID-19", "SARS-CoV2", "inflammation", "cytokines", and "immune responses" for research published in 2020, with no language restrictions. Additionally, we considered work by co-authors and colleagues on the subject of ARDS phenotyping. Two phenotypes of acute respiratory distress syndrome (ARDS) have consistently been identified in randomised controlled trials with divergent characteristics, clinical outcomes, and treatment responses. The hyperinflammatory phenotypes had more severe plasma inflammatory responses and worse outcomes. It has been hypothesised that the cytokine storm is integral to the pathogenesis of severe COVID-19. The prevalence of this phenotype in COVID-19-related ARDS was unknown.

Added value of this study

Using a previously validated parsimonious model and a point-of-care biomarker analyser, in this preliminary report, we classified 39 patients with COVID-19 ARDS into hypoinflammatory and hyperinflammatory phenotypes. Compared with a matched cohort of patients from the HARP-2 study of patients with ARDS due to causes other than COVID-19, the prevalence of the hyperinflammatory phenotype in the COVID-19 cohort was lower, and mortality at day 28 was higher in both phenotypes.

Implications of all the available evidence

The findings of this exploratory study suggest that the hyperinflammatory phenotype of ARDS is less prevalent in COVID-19 than in previous ARDS cohorts, undermining the theory that the cytokine storm is disproportionately characteristic of COVID-19. Future studies are needed to confirm these findings and to better understand the pathophysiology driving poor outcomes in patients with COVID-19-associated ARDS.

lower systemic inflammatory responses are about 20% and consistently 20% lower than in the hyperinflammatory phenotype. Further, in three of these randomised controlled trials, differential treatment responses to randomised interventions were observed in the two phenotypes.[8–10] These findings suggest that the hyperinflammatory phenotype might be useful for prognostic and predictive enrichment in ARDS.

See Online for appendix

LCA-derived phenotypes are usually identified using large datasets and the algorithms are dependent on research biomarkers. Parsimonious classifier models have been developed to identify ARDS phenotypes using a small number of variables.[12] We used these models and novel point-of-care assays[13] to identify ARDS phenotypes in patients with COVID-19 in real time. We aimed to describe the prevalence of ARDS phenotypes in COVID-19-associated ARDS; and to compare the clinical and biological characteristics of patients with COVID-19 and ARDS to a previously characterised population of patients with ARDS due to other causes—those enrolled in the Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction (HARP-2) clinical trial.[14]

For the **study protocol** see http://www.nictu.hscni.net/wp-content/uploads/2020/07/PHIND-Protocol-v6.0_Final_29.05.20.pdf

Methods

Study design and population

This was a prospective observational study done at two centres in Newport and London, UK. The study was a subset of an ongoing multicentre study, clinical evaluation of a point of care assay to identify PHenotypes IN the acute respiratory Distress syndrome (PHIND; NCT04009330). All patients were unable to provide consent themselves, so consent was gained using the appropriate emergency consent mechanisms in line with the ethical approval of the study by the Bromley Research Ethics Committee, UK (reference number 19/LO/0672). The study sites were the Royal Gwent Hospital, a district general hospital in Newport, Wales, and University College Hospital, a university hospital serving an inner-city population in London. Both intensive care units (ICUs) were operating at surge capacity for the duration of the study (appendix p 1).

Patients were eligible for recruitment if they were positive for SARS-CoV-2 and met the Berlin definition of ARDS.[15] Patients were excluded from the study if they were younger than 18 years; if onset of ARDS was more than 48 h before screening; if they were receiving extracorporeal membrane oxygenation; or if they had a do not resuscitate order in place. Diagnosis of ARDS was established by the attending physicians caring for the patient.

The study protocol is available online.

Data collection

Comprehensive data were collected at baseline, including demographics, chronic health conditions, vital signs, and ventilatory and laboratory investigations. In addition to standard laboratory investigations, data were also available for acute markers of inflammation widely described for COVID-19. These were D-dimer, ferritin, C-reactive protein, procalcitonin, lactate dehydrogenase, fibrinogen, and troponin. Biospecimens were also collected at baseline to quantify additional protein biomarker levels. The study was censored at day 28 and vital status was adjudicated at this point.

Protein biomarker quantification and phenotype classification

Probabilities for belonging to the hyperinflammatory phenotype were generated using a novel rapid point-of-care

platform. In a prespecified two-step process performed in real time, plasma samples were first used to quantify interleukin 6 (IL-6) and soluble tumour necrosis factor receptor superfamily member 1A (TNFR1) concentrations. Plasma levels of the two biomarkers were quantified at the time of study recruitment using a novel point-of-care assay measured using the Evidence Multistat Analyser (Randox Laboratories, Country Antrim, UK). Next, as per the PHIND study protocol,[12] a three-variable parsimonious classifier model comprised of IL-6, serum bicarbonate, and soluble TNFR1 was used to generate the probabilities of phenotype assignment (appendix p 2).[12] Values for serum bicarbonate were measured in clinical laboratories. Clinical staff at both sites were masked to the biomarker data and generated probabilities. The point-of-care platform-generated probabilities have been validated against probabilities generated using ELISA-based biomarker quantification and the same classifier model.[13] The study showed good correlation between the probabilities generated by the two methods, and both methods classified ARDS phenotypes accurately.[13] Details of assay-specific procedures are in the appendix (p 1).

As per the PHIND protocol, patients were classified into the hyperinflammatory phenotype using one of two prespecified probability cutoffs: (1) 0·5 or higher; and (2) the Youden index generated during model development (≥0·274). During previous model validation, classification based on a cutoff of 0·5 led to higher specificity, whereas the Youden index cutoff led to higher sensitivity.[12] Once classified, differences in measured baseline variables and mortality at day 28 were compared between the phenotypes.

Previous findings from the secondary analysis using LCA of a phase 2b randomised trial of simvastatin for treatment of ARDS (the HARP-2 study)[14] were used as a historical reference standard to compare proportions of phenotypes and clinical outcomes in the COVID-19 phenotypes. HARP-2 was specifically selected because data were available for IL-6 and soluble TNFR1 quantified by the Multistat analyser in a selection of patients and would allow direct comparison with the studied cohort. First, phenotype proportions, acute physiology and chronic health evaluation II (APACHE II) scores, ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air (PaO_2/FiO_2), and clinical outcomes from this study were compared with the entire HARP-2 cohort (n=539). For HARP-2, phenotypes described are those derived using LCA. It was not possible to use the parsimonious model used in the COVID-19 cohort in HARP-2 because bicarbonate was not measured. Next, biomarker levels, phenotype proportions, APACHE II scores, and clinical outcomes in the COVID-19 cohort were compared with an equivalent number of matched patients from HARP-2 that had IL-6 and soluble TNFR1 levels measured using the Evidence Multistat Analyser (herein referred to as the HARP-2 matched cohort). This matched analysis permitted comparison of biomarker levels quantified using the same assay across two independent populations. Of the entire HARP-2 cohort, Multistat biomarker analysis was available in 98 patients. In an

	Total population (n=39)	Survivors (n=22)	Non-survivors (n=17)	p value
Age, years	57 (52–61)	54 (45–57)	60 (56–64)	0·0036
Sex				0·0490*
Men	25 (64%)	11 (50%)	14 (82%)	
Women	14 (36%)	11 (50%)	3 (18%)	
Race				0·40*
White	19 (49%)	10 (45%)	9 (53%)	
Asian	9 (23%)	4 (18%)	5 (29%)	
Black	4 (10%)	2 (9%)	2 (12%)	
Other†	7 (18%)	6 (27%)	1 (6%)	
Diabetes	9 (23%)	6 (27%)	3 (18%)	0·70*
Hypertension	6 (15%)	2 (9%)	4 (24%)	0·37*
Heart rate, beats per min	103 (81–142)	106 (84–153)	98 (79–130)	0·34
Mean arterial pressure, mm Hg	64 (61–72)	64 (61–69)	65 (61–72)	0·60
PaO_2/FiO_2, kPa	18 (15–21)	20 (17–24)	15 (11–18)	0·0040
Minute ventilation, L/min	10·5 (9·4–12·1)	10·2 (9·3–12·2)	10·8 (9·8–11·2)	0·60
Plateau pressure, cm H_2O	31 (27–34)	30 (27–34)	31 (26–34)	0·82
Positive end-expiratory pressure, cm H_2O	12 (6–20)	13 (12–15)	12 (10–15)	0·37
Compliance, mL/cm H_2O	24 (20–28)	24 (21–28)	25 (20–29)	0·79
White blood cells, $\times 10^9$ per L	10 (8–12)	8·6 (7·8–12)	10·4 (9·7–14·2)	0·25
Lymphocytes, $\times 10^9$ per L	1 (0·6–1·1)	0·90 (0·6–1·1)	1 (0·6–1·4)	0·56
Platelets, $\times 10^9$ per L	272 (213–330)	285 (236–332)	244 (177–319)	0·16
Albumin, g/L	23 (20–26)	24 (20–27)	23 (20–25)	0·61
Bilirubin, µmol/L	10 (6–23)	8 (6–12)	23 (9–40)	0·0235
Bicarbonate, mmol/L	26 (24–30)	27 (24–31)	25 (23–27)	0·32
Creatinine, µmol/L	84 (65–172)	74 (63–165)	94 (74–201)	0·19
Troponin, ng/L	18 (5–37)	9 (5–21)	23 (12–58)	0·0549
Lactate dehydrogenase, units per L	458 (336–591)	439 (343–499)	530 (307–732)	0·24
Procalcitonin, ng/mL	1·2 (0·4–2·9)	1·2 (0·3–2·9)	1·7 (0·9–7·1)	0·28
Fibrinogen, g/L	6·6 (5·8–6·8)	6·4 (5·8–6·6)	6·6 (6·2–7·1)	0·0520
D-dimer, ng/mL	1622 (888–3742)	1089 (815–2262)	3730 (1604–5640)	0·0187
Ferritin, µg/L	1196 (421–2825)	806 (382–1613)	2178 (471–2947)	0·12
C-reactive protein, mg/L	214 (154–320)	199 (145–322)	277 (205–293)	0·19
Interleukin-6, pg/mL	192 (112–556)	149 (84–270)	457 (192–1042)	0·0048
Soluble TNFR1, pg/mL	3150 (2455–4405)	2735 (2323–3705)	4200 (3030–4590)	0·0197
Vasopressor use (baseline)	24 (62%)	14 (64%)	10 (59%)	0·99*
Invasive ventilation (baseline)	35 (90%)	21 (95%)	14 (82%)	0·44*
Sequential organ failure assessment score	6 (5–8)	6 (4–7)	7 (6–9)	0·09
APACHE II score	12 (10–16)	12 (10–15)	14 (11–16)	0·26

Data are median (IQR) and n (%). The cohort (a COVID-19 subset of the PHIND cohort of patients with ARDS) is stratified into groups of survivors and non-survivors. p values show comparison of survivors versus non-survivors and were calculated by Wilcoxon signed-rank test unless noted otherwise. APACHE II=acute physiology and chronic health evaluation II. ARDS=acute respiratory distress syndrome. PaO_2/FiO_2=ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air. PHIND=clinical evaluation of a point of care assay to identify PHenotypes IN the acute respiratory Distress syndrome. TNFR1=tumour necrosis factor receptor superfamily member 1A. *Fisher's exact test. †Includes Filipino and Romani.

Table 1: Baseline characteristics of the cohort

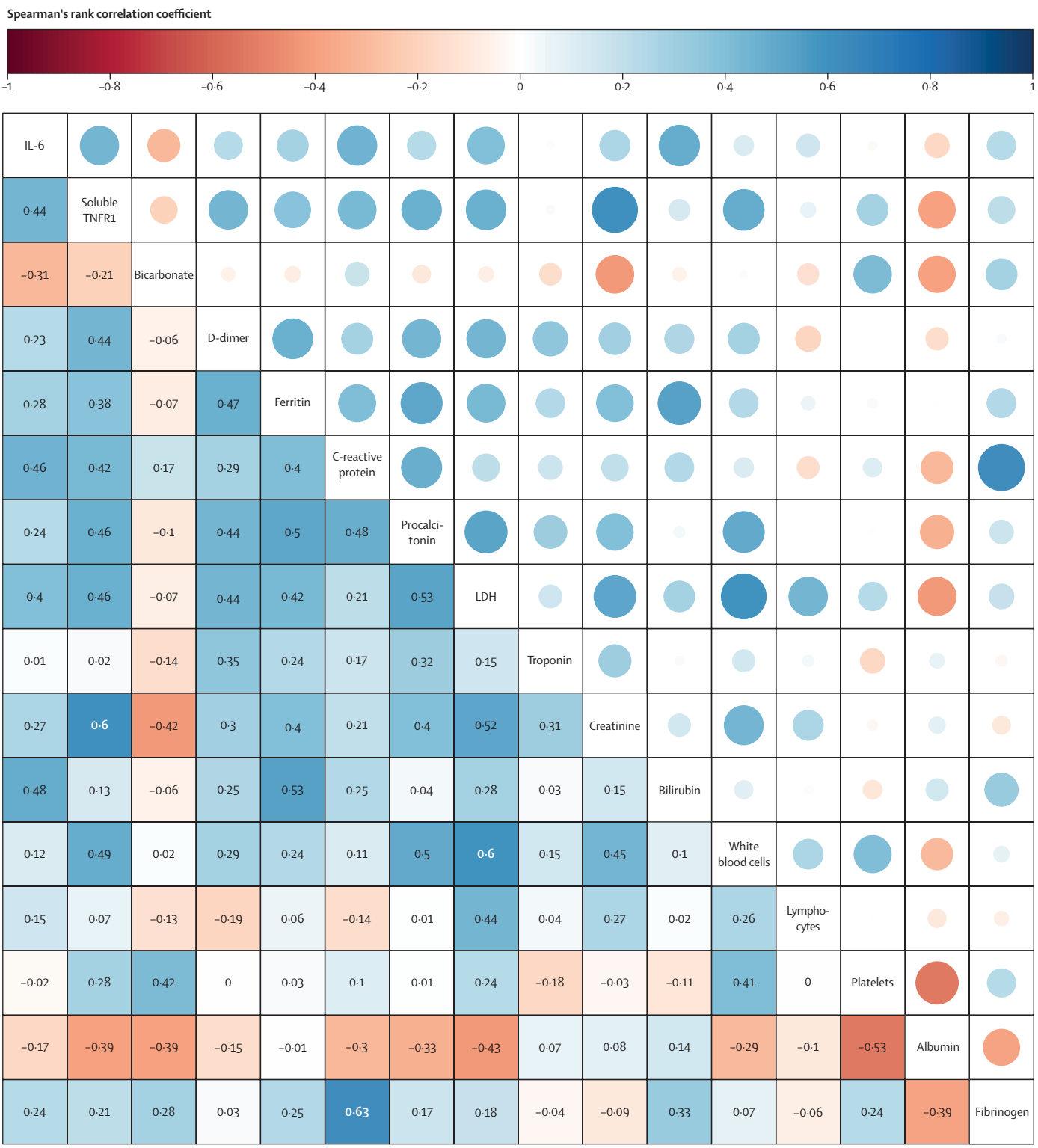

Figure 1: **Correlation matrix of the biomarkers measured at baseline in our cohort**
Increased size of the circles shows stronger correlation. Coefficients are derived using the Spearman's rank correlation coefficient. IL-6=interleukin 6. LDH=lactate dehydrogenase. TNFR1=tumour necrosis factor receptor superfamily member 1A.

effort to compare aetiologically similar groups to COVID-19, only patients with pneumonia as the primary risk factor for ARDS were selected for matching from this subset. Matching of patients to the COVID-19 cohort was done on the basis of a logistic regression-derived score using age, gender, and PaO_2/FiO_2 as predictor variables (appendix p 2).

Statistical analysis

Clinical data from the time of study enrolment were used for analysis. Given the small sample size in the analysed subgroups, data are presented as median (IQR) for all continuous variables. Characteristics between groups were compared using Wilcoxon signed-rank test or Fisher's exact test depending on the nature of the variable. Spearman's rank correlation coefficient was used to assess association between biomarkers. All analyses were done on R Studio, version 1.1.453, using R, version 3.4.1.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

39 patients were recruited to the study between March 17 and April 25, 2020. Of these, 32 were from Royal Gwent Hospital and seven were from University College Hospital. All samples were collected within 2 h of enrolment into the study and within 24 h of diagnosis of ARDS and meeting study enrolment criteria. The median time from the onset of symptoms to study enrolment was 10 days (IQR 7–13). 35 (90%) of 39 patients were receiving invasive mechanical ventilation and four patients were non-invasively ventilated at the time of recruitment to the study (table 1). All four patients receiving non-invasive ventilation were subsequently intubated during their stay in the ICU. 24 (62%) of 39 patients were on vasopressors at baseline (median dose 0·08 µg/kg per min). The median APACHE II score was 12 (IQR 10–16) and median PaO_2/FiO_2 was 18 kpa (15–21). At day 28, 17 (44%) of 39 patients had died. Of the survivors, seven remained in the ICU on day 28 of the study and have subsequently been discharged alive. 12 (38%) of 32 died in the Royal Gwent Hospital cohort and five (71%) of seven died in the University College Hospital cohort (appendix p 4).

Median age of survivors at baseline (54 years [IQR 45–57]) was significantly lower than that of non-survivors (60 years [56–64], p=0·0036). Of the baseline respiratory variables, only the PaO_2/FiO_2 was significantly different, with lower levels in non-survivors (p=0·0040). Of the baseline biomarkers, IL-6 (p=0·0048), soluble TNFR1 (p=0·0197), D-dimer (p=0·0187), and bilirubin (p=0·0235) were all significantly higher in non-survivors than in survivors (table 1). Significant correlations were noted between many of the measured biomarkers (figure 1). D-dimer, ferritin, C-reactive protein, lactate dehydrogenase, and procalcitonin showed association with one another with some correlation coefficients between 0·4 and 0·5 for the more highly correlated variables. The highest correlations were observed between fibrinogen and C-reactive protein (r=0·63) and soluble TNFR1 and creatinine (r=0·60).

Applying the parsimonious classifier model to the COVID-19 cohort resulted in a median probability for the

	Hypoinflammatory (n=31)	Hyperinflammatory (n=8)	p value
Age, years	57 (53–61)	57 (46–60)	0·55
Sex			0·69*
Men	19 (61%)	6 (75%)	..
Women	12 (39%)	2 (25%)	..
Race			0·38*
White	17 (55%)	2 (25%)	
Asian	6 (19%)	3 (38%)	..
Black	3 (10%)	1 (13%)	..
Other†	5 (16%)	2 (25%)	..
Diabetes	7 (23%)	2 (25%)	0·99*
Hypertension	6 (19%)	0	0·31*
Heart rate, beats per min	98 (77–141)	104 (97–144)	0·44
Mean arterial pressure, mm Hg	64 (61–71)	70 (60–75)	0·64
PaO_2/FiO_2, kPa	18 (16–22)	17 (11–21)	0·27
Minute ventilation, L/min	10·2 (9·4–11·3)	10·6 (9·3–13·0)	0·75
Plateau pressure, cm H_2O	31 (26–34)	31 (28–34)	0·98
Positive end-expiratory pressure, cm H_2O	12 (12–15)	12 (11–15)	0·83
Compliance, mL/cm H_2O	24 (20–28)	27 (21–29)	0·68
White blood cells, $\times 10^9$ per L	9·9 (7·6–12·2)	10·6 (9·1–12·7)	0·30
Lymphocytes, $\times 10^9$ per L	0·8 (0·6–1·1)	1·1 (1·0–1·4)	0·06
Platelets, $\times 10^9$ per L	272 (216–314)	259 (197–314)	0·48
Albumin, g/L	23 (20–27)	24 (22–25)	0·96
Bilirubin, µmol/L	10 (6–21)	12 (8–28)	0·55
Creatinine, µmol/L	78 (63–130)	216 (104–275)	0·0217
Troponin, ng/L	18 (5–29)	23 (8–220)	0·34
Lactate dehydrogenase, units per L	439 (315–534)	597 (534–758)	0·0392
Procalcitonin, ng/mL	0·9 (0·4–2·9)	2·6 (1·6–10·5)	0·14
Fibrinogen, g/L	6·6 (6·0–6·8)	5·8 (5·4–6·8)	0·39
D-dimer, ng/mL	1601 (873–4081)	1643 (1126–3226)	0·91
Ferritin, µg/L	807 (422–1855)	2878 (1229–4225)	0·21
C-reactive protein, mg/L	206 (145–304)	255 (145–348)	0·78
Vasopressor use (baseline)	19 (61%)	5 (63%)	0·99*
Invasive ventilation (baseline)	28 (90%)	7 (88%)	0·76
Sequential organ failure assessment score	6 (5–8)	8 (6–10)	0·10
APACHE II score	12 (10–15)	17 (16–18)	0·0223
Mortality at day 28	12 (39%)	5 (63%)	0·26*

p values calculated by Wilcoxon signed-rank test unless noted otherwise. APACHE II=acute physiology and chronic health evaluation II. PaO_2/FiO_2=ratio of partial pressure of arterial oxygen to fractional concentration of oxygen in inspired air. *Fisher's exact test. †Includes Filipino and Romani.

Table 2: **Difference in baseline characteristics between hypoinflammatory and hyperinflammatory phenotypes using a probability cutoff of 0·274 (Youden index) to assign class**

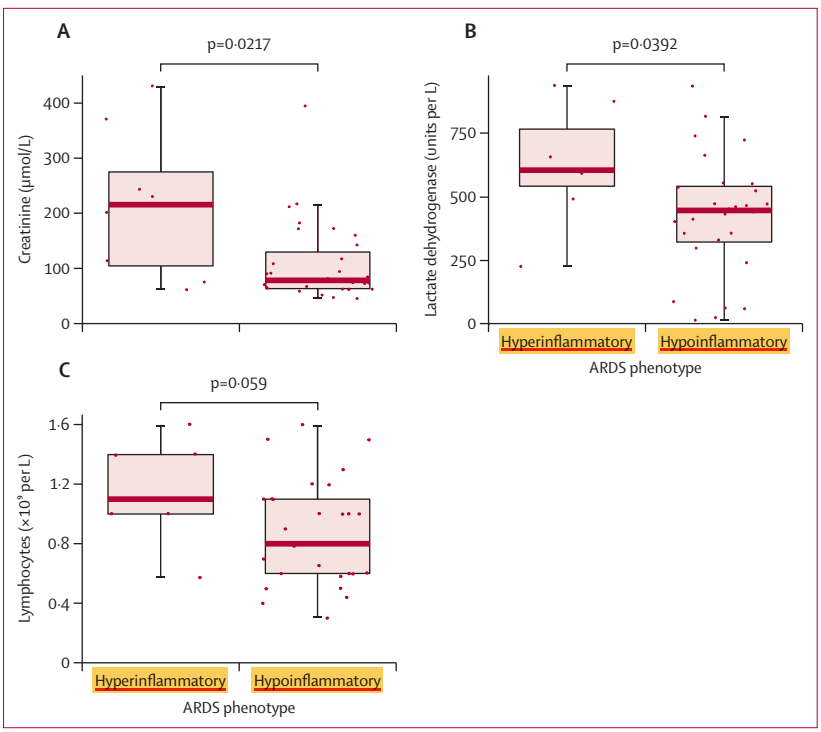

Figure 2: **Comparison of measures of creatinine, lactate dehydrogenase, and lymphocytes in the hyperinflammatory and hypoinflammatory phenotypes of COVID-19-associated ARDS**

Comparisons of creatinine (A), lactate dehydrogenase (B), and lymphocytes (C) between the hyperinflammatory and hypoinflammatory subgroups of the COVID-19 subset of the PHIND cohort. Phenotypes were assigned using the Youden index as the cutoff (≥0·274). Boxes show medians and IQRs; whiskers show the full range; and dots show individual observations. p values were calculated by Wilcoxon signed-rank test. ARDS=acute respiratory distress syndrome. PHIND=clinical evaluation of a point of care assay to identify PHenotypes IN the acute respiratory Distress syndrome.

hyperinflammatory classification of 0·03 (IQR 0·01–0·2), suggesting low prevalence of the phenotype in this population. Using a probability cutoff of 0·5 to assign phenotype, four (10%) of 39 patients were in the hyperinflammatory phenotype. With this cutoff, mortality at day 28 in the hyperinflammatory phenotype was 75% (three of four patients) and 40% (14 of 35 patients) in the hypoinflammatory phenotype (appendix p 5). Using the Youden index cutoff (0·274) to assign class led to eight patients (21%) being classified as the hyperinflammatory phenotype (table 2). It is worth noting that without LCA-derived phenotypes, it is not possible to ascertain which of the two cutoffs is more accurate. Given that more patients were in the hyperinflammatory phenotype using the Youden index cutoff, to enhance interpretability of comparative statistics, for the remainder of the manuscript only classifications using this cutoff are presented.

As with previous studies, baseline APACHE II score was higher in patients with the hyperinflammatory phenotype (17 [16–18]) than in those with the hypoinflammatory phenotype (12 [10–15]; p=0·0223). Five (63%) of eight individuals with the hyperinflammatory phenotype had died at day 28 compared with 12 (39%) of 31 individuals with the hypoinflammatory phenotype; the difference between the two groups was not statistically significant (p=0·26; table 2).

Baseline creatinine and lactate dehydrogenase were significantly higher in the hyperinflammatory than in the hypoinflammatory phenotype (figure 2A, B). Lymphocyte counts were not significantly different between the groups, but were slightly lower in individuals with the hypoinflammatory phenotype (figure 2C). Values of D-dimer (1601 ng/mL [873–4081] in the hypoinflammatory subgroup *vs* 1643 ng/mL [1126–3226] in the hyperinflammatory subgroup; p=0·91) and C-reactive protein (206 mg/dL [145–304] *vs* 255 mg/dL [145–348]; p=0·78) were similar between the phenotypes. Vital signs and respiratory variables at baseline were also similar between the two phenotypes (table 2). In contrast to previous studies, in which vasopressor use was consistently greater on the hyperinflammatory phenotype,[8–11] use was similar between the two phenotypes: five (63%) of eight patients in the hyperinflammatory subgroup used vasopressors versus 19 (61%) of 31 in the hypoinflammatory subgroup (p=0·99).

The entire HARP-2 cohort (n=539) had a similar age range (median 54 [IQR 42–66]) to the COVID-19 cohort (57 [52–61]). The median PaO_2/FiO_2 in HARP-2 was 15 kPa (11–21) compared with 18 kPa (15–21) in this study (p=0·07). Median APACHE II score in HARP-2 (18 [14–24]) was significantly higher than in this cohort (12 [10–16]; p<0·0001). Baseline PaO_2/FiO_2, sex, and age were used to match the COVID-19 cohort with patients in the HARP-2 cohort (n=39; appendix pp 6–7). Baseline characteristics of the entire HARP-2 cohort and the HARP-2 matched cohort are presented in the appendix (p 7). APACHE II score (p<0·0001; figure 3A) and soluble TNFR1 (p=0·0258; figure 3B) were significantly higher in the HARP-2 matched cohort than in our COVID-19 cohort; IL-6 (p=0·35; figure 3C) and creatinine (p=0·09; figure 3D) were similar between the two cohorts; and platelets (p=0·0068; figure 3E) were significantly higher in our cohort than in the HARP-2 matched cohort.

Despite the lower APACHE II score and similar PaO_2/FiO_2, mortality at day 28 in our COVID-19 cohort (17 [44%] of 39) was significantly higher than in the HARP-2 cohort (132 [24%] of 539; p=0·0128), and non-significantly higher than the HARP-2 matched cohort (11 [28%] of 39; p=0·16; table 3). Using the Youden index to assign phenotype, our COVID-19 cohort had a smaller proportion of patients classified in the hyperinflammatory phenotype (eight [21%] of 39) than both the entire HARP-2 cohort (186 [35%] of 539) and HARP-2 matched cohort (11 [28%] of 39). Mortality at day 28 in the hypoinflammatory phenotype in our COVID-19 cohort (12 [39%] of 31) was higher than in the two HARP-2 cohorts (59 [17%] of 353 in the whole cohort and six [21%] of 28 in the matched cohort; table 3). Notably, the mortality rate in the COVID-19 hypoinflammatory phenotype was similar to the rate in the hyperinflammatory phenotype in HARP-2 and HARP-2 matched

(table 3). By contrast, the hyperinflammatory phenotype in the COVID-19 cohort had higher mortality rates than all other groups (five [63%] of eight).

A sensitivity analysis was done by excluding the patients from University College Hospital and the findings were similar to those presented (data not shown).

Discussion

To our knowledge, this study is the first that has sought to identify the prevalence of previously described ARDS phenotypes in patients with COVID-19-associated ARDS. The findings of this preliminary study of 39 patients with COVID-19-associated ARDS suggest that the prevalence of the hyperinflammatory phenotypes was low in our cohort (10–21%). Mortality rates were about 20% higher in patients with the hyperinflammatory phenotype than in those with the hypoinflammatory phenotype, which is similar to previous findings for patients with ARDS. However, although the magnitude of difference in mortality between the phenotypes was consistent, the mortality rate for both phenotypes was considerably higher in the COVID-19 cohort than in historical ARDS data.[8–11] A second novel feature of the study was the use of a rapid point-of-care assay to quantify both IL-6 and soluble TNFR1, the levels of which were similar or lower in our patients with COVID-19-associated ARDS than in patients with ARDS in HARP-2.

The hyperinflammatory phenotype of ARDS is associated with higher circulating levels of proinflammatory biomarkers such as IL-6, IL-8, and soluble TNFR1 and lower levels of vitamin K-dependent protein C.[8–11] Further, this phenotype is associated with increased evidence of multiorgan failure and shock.[8–11] The low prevalence of the hyperinflammatory phenotype in COVID-19 ARDS challenges the hypothesis of the cytokine storm in its pathogenesis and suggests that it might not be as ubiquitous as purported, and might be less frequently encountered than in ARDS secondary to other causes.

The high mortality rate in the hypoinflammatory phenotype in COVID-19 is a notable and novel finding of this study. In previous studies, mortality in patients with the hypoinflammatory phenotype was about 20%.[8–11,16] However, the mortality of patients with COVID-19 and the hypoinflammatory phenotype in our study was nearly double that. Coupled with the lower burden of systemic inflammatory responses measured by IL-6 and TNFR1, the findings of higher mortality rates in COVID-19-associated ARDS suggests severity of pathogenesis not captured by these inflammatory biomarkers. The differences in mortality compared with patients with pneumonia in the HARP-2 matched cohort, in which the infective pathogen is more likely to be bacterial, might allude to the pathogenesis of SARS-CoV-2 and an absence of therapeutic options for source control in COVID-19 ARDS. A second factor to consider is whether attributable mortality in these patients differs. In ARDS unrelated to COVID-19, multiorgan failure is frequently encountered as the attributable factor for death,[17] whereas in COVID-19, reports suggest that a greater proportion of patients die because of respiratory failure,[13] a physiological abnormality that might be pathologically independent of systemic inflammation and subject to more localised injury to the lungs.

It is also worth noting that the APACHE II scores in our COVID-19 population were significantly lower than those in the HARP-2 cohort despite higher mortality in our cohort. All patients with COVID-19 in our study were managed in ICUs at surge capacity with a reduced nursing ratio, which might, in part, explain this finding. Overwhelmed ICU capacity might have an effect on

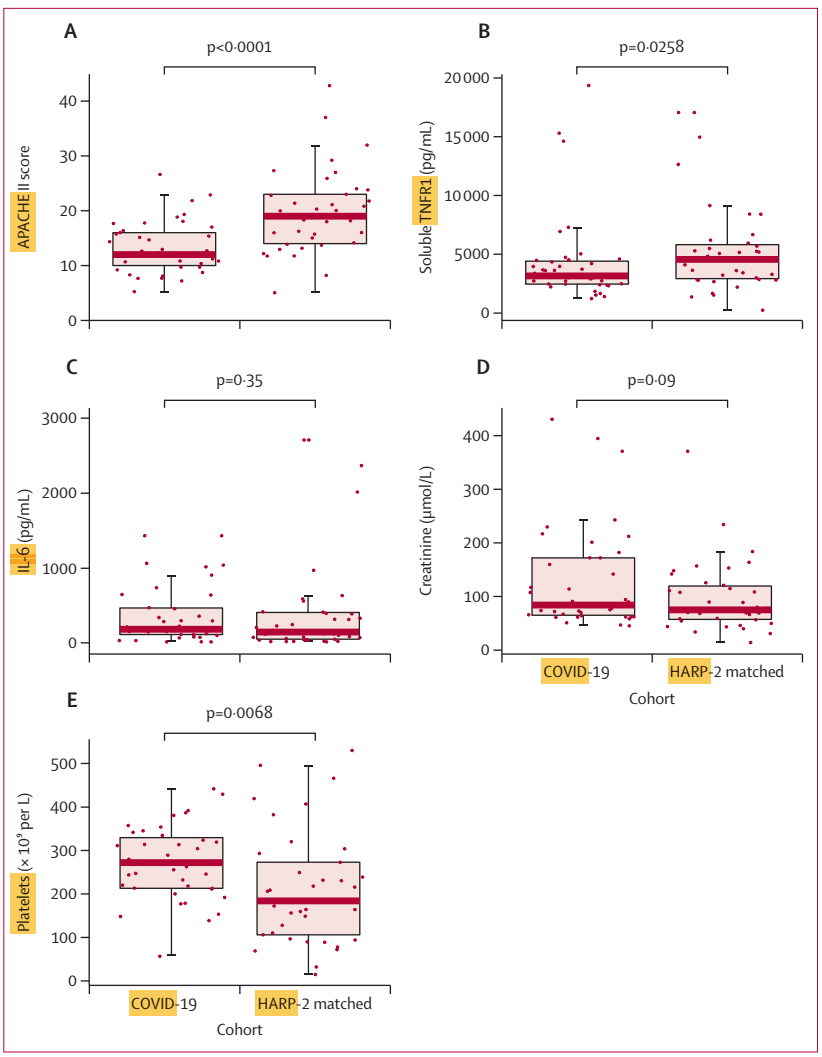

Figure 3: **Comparison of patient characteristics in the COVID-19-related ARDS cohort and the HARP-2[14] matched cohort**

Comparisons of APACHE II score (A) and measures of soluble TNFR1 (B), IL-6 (C), creatinine (D), and platelets (E) between the COVID-19 subset of the PHIND cohort and HARP-2 matched cohort. Boxes show medians and IQRs; whiskers show the full range; and dots show individual observations. p values were calculated by Wilcoxon signed-rank test. APACHE II=acute physiology and chronic health evaluation II. ARDS=acute respiratory distress syndrome. IL-6=interleukin 6. HARP-2=Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction. PHIND=clinical evaluation of a point of care assay to identify PHenotypes IN the acute respiratory Distress syndrome. TNFR1=tumour necrosis factor receptor superfamily member 1A.

	Total cohort		Hypoinflammatory		Hyperinflammatory	
	n	Mortality	n	Mortality	n	Mortality
HARP-2	539	132/539 (24%)	353/539 (65%)	59/353 (17%)	186/539 (35%)	73/186 (39%)
HARP-2 matched	39	11/39 (28%)	28/39 (72%)	6/28 (21%)	11/39 (28%)	5/11 (45%)
COVID-19	39	17/39 (44%)	31/39 (79%)	12/31 (39%)	8/39 (21%)	5/8 (63%)

Data are n or n/N (%). In HARP-2 and HARP-2 matched cohorts, the phenotypes were derived from the original latent class analysis studies. In the COVID-19 subset of the PHIND cohort, the phenotypes were derived using the parsimonious model using a probability cutoff of 0·274 (Youden index). HARP-2=Hydroxymethylglutaryl-CoA reductase inhibition with simvastatin in Acute lung injury to Reduce Pulmonary dysfunction. PHIND=clinical evaluation of a point of care assay to identify PHenotypes IN the acute respiratory Distress syndrome.

Table 3: **Comparison of mortality at day 28 between the HARP-2 cohort,[14] HARP-2 matched cohort, and COVID-19 PHIND cohort**

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outcomes in COVID-19 and lower mortality rates have been reported in ICUs that have operated under more conventional conditions and staffing ratios in patients with COVID-19 with similar APACHE II scores.[18,19] The low APACHE II scores are also in keeping with those reported by the Intensive Care National Audit and Research Centre in 9777 patients admitted to the ICU in the National Health Service hospitals in the UK,[20] where the median APACHE II score in patients with COVID-19 was 14 (IQR 11–18) and the mortality rate was greater than 40%. These consistent findings suggest that the APACHE II score might not be valid for prognostication in COVID-19. Taken together, the findings of the low APACHE II score and high mortality suggest that alternative phenotyping approaches might be needed to identify biologically and clinically homogeneous clusters using novel biomarkers that might, in turn, enhance our understanding of pathogenesis and improve prognostication in COVID-19-related ARDS.

One advantage of specifically studying the COVID-19 population is that the heterogeneity of the cause, a common feature of ARDS unrelated to COVID-19, is largely negated. Notably, the prevalence of vasopressor use at baseline was similar between patients with the hyperinflammatory phenotype and those with the hypoinflammatory phenotype, whereas in previous studies of ARDS unrelated to COVID-19, vasopressor use was significantly higher in those with the hyperinflammatory phenotype.[8–11] This might in part be explained by the fact that in previous studies, the risk factor for ARDS differed between the phenotypes with sepsis predominantly featuring in the hyperinflammatory phenotype. In COVID-19, given the uniformity of cause, it might be that there are additional drivers of vasopressor use that are disease specific and extraneous to inflammatory phenotypes, such as cardiovascular complications.[21]

It is also known that cause is an important determinant of the signature of circulating biomarkers.[22] For example, indirect causes of lung injury, such as sepsis, are associated with higher levels of endothelial injury, whereas direct lung injury is associated with higher levels of markers of epithelial injury.[23] Biomarkers pertaining to severity of epithelial injury and cell death might be more informative in COVID-19-associated ARDS because the primary source of injury is presumed to be a viral pneumonitis. In two recent case series of autopsies of patients with severe COVID-19, the only common findings in all patients across both studies was diffuse alveolar damage.[24,25] However, this theory remains speculative, and it stands to reason that before phenotyping, comprehensive typing of COVID-19 and its biological signature using data is needed, preferably from large multinational collaboratives such as ISARIC 4C by the International Severe Acute Respiratory and Emerging Infection Consortium.

Another strength of this study has been to show the logistical feasibility of rapid point-of-care phenotyping of patients in a busy ICU using a novel bioanalyser. Precision-based care has been a promising yet elusive opportunity in critical care medicine.[26] Although other specialties have more time, in the ICU, any phenotype-based decisions need to be made rapidly. The time taken to do ELISA-based assays is prohibitive in the clinical implementation of biomarker-driven phenotypes.[22] Using this novel solid state-based analysing technology, we were able to classify patients into biomarker-driven phenotypes in less than 1 h from sample acquisition. Bicarbonate can be easily measured using standard clinical laboratory assays. The availability of such assays has important implications for future precision medicine studies in critical care.

Paradoxically, this strength is also a limitation of the study. The larger PHIND study, from which this COVID-19 subset was derived, was designed to further validate the point-of-care platform. The platform has only been validated using stored plasma samples, and its performance using real samples from patients in the ICU is yet to be formally validated. Given this uncertainty, the findings of this study should be interpreted with caution. The clinically measured biomarker component of the model, namely bicarbonate, can often be informative of the validity of the distribution of the phenotypes. In a previous ARDS cohort,[11] in which the prevalence of the hyperinflammatory phenotype was 37%, the mean serum bicarbonate level was 22 mmol/L (SD 6) compared with the 27 mmol/L (6) in our COVID-19 cohort. On the basis of this comparison, the estimated prevalence of the hyperinflammatory phenotype between 10% and 20% in this cohort seems accurate.

The key limitation of this study is the small sample size. The even smaller number in the hyperinflammatory phenotype and the observed sample size imbalance when comparing phenotypes makes comparative statistics difficult to interpret, and differences between groups must be interpreted with caution. A further limitation of the study is that it is focused on baseline data only for phenotype classification. The natural progression of COVID-19 over time might lead to changing phenotypes and requires further study. Another important limitation is that only circulating levels of two biomarkers were studied, whereas in previous work we studied six to eight protein biomarkers. Inflammatory markers might differ more substantially in the lungs. In addition, if a larger number of plasma inflammatory biomarkers were studied in a larger population, more distinct patterns of differences in the inflammatory response might have been detected. Further, we were unable to validate the biomarkers quantified using the Multistat analyser against conventional ELISAs because of an absence of stored plasma samples from patients with COVID-19. Future studies of COVID-19 pneumonia, where feasible, should study the circulating plasma and lung compartments simultaneously and over the course of COVID-19 critical illness.

In summary, in this small exploratory analysis of 39 patients, the prevalence of the hyperinflammatory phenotype in patients with COVID-19-associated ARDS was lower than in patients with ARDS unrelated to COVID-19 in a previous study. This finding suggests that, compared with other causes of ARDS, the excessive mortality in COVID-19-related ARDS is unlikely to be due to upregulation of the inflammatory pathways described by the parsimonious model. Finally, with the caveat that the findings require validation with LCA-derived phenotypes, the point-of-care platform used to classify phenotypes at the bedside shows the feasibility of phenotype-informed trials in the ICU.

Contributors

All authors conceived and designed the study. SCh, DB, SCu, CKin, CKil, OR, YC, CB, and TS collected the data. All authors contributed to data analysis and interpretation. PS drafted the manuscript and all authors contributed to revisions and approved the final version.

Declaration of interests

CSC reports grants from the US National Institutes of Health (NIH), during the conduct of the study; grants from Roche/Genentech and Bayer, outside the submitted work; and personal fees for consultancy from Quark Pharmaceuticals, Vasomune, and Gen1e Life Sciences, outside the submitted work. ACG reports funding through a UK National Institute for Health Research (NIHR) Research Professorship, during the conduct of the study; and fees for consultancy, paid to his institution, from Bristol-Meyers Squibb and GlaxoSmithKline (GSK), outside the submitted work. CMO'K reports grants from Innovate UK, during the conduct of the study; and grants from NIHR, the Wellcome Trust, the UK Medical Research Council, Northern Ireland (NI) Health and Social care R&D Division, NI Chest Heart and Stroke, and Medical Research Council, outside the submitted work. DFM reports a grant from Innovate UK for the conduct of the phenotypes in the acute respiratory distress syndrome (PHIND) study; and personal fees for consultancy from GSK, Boehringer Ingelheim, and Bayer, outside the submitted work. DFM's institution has received grants from the NIHR, the Wellcome Trust, NI Health and Social care R&D Division, NI Chest Heart and Stroke, and Medical Research Council; DFM has a patent issued to his institution for a treatment for acute respiratory distress syndrome. DFM is a Director of Research for the Intensive Care Society and NIHR Efficacy and Mechanism Evaluation Programme Director. All other authors declare no competing interests.

Data sharing

Requests for data will be reviewed on an individual basis by the PHIND chief investigator (DFM) and the Clinical Trials Unit (CTU). After the publication of primary and secondary outcomes from the overall PHIND trial, requests for data should be made in writing to the chief investigator via the CTU, who will discuss such requests with the study sponsor.

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