Acute respiratory distress syndrome phenotypes with distinct clinical outcomes in PHARLAP trial cohort

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Distinct phenotypes of acute respiratory distress syndrome (ARDS) have been identified (hypo-inflammatory and hyperinflammatory), and are associated with different outcomes and treatment responses in secondary analyses of completed clinical trials.[1-3] More recently, parsimonious models for classifying ARDS phenotypes have been described; three randomised controlled trial (RCT) cohorts from the National Lung, Heart, and Blood Institute ARDS Network were used as the derivation dataset (n = 2022), and a fourth RCT was used as the validation test set (n = 715).[4] From the nested models, three-variable (interleukin-8 [IL-8], bicarbonate and protein C) and four-variable (IL-8, bicarbonate, protein C and vasopressor use) models were adjudicated to be the best performing. Identification of phenotypes in ARDS may improve our understanding of the syndrome and inform design of future clinical trials and clinical management.

The Permissive Hypercapnia, Alveolar Recruitment and Low Airway Pressure (PHARLAP) study was an RCT that compared open lung ventilation (OLV) with maximal lung recruitment against control ventilation in patients with moderate-to-severe ARDS. It found that maximal lung recruitment did not reduce the number of ventilator-free days (VFDs) or mortality, and was associated with increased cardiovascular adverse events but lower use of hypoxaemic adjuvant therapies.[5] The study was stopped early (after enrolling 115 of the planned 340 patients) following release of results from the Alveolar Recruitment for Acute Respiratory Distress Syndrome Trial, which showed increased mortality with OLV and lung recruitment, in contrast with previously available analysis showing possible benefits with OLV. [6-8] However, no mechanistic explanation of these potentially contrasting findings was reported.

The PHARLAP trial included a pre-planned substudy that would examine epithelial,

ABSTRACT

Background: The Permissive Hypercapnia, Alveolar Recruitment and Low Airway Pressure (PHARLAP) randomised controlled trial compared an open lung ventilation strategy with control ventilation, and found that open lung ventilation did not reduce the number of ventilator-free days (VFDs) or mortality in patients with moderate-to-severe acute respiratory distress syndrome (ARDS). Parsimonious models can identify distinct phenotypes of ARDS (hypo-inflammatory and hyperinflammatory) which are associated with different outcomes and treatment responses.

Objective: To test the hypothesis that a parsimonious model would identify patients with distinctly different clinical outcomes in the PHARLAP study.

Design, setting and participants: Blood and lung lavage samples were collected in a subset of PHARLAP patients who were recruited in Australian and New Zealand centres. A previously validated parsimonious model (interleukin-8, soluble tumour necrosis factor receptor-1 and bicarbonate) was used to classify patients with blood samples into hypo-inflammatory and hyperinflammatory groups. Generalised linear modelling was used to examine the interaction between inflammatory phenotype and treatment group (intervention or control).

Main outcome measure: The primary outcome was number of VFDs at Day 28.

Results: Data for the parsimonious model were available for 56 of 115 patients (49%). Within this subset, 38 patients (68%) and 18 patients (32%) were classified as having hypo-inflammatory and hyperinflammatory phenotypes, respectively. Patients with the hypo-inflammatory phenotype had more VFDs at Day 28 when compared with those with the hyperinflammatory phenotype (median [IQR], 19.5 [11–24] versus 8 [0–21]; P = 0.03). Patients with the hyperinflammatory phenotype had numerically fewer VFDs when managed with an open lung strategy than when managed with control "protective" ventilation (median [IQR], 0 [0–19] versus 16 [8–22]).

Conclusion: In the PHARLAP trial, ARDS patients classified as having a hyperinflammatory phenotype, with a parsimonious three-variable model, had fewer VFDs at Day 28 compared with patients classified as having a hypo-inflammatory phenotype. Future clinical studies of ventilatory strategies should consider incorporating distinct ARDS phenotypes into their trial design.

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endothelial and inflammatory markers with serial measurements of lung lavage samples and serum. Although the lack of observed clinical benefit in the PHARLAP study may reflect no difference between groups, or inadequate power to detect a difference, we hypothesised that differences in phenotypic responses could have contributed to this result.

To date, the biomarker characteristics of the two phenotypes have only been studied in plasma. The differences in the biomarker signatures of the phenotypes in the lung compartment are unknown. We hypothesised that inflammatory burden in bronchoalveolar lavage (BAL) samples would reflect that observed in plasma. To test this, we used serum and BAL samples from the pre-planned PHARLAP substudy cohort to define hypo-inflammatory and hyperinflammatory phenotypes and examine outcomes.

Methods

The PHARLAP trial was a randomised, controlled, parallel group trial conducted in patients with moderate-to-severe ARDS who were randomly assigned to maximal lung recruitment with titrated positive end expiratory pressure and further tidal volume limitation, or to control "protective" ventilation. The trial conduct and design have been described previously.[9] This substudy was conducted only in participants recruited in Australian and New Zealand centres. Informed consent was obtained from each patient's next of kin as part of consent for the study. Blood samples for this pre-specified substudy were collected before randomisation (for 58 out of the 115 patients) and at Day 3 (for 49 patients). Lung lavage samples were collected on Day 1 and Day 3 for 34 and 22 patients, respectively. The primary outcome of this analysis was number of VFDs at Day 28, as in the main study. Ethics approval for this substudy was obtained from Monash University and local ethics committees at trial sites.

Sample collection

For each patient, a 10 mL blood sample was obtained and collected in a serum-separating tube (Greiner Bio-One) on Day 1 and Day 3 following enrolment. In addition, these patients underwent a bronchoscopic lavage or mini-bronchoscopy using the KimVent bronchial aspirate sampling catheter (Kimberley-Clark), with 60 mL of sterile saline instilled into a wedged subsegmental middle lobe or lingula bronchus, while under sedation and mechanically ventilated in the intensive care unit (ICU). BAL samples were stored at 4°C for less than 1 h before processing. Blood was allowed to clot at room temperature for 20–30 min before centrifugation at 1000 g for 10 min at 4°C. BAL samples were centrifuged at 150 g for 5 min at 4°C to pellet cells. Supernatant from serum samples and BAL samples was aliquoted and stored at –80°C at each participating site until transfer. When study sample collection was complete, aliquots were transported on dry ice by commercial carrier to the Intensive and Critical Care Unit laboratory at Flinders Medical Centre, Adelaide, Australia, where they were stored at –80°C until a blinded, random, batch analysis of all samples was conducted.

Cytokine measurements

Supernatant from serum samples and BAL samples was assayed for angiopoietin-1, angiopoietin-2, interleukin 6 (IL-6), IL-8, tumour necrosis factor-- (TNF-α), soluble tumour necrosis factor receptor-1 (sTNFR1) (R&D Systems) and prosurfactant protein B (pSP-B) (CPR Pharma Services) using individual double antibody sandwich enzyme-linked immunosorbent assays (ELISAs), as per the manufacturer's instructions, as previously described.[10] BAL sample supernatant was also analysed for total soluble protein (Micro BCA Protein Assay Kit, Thermo Fisher Scientific). Limits of detection were 10, 45, 12, 3, 2, 2, 2 and 15 pg/mL, respectively. Bicarbonate levels were taken from the first blood gas analysis performed after randomisation.

Statistical analysis

We performed all analyses using SAS 9.4 (SAS Institute). We used a validated parsimonious model to identify ARDS phenotypes in the PHARLAP dataset.[4] As the use of vasopressors was protocolised in the PHARLAP study, we did not analyse the models that used vasopressor use as a classifier variable. The three classifier variables that we included in this study were IL-8, bicarbonate and sTNFR1. We applied model coefficients of these variables to the PHARLAP dataset to estimate predicted probabilities via logistic regression, which we then used to identify two distinct phenotypes. We used a probability cut-off of ≥ 0.5 to assign hyperinflammatory phenotype, and we used probabilities of < 0.5 to assign hypo-inflammatory phenotype. We assessed the interaction between inflammatory phenotype and treatment group on number of VFDs using generalised linear models fitting main effects for treatment group, phenotype and their two-way interactions. We compared baseline characteristics, cytokine measurements and outcome variables between groups using the student *t* test for normally distributed continuous variables, Wilcoxon rank-sum test for non-normally distributed continuous variables, and -χ2 or Fisher exact test as appropriate for categorical variables. We present results as mean (standard deviation), median (interquartile range) and number (percentage), respectively. We did not make adjustments to account for

Table 1. Baseline characteristics of patients included in the study when categorised as having a hyperinflammatory or hypo-inflammatory phenotype*

	Hyperinflammatory (n = 18)	Hypo-inflammatory (n = 38)	*P*
Age (years)	53.4 (12.9)	51.2 (15.0)	0.61
Height (cm)	168 (7.3)	173 (9.3)	0.08
Weight (kg)	81.1 (14.7)	90.2 (24.7)	0.16
APACHE II score	24.6 (6.5)	21.2 (6.7)	0.08
Blood gas pH	7.28 (0.10)	7.38 (0.09)	0.001
Blood gas PaO_2 (mmHg)	73.2 (8.3)	73.4 (14.0)	0.96
Blood gas $PaCO_2$ (mmHg)	47.8 (11.6)	48.4 (13.8)	0.87
Blood gas SaO_2	93.2 (2.86)	93.9 (2.91)	0.42
Blood gas FiO_2	0.60 (0.16)	0.58 (0.19)	0.80
Blood gas lactate (mmol/L)	2.15 (1.70–2.90)	1.30 (1.00–1.40)	< 0.0001
Blood gas base excess (mmol/L)	–6.0 (–7.2 to –2.0)	1.5 (–2.0–5.0)	< 0.0001
PaO_2 to FiO_2 ratio	129 (32.9)	134 (33.8)	0.63
SOFA score	10.7 (2.76)	6.68 (2.61)	< 0.0001
ARDS – pulmonary	13 (72%)	30 (79%)	0.58
Time since ARDS onset	12.10 (1.99–21.90)	13.30 (4.21–29.00)	0.31
Respiratory system compliance (mL/cmH_2O)†	49 (38–66)	31 (25–43)	0.22
Radiological subtype focal	14 (78%)	25 (66%)	0.75

APACHE = Acute Physiology and Chronic Health Evaluation. ARDS = acute respiratory distress syndrome. FiO_2 = fraction of inspired oxygen. PaO_2 = partial pressure of oxygen (arterial). $PaCO_2$ = partial pressure of carbon dioxide (arterial). SaO_2 = oxygen saturation (arterial). SOFA = Sequential Organ Failure Assessment. * Data are presented as mean (SD), median (interquartile range) or number (%) based on their distribution; the Fisher exact test, $\chi 2$ square test, Wilcoxon rank-sum test and student *t* test were used for comparisons. † Data were only available for seven patients.

Table 2. Comparison of patients with the hypo-inflammatory phenotype and the hyperinflammatory phenotype*

	Hyperinflammatory (n = 17)	Hypo-inflammatory (n = 38)	*P*
Ventilator-free days at Day 28	8 (0–21)	19.5 (11–24)	0.03
Total duration of mechanical ventilation (h)	173 (96–475)	140 (90–305)	0.35
Intensive care unit length of stay (h)	212 (99–601)	214 (123–362)	0.96
Hospital length of stay (days)	11.0 (4.1–44.3)	14.8 (7.2–22.1)	0.91
Intensive care unit death	5 (29%)	5 (13%)	0.25
Hospital death	5 (29%)	6 (16%)	0.29
Mortality at Day 90	5 (29%)	6 (16%)	0.29

* Data are median (IQR) or number (%) based on their distribution; the Fisher exact test, $\chi 2$ square test, Wilcoxon rank-sum test and student *t* test were used for comparisons.

multiple outcome comparisons. We also compared blood and BAL sample markers in patients based on a median number of VFDs of 15 (good versus bad outcome). We used a two-sided *P* value of 0.05 to indicate statistical significance.

Results

We found no significant baseline differences between the 58 patients for whom Day 1 blood samples were available and the 56 for whom Day 1 samples were not available (Supporting Information, Table 1). Data for the parsimonious model (IL-8, bicarbonate and sTNFR1) were available for 56 patients. Data for the parsimonious model and clinical outcomes (VFDs) were available for 55 patients, of whom 28 had been randomly assigned to the PHARLAP intervention and 27 had been assigned to the control group (Supporting Information, Figure 1).

Using the parsimonious model with three variables (IL-8, bicarbonate and sTNFR1), 38 patients (68%) were categorised as having the hypo-inflammatory phenotype and 18 patients (32%) were categorised as having the hyperinflammatory phenotype. Comparing the baseline characteristics of these groups (Table 1), patients in the hyperinflammatory group had lower blood gas pH, higher blood gas lactate levels and higher Sequential Organ Failure Assessment scores. However, there were no significant differences between groups for any of the following: Acute Physiology and Chronic Health Evaluation II score at admission, pulmonary and extrapulmonary subtypes, respiratory system compliance, and presence of focal versus diffuse radiological subtypes.

Table 3. Serum sample results for patients with the hypo-inflammatory and hyperinflammatory phenotypes, based on a three-variable parsimonious model (interleukin-8, bicarbonate and sTNFR1)*

	Hyperinflammatory	Hypo-inflammatory	*P*
Day 1			
Number of patients	18	38	–
Angiopoietin-1 (ng/mL)	26.2 (14.4–34.9)	30.4 (19–50.5)	0.18
Angiopoietin-2 (ng/mL)	21.3 (13.4–33.6)	8.2 (5.4–15)	< 0.001
Angiopoietin-1 to angiopoietin-2 ratio	1.42 (0.43–2.76)	3.69 (1.82–6.33)	0.001
Interleukin-6 (pg/mL)	1097 (326–2075)	131 (47–294)	< 0.0001
Interleukin-8 (pg/mL)†	274 (188–807)	57.5 (36–96)	< 0.0001
Tumour necrosis factor-α (pg/mL)	30 (20–53)	16.5 (8–43)	0.07
pSP-B (µg/mL)	113 (38.1–218)	329 (100–518)	0.019
sTNFR1 (pg/mL)†	11 900 (5 100–17 500)	3 950 (2 700–5 700)	< 0.0001
Day 3			
Number of patients	12	33	–
Angiopoietin-1 (ng/mL)	19.2 (7.5–40.2)	40.2 (20.2–56.8)	0.06
Angiopoietin-2 (ng/mL)	12.6 (8.6–22.4)	6.5 (4.2–8.9)	0.022
Angiopoietin-1 to angiopoietin-2 ratio	1.64 (0.63–3.06)	4.93 (1.99–8.20)	0.004
Interleukin-6 (pg/mL)	116 (56.5–175)	69.0 (27.0–234)	0.60
Interleukin-8 (pg/mL)†	118 (49.5–166)	48.0 (26.0–88.0)	0.048
Tumour necrosis factor-α (pg/mL)	17.5 (10.5–35.5)	12 (6–67)	0.45
pSP-B (µg/mL)	90.8 (29.8–272)	287 (112–567)	0.018
sTNFR1 (pg/mL)	10 600 (5 300–20 500)	3 850 (2 600–6 250)	0.001

pSP-B = prosurfactant protein B. sTNFR1 = soluble tumour necrosis factor receptor-1. * Data are median (interquartile range) based on their distribution, unless otherwise specified, and the Wilcoxon rank-sum test was used for comparisons. † Included in the parsimonious model.

Differences between phenotypes in terms of numbers of VFDs

Patients with the hypo-inflammatory phenotype had more VFDs at Day 28 compared with those in the hyperinflammatory group (median [IQR], 19.5 [11–24] *v* 8 [0–21]; *P* = 0.03). Mortality at Day 90 was 29% in the hyperinflammatory group compared with 16% in the hypo-inflammatory group (*P* = 0.29). There were no differences in ICU or hospital length of stay between phenotypes (Table 2).

We found no interaction between the treatment group (PHARLAP intervention *v* control) and inflammatory phenotype (*P* = 0.08). Patients with the hyperinflammatory phenotype who were managed with an open lung strategy had numerically fewer VFDs than those with the hyperinflammatory phenotype who were managed with control protective ventilation (median [IQR], 0 [0–19] *v* 16 [8–22] VFDs). In the hypo-inflammatory phenotype, numbers of VFDs were similar in both groups (median [IQR], 19.5 [11–23] *v* 20 [12–24]) (Supporting Information, Table 2).

Differences between phenotypes on Days 1 and 3

Data for the parsimonious model were available for 56 and 45 participants for Days 1 and 3, respectively. The hyperinflammatory group had higher Day 1 angiopoietin-2 levels, corresponding lower angiopoietin-1 to angiopoietin-2 ratios, and higher IL-6 levels; however, pSP-B levels were lower in the hyperinflammatory group. Similar findings were seen on Day 3 (Table 3). When comparing changes in values from Day 1 to Day 3, there were larger percentage decreases in IL-6, IL-8 and TNF-α levels in the hyperinflammatory group compared with the hypo-inflammatory group (Supporting Information, Table 3).

BAL samples were available for 34 and 22 participants for Days 1 and 3, respectively. BAL levels of angiopoietin-2 and sTNFR1 at Day 1 were higher in the hyperinflammatory group, and by Day 3, IL-8 levels were higher in the hyperinflammatory group (Table 4). There were no differences in any of the other examined markers including total protein. There were also no differences between groups in the percentage changes of these markers from Day 1 to Day 3 (Supporting Information, Table 4).

Blood and BAL sample markers examined based on numbers of VFDs

When compared based on the study primary outcome (a median of 15 VFDs), patients who had a lower number of VFDs (< 15 days) had higher serum IL-8 levels on Day 1 (Supporting Information, Table 5). However, IL-6 and TNF-α levels from BAL samples taken on Day 1 were higher in patients who had a higher number of VFDs (Supporting Information, Table 6).

Table 4. Bronchoalveolar lavage sample results for patients with the hypo-inflammatory and hyperinflammatory phenotypes, based on a three-variable parsimonious model (interleukin-8, bicarbonate and sTNFR1)*

	Hyperinflammatory	Hypo-inflammatory	*P*
Day 1			
Number of patients	11	23	–
Angiopoietin-1(ng/mL)	0.19 (0.04–1.73)	0.12 (0.02–0.46)	0.33
Angiopoietin-2 (ng/mL)	0.84 (0.44–3.04)	0.29 (0.03–0.57)	0.02
Interleukin-6 (pg/mL)	4542 (647–9690)	724 (405–1860)	0.14
Interleukin-8 (pg/mL)	1011 (824–2242)	824 (272–1307)	0.09
Tumour necrosis factor-α (pg/mL)	24 (4–321)	4 (4–27)	0.28
pSP-B (µg/mL)	115 (51.6–215)	75.3 (11.3 – 148)	0.32
sTNFR1 (pg/mL)	1920 (595–4822)	668 (256–973)	0.02
Total protein (µg/mL)	1647 (1194–8318)	1550 (995–3351)	0.48
Day 3			
Number of patients	7	15	
Angiopoietin-1(ng/mL)	0.16 (0.06–0.46)	0.08 (0.02–0.66)	0.57
Angiopoietin-2 (ng/mL)	0.175 (0.09–0.70)	0.025 (0.025–0.416)	0.11
Interleukin-6 (pg/mL)	107 (29–3286)	182 (3–721)	0.62
Interleukin-8 (pg/mL)	899 (397–1167)	349 (50–488)	0.03
Tumour necrosis factor-α (pg/mL)	4 (4–20)	4 (2–22)	0.53
pSP-B (µg/mL)	50.6 (10.7–94.8)	42.6 (6.3–78.5)	0.44
sTNFR1 (pg/mL)	177 (127–6412)	139 (66–521)	0.22
Total protein (µg/mL)	1191 (212–6116)	490 (111–2286)	0.48

pSP-B = prosurfactant protein B. sTNFR1 = soluble tumour necrosis factor receptor-1. * Data are median (interquartile range) based on their distribution, unless otherwise specified, and the Wilcoxon rank-sum test was used for comparisons.

Discussion

By using a previously validated parsimonious model, we identified two ARDS phenotypes — in a subset of patients from Australian and New Zealand PHARLAP study sites — that had distinct clinical characteristics and potentially divergent clinical outcomes. These findings complement the results of previous studies of inflammatory ARDS phenotypes.[1-4] Inflammatory biomarker levels were higher in BAL samples of the patients with hyperinflammatory phenotype.

Two findings from our study are particularly important. The first is the finding that ARDS phenotypes may be generalisable to the Australian ARDS population with similar biological characteristics and prognostic information. Second, from a biological standpoint, the inflammatory circulating responses that differentiate ARDS phenotypes are also detectable in the alveolar compartment. Incorporating these findings of distinct ARDS phenotypes into the designs of future clinical trials is therefore worth considering.

Previous studies have explored evidence of differential treatment response between ARDS subgroups. Physiological approaches include stratification based on ventilatory parameters and PaO_2/FiO_2 ratio (ratio between partial pressure of oxygen [arterial] and fraction of inspired oxygen),[11,12] and PaO_2/FiO_2 ratio has already been employed in clinical trials.[13] Clinical approaches include stratification based on ARDS risk factors, pulmonary versus extrapulmonary ARDS,[14] and direct versus indirect ARDS.[15] Moreover, radiological subtypes have been described, with reports of focal and diffuse ARDS differing in terms of clinical outcomes and response to PEEP.[16-18] More recently, distinct physiologically derived phenotypes such as low lung weight and low lung elastance (L phenotype) and high lung weight and high elastance (H phenotype) with and without coronavirus disease 2019 (COVID-19) ARDS have been suggested,[19-22] although this awaits future validation.

Examining patients in more biologically homogeneous subsets may provide the opportunity to target specific biological mechanisms and thereby enrich clinical studies.[23] The ARDS phenotypes described by the parsimonious model that we applied in our analysis have been reported to have different treatment responses in secondary analyses of randomised interventions, namely PEEP and fluid-management strategies in the ALVEOLI (Assessment of Low Tidal Volume and Elevated End-Expiratory Volume to Obviate Lung Injury) trial and FACTT (Fluid and Catheter Treatment Trial), respectively.[12,24,25] Although the interaction was not significant, the PHARLAP findings are potentially consistent with a differential response to PEEP and recruitment in patients with the hyperinflammatory subtype, which requires further investigation. Similar findings have been seen with the use of statins in ARDS, where simvastatin was found to be beneficial only in the hyperinflammatory group on secondary analysis of the completed HARP-2 trial.[26] However, no treatment effect was seen with the use of rosuvastatin for acutely injured lungs due to sepsis,

which may indicate different effects with different statins.[27] Our findings are also similar to those reported following the application of latent class analysis to two National Heart, Lung, and Blood Institute ARDS Network trials, ARMA and ALVEOLI,[12,28] where the hyperinflammatory phenotype constituted about 30% of the population and was characterised by amplified inflammatory signals, increased prevalence of shock, fewer VFDs and higher mortality.

Biomarker patterns are known to differ between subgroups, with lower levels of circulating markers of endothelial injury (eg, angiopoietin-2) and higher levels of markers of epithelial injury (eg, surfactant protein D) in direct ARDS.[15] Similarly, soluble receptor for advanced glycation end products (sRAGE) and plasminogen activator inhibitor-1 (PAI-1) levels were significantly higher in patients with non-focal ARDS.[29] Although we did not find any difference in focal radiological subtype of ARDS in our study, we did find an increase in angiopoietin-2 but a lower level of pSP-B in the hyperinflammatory phenotype.

We also report novel findings of higher angiopoietin-2, sTNFR1 and IL-8 levels in lung lavage samples in the hyperinflammatory group, which provides insights into the inflammatory phenotypes in the lung compartment. Moreover, we report that levels of angiopoietin-2, a marker of endothelial injury and inflammation, were higher in the hyperinflammatory group, but epithelial injury as indicated by pSP-B levels was higher in the hypo-inflammatory group.[30-33] Angiopoietin-2 levels increase during liberal fluid therapy, which is associated with worse outcomes in patients with acute lung injury,[34] while the ratio of angiopoietin-1 to angiopoietin-2 in serum predicts mortality in patients with lung injury.[35,36] This increase in angiopoietin-2 has previously been reported in serum samples of a hyperinflammatory group.[25] Conversely, pSP-B, a lung-specific protein secreted into the alveolar epithelial lining fluid by pulmonary epithelial type II cells, appears in the bloodstream in increased amounts in patients with ARDS,[32,33] suggesting epithelial injury.[37] The finding of higher pSP-B in the hypo-inflammatory group may therefore indicate local alveolar injury, rather than systemic inflammation as seen in the hyperinflammatory group, which may be perpetuated by the use of recruitment manoeuvres. However, the difference in pSP-B levels was not evident in the alveolar compartment (BAL), necessitating examination of other markers of epithelial injury (eg, Club cell secretory protein [CC16]) and apoptosis (eg, Fas and Fas ligand) in future studies.[38-40]

Changes in these biomarkers from Day 1 to Day 3 were also different between groups. Larger percentage changes in IL-6, IL-8 and TNF-α levels in the hyperinflammatory group compared with the hypo-inflammatory group suggest a difference in temporal trajectory between the groups.

Our study has multiple strengths. We have: confirmed the presence of hyperinflammatory and hypo-inflammatory phenotypes in a novel and diverse cohort of ARDS patients with lower mortality; provided insights into alveolar inflammation by examining the lung lavage fluid in these phenotypes; examined the temporal changes in these markers in blood; and examined the effect of an intervention with these patients. However, our study also has some limitations. As the PHARLAP study was terminated early, the available sample size was small, and blood and BAL samples were collected only from patients recruited in Australia and New Zealand, although there were minimal differences between the patients' characteristics when those with and without available samples were compared. Finally, the lavage timing and protein levels indicate that patients included in our study were in the early phase of ARDS, so our findings are not applicable to patients in other phases of ARDS.[41] Future studies should explore differential effects of ventilatory strategy by ARDS phenotype, and development of point-of-care testing should be examined for future testing with these variables.[2]

Conclusion

In the PHARLAP study, ARDS patients classified as having a hyperinflammatory phenotype with a parsimonious three-variable model had fewer VFDs at Day 28 compared with patients classified as having a hypo-inflammatory phenotype. When future clinical studies of ventilatory strategies are being planned, researchers should consider incorporating these findings of distinct ARDS phenotypes into their trial designs.

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Competing interests

No relevant disclosures.

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