

Bedside identification of subphenotypes in acute respiratory failure (PHIND): a multicentre, observational cohort study



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Summary

Background Acute respiratory distress syndrome (ARDS) is a clinically defined, biologically heterogeneous condition with no proven disease-modifying therapies. Retrospective analyses have identified two biologically distinct subphenotypes (hyperinflammatory and hypoinflammatory) of ARDS, with differing outcomes and responses to therapy. Rapid identification of these subphenotypes in an actionable timeframe has previously not been possible. The PHIND study aimed to prospectively identify these subphenotypes and to demonstrate differing 60-day mortality.

Methods The PHIND study was a prospective, multicentre, observational cohort study conducted in intensive care units (ICUs) within the National Health Service in the UK and the Health Service Executive in Ireland. Adult patients aged 18 years and older with ARDS or acute hypoxaemic respiratory failure (AHRF) were enrolled within 72 h of onset of the syndrome. Eligible patients were required to be receiving invasive mechanical ventilation, non-invasive ventilation, or high-flow nasal oxygen. Plasma interleukin (IL-6) and soluble TNF receptor-1 (TNFR1) were quantified at enrolment using a near-patient benchtop immunoanalyser (Randox multiSTAT) with a run time of approximately 1 h. Together with plasma bicarbonate measured from an arterial blood sample, these values were used to prospectively determine subphenotypes on an individual patient basis using a validated parsimonious logistic regression model. The primary outcome was 60-day mortality. The study was registered on ClinicalTrials.gov, NCT04009330.

Findings Between Nov 22, 2019, and Sept 28, 2023, 1853 patients from 30 centres were screened for eligibility. Of these, 1328 were excluded and 525 were recruited into the study, with 512 individuals included. 308 (60%) patients were male, 204 (40%) were female, and mean age was 57·0 years (SD 15·1). 443 (87%) patients were white, 18 (4%) were Black, and 16 (3%) were Asian. 490 were subphenotyped using the near-patient assay: 89 (18%) were classified as hyperinflammatory and 401 (82%) as hypoinflammatory. The primary outcome of 60-day mortality was measured in 486 patients after four patients withdrew consent for confirmation of vital status. 60-day mortality was significantly higher in the hyperinflammatory group (45 [51%] of 88) than in the hypoinflammatory group (111 [28%] of 398; risk ratio 1·8 [95% CI 1·4–2·4], $p < 0·0001$). After adjustment, hyperinflammatory patients had increased odds of 60-day mortality (adjusted odds ratio 2·7 [95% CI 1·6–4·4], $p = 0·0002$).

Interpretation Rapid identification of ARDS inflammatory subphenotypes using a near-patient assay was feasible and associated with many clinical characteristics and outcomes consistent with those described in earlier retrospective studies, including mortality, prevalence of sepsis, and incidence of metabolic acidosis. These findings support the implementation of precision medicine approaches in ARDS and the urgent need for prospective, subphenotype-stratified interventional trials.

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Introduction

Despite extensive investigation, no pharmacological therapy has yet been proven effective for acute respiratory distress syndrome (ARDS) outside of COVID-19. A probable explanation for the lack of benefit shown in pharmacological trials in ARDS is the underlying heterogeneity of the syndrome, which is defined by clinical criteria^{1,2} rather than underlying biological pathways.³ As a result, ARDS is treated as a single condition in trials, diluting treatment effects across heterogeneous responders and non-responders, underscoring the need for a precision

medicine approach. Efforts to parse ARDS into meaningful subgroups are longstanding. The hyperinflammatory and hypoinflammatory subphenotypes have been identified and replicated in retrospective analyses of large interventional trials and observational cohorts.^{4–14} The hyperinflammatory subphenotype, seen in 26–40% of patients, is characterised by a higher level of circulating plasma markers of inflammation, increased use of vasopressors, increased metabolic acidosis, higher prevalence of sepsis, and substantially worse outcomes (44–62% mortality). Conversely, the hypoinflammatory

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Research in context

Evidence before this study

Acute respiratory distress syndrome (ARDS) is a common and often fatal disease in the intensive care unit with no pharmacotherapeutic treatment. Finding new treatments will involve improving our understanding of its heterogeneous pathobiology and identifying treatment-responsive subgroups, termed subphenotypes. We searched PubMed/MEDLINE and Embase for articles published in English from Jan 1, 2000, to Jan 22, 2026. Search terms included combinations of: acute respiratory distress syndrome, ARDS, acute hypoxaemic respiratory failure, inflammatory subphenotype, hyperinflammatory, hypoinflammatory, latent class analysis, biomarkers, IL-6, TNF receptor, and precision medicine. A complete list of search terms is presented in the appendix (pp 3–4). Reference lists of relevant reviews and key primary studies were also screened. Previous studies consistently identified hyperinflammatory and hypoinflammatory ARDS subphenotypes and demonstrated prognostic differences and heterogeneity of treatment effect; however, all such findings derived from retrospective analyses, and prospective real-time identification at the bedside had not yet been shown.

subphenotype, comprising 60–73% of patients, is associated with a lower mortality rate (19–30%) than the hyperinflammatory subphenotype. Heterogeneity of treatment effect by subphenotype has been demonstrated for ventilation⁴ and fluid strategies,⁵ simvastatin⁶ in ARDS, as well as activated protein C in sepsis.¹² However, all heterogeneity of treatment effect findings to date derive from retrospective analyses, and prospective identification of ARDS inflammatory subphenotypes has not yet been shown.

The first challenge for prospective identification of the hyperinflammatory and hypoinflammatory ARDS subphenotypes is that latent class analysis models used to identify the subphenotypes in previous studies are trained to predict on standardised values of the input variables, rendering them inappropriate for prospective validation. Furthermore, latent class analysis models require large datasets and knowledge of the population-level mean for each input variable. To address these issues, several parsimonious logistic regression models have been developed that can accurately identify the subphenotypes in individual patients (area under the curve [AUC] 0.92–0.96).¹⁵ These models incorporate three or four of the following biomarker and clinical variables: interleukin (IL)-6, IL-8, protein C, soluble tumour necrosis factor receptor-1 (TNFR1), bicarbonate, and vasopressor use.¹⁵ However, the absence of a near-patient diagnostic to rapidly quantify these biomarkers remains a key barrier to the bedside classification of ARDS inflammatory subphenotypes. To address this gap, we undertook the PHenotypes IN the Acute

Added value of this study

This large, multicentre, prospective observational study shows, for the first time, that inflammatory subphenotypes of ARDS can be identified in real time at the bedside using a near-patient immunoassay and a parsimonious logistic regression model. Prospectively identified patients with hyperinflammatory ARDS had substantially higher 60-day mortality and worse clinical outcomes, confirming the prognostic relevance of these subphenotypes when adjudicated during routine ICU care. The study also establishes the feasibility and reliability of near-patient cytokine measurement for prospective subphenotyping across multiple centres.

Implications of all the available evidence

Taken together, existing retrospective evidence and these prospective data support inflammatory subphenotypes as clinically meaningful and actionable constructs in ARDS. Real-time bedside identification enables enrichment strategies for future precision medicine trials and provides a foundation for testing subphenotype-targeted therapies. These findings represent a crucial step toward moving ARDS therapeutics away from a one-size-fits-all approach and toward biologically informed, stratified clinical trials.

Respiratory Distress Syndrome (PHIND) study, which is the first large, multicentre, observational study with the aim of prospectively identifying inflammatory subphenotypes in a real-world clinical setting using a parsimonious logistic regression model (ie, IL-6, soluble TNFR1, bicarbonate) and a near-patient immunoanalyser (Radox multiSTAT). We hypothesised that these subphenotypes can be identified in ARDS in real-time and show differing 60-day mortality, providing a foundation for future precision medicine trials that target pharmacotherapeutics to subphenotypes. We also conducted an exploratory analysis in patients with acute hypoxaemic respiratory failure (AHRF) without ARDS to assess whether these subphenotypes extend beyond ARDS.

Methods

Study design and participants

We did a multicentre, prospective, observational cohort study in adult general intensive care units (ICUs) within the National Health Service (NHS) in the UK and within the Health Service Executive (HSE) in Ireland. Two patient cohorts were recruited: the ARDS cohort (which PHIND was primarily powered to study), and a cohort of patients with AHRF but without ARDS. Patients who were admitted in participating ICUs were eligible for inclusion if they were receiving invasive mechanical ventilation, non-invasive ventilation, or high-flow nasal oxygen, and had ARDS or AHRF. ARDS was defined by the Berlin definition,¹ but additionally included patients receiving high-flow nasal oxygen.

Patients receiving high-flow nasal oxygen were assumed to have positive end-expiratory pressure of at least 5 cmH₂O for the purposes of eligibility. The method of delivery of high-flow nasal oxygen was not standardised across sites and was left to clinician discretion. The AHRF cohort (without ARDS) was defined using the same PaO₂/FiO₂ ratio criterion as ARDS, but this cohort had only unilateral infiltrates on chest imaging rather than bilateral infiltrates. Exclusion criteria included: age younger than 18 years; more than 72 h after onset of ARDS or AHRF (which was amended during the course of the study from more than 48 h after onset of ARDS to facilitate patient recruitment and justified based on evidence supporting subphenotype stability¹⁶); receiving extracorporeal membrane oxygenation at the time of recruitment; treatment withdrawal imminent within 24 h; a do not attempt resuscitation order in place; anyone who declined consent; or people who were incarcerated. Full inclusion and exclusion criteria are provided in the study protocol in the appendix (pp 69).

Sex and ethnicity data were collected either via self-report or from next of kin when the patient was incapacitated. Options for sex were male or female and options for ethnicity were Black, White, Asian, or Other.

The study was coordinated by the Northern Ireland Clinical Trials Unit and was sponsored by Queen's University Belfast. The study protocol was prospectively published online and was registered on ClinicalTrials.gov, NCT04009330. The protocol and statistical analysis plan are provided in the appendix (pp 51–142). The protocol was approved by research ethics committees in England, Wales, Northern Ireland (19/LO/0672), Scotland (19/SS/0073), and Ireland (Ca. 2344). An independent steering committee was convened and oversaw the conduct of the study. The study was conducted in accordance with Good Clinical Practice guidelines, local regulations, and the ethical principles described in the Declaration of Helsinki. Written informed consent was obtained from patients' surrogates before recruitment. As this was deemed a low-risk study, when a surrogate decision-maker was not available, a deferred consent model was utilised in England, Wales, and Northern Ireland (but not in Scotland or Ireland). If deferred consent was not obtained, the patient was withdrawn from the study, samples were destroyed, and all data were removed. Written consent to continue participation in the study was sought for all patients who regained capacity following intensive care discharge.

In December, 2021, the study protocol was substantially amended to additionally recruit the exploratory cohort of patients with acute hypoxaemic respiratory failure (AHRF) and to extend the eligibility timeframe from less than 48 h from ARDS onset to less than 72 h from ARDS or AHRF onset. Patient and public representatives were consulted and had input throughout the design, conduct, and reporting of the study. A patient representative was a key member of the study steering committee.

Procedures

Patient blood samples for near-patient subphenotyping were collected immediately at the time of recruitment from an indwelling arterial or central venous line. Sample collection and subphenotype allocation was within approximately 1 h. 4 mL of blood collected immediately in a lithium heparin tube was used for the near-patient assay at the recruiting site. Following centrifugation, plasma was transferred to a precoated assay plate (InflammiSTAT, Randox Laboratories, County Antrim, Northern Ireland, UK) and quantitatively analysed in duplicate for IL-6 and soluble TNFR1 using a multiSTAT device (Randox Laboratories, UK). The upper limit of quantification for IL-6 was 1603·74 pg/mL and for soluble TNFR1 was 27·97 ng/mL. When the concentration of IL-6 or soluble TNFR1 in the patient sample exceeded the upper limit of quantification, the multiSTAT device truncated the concentration as equal to the upper limit of quantification. Similarly, the device truncated the concentration as equal to the lower limit of quantification when the concentration was lower than the specified limit (13·10 pg/mL for IL-6 and 150 pg/mL for soluble TNFR1). Following successful calibration, the multiSTAT device took approximately 1 h to produce successful results for IL-6 and soluble TNFR1, which could be used immediately in the subphenotyping model.

The remaining blood was centrifuged at the recruitment sites and plasma or serum was aliquoted and frozen at –80°C. These samples were transferred to a central processing facility (Wellcome–Wolfson Institute for Experimental Medicine, Queen's University Belfast).

At the end of the study, plasma was analysed by ELISA for IL-6 and soluble TNFR1. IL-6 and soluble TNFR1 were also measured by automated immunoassay using an ELLA device (BioTechne, Minneapolis, MN, USA). Quantikine ELISA (BioTechne) cytokine measurements were taken as the reference standard due to their use in previous studies identifying ARDS inflammatory subphenotypes.^{4,7} Further details of assay procedures are presented in the appendix (p 5).

The probability of hyperinflammatory subphenotype assignment was determined using a previously described, publicly available parsimonious logistic regression model incorporating IL-6, soluble TNFR1, and bicarbonate (AUC 0·94 [95% CI 0·92–0·95]).¹⁵ The complete regression model that was used is provided in the appendix (p 10). Following measurement on the multiSTAT near-patient immunoanalyser (Randox Laboratories), values for IL-6 and soluble TNFR1 were entered into the case report form by site staff, along with the lowest arterial bicarbonate value on the same day of recruitment but before the subphenotyping sample was taken. Bicarbonate values were exclusively arterial. The parsimonious logistic regression model was embedded in the study database and calculated the probability of assignment to the hyperinflammatory subphenotype

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See Online for appendix

For the study protocol see
<https://nictu.hscni.net/service/phind-trial>

(between 0 and 1). A probability of 0.5 or higher was used to classify the patient to the hyperinflammatory subphenotype and the remaining patients were classified as the hypoinflammatory subphenotype. Site investigators were masked to subphenotype allocation, but not to individual biomarker values that comprised the parsimonious model inputs (ie, IL-6, soluble TNFR1, and bicarbonate). Although unlikely, knowledge of biomarker values might have influenced patient management. For Quantikine ELISA and ELLA retrospective subphenotype allocation, the same parsimonious logistic regression model and dichotomisation schema were used.

Outcomes

The primary outcome was all-cause mortality at 60 days. Secondary clinical outcome measures were mortality at 28 days, ventilator-free days at day 28, duration of ventilation, time to extubation, re-intubation rate, intubation rate for patients not receiving invasive mechanical ventilation at recruitment, length of ICU stay, and length of hospital stay. Patients were followed up to discharge or death in hospital. If discharged, patients were followed up to day 60 for mortality. Patient survival after hospital discharge was determined from either: hospital or regional information systems, NHS Digital records in the UK, or by contacting the patient's general practitioner, which was undertaken centrally by core trials unit staff.

Statistical analysis

For sample size calculations, the following assumptions were made: 25% prevalence of the hyperinflammatory subphenotype, 17% mortality in the hypoinflammatory subphenotype, and a difference in mortality of 15% between the hyperinflammatory and hypoinflammatory subphenotypes. Based on these assumptions and a two-group χ^2 test, a sample size of 347 patients with the hypoinflammatory subphenotype and 116 patients with the hyperinflammatory subphenotype would achieve a 90% power at a two-sided significance level of 0.05 to detect a 15% mortality difference. The sample size was initially inflated by 3% to allow for technical failure of the multiSTAT device, drop-out, and loss to follow-up, resulting in a total sample size of 480 patients with ARDS. This was later amended to increase the sample size to include a 10% inflation due to slightly greater than anticipated rates of dropout and technical failure, resulting in a total sample size of 515 patients with ARDS. For the exploratory AHRF cohort, no previous data existed, and no formal sample size calculation was undertaken. As such, a convenience sample size of 300 patients with AHRF was planned.

Baseline characteristics and clinical measurements were described separately in the ARDS cohort and AHRF cohorts, using absolute values with percentages for categorical data and means with SDs for continuous data. Biomarker measurements were described using medians with IQRs. Between-group differences were

evaluated using the χ^2 test for categorical data, two sample *t*-tests for continuous clinical data, and the Mann–Whitney U test for biomarker data.

The primary outcome of 60-day mortality in the ARDS cohort was reported as an unadjusted percentage point difference with 95% CI and risk ratio (RR) with 95% CI. Pearson's χ^2 test was used to compare the hyperinflammatory and hypoinflammatory subphenotypes and the *p* value was reported. Additionally, the primary outcome was analysed using a mixed effect general logistic regression model adjusted for age, sex, and site as a random effect and the output reported as an adjusted odds ratio (OR) with 95% CI. Post-hoc, a mixed effects logistic regression model that additionally adjusted for Acute Physiology and Chronic Health Evaluation (APACHE) II score and worst PaO₂/FiO₂ ratio at enrolment was also estimated, as well as Poisson regression models for select secondary outcomes that adjusted for the same covariates. Kaplan–Meier survival curves were constructed and the *p* value from the log-rank test as well as the hazard ratio (HR) from the estimate Cox proportional hazard model were reported.

For secondary outcomes in the ARDS cohort, those based on dichotomous variables (ie, re-intubation rate, intubation rate on high-flow nasal oxygen, and 28-day mortality) were reported as absolute number and percentage by subphenotype, with *p* values calculated using Pearson's χ^2 test. For continuous secondary outcomes, such as ventilator-free days at day 28, duration of ventilation, length of ICU stay, and length of hospital stay, data were reported as median with IQR, with a *p* value calculated using the Mann–Whitney U test. A cumulative incidence function for time to successful extubation in the presence of the competing risk of death was calculated from a semiparametric regression model. Subhazard ratio and 95% CIs were also reported from this model.

To compare subphenotype allocations by different methods (multiSTAT near-patient, Quantikine ELISA, and ELLA), 2×2 contingency tables were constructed. McNemar's χ^2 and Cohen's κ were estimated, and the results were reported. The probability of subphenotype allocation from two methods was plotted on a scatter plot. Subphenotype probabilities and raw biomarker measurements across assay methods were also compared by Bland–Altman analysis and by estimation of two-way random, absolute agreement, single measurement intraclass correlation coefficients with bootstrapped 95% CIs. Raw biomarker measurements were additionally compared by the Kruskal–Wallis test and pairwise Mann–Whitney U test, with Benjamini–Hochberg correction for multiple comparisons.

There were no missing data for the primary outcome. Missing data for demographic, clinical, biomarker, and secondary outcome data were minimal and missing values were omitted without imputation. Outcomes were analysed separately for the AHRF cohort, using the same

methods as the ARDS cohort. All statistical analyses were performed using STATA version 15.1 and R version 4.3.1.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From Nov 22, 2019, to Sept 28, 2023, 1853 patients with suspected ARDS from 30 centres were screened for eligibility and 525 (28%) were recruited. Of these, 13 (3%) patients subsequently withdrew consent for all study involvement and 22 (4%) could not be allocated to a subphenotype due to near-patient assay technical failure, leaving 490 patients included. Assay failures were related to failure of device calibration or failure to measure reproducible biomarker concentrations. The most common reasons for patient exclusion were more than 48 h or 72 h since ARDS onset (earlier protocol version excluded patients with more than 48 h since ARDS onset; this was amended to >72 h in 2021), a do not attempt cardiopulmonary resuscitation order in place, declined consent, and patient receiving extracorporeal membrane oxygenation. 724 (39%) patients were excluded for other reasons. Most commonly, this occurred during the COVID-19 pandemic when a patient tested positive for SARS-CoV-2 and the site was unable to collect biological samples. Four patients withdrew consent for confirmation of vital status only (but not for biological sampling or study inclusion) and no patients were lost to follow-up, leaving 486 patients included in the analysis of the primary outcome (figure 1).

Of 512 patients (including 22 for whom subphenotype could not be determined), 308 (60%) patients were male, 204 (40%) were female, and mean age was 57.0 years (SD 15.1). 443 (87%) patients were white, 18 (4%) were Black, and 16 (3%) were Asian (table 1). Of 490 patients who had subphenotype data available, for ARDS, 89 (18%) patients were classified as the hyperinflammatory subphenotype based on cytokine measurement with the near-patient assay, with the remaining 401 (82%) being classified as the hypoinflammatory subphenotype (figure 2). The characteristics of the hyperinflammatory and hypoinflammatory subphenotypes were similar to previous datasets.⁴⁻⁷ Hyperinflammatory patients were more severely ill as measured by APACHE II and SOFA score. They also had higher heart rates, lower systolic blood pressures, worse renal and liver function, more coagulopathy, and more metabolic acidosis. Hyperinflammatory patients were more likely to be on renal replacement therapy and vasopressors at study recruitment, although markers of lung injury and ARDS severity (ie, Murray lung injury score, PaO₂/FiO₂ ratio, and oxygenation index) were not significantly different between subphenotypes. Complete baseline characteristics are presented in the appendix (pp 11–13).

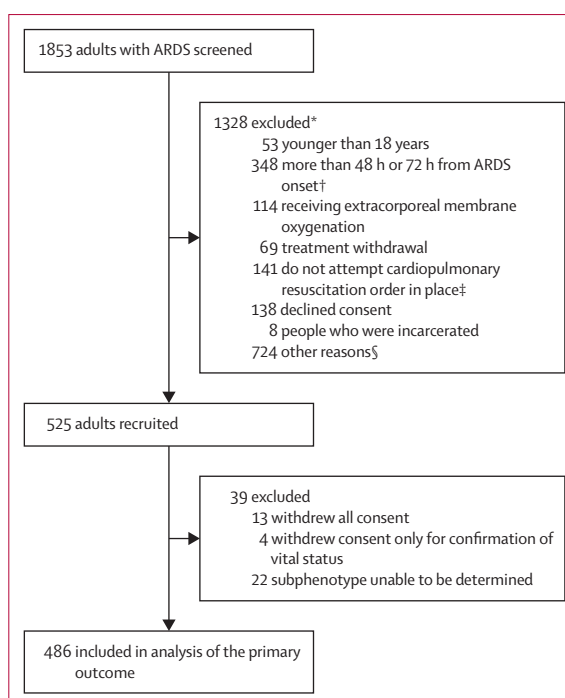


Figure 1: Study profile

ARDS=acute respiratory distress syndrome. *Patients could meet more than one exclusion criterion. †Before 2021, patients were excluded if more than 48 h had elapsed since ARDS onset, after 2021, this exclusion criterion was amended to 72 h. ‡Do not attempt cardiopulmonary resuscitation order in place; intended only to exclude patients not receiving active medical management and who were receiving palliative care. §Other was used when the reason for exclusion was not among those predefined in the protocol; the most specified explanation was patient testing positive for SARS-CoV-2 at a site that was unable to collect samples from patients with COVID-19.

In ARDS, when limited to the 374 patients receiving invasive mechanical ventilation, 298 (80%) were hypoinflammatory and 76 (20%) were hyperinflammatory (figure 2). Of 95 patients with ARDS on high-flow nasal oxygen, 84 (88%) were hypoinflammatory and 11 (12%) were hyperinflammatory. Of 86 patients with ARDS on high-flow nasal oxygen with flow rates of at least 30 L/min (consistent with the Global definition of ARDS⁸), 75 (87%) were hypoinflammatory and 11 (13%) were hyperinflammatory. Very few patients with ARDS were on non-invasive ventilation. Of these 21 patients, 19 (90%) were hypoinflammatory and two (10%) were hyperinflammatory. When patients with COVID-19 were excluded, 458 patients remained, and of these, 372 (81%) were hypoinflammatory and 86 (19%) were hyperinflammatory.

Mortality at 60 days in the hyperinflammatory subphenotype was significantly higher than observed with the hypoinflammatory subphenotype. The 60-day mortality rate was 51% (45 of 88) in the hyperinflammatory subphenotype versus 28% (111 of 398) in the hypoinflammatory subphenotype (RR 1.8 [95% CI 1.4–2.4]; unadjusted percentage difference 23.2% [95% CI 11.9–34.6], $p<0.0001$). In the adjusted analysis, the

	Total (N=512)	Hyperinflammatory (n=89)	Hypoinflammatory (n=401)	p value
Age, years	57.0 (15.1)	58.0 (14.0)	56.6 (15.5)	0.42
Sex				0.18
Male	308 (60%)	47 (53%)	243 (61%)	..
Female	204 (40%)	42 (47%)	158 (39%)	..
Ethnicity				..
White	443 (87%)	73 (82%)	352 (88%)	0.36
Black	18 (4%)	6 (7%)	11 (3%)	..
Asian	16 (3%)	3 (3%)	12 (3%)	..
Unknown	21 (4%)	5 (6%)	15 (4%)	..
Other	14 (3%)	2 (2%)	11 (3%)	..
Aetiology of ARDS				
Pneumonia	360 (70%)	56 (63%)	291 (73%)	0.070
Sepsis	197 (39%)	51 (57%)	137 (34%)	<0.0001
Gastric content aspiration	88 (17%)	17 (19%)	68 (17%)	0.63
Pancreatitis	26 (5%)	11 (12%)	13 (3%)	<0.0001
Thoracic trauma	23 (5%)	0	22 (6%)	0.024
APACHE II score	17.9 (6.0)	22 (6)	17 (6)	<0.0001
SOFA score	11.5 (3.4)	14 (3)	11 (3)	<0.0001
Murray lung injury score	2.7 (0.6)	2.7 (0.6)	2.7 (0.6)	0.65
PaO ₂ /FiO ₂ ratio, mm Hg	17.1 (7.3)	17.1 (7.0)	17.3 (7.4)	0.81
ARDS severity				
Mild	48/508 (9%)	10 (11%)	38/397 (10%)	0.50
Moderate	294/508 (58%)	48 (54%)	235/397 (59%)	..
Severe	166/508 (33%)	31 (35%)	124/397 (31%)	..
Oxygenation index	29.0 (39.0)	89 (46)	77 (48)	0.24
PaCO ₂	5.9 (1.5)	6.0 (1.7)	6.0 (1.4)	0.97
Bicarbonate, mmol/L	23.0 (4.9)	17.5 (4.3)	24.2 (4.2)	<0.0001
Respiratory rate, breaths per min	22 (6)	22 (6)	22 (6)	0.93
Plateau pressure, cmH ₂ O	25.0 (5.0)	25.2 (5.0)	24.8 (5.0)	0.69
Positive end-expiratory pressure, cmH ₂ O	9.1 (3.0)	9.0 (2.8)	9.1 (3.0)	0.81
Tidal volume per predicted body weight, mL/kg	7.1 (2.1)	7.4 (1.9)	7.1 (2.1)	0.29
Heart rate, bpm	91.7 (21.9)	103 (22)	89 (21)	<0.0001
Systolic blood pressure, mm Hg	118 (23)	109 (21)	120 (23)	<0.0001
Albumin, g/L	25 (6)	25 (6)	26 (6)	0.10
Aspartate aminotransferase, U/L	104 (174)	204 (301)	83 (126)	0.0056
Alanine transaminase, U/L	143 (520)	414 (1070)	82 (233)	<0.0001
White cell count, ×10 ⁹ /L	13.8 (8.7)	14.8 (11.5)	13.7 (8.1)	0.28
Prothrombin time, s	15.9 (8.4)	19 (12.0)	15 (6.9)	0.0008
Fibrinogen, g/L	5.5 (2.3)	5.0 (2.8)	5.5 (2.1)	0.10
Vasopressor use at enrolment	315 (62%)	74 (83%)	230 (57%)	<0.0001
Renal replacement therapy at enrolment	64 (13%)	35 (39%)	27 (7%)	<0.0001
Use of corticosteroids at enrolment	194 (38%)	43 (48%)	141 (35%)	0.020
Adjunctive therapies at enrolment				
Prone positioning	41 (8%)	8 (9%)	30 (8%)	0.79
Neuromuscular blockade	88 (17%)	19 (21%)	64 (16%)	0.28
Inhaled nitric oxide	11 (2%)	3 (3%)	8 (2%)	0.69
Other	9 (2%)	4 (5%)	5 (1%)	0.10
IL-6 (R&D Quantikine ELISA), pg/mL	186 (58–497)	747 (425–1572)	135 (43–298)	<0.0001

(Table 1 continues on next page)

OR for 60-day mortality for the hyperinflammatory subphenotype versus the hypoinflammatory subphenotype was 2.7 (95% CI 1.6–4.4; $p=0.0002$).

ARDS survival curves are shown in figure 3A and AHRF survival curves are shown in figure 3B. Log-rank test and estimation of a Cox proportional hazard model demonstrated increased relative risk of mortality in the hyperinflammatory subphenotype (log-rank $p<0.0001$; HR 2.29 [95% CI 1.62–3.23], $p<0.0001$; figure 3A). Secondary outcomes are presented in table 2. In the hyperinflammatory subphenotype, compared with the hypoinflammatory subphenotype, patients were more likely to die by day 28 (39 [44%] of 88 vs 91 [23%] of 398; 21.5% point difference [95% CI 6.8–36.1], $p<0.0001$) and had fewer ventilator-free days at day 28 (0.0 days [0.0–16.0] vs 14.0 days [0.0–24.0]; $p<0.0001$). Among survivors beyond 60 days, patients in the hyperinflammatory subphenotype had a prolonged duration of invasive ventilation (median 11.5 days [IQR 5.0–25.0] versus 8.0 days [1.0–15.0]; $p=0.011$), prolonged ICU stay (median 17.0 days [10.0–32.0] vs 14.0 days [8.0–24.5]; $p=0.038$), and prolonged hospital stay (median 42.5 days [23.0–67.0] vs 27.0 days [15.0–48.0]; $p=0.0031$). Among hyperinflammatory patients who were receiving high-flow nasal oxygen at recruitment, progress to intubation during the study period was significantly higher than in hypoinflammatory patients (seven [64%] of 11 vs 21 [26%] of 82; 38.0% difference [95% CI 8.1–68.0]; $p=0.026$). Re-intubation rates were similar across subphenotypes. Time to successful extubation was longer in the hyperinflammatory subphenotype when accounting for the competing risk of death (11.0 days [IQR 5.5–23.5] vs 9.0 days [4.0–16.0]; subhazard ratio 0.54 [95% CI 0.40–0.74]; $p<0.0001$; figure 4).

Primary and secondary outcomes for sensitivity analyses that were prespecified in the statistical analysis plan are reported in the appendix and include the following subgroups: patients with ARDS on invasive mechanical ventilation, patients with ARDS on high-flow nasal oxygen, patients with ARDS on high-flow nasal oxygen with a flow rate of at least 30 L/min as per the Global definition of ARDS,² ARDS cohort excluding patients with COVID-19, patients with ARDS who had samples obtained within 48 h of ARDS diagnosis, and patients with ARDS with samples obtained within 24 h of ARDS diagnosis (appendix pp 14–25). These results were generally consistent with outcomes in the overall ARDS cohort. In a post-hoc analysis, we also estimated a multivariate model for 60-day mortality in the overall ARDS cohort that adjusted for additional covariates not prespecified in the statistical analysis plan, this was done to address the question of whether increased severity of illness could be responsible for worsened mortality in the hyperinflammatory subphenotype. These additional covariates were the worst PaO₂/FiO₂ ratio on the day of enrolment and APACHE II score. The results of this

model yielded an OR for worsened 60-day mortality in the hyperinflammatory subphenotype of 2·33 (95% CI 1·36–3·97; $p=0\cdot0020$), largely consistent with the primary analysis. We also estimated multivariate Poisson regression models for ventilator-free days at day 28, duration of ventilation, and ICU length of stay, the results of which remain consistent with the unadjusted analysis (appendix p 26).

From Dec 1, 2021, to Sept 28, 2023, 497 patients with AHRF (who did not have ARDS) from 28 centres were screened for eligibility and 143 (29%) were recruited. Of these, five (4%) patients subsequently withdrew consent for participation in the study and seven (5%) could not be allocated to subphenotype due to near-patient assay technical failure, leaving 131 patients included in the study (appendix p 27). The most common reasons for exclusion were more than 72 h since AHRF onset, declined consent, a do not attempt cardiopulmonary resuscitation order in place, and imminent treatment withdrawal. 163 patients were excluded for other reasons.

Of 131 patients with AHRF who were recruited and included in the analysis of the primary outcome, 109 (83%) were classified as hypoinflammatory and 22 (17%) as the hyperinflammatory subphenotype (figure 2). Baseline demographics of the AHRF cohort are shown in the appendix (pp 28–30). In the AHRF cohort, hyperinflammatory patients were more severely ill, with higher APACHE II and SOFA scores; higher heart rates; greater derangements in liver function, coagulation, and metabolic acidosis; and a markedly higher likelihood of requiring renal replacement therapy and vasopressors at enrolment. Unlike in ARDS, the Murray lung injury score was also significantly worse in the hyperinflammatory subphenotype. Notably median IL-6 and soluble TNFR1 concentrations were slightly lower in hyperinflammatory and hypoinflammatory AHRF than in hyperinflammatory and hypoinflammatory ARDS. In AHRF, mortality at 60 days in the hyperinflammatory subphenotype was numerically higher but not significantly different between the inflammatory subphenotypes (six [27%] of 22 vs 23 [21%] of 109; RR 1·3 [95% CI 0·6 to 2·8]; unadjusted percentage difference 6·2% [95% CI –14·0 to 26·3]; $p=0\cdot53$). In the adjusted analysis, the OR for 60-day mortality for the hyperinflammatory subphenotype versus the hypoinflammatory subphenotype was 2·2 (95% CI 0·7 to 7·1; $p=0\cdot21$). Survival curves for AHRF are shown in figure 3B. Log-rank test and estimation of a Cox proportional hazard model did not demonstrate a differential relative risk of mortality across subphenotypes in AHRF (log-rank $p=0\cdot45$; HR 1·41 [95% CI 0·57 to 3·46], $p=0\cdot45$). In AHRF, no significant differences were seen in secondary clinical outcomes except for increased hospital length of stay in survivors beyond 60 days (43·0 days [IQR 21·0 to 58·0] vs 22·0 days [11·5 to 41·0], $p=0\cdot037$; appendix p 31). Time to successful extubation was 7·5 days (IQR 5·0 to 10·0) in the hyperinflammatory subphenotype and 7·0 days

	Total (N=512)	Hyperinflammatory (n=89)	Hypoinflammatory (n=401)	p value
(Continued from previous page)				
IL-6 (R&D ELLA), pg/mL	144 (48–434)	860 (377–2981)	98 (34–263)	<0·0001
Soluble TNFR1 (R&D Quantikine ELISA), pg/mL	5127 (3140–10 147)	14 291 (9688–21 334)	4216 (2803–6726)	<0·0001
Soluble TNFR1 (R&D ELLA), pg/mL	3101 (1926–4846)	6356 (3453–17 549)	2909 (1772–4195)	<0·0001

Data are n (%), n/N (%), mean (SD), or median (IQR), unless otherwise specified. Complete baseline demographics (with missingness) are presented in the appendix (pp 11–13). The total N=512 includes 22 patients for whom subphenotype could not be determined. Bicarbonate values and PaO₂/FIO₂ ratios are the worst taken on the day of study enrolment. ARDS aetiology was adjudicated at the discretion of site investigators and criteria for pneumonia or sepsis were not defined in the protocol. Multiple aetiologies could be chosen. ARDS severity was as per PaO₂/FIO₂ ratio thresholds from the Berlin definition (severe, $\leq 13\cdot3$ kPa; moderate, $>13\cdot3$ to $\leq 26\cdot7$ kPa; mild, $>26\cdot7$ to ≤ 40 kPa). For categorical variables, data are n (%) with p values calculated from the χ^2 test. For continuous variables other than inflammatory biomarkers, data are mean (SD) and p values were calculated from two sample t-tests. For inflammatory biomarkers (IL-6 and soluble TNFR1) data are median (IQR) and p values were calculated from the Mann-Whitney U test. Significance was defined as a two-sided significance level of 0·05. ARDS=acute respiratory distress syndrome. IL-6=interleukin-6. Soluble TNFR1=soluble tumour necrosis factor receptor 1.

Table 1: Selected baseline demographics of patients with ARDS

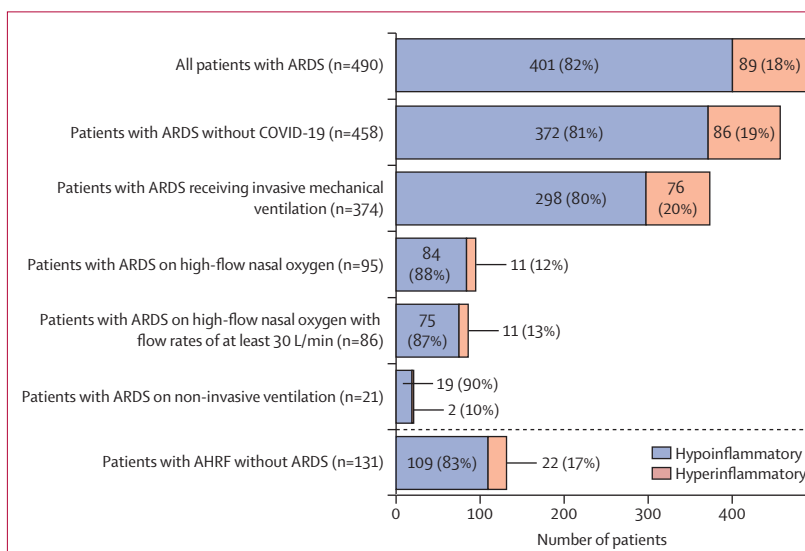


Figure 2: Proportion of patients classified as hypoinflammatory and hyperinflammatory subphenotypes by the near-patient assay across the ARDS cohort, the AHRF cohort, and pre-defined subgroups of the ARDS cohort

22 patients with ARDS and seven patients with AHRF were unclassified due to assay failure and are not included in this table. AHRF=acute hypoxaemic respiratory failure. ARDS=acute respiratory distress syndrome.

(4·0 to 13·0) in the hypoinflammatory subphenotype (subhazard ratio 0·86 [95% CI 0·50 to 1·48]; $p=0\cdot59$; appendix p 32).

In the ARDS cohort, we compared prospective subphenotype classification using cytokine measurements from the multiSTAT near-patient assay with classification based on cytokine measurement using Quantikine ELISA and the ELLA device. Intraclass agreement between near-patient and Quantikine ELISA subphenotype probability was excellent (intraclass correlation coefficient 0·94 [95% CI 0·92–0·95]). MultiSTAT classification more closely matched Quantikine classification than did ELLA classification, although both under-identified the

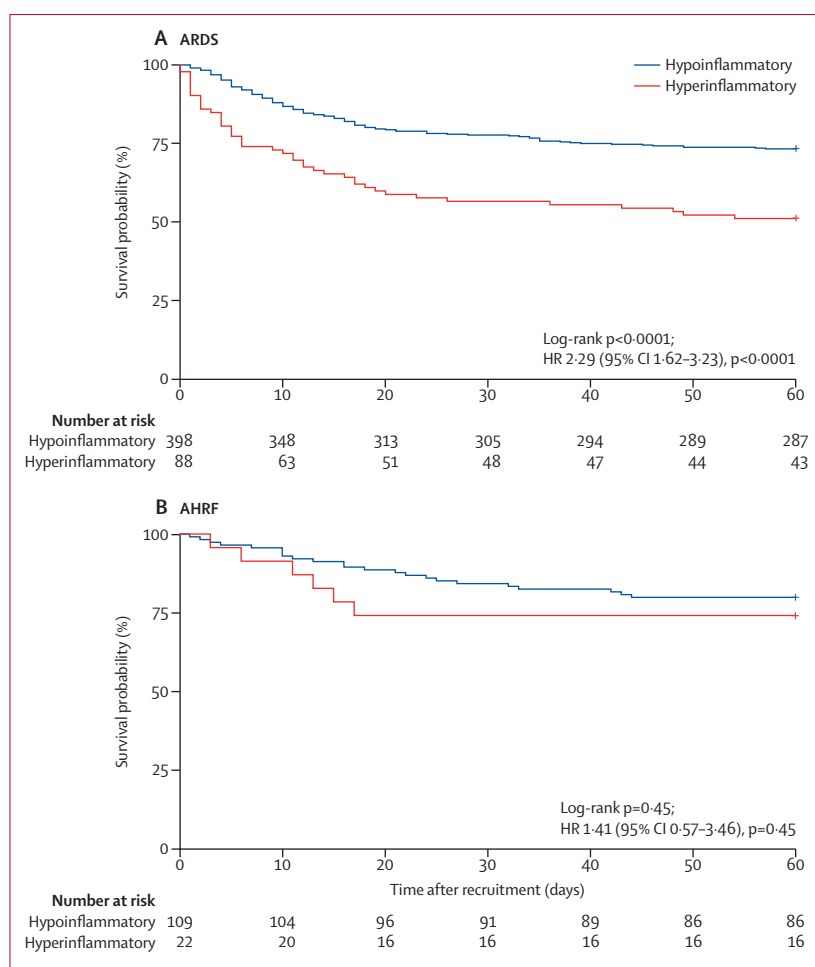


Figure 3: Kaplan-Meier survival curves for the hypoinflammatory and hyperinflammatory subphenotypes in ARDS (A) and AHRF (B)

AHRF=acute hypoxaemic respiratory failure. ARDS=acute respiratory distress syndrome.

hyperinflammatory subphenotype, with this effect being more pronounced for the ELLA device. Discrepancies across subphenotyping methods seemed to be largely driven by differing concentrations of soluble TNFR1, as measured by the various assays. By contrast, IL-6 concentrations were relatively reproducible across measurement methods (appendix pp 6–8, 33–48).

Results from a subset of patients with COVID-19-associated ARDS were previously published and are not included in this cohort.¹⁷

Discussion

This large, prospective, multicentre observational study demonstrates that the hyperinflammatory and hypoinflammatory subphenotypes of ARDS, previously characterised only in retrospective analyses, can be identified in real time at the bedside using a near-patient immunoassay and a parsimonious logistic regression model. Notably, these prospectively identified subphenotypes have very different clinical outcomes, with the

hyperinflammatory subphenotype having significantly higher 60-day mortality. These findings validate and extend previous retrospective work that has characterised these subphenotypes across ARDS, sepsis, and patients on mechanical ventilation.^{4–13} Hyperinflammatory ARDS patients on high-flow nasal oxygen were more likely to require intubation, a finding replicated in the contemporaneous SPARC study,¹⁸ suggesting potential utility for guiding escalation of respiratory support if further validated.

The clinical characteristics and outcomes observed in the PHIND study were broadly consistent with those reported in previous secondary analyses. However, the prevalence of the hyperinflammatory subphenotype in PHIND was 18%, which was less than the expected 25%. There are a few potential explanations for this finding. ARDS inflammatory subphenotypes were originally identified in trial cohorts of patients on invasive mechanical ventilation,^{4–7} while PHIND was an observational study that additionally included patients on high-flow nasal oxygen and non-invasive ventilation. In this observational study, it is possible that differences in eligibility criteria, illness severity, or current ICU practices might have meant we recruited a cohort with better outcomes than in historical moderate-to-severe ARDS randomised controlled trials. In ARDS, the hyperinflammatory subphenotype represented 12% of patients on high-flow nasal oxygen as opposed to 20% on invasive mechanical ventilation, suggesting that the inclusion of patients on high-flow nasal oxygen, which might be a less severely ill cohort, might account in part for the lower proportion of hyperinflammatory patients compared with historical data, somewhat limiting comparability to previous cohorts. The dynamic range of the near-patient immunoassay did not substantially influence subphenotype allocation, although systemic bias in measurement of soluble TNFR1 might have influenced subphenotype proportions across methods. Furthermore, since PHIND exclusively employed arterial bicarbonate from arterial blood gas rather than using serum bicarbonate from biochemistry panels in the parsimonious subphenotyping model, the extent to which variability across bicarbonate measurement methods might influence subphenotype proportions remains unclear. However, data suggest although there is a bias between ABG-derived bicarbonate and paired plasma bicarbonate calculations, the difference is small and unlikely to affect subphenotype allocations.¹⁹

Another possible explanation for the reduced proportion of hyperinflammatory patients is that PHIND allowed recruitment of patients within 48 h of ARDS diagnosis, which was later amended to within 72 h of meeting eligibility criteria. The subphenotype to which a patient is allocated can change longitudinally when identified prospectively. PHIND also aimed to collect longitudinal subphenotyping samples to address this question, the results of which will be reported in future work. The decision to amend the recruitment window to 72 h was

based on previous evidence from latent transition analysis, which demonstrated that ARDS inflammatory subphenotypes are stable over a 72-h window.¹⁶ However, by use of a parsimonious subphenotyping model, it has been shown that ARDS inflammatory subphenotypes are more dynamic in the first 72 h, with the proportion of hyperinflammatory patients decreasing as time progresses due to a decrease in plasma cytokines.²⁰ The longer window for recruitment used in PHIND might have resulted in the selection of some patients who had previously been hyperinflammatory earlier in their disease course but had transitioned to hypoinflammatory by the time of recruitment and biological sampling. In the survival analysis, it was apparent that the difference in mortality between hyperinflammatory and hypoinflammatory subphenotypes is driven by early deaths. This is consistent with findings from post-hoc subphenotyping of the Re-evaluation of Systemic Early Neuromuscular Blockade (ROSE) trial,¹⁴ in which most deaths in the hyperinflammatory subphenotype occurred in the first 7 days. However, median time to recruitment in ROSE was much earlier at 7.6 h, the proportion of hyperinflammatory patients was higher at 39.6%, and mortality in the hyperinflammatory subphenotype was also higher, at 61% at 90-days. Taken together, these data suggest that discrepancies between subphenotype proportions in PHIND and historical data might at least in part be driven by a longer median time to recruitment. Future trials targeting the hyperinflammatory subphenotype should incorporate a more stringent recruitment window (eg, ≤ 48 h from illness onset) to maximise enrichment and potential therapeutic benefit.

This work demonstrates the feasibility of implementing a near-patient platform (Radox multiSTAT) for quantifying cytokines used for ARDS inflammatory subphenotyping (IL-6 and soluble TNFR1). To date, identifying ARDS subphenotypes has been constrained by reliance on time-intensive laboratory methods such as ELISA, which necessitate batching and centralised processing, rendering them impractical for clinical use. The PHIND study shows that rapid bedside subphenotyping is not only technically feasible but also yields results that are consistent with Quantikine ELISA. Although some discrepancies were observed in classifications using the two methods to quantify cytokines, misclassification rates were generally low. Furthermore, given the convenience and rapidity of the near-patient assay, and based on the consistency of clinical outcomes in phenotypes classified using this assay, we believe this approach to be robust for practical patient-level prospective phenotype classification. These results provide an important foundation for prospective, stratified clinical trials that will test whether tailoring interventions to biological subphenotypes can improve outcomes, transforming the paradigm from a one-size-fits-all approach to a precision approach.

	Hyperinflammatory (n=88)	Hypoinflammatory (n=398)	Unadjusted percent point difference (95% CI)	p value
28-day mortality	39 (44%)	91 (23%)	21.5 (6.8 to 36.1)	<0.0001
Intubation rate on high-flow nasal oxygen	7/11 (64%)	21/82 (26%)	38.0 (8.1 to 68.0)	0.026
Re-intubation rate	4/47 (9%)	33/254 (13%)	-4.5 (-13.5 to 4.5)	0.39
Ventilator-free days at day 28	0.0 (0.0 to 16.0)	14.0 (0.0 to 24.0)	..	<0.0001
Duration of ventilation, days	9.0 (3.0 to 19.0)	8.0 (3.0 to 15.0)	..	0.19
Duration of ventilation, survivors, days	11.5 (5.0 to 25.0)	8.0 (1.0 to 15.0)	..	0.011
Duration of ventilation, non-survivors, days	6.0 (3.0 to 14.0)	10.0 (6.0 to 16.5)	..	0.012
Length of ICU stay, days	13.0 (6.0 to 21.0)	13.0 (7.0 to 22.0)	..	0.54
Length of ICU stay, survivors, days	17.0 (10.0 to 32.0)	14.0 (8.0 to 24.5)	..	0.038
Length of ICU stay, non-survivors, days	6.0 (5.0 to 17.0)	12.0 (7.0 to 19.0)	..	0.0093
Length of hospital stay, days	20.5 (6.5 to 44.5)	22.0 (12.0 to 40.0)	..	0.35
Length of hospital stay, survivors, days	42.5 (23.0 to 67.0)	27.0 (15.0 to 48.0)	..	0.0031
Length of hospital stay, non-survivors, days	6.5 (4.5 to 17.5)	12.0 (7.0 to 19.0)	..	0.012

For categorical variables, data are n (%) with p values calculated from the χ^2 test. For continuous variables, data are median (IQR) with p values calculated from the Mann-Whitney U test. Significance was defined as a two-sided significance level of 0.05. ARDS=acute respiratory distress syndrome.

Table 2: Secondary outcomes in the ARDS cohort

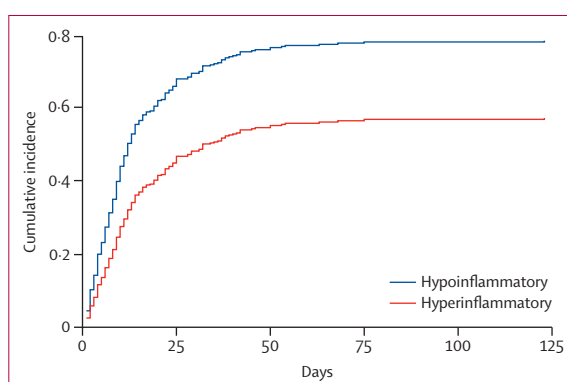


Figure 4: Cumulative incidence function from a semiparametric regression model for time to successful extubation in the presence of the competing risk of death in the acute respiratory distress syndrome cohort. Subhazard ratio 0.54 (95% CI 0.40–0.74; $p < 0.0001$) for hyperinflammatory subphenotype versus hypoinflammatory subphenotype.

As we move to the practical prospective application of subphenotypes, we need to consider that the distribution of subphenotype probabilities generated from parsimonious classification models will become less bimodal (ie, more patients fall close to the classification threshold). Our binary classification of hyperinflammatory and hypoinflammatory subphenotypes is probably overly simplistic and subphenotype membership is more likely to be a continuum. As more evidence is generated, we are

likely to refine new optimal classification thresholds in subphenotyping models that vary based on the treatment of interest.

A key strength of the PHIND study is its pragmatic design. By enrolling participants based on broad inclusion criteria and those receiving a range of advanced respiratory supports (eg, high-flow nasal oxygen, non-invasive ventilation, and invasive mechanical ventilation), the PHIND study reflects a heterogeneous real-world population of patients with ARDS and AHRF, rather than a highly selected typical trial population. The use of inclusion criteria consistent with the updated Global Definition of ARDS,² which incorporates patients on high-flow nasal oxygen, further enhances the external validity and modern applicability of these findings.

The inclusion of a separate exploratory AHRF cohort tested the hypothesis that these inflammatory subphenotypes extend beyond ARDS. Notably, in PHIND, patients with hyperinflammatory AHRF did not have any significantly worsened outcomes than did hypoinflammatory patients, other than increased hospital length of stay in survivors beyond 60 days and the frequency of the hyperinflammatory subphenotype was slightly lower (17% in AHRF and 18% in ARDS). The lack of significantly different clinical outcomes might have been a result of this exploratory cohort being underpowered, and indeed the adjusted OR for 60-day mortality in the hyperinflammatory subphenotype of AHRF, while not significant (OR 2.2 [95% CI 0.7–7.1]; $p=0.21$), was similar to that found in ARDS. This result contrasts with the contemporaneous SPARC study, which did not find a significant difference in mortality for patients with AHRF, but found worse organ-support free days, less ventilator-free days, and higher rates of intubation on non-invasive ventilation and high-flow nasal oxygen in patients with hyperinflammatory AHRF.¹⁸

The PHIND study has some limitations. We recognise that including patients on high-flow nasal oxygen and assuming a positive end-expiratory pressure of at least 5 cm H₂O in patients receiving high-flow nasal oxygen deviates from the Berlin definition. These modifications were an attempt to ensure generalisability of the study population in the setting of the growing use of high-flow nasal oxygen, and the increasing recognition that patients receiving high-flow nasal oxygen should be included in the definition of ARDS.²¹ Our study population is more closely aligned to the recently published Global definition of ARDS, which includes patients on high-flow nasal oxygen.² Notably, a significant number of patients were excluded at clinician discretion for other reasons not specified in the protocol. Most of these were excluded due to pandemic-related concerns between 2020 and 2022, when recruiting sites could not collect biological samples from patients with COVID-19. This factor did not seem to produce significant selection bias, as the clinical characteristics (eg, aetiology, SOFA score, PaO₂/FiO₂ ratio) of patients with ARDS in our study largely corresponded to

characteristics of a large pre-pandemic global epidemiological study.²² Next, although the near-patient immunoanalyser performed reliably in most cases, a small proportion of patients could not be subphenotyped due to technical issues (22 [4%] of 525). These issues were related to failure of device calibration or failure to measure reproducible biomarker concentrations, which were potentially secondary to operator pipetting error or defective assay plates. These issues were not associated with any identifiable patient characteristics. Throughout the study, subphenotyping failures were addressed by providing additional training at sites with increased failure rates and by rapidly replacing assay plate batches when failures were encountered. Additionally, subphenotype adjudication in PHIND was based on a cutoff probability from the parsimonious logistic regression equation of $p \geq 0.5$, which is arbitrary. A Youden-index derived threshold of 0.274 has been developed¹⁵ and would have probably allocated more patients to the hyperinflammatory subphenotype in PHIND. As this area of research progresses, we will undoubtedly discover other meaningful ways to subgroup these syndromes, which might be combined with or used in tandem with the hyperinflammatory and hypoinflammatory subphenotypes. Although PHIND presents strong evidence for the prognostic enrichment potential of a subphenotyping approach that splits ARDS into hyperinflammatory and hypoinflammatory subphenotypes, future work might possibly identify further biological heterogeneity.

As an observational study, PHIND was unable to prospectively assess the predictive or prognostic enrichment potential of inflammatory subphenotypes (ie, whether they identify patients more or less likely to benefit from pharmacological therapies). However, PHIND establishes the infrastructure and justification for future stratified trials to test the enrichment potential of ARDS subphenotypes. This is the first work (along with SPARC¹⁸) to demonstrate that subphenotypes exist prospectively and can be targeted with pharmacotherapeutics. The Precision medicine Adaptive platform Network Trial in Hypoxaemic acuteE respiratory failure (PANTHER; ISRCTN81435672) is a prospective, open-label, parallel-arm, adaptive platform trial that is enabled by the capacity to identify inflammatory subphenotypes in real time at the bedside.²³ PANTHER will focus on testing disease-modifying pharmacotherapeutic agents in a phase 2 subphenotype-stratified design; it began recruiting in November, 2025, and might lead to the development of subphenotype-targeted therapies in ARDS.

The PHIND study represents an important advance in the operationalisation of biological subphenotypes of ARDS into clinical care, providing prospective evidence that these subphenotypes can be identified in real time using a clinically feasible, near-patient assay and that are associated with differences in mortality and other clinically relevant outcomes.

Contributors

KR was responsible for conceptualisation, data curation, formal analysis, investigation, methodology, project administration, software, validation, visualisation, writing of the original draft, and review and editing. PS was responsible for conceptualisation, supervision, and review and editing. DBA was responsible for conceptualisation, supervision, and review and editing. CMCd was responsible for data curation, formal analysis, methodology, project administration, software, and review and editing. PAB was responsible for data curation, project administration, and review and editing. LB was responsible for data curation, project administration, and review and editing. LM was responsible for data curation, project administration, and review and editing. JB and JC were responsible for formal analysis, investigation, and review and editing. LC, MO, PH, TS, SC, IW, DB, DP, AJR, and AN were responsible for investigation and review and editing. LDJB was responsible for conceptualisation, supervision, and review and editing. MS-H and ACG were responsible for conceptualisation, supervision, investigation, and review and editing. KD was responsible for conceptualisation, methodology, supervision, investigation, validation, and review and editing. CMO'K was responsible for conceptualisation, methodology, funding acquisition, supervision, investigation, validation, and review and editing. MAM and CSC were responsible for conceptualisation, supervision, investigation, and review and editing. DFMCA was responsible for conceptualisation, funding acquisition, investigation, methodology, resources, supervision, validation, writing of the original draft, and review and editing. All authors had access to the raw data. Data were verified by KR, CMCd, PAB, LB, LM, CMO'K, and DFMCA. All authors accept responsibility for the decision to submit for publication, with the final decision made by KR and DFMCA.

Declaration of interests

KR reports funding for the submitted work from the Wellcome Trust ICAT Programme (203930/B/16/Z), Innovate UK (reference, 18045), Randox Laboratories, and the Belfast Health and Social Care Trust. Outside of the submitted work, he reports grants from the National Institute for Health and Care Research (NIHR), the Health Research Board, and the Association of Anaesthetists. He also reports travel support from the American Thoracic Society and membership of both the Irish Critical Care Clinical Trials Network and Irish Critical Care Clinical Trials Group executive committees. PS reports grants from the National Institutes of Health (NIH; R35GM142992; R01HL173531) and the Department of Defense (PR241420), outside of the submitted work. He also reports consulting fees from AstraZeneca and Prenosis. DBA reports grants from the NIHR, outside of the submitted work. LM reports funding for the submitted work from Innovate UK. MO reports grants from Biomerieux and Baxter, outside of the submitted work. She also reports her roles as Chair of the AKI Working Group of the European Renal Association, President-Elect of the European Society of Intensive Care Medicine, and Member of the Council of the Intensive Care Society UK. TS reports grants from the NIHR, outside of the submitted work. He reports honoraria from Biotest AG and a stake in a relevant patent EP3519594B1. He also reports his roles as Trustee for ICNARC and Editor-in-Chief for *Critical Care Explorations*. DB reports infrastructure support from the NIHR University College London Hospitals Biomedical Research Centre. DP reports grants from the NIHR, outside of the submitted work. He also reports his role as Faculty Member of the Intensive Care Medicine Board. LDJB reports grants from Longfords, the Innovative Medicine Initiative, Amsterdam UMC, Health Holland via Longfords, ZonMW, and Volition, outside of the submitted work. He reports participation on advisory boards for AstraZeneca and CSL Behring, and participation on a Data Safety Monitoring Board for Aptarion. MS-H reports grants from the Chief Scientist's Office of Scotland (PMAS/21/08), the NIHR, the Wellcome Trust, and the Medical Research Council (MRC), outside of the submitted work. He declares his roles as Director of Research for the Intensive Care Society, Member of the MRC–NIHR Programme, Chair of the EME–NIHR Advanced Fellowship Committee, and Member of the Council of the International Sepsis Forum. He also declares advisory board activity either directly or indirectly through the International Sepsis Forum. ACG reports infrastructure support from the NIHR Imperial Biomedical Research Centre. He reports consulting fees from AstraZeneca, Beckman Coulter, and Partner Therapeutics. He also reports his role as director of the NIHR

Health Technology Assessment Programme. CMO'K reports funding for the submitted work from Innovate UK (reference, 18045), Randox Laboratories, and the Belfast Health and Social Care Trust. She reports grants from the MRC, the Northern Ireland Health and Social Care Research and Development Division, and the Wellcome Trust, outside of the submitted work. She reports consulting fees and honoraria from Insmed. She reports her role as Member of the MRC grant Committee and Member of the Grant Working Group for the California Institute of Regenerative Medicine. She also reports that her spouse has received consultancy fees from Bayer, Aptarion, Direct Biologics, Helios, Novartis, and MSD. Her spouse has also received payment in relation to a patent for a novel treatment for inflammatory disease (USB962032). MAM reports grants from the NIH, National Heart, Lung, and Blood Institute; National Institute of Allergy and Infectious Disease; Department of Defense; the California Institute of Regenerative Medicine; Roche Genentech; and Quantum Therapeutics, outside of the submitted work. He also reports consulting fees from Merck and Healios. CSC reports grants from the NIH (R35HL140026; R35HL177135). She reports grants from Roche Genentech, NIH, the Department of Defense, and the Quantum Leap Healthcare Collaborative, outside of the submitted work. She reports consulting fees from Vasomune, Gen1E Life Sciences, NGM Bio, Cellenkos, Calcimedica, Arrowhead, EnlITISA, Novartis, Merck, Healios, Matisse, and Boehringer Ingelheim. She reports honoraria from Fisher-Paykel. She reports a patent on metagenomic sequencing for sepsis diagnosis issued to the Regents of University of California and the Chan Zuckerberg BioHub. She also reports her role as Council Member of the International Sepsis Forum. DFMCA reports funding for the submitted work from Innovate UK (reference, 18045), Randox Laboratories, and the Belfast Health and Social Care Trust. He reports grants from the NIHR, the MRC, the Northern Ireland Health and Social Care Research and Development Division, and the Wellcome Trust, outside of the submitted work. He reports consulting fees from Bayer, Aptarion, Direct Biologics, Healios, Novartis, and Meso Scale Discovery. He reports a patent for a novel treatment for inflammatory disease (USB962032), issued to his institution. He also reports his roles as the Director of the MRC–NIHR Programme and Scientific Director for Research Programmes for the NIHR. All other authors declare no competing interests.

Data sharing

Requests for access to de-identified participant data can be made to the corresponding author. Data sharing will be considered following the approval of the research proposal by the study authors, the study sponsor, and an appropriate ethical review board.

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