Identification of molecular subphenotypes in two cohorts of paediatric ARDS

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ABSTRACT

Background: Two subphenotypes of acute respiratory distress syndrome (ARDS), hypoinflammatory and hyperinflammatory, have been reported in adults and in a single paediatric cohort. The relevance of these subphenotypes in paediatrics requires further investigation. We aimed to identify subphenotypes in two large observational cohorts of paediatric ARDS and assess their congruence with prior descriptions.

Methods: We performed latent class analysis (LCA) separately on two cohorts using biomarkers as inputs. Subphenotypes were compared on clinical characteristics and outcomes. Finally, we assessed overlap with adult cohorts using parsimonious classifiers.

Findings: In two cohorts from the Children’s Hospital of Philadelphia (n=333) and from a multicentre study based at the University of California San Francisco (n=293), LCA identified two subphenotypes defined by differential elevation of biomarkers reflecting inflammation and endotheliopathy. In both cohorts, hyperinflammatory subjects had greater illness severity, more sepsis and mortality in ‘hyperinflammatory’ subjects.8 More recently, LCA was used to identify subphenotypes in 304 children enrolled in the Biomarkers in Children with Acute Lung Injury (BALI) study,9 replicating the methodology used in adult ARDS. As in adults, hypoinflammatory and hyperinflammatory subphenotypes were identified in this paediatric cohort.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ We explored PubMed without age or date limitations using terms related to acute respiratory distress syndrome (ARDS) (eg, ‘acute respiratory distress’ and ‘ARDS’) and subphenotyping (eg, ‘subphenotype’, ‘subtype’ and ‘endotype’). Overall, the data strongly confirmed the presence of two subphenotypes of ARDS based on reanalyses of multiple adult ARDS trials and cohorts, including recent evidence in COVID-19. Most studies suggested ‘hypoinflammatory’ and ‘hyperinflammatory’ subphenotypes identified using latent class analysis, including a single paediatric study. The paediatric study has not been replicated.

WHAT THIS STUDY ADDS

⇒ We report biomarker-based subphenotypes in two independent cohorts of paediatric ARDS consistent with the hypoinflammatory and hyperinflammatory described primarily in adults. Both cohorts demonstrated overlap with adult subphenotypes when assessed using various parsimonious classifiers.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Hypoinflammatory and hyperinflammatory subphenotypes of paediatric ARDS are consistently identifiable and have clinical relevance. Subphenotypes should be leveraged for prognostic and predictive enrichment in future paediatric ARDS trials.

INTRODUCTION

In adults with acute respiratory distress syndrome (ARDS), reanalyses of clinical trials using protein biomarkers1–4 have led to the discovery of subphenotypes with presumed shared pathophysiology.5–8 Two distinct subphenotypes, dubbed ‘hypoinflammatory’ and ‘hyperinflammatory’, have consistently been described using latent class analysis (LCA) with a combination of biomarkers and clinical variables as inputs. Hyperinflammatory ARDS was characterised by higher rates of shock and sepsis, higher levels of inflammatory biomarkers and greater mortality. The subphenotypes demonstrated differential response to higher positive end-expiratory pressure,1 fluid strategies2 and simvastatin,3 suggesting the potential for predictive enrichment. More recently, these subphenotypes were identified in ARDS caused by COVID-19,7 with a suggestion of differential response to corticosteroids.

Investigations of whether clinically relevant subphenotypes exist in paediatric ARDS are in their infancy. Molecular subtypes defined by LCA of circulating matrix metalloproteinases (MMPs) were reported in 235 children with ARDS, with higher mortality in ‘hyperinflammatory’ subjects.8 More recently, LCA was used to identify subphenotypes in 304 children enrolled in the Biomarkers in Children with Acute Lung Injury (BALI) study,9 replicating the methodology used in adult ARDS. As in adults, hypoinflammatory and hyperinflammatory subphenotypes were identified in this paediatric
ARDS cohort, with greater vasopressor use, more sepsis, higher levels of inflammatory biomarkers and higher mortality in hyper-inflammatory subjects. However, as BALI was an ancillary of the Randomized Evaluation of Sedation Titration for Respiratory Failure (RESTORE) clinical trial, some limitations of the BALI cohort may limit generalisability. For example, bronchiolitis was over-represented as an aetiology of ARDS in BALI. Additionally, while these subphenotypes mirror the prognostic significance seen in adults, there was no differential effect of the sedation protocols tested in the RESTORE trial, and thus the predictive utility of these subphenotypes remains unclear in paediatrics. Finally, the findings from BALI were restricted to a single cohort and lacked external validation.

Thus, while the premise of hypoinflammatory and hyper-inflammatory ARDS has been established in adults and suggested in paediatrics, additional exploration is necessary. Therefore, we aimed to identify subphenotypes independently in two large observational cohorts of paediatric ARDS, hypothesising the existence of at least two classes. Given the recent interest in differential efficacy of corticosteroids in ARDS according to subphenotype, we assessed whether non-randomised methylprednisolone use showed heterogeneous treatment effect. As we were interested in whether these subphenotypes represented distinct molecular subtypes of ARDS and because of differential availability of clinical variables in the two cohorts, we purposefully used only circulating biomarkers as inputs for our analysis.

**METHODS**

**Study design and overview**

Detailed methods are provided in the online supplemental data. This was a secondary analysis of two separate paediatric ARDS cohorts, both with distinct eligibility criteria, timeframes and biomarker measurements.

**Participants**


**Measurements**

In both cohorts, plasma was collected within 24 hours of ARDS diagnosis and biomarkers measured using a combination of single and multiplex ELISAs. At CHOP, Olfactomedin 4, granzyyme B, heat shock protein 70, interleukin-1α (IL-1α), IL-8, C-C motif chemokine ligand 3/macrophage inflammatory protein-1α (CCL3/MIP-1α), MIP-1β and MMP8 were measured on a Luminex platform. CCL7, CCL22, IL-6, soluble tumour necrosis factor receptor 1 (sTNFRF1) and TNFα were measured using a custom Ella (Bio-Technne) multiplex. Nucleosomes were measured using a singleplex ELISA (Sigma-Aldrich). Angiopoietin 2 (ANG2), the soluble receptor for advanced glycation endproducts (sRAGE) and surfactant protein D (SPD) were measured using singleplex ELISAs (R&D Systems). At UCSF, the Human InflammationMAP panel was used to measure 46 biomarkers of inflammation (Myriad RBM). In addition, singleplex ELISAs measured SPD (Tamaea Corp), ANG2 and sRAGE (R&D Systems), soluble thrombomodulin (sTM; Diagnostica Stago), fibroblast growth factor 23 (FGF23; Quidel) and MMP1, 2, 7, 8, 9a and tissue inhibitor of metalloproteinase-2 (Myriad RBM) in a subset of patients with residual plasma.

**Definitions**

Oxygenation was quantified using \( \text{PaO}_2/\text{FiO}_2 \) and oxygenation index (OI: mean airway pressure×\( \text{FiO}_2 \)×100/\( \text{PaO}_2 \)). The vasopressor-inotrope score quantified degree of vasoactive support. Non-pulmonary organ failures at the time of ARDS diagnosis were identified using accepted definitions.

Severity of illness was scored using the Pediatric Risk of Mortality III at 12 hours. The designation ‘immunocompromised’ required presence of an immunocompromising diagnosis and active immunosuppressive chemotherapy or a congenital immunodeficiency.

Ventilator-free days (VFDs) at 28 days were defined by subtracting the number of invasive ventilator days from 28, with non-survivors and ≥28 ventilator days assigned 0 VFDs. Mortality was censored at 90 days.

**Latent class analyses in CHOP and UCSF cohorts**

In both cohorts, biomarkers were log-transformed, standardised (mean=0, SD=1) and checked for collinearity, with highly correlated biomarkers removed to improve model performance. A priori, both CHOP and UCSF performed separate LCA solely using biomarkers, and not clinical variables, agnostic to outcomes. Model performance was assessed using Bayesian Information Criterion, Vuong-Lo-Mendell-Rubin likelihood ratio test and entropy. For the UCSF cohort, missingness was addressed using full information maximum likelihood. As a sensitivity analysis, we repeated LCA removing biomarkers with missingness for the UCSF cohort. There was no missingness in the CHOP cohort. LCA was performed in Mplus V.8 and additional analyses using Stata V.14.

Clinical characteristics between subphenotypes were compared using non-parametric statistics. As the CHOP cohort collected detailed medication data, as an exploratory analysis, we assessed the association between methylprednisolone use in the first 72 hours of ARDS with 90-day mortality using Cox regression, stratified by subphenotype, and testing for significance of the interaction term. As methylprednisolone use was non-randomised, we repeated this analysis adjusting for age, sex, OI, non-pulmonary organ failures, vasopressor score and immunocompromised status. We performed an additional analysis adjusting for the above variables and hydrocortisone exposure. Next, we assessed the association between any corticosteroid exposure (methylprednisolone and/or hydrocortisone) with 90-day mortality.

As a final check on the association between methylprednisolone and mortality stratified by subphenotype, we generated a propensity score for the probability of receiving methylprednisolone and repeated the Cox regression after inverse probability treatment weighting (IPTW) of the stabilised propensity score.

**Validation of existing subphenotype classifications**

Certain biomarkers or clinical variables used in the development of previously described parsimonious predictive models were not available in both cohorts. This necessitated a multi-step procedure, wherein we (1) first validated existing models in the CHOP cohort, (2) developed a predictive model within the CHOP cohort using biomarkers available at both CHOP and UCSF and (3) tested this new model in the UCSF cohort. First, we applied 3-variable and 4-variable models to the CHOP cohort using IL-6, IL-8, sTNFR1, bicarbonate and use of vasopressors using the equations in Table S4 from Sinha et al. We...
then reported sensitivity, specificity, accuracy and the area under the receiver operating characteristic (AUROC) curve. Second, we identified biomarkers common to both cohorts in order to build a model that discriminated between subphenotypes. We first performed the least absolute shrinkage and selection operator, subsequently testing multiple separate models with the remaining biomarkers, assessing Bayesian Information Criterion, AUROC and calibration. Nested models were directly compared overlapping (91% concordance), suggesting stability of the initial modelling approach.

**RESULTS**

**Description of the cohorts**

There were 333 children with Berlin ARDS from CHOP and 293 with AECC ALI from UCSF (table 1; online supplemental figures 1 and 2). The CHOP cohort had a median age of 5.8 years, with pneumonia (49%) and non-pulmonary sepsis (25%) representing the main ARDS aetiologies. Approximately one-quarter of the CHOP cohort was immunocompromised, half of whom had undergone stem cell transplant. Mortality in the CHOP cohort was 20%. The UCSF cohort had a median age of 5.1 years, and also had pneumonia (52%) and non-pulmonary sepsis (24%) as the main ARDS aetiologies. The UCSF cohort had a similar percentage of subjects with immunocompromising diagnoses and stem cell transplant, with a 13% overall mortality rate.

**Identification of subphenotypes**

For both cohorts, LCA was performed separately using biomarkers as the only input. After removing colinear biomarkers in the CHOP cohort (online supplemental figure 3), CCL3, IL-6, MMP8, CCL7, TNFR1, ANG2, sRAGE and SPD were used as class-defining variables. For the UCSF cohort, missingness ranged between 0% and 36% (online supplemental table 1; online supplemental figure 4). After removing colinear biomarkers and those consistently below the lower limits of multiplex detection, LCA was performed on 23 biomarkers, of which 10 had missingness ranging between 17% and 36%. Biomarkers included for LCA common to both cohorts were ANG2, IL-6, MMP8, sRAGE and SPD.

In both cohorts, a two-class model showed best fit (figure 1; online supplemental table 2). The classes showed the greatest separation in biomarkers reflecting innate and myeloid immunity (IL-6, IL-8, sTNFR1, sTNFR2, granzyme B), endothelial injury (ANG2, sRAGE) and stromal injury (MMP8), with higher values in hyperinflammatory ARDS. In both cohorts, the hyperinflammatory subphenotype was less prevalent (31% at CHOP and 27% at UCSF). In both cohorts (figure 2), mortality was higher in the hyperinflammatory (41% at CHOP and 28% at UCSF) than in the hypoinflammatory subphenotype (11% at CHOP and 7% at UCSF). In both cohorts, the use of the full panel of biomarkers resulted in identical class identification and assignments (online supplemental figures 5 and 6).

In sensitivity analyses for the UCSF cohort, when using 17 biomarkers (removing 6 with high missingness: endothelial protein C receptor (EPCR), FGF23i, MMP1, MMP2, MMP7, MMP8), subphenotype assignment was 98% concordant with the main analysis using all 23 biomarkers. In an analysis using 13 biomarkers with 0% missingness (removing ANG2, sRAGE, SPD, sTM), subphenotype assignment remained highly overlapping (91% concordance), suggesting stability of the initial modelling approach.

**Clinical characterisation of subphenotypes**

We stratified patients within each cohort according to most likely subphenotype. In the CHOP cohort, hyperinflammatory subjects had higher illness severity, more organ failures, worse oxygenation at 24 hours and were more likely to be immunocompromised (online supplemental table 3). Non-pulmonary sepsis was the predominant aetiology of ARDS (50%) in hyperinflammatory ARDS, whereas pneumonia was most common in hypoinflammatory ARDS (59%). Inhaled nitric oxide (51% vs 31%, p=0.001) and vasopressor use (90% vs 61%, p<0.001) were more common in hyperinflammatory ARDS at CHOP. The UCSF cohort had similar clinical characteristics (online supplemental table 4), with greater severity of illness, worse hypoxemia, more immunocompromised subjects, greater prevalence of

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**Table 1** Description of the cohorts.

<table>
<thead>
<tr>
<th>Variables</th>
<th>CHOP cohort (n=333)</th>
<th>UCSF cohort (n=293)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>5.8 (1.8–13.1)</td>
<td>5.1 (1.1–12.7)</td>
</tr>
<tr>
<td>Female (%)</td>
<td>149 (45)</td>
<td>137 (47)</td>
</tr>
<tr>
<td>Severity of illness</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRISM III at 12 hours</td>
<td>11 (6–17)</td>
<td>11 (5–18)</td>
</tr>
<tr>
<td>Non-pulmonary organ failures</td>
<td>2 (1–3)</td>
<td>2 (1–3)</td>
</tr>
<tr>
<td>Vasopressor score</td>
<td>8 (0–18)</td>
<td>0 (0–5)</td>
</tr>
<tr>
<td>Comorbidities (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Immunocompromised</td>
<td>84 (25)</td>
<td>70 (24)</td>
</tr>
<tr>
<td>Stem cell transplant</td>
<td>39 (12)</td>
<td>36 (13)</td>
</tr>
<tr>
<td>Cause of ARDS (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infectious pneumonia</td>
<td>162 (49)</td>
<td>151 (52)</td>
</tr>
<tr>
<td>Non-pulmonary sepsis</td>
<td>83 (25)</td>
<td>68 (24)</td>
</tr>
<tr>
<td>Aspiration pneumonia</td>
<td>50 (15)</td>
<td>20 (7)</td>
</tr>
<tr>
<td>Other</td>
<td>38 (11)</td>
<td>54 (18)</td>
</tr>
<tr>
<td>ARDS onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2/FIO2</td>
<td>153 (99–225)</td>
<td>138 (85–223)</td>
</tr>
<tr>
<td>Oxygenation index</td>
<td>11.1 (7.3–19.3)</td>
<td>9.4 (4.8–12.9)</td>
</tr>
<tr>
<td>ΔP (PIP minus PEEP)</td>
<td>21 (17–25)</td>
<td>20 (16–24)</td>
</tr>
<tr>
<td>24 hours after onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaO2/FIO2</td>
<td>223 (155–288)</td>
<td>165 (102–246)</td>
</tr>
<tr>
<td>Oxygenation index</td>
<td>7.4 (4.8–12.9)</td>
<td>8.2 (5.3–15.3)</td>
</tr>
<tr>
<td>ΔP (PIP minus PEEP)</td>
<td>17 (14–21)</td>
<td>19 (14–22)</td>
</tr>
<tr>
<td>Outcomes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>90-day mortality (%)</td>
<td>68 (20)</td>
<td>38 (13)</td>
</tr>
<tr>
<td>28-day VFDs</td>
<td>17 (0–22)</td>
<td>21 (11–24)</td>
</tr>
</tbody>
</table>

Variables are presented as median (IQR) and N (%). ARDS, acute respiratory distress syndrome; CHOP, Children’s Hospital of Philadelphia; PEEP, positive end-expiratory pressure; PIP, peak inflating pressure; PRISM, Pediatric Risk of Mortality; UCSF, University of California San Francisco; VFDs, ventilator-free days.

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non-pulmonary sepsis and more vasopressor use (59% vs 35%, p<0.001) in hyperinflammatory ARDS.

Exploratory analysis of heterogeneity of treatment effect of methylprednisolone

We assessed whether there was heterogeneity of treatment effect of early methylprednisolone exposure on mortality according to subphenotype in the CHOP cohort. We chose methylprednisolone use within the first 72 hours of ARDS onset, rather than all corticosteroids, as hydrocortisone was used exclusively for shock or known adrenal insufficiency, rather than for pulmonary indications such as pneumonia or ARDS. We have previously shown that 89% of methylprednisolone use at CHOP occurred within 3 days, with a median of 0 (IQR 0, 1) days until initiation. Methylprednisolone use was non-randomised (online supplemental table 5) and more prevalent in hypoinflammatory ARDS (33% hypoinflammatory vs 13% hyperinflammatory). In unadjusted analysis (table 2; online supplemental table 6), there was effect modification by subphenotype (interaction p=0.017) on the association between methylprednisolone and mortality, with no benefit in hypoinflammatory subjects (HR 0.61, 95% CI 0.24 to 1.52) and suggestion of harm in hyperinflammatory (HR 2.46, 95% CI 1.21 to 5.02). To account for non-randomised exposure to methylprednisolone, we repeated this analysis adjusted for confounders (online supplemental figure 7; table 2). We found possible benefit in hypoinflammatory (HR 0.31, 95% CI 0.10 to 0.96) and harm in hyperinflammatory ARDS (HR 3.13, 95% CI 1.36 to 7.21; interaction p=0.0017). Conclusions were unchanged when also adjusting for hydrocortisone (table 2). In sensitivity analyses, conclusions remained consistent when we performed IPTW using the propensity score (online supplemental figures 8 and 9). While the p value for the interaction was p=0.083 in this analysis (table 2), in stratified analysis the IPTW confirmed possible benefit for methylprednisolone in hypoinflammatory ARDS (HR 0.22, 95% CI 0.06 to 0.80) and suggested harm in hyperinflammatory ARDS (HR 1.93, 95% CI 0.97 to 3.94).

Figure 1 Differences in standardised variables between subphenotypes in the CHOP and UCSF cohorts. Biomarkers were standardised (mean=0, SD=1) and ranked according to degree of separation between subphenotypes. ARDS, acute respiratory distress syndrome; CHOP, Children’s Hospital of Philadelphia; UCSF, University of California San Francisco.

Figure 2 Kaplan-Meier survival curves for the subphenotypes in the CHOP and UCSF cohorts. ARDS, acute respiratory distress syndrome; CHOP, Children’s Hospital of Philadelphia; UCSF, University of California San Francisco.
to 3.84). There was no evidence for heterogeneous treatment effect according to subphenotype when assessing the association of any corticosteroid (rather than solely methylprednisolone) with mortality (online supplemental table 7).

Comparison with adult subphenotypes

When applying parsimonious models for discriminating subphenotypes described in adults1–7 in the CHOP cohort, both 3-variable and 4-variable models demonstrated excellent discrimination (online supplemental tables 8 and 9). Using CHOP-derived LCA subphenotypes as the gold standard, >80% of subjects were assigned to the correct class in all models assessed. Then, using biomarkers common to both CHOP and UCSF cohorts, we developed models within the CHOP cohort to discriminate between subphenotypes. Two separate 4-variable models including both IL-6 and IL-8 were optimal (table 3; online supplemental table 10; online supplemental figure 10). As some adult studies report 3-variable parsimonious models, we also assessed the performance of two separate 3-variable models using IL-6. We applied these CHOP-derived classifiers to the UCSF cohort (table 4). Both 3-variable and 4-variable models demonstrated excellent discrimination in the UCSF cohort, with the 4-variable classifiers performing better. Finally, when we repeated the LCA de novo using a combination of clinical variables and biomarkers, we found 95% overlap of subjects assigned to hypoinflammatory and hyperinflammatory ARDS when we used all available biomarkers and clinical variables (online supplemental table 11), and 87% overlap when we restricted to biomarkers and clinical variables used in prior descriptions of subphenotyping adult and paediatric ARDS (online supplemental table 12).

DISCUSSION

In two cohorts of paediatric ARDS, we found evidence for subphenotypes consistent with hypoinflammatory and hyperinflammatory ARDS previously reported, with substantial overlap with those described in adults using parsimonious predictive models, suggesting that we likely replicated these subphenotypes. Subphenotypes were characterised by differences in inflammatory biomarkers and the tissue damage markers ANG2 and sRAGE. In both cohorts, the subphenotypes had divergent outcomes, suggesting utility for prognostic enrichment.

The subphenotypes reported in the CHOP and UCSF paediatric ARDS cohorts reproduce in composition, prevalence (hypo:hyper~2:1) and clinical characteristics what has been reported in five trials and three observational cohorts of adult ARDS1–4 7 23 and in a single paediatric study.7 The biomarkers driving class assignments in our study were similar (sTNFR1 and sTNFR2) or identical (IL-6, IL-8) to those with the largest differences between subphenotypes in prior reports. Notably, there were differences between our CHOP and UCSF ARDS cohorts and the paediatric reanalysis of BALI.9 First, BALI was a substudy of a trial of intubated subjects with varying degrees of lung disease, including those with bronchiolitis and asthma, with ARDS assigned post hoc. Subjects in BALI had lower mortality (7%), potentially due to less severe ARDS. By contrast, both CHOP and UCSF cohorts had a higher prevalence of more typical ARDS aetiologies (pneumonia and non-pulmonary sepsis) and worse mortality (20% and 13%, respectively), consistent with modern paediatric cohorts.24 Thus, the discovery of hypoinflammatory and hyperinflammatory ARDS in our study is reassuring of the generalisability of these subtypes in both adults and paediatrics. Alternatively, the ability to identify these subphenotypes in both typical ARDS cohorts (CHOP and UCSF) as well as in less severe lung injury (BALI) suggests that the identified subphenotypes are not necessarily specific to ARDS. Indeed, these subphenotypes have been reported in non-ARDS respiratory failure in adults25 and in children.26

Despite discovery of hypoinflammatory and hyperinflammatory subphenotypes in multiple ARDS cohorts, it is notable that the variables driving class assignment reflect innate immune activation (IL-6, IL-8, sTNFR1) or endotheliopathy (ANG2), rather than biomarkers specific to lung injury (eg, SPD). Recently,

### Table 2

<table>
<thead>
<tr>
<th>Methylprednisolone effect</th>
<th>Hypoinflammatory</th>
<th>Hyperinflammatory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unadjusted</td>
<td>HR (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>Unadjusted</td>
<td>0.61 (0.24 to 1.52)</td>
<td>0.289</td>
</tr>
<tr>
<td>Adjusted*</td>
<td>0.31 (0.10 to 0.96)</td>
<td>0.043</td>
</tr>
<tr>
<td>Additionally adjusted for hydrocortisone*</td>
<td>0.29 (0.08 to 1.04)</td>
<td>0.058</td>
</tr>
<tr>
<td>IPTW</td>
<td>0.22 (0.06 to 0.80)</td>
<td>0.021</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, oxygenation index, non-pulmonary organ failures, vasopressor score and immunocompromised status.

### Table 3

<table>
<thead>
<tr>
<th>Model</th>
<th>Intercept</th>
<th>IL-6</th>
<th>CCL3</th>
<th>ANG2</th>
<th>MMP8</th>
<th>IL-8</th>
<th>AUROC (95% CI)</th>
<th>Calibration belt p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three variables</td>
<td>16.262</td>
<td>1.111</td>
<td>1.620</td>
<td>–</td>
<td>0.333</td>
<td>–</td>
<td>0.95 (0.93 to 0.97)</td>
<td>0.223</td>
</tr>
<tr>
<td>29.439</td>
<td>1.232</td>
<td>1.598</td>
<td>1.747</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.97 (0.96 to 0.99)</td>
<td>0.483</td>
</tr>
<tr>
<td>Four variables</td>
<td>23.285</td>
<td>0.382</td>
<td>0.841</td>
<td>–</td>
<td>0.642</td>
<td>2.010</td>
<td>0.98 (0.97 to 0.99)</td>
<td>0.424</td>
</tr>
<tr>
<td>39.766</td>
<td>0.616</td>
<td>1.086</td>
<td>2.149</td>
<td>–</td>
<td>2.178</td>
<td>–</td>
<td>0.99 (0.98 to 1.00)</td>
<td>0.789</td>
</tr>
</tbody>
</table>

Coefficients were derived using the (natural)log-transformed biomarker, with 1 added to each measured value prior to log-transforming. AUROC, area under the receiver operating characteristic; CHOP, Children’s Hospital of Philadelphia; IL-6, interleukin 6.

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The subphenotypes reported in the CHOP and UCSF paediatric ARDS cohorts reproduce in composition, prevalence (hypo:hyper~2:1) and clinical characteristics what has been reported in five trials and three observational cohorts of adult ARDS1–4 7 23 and in a single paediatric study.7 The biomarkers driving class assignments in our study were similar (sTNFR1 and sTNFR2) or identical (IL-6, IL-8) to those with the largest differences between subphenotypes in prior reports. Notably, there were differences between our CHOP and UCSF ARDS cohorts and the paediatric reanalysis of BALI.9 First, BALI was a substudy of a trial of intubated subjects with varying degrees of lung disease, including those with bronchiolitis and asthma, with ARDS assigned post hoc. Subjects in BALI had lower mortality (7%), potentially due to less severe ARDS. By contrast, both CHOP and UCSF cohorts had a higher prevalence of more typical ARDS aetiologies (pneumonia and non-pulmonary sepsis) and worse mortality (20% and 13%, respectively), consistent with modern paediatric cohorts.24 Thus, the discovery of hypoinflammatory and hyperinflammatory ARDS in our study is reassuring of the generalisability of these subtypes in both adults and paediatrics. Alternatively, the ability to identify these subphenotypes in both typical ARDS cohorts (CHOP and UCSF) as well as in less severe lung injury (BALI) suggests that the identified subphenotypes are not necessarily specific to ARDS. Indeed, these subphenotypes have been reported in non-ARDS respiratory failure in adults25 and in children.26

Despite discovery of hypoinflammatory and hyperinflammatory subphenotypes in multiple ARDS cohorts, it is notable that the variables driving class assignment reflect innate immune activation (IL-6, IL-8, sTNFR1) or endotheliopathy (ANG2), rather than biomarkers specific to lung injury (eg, SPD). Recently,
two separate studies using LCA have identified hypoinflammatory and hyperinflammatory subtypes of acute kidney injury, with class assignments driven by ANG2, IL-8 and sTNFR1. More recently, these subphenotypes have also been described in COVID-19 and in children with shock, respiratory failure or severe burns. It is possible that subtypes are not specific to ARDS, but reflect subjects with and without endotheliopathy and hyperinflammation generalisable to other critical illness syndromes. This requires further evaluation in cohorts with a broad range of biomarkers. The discovery-based panel used in UCSF confirms endotheliopathy in hyperinflammatory ARDS beyond elevated ANG2, with higher levels of stem cell factor, intracellular and vascular cell adhesion molecules, EPCR and sTM. Several additional targetable pathways were implicated in hyperinflammatory ARDS, including elevations in inflammasome-related IL-18 and depression of the T cell marker CCL5/RANTES, which may indicate lymphosuppression in early sepsis, or may be due to a lower proportion of viral infections in hyperinflammatory ARDS.

We found preliminary evidence for differential efficacy of methylprednisolone, with benefit in hypoinflammatory (primarily pneumonia and direct ARDS) and harm in hyperinflammatory (primarily non-pulmonary sepsis) ARDS. Methylprednisolone has shown benefit in severe community acquired pneumonia, suggesting biologic plausibility favouring methylprednisolone in hypoinflammatory ARDS, which was primarily caused by pneumonia. However, the non-randomised nature of the intervention risks significant residual confounding, and the total number of non-survivors is small, suggesting that this result should be interpreted cautiously, as they may result from unmeasured bias inherent to cohort studies. Notably, these results contrast with what was reported with corticosteroids in COVID-19, where corticosteroids were associated with benefit in hyperinflammatory subjects and harm in hypoinflammatory. We note that the COVID-19 analysis was also non-randomised, and that results from a homogeneous aetiology of adult respiratory failure (eg, COVID-19) may not generalise to a heterogeneous paediatric ARDS cohort. Overall, given the small sample size, the lack of randomisation, and the propensity of using corticosteroids in the most severely ill patients, the finding of heterogeneous efficacy of methylprednisolone between the classes should be treated highly cautiously and warrants further evaluation in larger studies and in the constraints of a randomised trial. Our results do, however, demonstrate the potential for leveraging biomarker-based predictive enrichment in future trials in paediatric ARDS.

Our study has several strengths. We independently analysed two cohorts, separated both temporally and geographically. The results did not substantially change when we removed biomarkers with high missingness. We used 90-day mortality as our primary outcome, a longer timeframe than most paediatric studies. Finally, our findings are congruent with what has previously been reported in adult and paediatric ARDS, as well as other critical illness syndromes, with substantial overlap when using simplified prediction models and repeating the methodology of prior publications.

However, our study has limitations. While sample sizes were large for paediatric ARDS, which is less prevalent than adult ARDS, they were only ~300, which could affect modelling. Notably, the BALI reanalysis also found the same subtypes with n=304, and our fit statistics robustly identified two subphenotypes. The CHOP and UCSF cohorts did not collect the same biomarkers, making a direct comparison less straightforward. Our study thus did not formally validate the subphenotypes found in one cohort in the second cohort using the same biomarkers. Nevertheless, significant similarities were found in the biomarker, clinical characteristics and outcomes, as well as good discrimination when using simplified prediction algorithms. The two cohorts did not collect data on the same treatments, precluding a validation of the heterogeneity of treatment effect of methylprednisolone. Importantly, neither cohort was from a randomised trial, and treatments reflected provider decisions, and thus subject to confounding. Thus, the differential efficacy of methylprednisolone should be interpreted very cautiously as hypothesis generating.

CONCLUSIONS

We identified two subphenotypes of paediatric ARDS from two separate cohorts, consistent with hypoinflammatory and hyperinflammatory subphenotypes reported previously. The hyperinflammatory subphenotypes had worse outcomes, suggesting utility for prognostic enrichment. Given their apparent biologic relevance, future paediatric ARDS trials should leverage biomarker-defined subphenotypes in their analysis.

Table 4 Performance of 3-variable and 4-variable models to predict hyperinflammatory subphenotypes derived from the CHOP cohort (from online supplemental table 5) in the UCSF cohort

<table>
<thead>
<tr>
<th>Variables</th>
<th>AUROC (95% CI)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Three variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6, CCL3, MMP8</td>
<td>0.84 (0.77 to 0.90)</td>
<td>0.63</td>
<td>0.90</td>
<td>0.75</td>
</tr>
<tr>
<td>IL-6, sTNFR1, ANG2</td>
<td>0.89 (0.84 to 0.94)</td>
<td>0.68</td>
<td>0.90</td>
<td>0.84</td>
</tr>
<tr>
<td>Four variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6, CCL3, MMP8, IL-8</td>
<td>0.90 (0.85 to 0.94)</td>
<td>0.70</td>
<td>0.91</td>
<td>0.86</td>
</tr>
<tr>
<td>IL-6, CCL3, ANG2, IL-8</td>
<td>0.93 (0.90 to 0.97)</td>
<td>0.75</td>
<td>0.93</td>
<td>0.88</td>
</tr>
</tbody>
</table>

Test characteristics were determined using a cut-off of ≥0.5 to identify the hyperinflammatory subtype. AUROC, area under the receiver operating characteristic; CHOP, Children’s Hospital of Philadelphia; IL-6, interleukin 6; UCSF, University of California San Francisco.
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Contributors NY, MS2, PS and AS conceived and designed the study and performed the bulk of the data analysis. JT, MJ1, MRH and MFA performed biomarker measurements and related analyses at UCSF and UCLA. HW, MNA, DJ1M and ESH performed the biomarker measurements and related analyses at CHOP, CCHMC and Penn State Hershey. All authors contributed to the manuscript. NY is guarantor of the manuscript.

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Patient consent for publication Not applicable.

Ethics approval This study involves human participants. Both studies were approved by their local institutional review boards (IRBs), and informed consent obtained prior to sample and data collection (CHOP IRB 13-010578 and UCSF IRB 10-00206), consistent with the Helsinki Declaration of 1975. Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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