BRIEF REPORT

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Expression Patterns of Airway Fluid Cytokines From Intubated Children With Pediatric Acute Respiratory Distress Syndrome

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OBJECTIVES: Pediatric acute respiratory distress syndrome (PARDS) is a heterogeneous illness affecting 6% of mechanically ventilated children and with an overall mortality of 17%. Studies in PARDS have mainly focused on plasma biomarkers which may not reflect airway biomarkers. We lack adequate understanding of the inflammatory mediators and underlying immune responses in the airways of PARDS patients. Our objective was to compare the levels of cytokines in the airway fluid of intubated children with severe versus nonsevere acute respiratory distress syndrome.

DESIGN: Prospective observational cohort study.

SETTING: Single 36-bed quaternary care academic safety-net hospital PICU.

PATIENTS: Children intubated for acute respiratory failure between January 2018 and November 2021 stratified by Pediatric Acute Lung Injury Consensus Conference-1 criteria for PARDS.

INTERVENTIONS: None.

MEASUREMENTS AND MAIN RESULTS: We measured levels of 23 cytokines, chemokines, and protein biomarkers in the tracheal aspirate from 82 intubated children, between 14 days and 17 years old, at risk for or with PARDS. Levels of interleukin-4, -5, -7, -8, -12(p-70), -17a, -21, and fractalkine were higher in patients with severe versus nonsevere PARDS. There were no associations between airway and plasma cytokines.

CONCLUSIONS: Proinflammatory cytokines are elevated in the airway fluid from intubated children with severe PARDS and reflect diverse patterns of airway inflammation.

KEY WORDS: biomarker; cytokine; intubation; mechanical ventilation; pediatric acute respiratory distress syndrome; tracheal aspirate

Pediatric acute respiratory distress syndrome (PARDS) is a heterogeneous condition characterized by damage to alveolar epithelial and vascular endothelial cells lining the alveolar-capillary units of the lung. Breakdown of the alveolar epithelial/endothelial barriers results in proteinaceous fluid filling the alveolar airspace which leads to poor gas exchange and severe refractory hypoxemia (1). Six percent of mechanically ventilated children develop PARDS; approximately 25% of these children develop new functional deficits, and overall mortality is approximately 20% (2–4). To date, the inflammatory mechanisms associated with PARDS are not well understood.

Recent studies have used biomarkers, namely systemic ones, to examine PARDS heterogeneity (5–9). For example, elevated concentrations of plasma interleukin (IL)-6, IL-8, IL-18, macrophage inflammatory protein (MIP)–1β, IL-10, and tumor necrosis factor (TNF) receptors 1 and 2 are associated with

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KEY POINTS

Question: What are the levels of cytokines in the airway fluid of intubated children with severe versus nonsevere acute respiratory distress syndrome (ARDS)?

Findings: Levels of interleukin-4, -5, -7, -8, -12(p-70), -17a, -21, and fractalkine were higher in patients with severe versus nonsevere pediatric ARDS (PARDS). There were no associations between airway and plasma cytokines.

Meaning: Tracheal aspirate sampling in children is feasible and may permit better understanding of the airway immune response in severe PARDS.

PARDS severity, fewer ventilator-free days, nonpulmonary organ dysfunctions, and death (5, 10). However, these systemic biomarkers do not reflect the state of the airways (11). Given the paucity of data on airway cytokine concentrations in PARDS patients, the primary objective of this study was to measure a panel of common T-cell–expressed cytokines in tracheal aspirate samples from intubated children with PARDS. We hypothesized that children with severe PARDS would have higher concentrations of proinflammatory cytokines compared with children with nonsevere PARDS and that these concentrations would not be associated with plasma values.

METHODS

The Emory University School of Medicine Institutional Review Board (IRB00034236: Prevalence of oxidative stress in critically ill children and its relationship to immune function; a pilot study, Approved: February 14, 2018 and IRB00113035: Airway Immune Response in Critically ill Children: Precision Medicine in Children at Risk for Acute Respiratory Distress Syndrome, Approved: July 29, 2019) approved the study. Informed consent from a parent or legal guardian was performed by a trained study coordinator prior to enrollment. All study procedures were performed according to the relevant guidelines and regulations in the Declaration of Helsinki. Detailed methods are provided in the **Online Supplement** (http://links.lww.com/CCX/B106). Briefly, children between 14 days, with a corrected gestational age of 40 weeks, and 17 years were eligible if they were endotracheally intubated in a PICU within the preceding 72 hours. Immunosuppressed children were excluded. PARDS was assigned based on Pediatric Acute Lung Injury Consensus Conference (PALICC)-1 criteria including the unilateral infiltrate on chest radiograph. Children not meeting PALICC-1 criteria were assigned to the "at-risk" group. PARDS patients were further stratified by severity based on oxygenation index (OI) or oxygen saturation index (OSI), if an arterial catheter was not in place, on the day of sample collection according to the PALICC-1 definition (3). An OI of 16 or greater (OSI ≥ 12.3) was defined as severe PARDS. An OI of 4–16 (OSI 5–12.3) was defined as nonsevere PARDS.

Tracheal aspirates were obtained and processed (12–14), and airway fluid was analyzed for 21 T-cell–expressed cytokines (fractalkine; granulocyte-macrophage colony-stimulating factor [GM-CSF]; interferon gamma; IL-1b, 2, 4, 5, 6, 7, 8, 10, 12 (p70), 13, 17, 21, 23; interferon-inducible T-cell alpha chemoattractant; MIP-1A, MIP-1B, MIP-3A; and TNF-α), soluble TNF receptor 1 (sTNFr1), and receptor for advanced glycation end products (RAGE). These assays were also performed on plasma samples from a subset of participants. Analyses were performed using IBM SPSS Statistics software (IBM Corp. Released 2021. IBM SPSS Statistics for Windows, Version 28.0; Armonk, NY, IBM Corp) after logarithmic transformation of nonnormally distributed data. A p value of less than 0.5 was considered statistically significant.

RESULTS

Eighty-two children were enrolled, including 47 children with PARDS (nonsevere, n = 34; severe, n = 13) (**Table 1**). Children with severe PARDS had higher Pediatric Logistic Organ Dysfunction scores, longer hospital and PICU lengths of stays, longer duration of mechanical ventilation, and increased extracorporeal membrane oxygenation requirements.

Airway fluid cytokine assay performance characteristics and raw data values are shown in **Table E1** (http://links.lww.com/CCX/B106). GM-CSF and IL-2 fell below the limit of detection in 54 children

TABLE 1.
Features of the Participants

Feature	At-Risk for PARDS, *N* = 35	Nonsevere PARDS, *N* = 34	Severe PARDS, *N* = 13
Age, mo	10 (3–28)	11 (3–21)	23 (4–28)
Male	20 (57.1)	20 (58.8)	8 (61.5)
Ethnicity			
Hispanic/Latino	2 (5.7)	0	1 (7.7)
Not Hispanic/Latino	32 (91.4)	34 (100)	12 (92.3)
Unknown/not reported	1 (2.9)	0	0
Race			
White or White-Hispanic	13 (37.1)	10 (29.4)	4 (30.8)
Black or Black-Hispanic	20 (57.1)	22 (64.7)	6 (46.2)
Multiple races	0	2 (5.9)	3 (23.1)
Other	1 (2.9)	0	0
Unknown/not reported	1 (2.9)	0	0
Pediatric Risk of Mortality-III score	12 (8–17)	14 (9–17)	16 (12–20)
Pediatric Logistic Organ Dysfunction score	5 (4–8)	6 (5–6)	9 (5–12)[a,b]
Viral respiratory culture			
Not performed	7 (20.0)	5 (14.7)	0
No virus	3 (8.6)	4 (11.8)	3 (23.1)
Positive virus	21 (60.0)	21 (61.8)	8 (61.5)
Positive multiple virus	4 (11.4)	4 (11.8)	2 (15.4)
Bacterial respiratory culture			
Not performed	17 (48.6)	4 (11.8)	3 (23.1)
No bacteria	1 (2.9)	2 (5.9)	0
Positive bacteria or yeast	17 (48.6)	28 (82.4)[a]	10 (76.9)[a]
Hours to sample collection	17 (12–22)	21 (11–37)	23 (11–43)
Hospital days	10 (7–13)	16 (7–19)	21 (15–38)[a,b]
PICU days	7 (4–10)	8 (6–13)	15 (10–32)[a,b]
Ventilator days	4 (2–6)	5 (3–8)[a]	7 (6–20)[a,b]
28-d ventilator-free days	25 (22–26)	23 (19–24)[a]	19 (5–22)[a,b]
Extracorporeal membranous oxygenation	0	1 (2.9)	5 (38.5)[a,b]
Died	1 (2.9)	1 (2.9)	1 (7.7)

PARDS = pediatric acute respiratory distress syndrome.
[a]$p < 0.05$ vs at-risk.
[b]$p < 0.05$ vs nonsevere.
Data are shown as the median (25–75th percentile) or the number of participants (*n*) (%).

(66%) and 36 children (44%) and were excluded from further analyses. Discriminant analysis of the linear combination of all other raw cytokines revealed significant differences in children with severe PARDS (**Fig. E1*A***, http://links.lww.com/CCX/B106). However, children with severe PARDS also had significantly lower airway total protein concentrations (median [25–75th percentile] for

at-risk vs nonsevere vs severe PARDS: 1.42 [0.79–2.63] vs 1.74 [0.66–3.75] vs 0.30 mg/mL [0.12–2.63 mg/mL]; $p = 0.011$). Therefore, cytokines were normalized to total protein for further analyses. Discriminant analysis of the linear combination of protein-normalized cytokines also revealed significant differences in the cytokine expression pattern of children with severe PARDS (**Fig. E1*B***, http://links.lww.com/CCX/B106). Further inspection of individual cytokines demonstrated no differences between at-risk children and children with nonsevere PARDS, with the exception of fractalkine (**Table 2**). However, children with severe PARDS, compared with children with nonsevere PARDS, had significantly higher airway concentrations of IL-4 ($p = 0.046$), IL-5 ($p = 0.035$), IL-7 ($p = 0.012$), IL-8 ($p = 0.019$), IL-12p70 ($p = 0.031$), IL-13 ($p = 0.013$), IL-17A ($p = 0.007$), IL-21 ($p = 0.019$), and fractalkine ($p < 0.001$) (**Table 2**).

We then assessed associations between IL-4, IL-5, IL-7, IL-8, IL-12p70, IL-13, IL-17A, IL-21 and fractalkine in airway fluid and paired plasma. For this analysis, blood and airway samples were collected over a 21-day period in a subset of participants (paired samples, at-risk, $n = 6$; nonsevere, $n = 13$; severe, $n = 9$). There were no significant correlations between airway and plasma cytokines in this subset (**Table E2**, http://links.lww.com/CCX/B106).

TABLE 2.
Protein-Normalized Cytokine Concentrations in the Airway Fluid

Cytokine (pg/mg Protein)	At-Risk for PARDS, *N* = 35	Nonsevere PARDS, *N* = 34	Severe PARDS, *N* = 13
IL-1β	73.3 (9.6–433.8)	48.1 (7.6–146.2)	26.8 (3.7–107.1)[a]
IL-4	7.3 (4.6–13.7)	4.8 (3.3–12.4)	11.1 (4.5–23.7)[b]
IL-5	0.3 (0.1–0.5)	0.2 (0.1–0.4)	0.4 (0.3–2.5)[a,b]
IL-6	93.3 (37.7–158.5)	99.1 (34.2–146.0)	157.2 (45.3–304.8)
IL-7	3.8 (2.4–6.7)	2.9 (0.9–10.4)	6.8 (3.6–17.6)[b]
IL-8	752.8 (281.5–1433)	569.5 (283.2–973.6)	1,715 (372.9–4,192)[b]
IL-10	16.9 (7.1–49.6)	12.6 (7.3–41.6)	10.3 (3.6–34.2)
IL-12p70	0.4 (0.3–0.6)	0.3 (0.2–0.7)	0.5 (0.4–1.0)[b]
IL-13	0.3 (0.2–0.8)	0.3 (0.1–0.8)	0.7 (0.5–1.3)[b]
IL-17A	4.6 (2.8–6.9)	3.6 (1.5–8.1)	15.0 (3.3–20.9)[b]
IL-21	5.6 (3.2–10.3)	5.4 (1.3–8.8)	12.3 (5.3–17.8)[b]
IL-23	15.2 (8.5–29.5)	11.6 (7.9–20.1)	12.3 (9.3–28.7)
IFN-inducible T-cell alpha chemoattractant	20.7 (9.1–123.9)	17.7 (8.4–363.7)	65.5 (16.7–174.7)
Fractalkine	78.5 (39.0–132.2)	39.7 (12.3–100.3)[a]	186.9 (59.3–300.1)[b]
IFN gamma	4.7 (2.0–8.1)	4.3 (2.3–11.2)	5.9 (3.9–13.4)
MIP-1α	69.6 (22.8–195.5)	66.3 (20.5–109.0)	185.3 (13.8–273.0)
MIP-1β	62.0 (31.2–127.7)	59.6 (24.8–145.6)	109.9 (53.0–245.2)
MIP-3α	90.2 (22.2–183.9)	40.2 (14.5–209.9)	75.4 (26.9–544.4)
Tumor necrosis factor-α	56.8 (7.4–176.3)	17.2 (6.7–58.3)	24.9 (8.5–151.9)
Receptor for advanced glycation end product	20.9 (2.4–196.4)	47.3 (2.6–391.4)	25.8 (8.4–12,409)
soluble tumor necrosis factor receptor 1	517.4 (380.5–923.7)	647.8 (447.4–812.6)	511.4 (411.1–833.0)

IFN = Interferon, IL = interleukin, MIP = macrophage inflammatory protein, PARDS = pediatric acute respiratory distress syndrome.
[a]$p < 0.05$ vs at-risk.
[b]$p < 0.05$ vs nonsevere.
Data are shown as the median (25–75th percentile) and were logarithmically transformed prior to analysis.

DISCUSSION

Previous studies of children with PARDS have reported plasma cytokines due to the ease of collection compared with tracheal aspirate sampling. Herein we report airway cytokine levels in intubated children with PARDS to deepen our understanding of the factors that instigate and intensify alveolar inflammation. We found elevated concentrations of nine cytokines in children with severe versus nonsevere PARDS reflecting global inflammation that is not specific to a single T-cell subset. Instead, the cytokines that were elevated signify Type 1 inflammation (i.e., IL-12p70), eosinophilic Type 2 inflammation (i.e., IL-4, IL-5, IL-13), neutrophilic inflammation (i.e., IL-8), and also Type 17 inflammation (i.e., IL-17A). However, Type 17 inflammation was most pronounced and was reflected by more than five-fold elevated concentrations of IL-17A in children with severe versus nonsevere PARDS. Although we found several cytokines from our tracheal aspirate sampling that have also been shown to be elevated in plasma samples of PARDS patients, there were no significant associations between airway and plasma cytokines in the present study. This finding suggests that components described in the plasma may not represent ongoing pathophysiologic processes at the lung epithelial border. However, this study is small, and our findings warrant further investigation.

Our findings are consistent with a prior study of 16 children with pneumonia-triggered PARDS that reported elevated airway concentrations of IL-12 (p70) and IL-17A (15). IL-12 is a proinflammatory cytokine that promotes differentiation of T helper (Th) 1 cells and activation of cytotoxic $CD8^+$ T cells (16). In adults with ARDS, elevated circulating and alveolar levels of IL-17A are associated with increased alveolar neutrophils, permeability, and organ dysfunction (17). A higher ratio of IL-17–secreting Th17 to regulatory T cells in the peripheral blood of adults with ARDS was associated with death (18). IL-21, which was also elevated in the severe PARDS patients in our cohort, is required for Th17 cell maintenance via a Signal transducer and activator of transcription3 signaling (19).

IL-4, IL-8, and fractalkine play key roles in activation and recruitment of neutrophils, monocytes, and lymphocytes to sites of infection. Elevated levels of IL-4 in PARDS airways may promote repolarization of a proinflammatory (M1) alveolar macrophage toward an alternatively activated (M2) anti-inflammatory alveolar macrophage to aid in resolution of acute lung injury (ALI) (20). IL-8 is elevated in the bronchoalveolar lavage fluid of children with ALI (21). In children critically ill with influenza, IL-8 was also higher in nonsurvivors and in those with *Staphylococcus aureus* secondary infections (22). Mice with knock-out in the fractalkine receptor ($C\text{-}X_3\text{-}C$ motif chemokine receptor 1) in a lipopolysaccharide model of acute pulmonary inflammation had elevated levels of fractalkine that led to the release of damage-associated molecular patterns that worsened lung inflammation (23).

Interestingly, we did not detect differences in tracheal aspirate concentrations of soluble RAGE (sRAGE) and sTNFr1 between severe and nonsevere PARDS. sRAGE in plasma has previously discriminated systemic organ injury and death among patients with indirect lung injury (24). However, the patients in our study have predominantly direct lung injury which may account for this discrepancy. A recent systematic review and meta-analysis of ARDS/ALI biomarkers also showed that elevated serum and bronchoalveolar lavage levels of TNF-α were associated with ARDS/ALI; however, there was marked heterogeneity in measurements (25).

Other limitations include a relatively small convenience sample of intubated children with PARDS from a single center, which prohibited associations with clinical outcomes such as ventilator-free days due to limited power. We also focused on a single time point and cannot correlate clinical trajectory with changes in airway cytokines. Tracheal aspirate fluid may also not reflect the cytokine response from the alveolar air spaces, but bronchoalveolar is not routinely performed in PARDS. Finally, we chose to normalize cytokine levels to total protein measured in the tracheal aspirate fluid to account for dilution differences and variability in sampling. Normalization to total proteins in the airway fluid may introduce other variability related to the sample collection procedure. Nevertheless, we attempted consistency in the lavage procedure, report the raw cytokine values, and acknowledge that there are no better alternatives available in clinical practice. Future studies should ideally focus on longitudinal and simultaneous sampling of the systemic and lung compartments using clinically accessible and practical sampling of the airway environment.

CONCLUSIONS

Severe PARDS is associated with elevation of multiple airway proinflammatory cytokines. This study

suggests that tracheal aspirate sampling in this population is feasible and may permit better understanding of the airway immune response in severe PARDS, to ultimately develop targeted therapies to improve functional morbidity and mortality in these children.

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