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ORIGINAL

Circulating markers of endothelial and alveolar epithelial dysfunction are associated with mortality in pediatric acute respiratory distress syndrome

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Abstract

Purpose: Angiopoietin 2 (Ang2) and soluble receptor for advanced glycation end products (sRAGE) are markers of endothelial and pulmonary epithelial damage with prognostic implications in adult acute respiratory distress syndrome (ARDS), but unclear significance in pediatric ARDS (PARDS).

Methods: This was a prospective, observational study in children with PARDS (2012 Berlin and 2015 PALICC definitions) at the Children's Hospital of Philadelphia. Plasma was collected within 48 h of PARDS onset and biomarkers quantified by enzyme-linked immunosorbent assay.

Results: In 82 children with PARDS (12 deaths, 15 %), Ang2 and sRAGE were higher in non-survivors than survivors ($p < 0.01$ for both). Mortality was highest in patients with Ang2 and sRAGE levels both above median values. Ang2 and sRAGE correlated with the number of non-pulmonary organ failures (both $p < 0.001$). Ang2 was higher in indirect lung injury and in immunocompromised children. In stratified analysis, both Ang2 and sRAGE were associated with mortality only in direct lung injury and in immunocompetent children, with no association evident in indirect lung injury or in immunocompromised children.

Conclusions: Ang2 and sRAGE in early PARDS were higher in non-survivors than survivors and strongly correlated with number of non-pulmonary organ failures. When stratified by type of lung injury, Ang2 and sRAGE were associated with mortality only in direct lung injury. Similarly, when stratified by immunocompromised status, Ang2 and sRAGE were associated with mortality only in immunocompetent children. The utility of these biomarkers for prognostication and risk stratification requires investigation.

Keywords: ARDS, PARDS, Angiopoietin 2, Ang2, Soluble receptor for advanced glycation end products, sRAGE

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Take-home message: Biomarkers of endothelial (Ang2) and alveolar epithelial dysfunction (sRAGE) were associated with increased mortality in pediatric ARDS, with biomarker levels strongly correlating with organ failures. When stratified by immunocompromised status, Ang2 and sRAGE were associated with mortality only in immunocompetent children.

Introduction

Pediatric acute respiratory distress syndrome (PARDS) is a syndrome of respiratory failure without directed therapies. Historically defined using adult ARDS criteria [1, 2], a pediatric-specific definition for PARDS was developed [3] by the Pediatric Acute Lung Injury Consensus Conference (PALICC) with stratification by oxygenation index (OI), rather than Pao_2/FIo_2. Both Berlin and PALICC rely on recognition of risk factors, imaging, and hypoxemia.

Biomarkers have been proposed to aid in diagnosis, improve risk stratification, and identify sub-phenotypes. Adult ARDS studies [4] have implicated inflammatory mediators [5, 6], damage-associated molecular patterns [7], and markers of endothelial [8–10] and epithelial damage [10–12]. Fewer comparable studies have examined the clinical utility of biomarkers in PARDS [13–18].

As endothelial dysfunction is a hallmark of ARDS, attention has focused on angiopoietin 2 (Ang2), which worsens endothelial permeability. Ang2 discriminates ARDS in at-risk adults [19], is elevated in non-survivors [8, 9], and predicts subsequent development of ARDS [20]. Similarly, as alveolar damage is central to ARDS, the soluble receptor for advanced glycation end product (sRAGE), a marker of type I pneumocyte injury [21], is higher in adults with ARDS relative to those at risk [21], and in non-survivors [22]. While a single study has investigated Ang2 in PARDS [16], sRAGE has not been studied. Given the presumed shared pathophysiology between adults and children, we tested association of these two markers with mortality in children with PARDS. We hypothesized that Ang2 and sRAGE would be higher in non-survivors.

Methods

Study design and patient selection

This prospective cohort study was approved by the Children's Hospital of Philadelphia's (CHOP) Institutional Review Board, and informed consent was obtained from caregivers prior to enrollment. Clinical data were collected prospectively.

Consecutive patients in the pediatric intensive care unit (PICU) were screened for Berlin-defined ARDS between July 1, 2014 and December 30, 2015. Inclusion criteria were (1) acute (≤7 days of known risk factor) respiratory failure requiring invasive (endotracheal) mechanical ventilation, (2) invasive arterial access, (3) age >1 month (to avoid confounding by neonatal physiology) and <18 years, (4) PaO_2/FIO_2 ≤300 on two consecutive arterial blood gases separated by ≥1 h on positive end-expiratory pressure (PEEP) ≥5 cmH_2O, and (5) bilateral infiltrates on radiograph. Exclusion criteria were (1) respiratory failure from cardiac failure (by echocardiography), (2) exacerbation of underlying chronic lung disease, (3) chronic ventilator dependence, (4) cyanotic heart disease, (5) ventilation for >7 days before PaO_2/FIO_2 ≤300, (6) ARDS established outside of the CHOP PICU, (7) inability to obtain consent, or (8) prior enrollment.

Determination of bilateral infiltrates was made independently by a PICU attending and a pediatric radiologist; only cases agreed upon by both as consistent with Berlin criteria were included. Since the study was initiated prior to the 2015 PALICC definitions of PARDS, we did not screen patients on the basis of OI; however, all patients met PARDS criteria in addition to Berlin.

At-risk intubated controls

To facilitate comparisons to patients without PARDS, a convenience sample of ventilated children with risk factors for PARDS who were screened for the study, but did not meet Berlin oxygenation criteria (PaO_2/FIO_2 > 300), were used as intubated at-risk controls.

Plasma collection and measurements

Blood was collected within 48 h of PARDS onset (defined as time of meeting all Berlin criteria) in citrated tubes (Becton, Dickinson and Company, Franklin Lakes, NJ, USA), centrifuged within 30 min of collection (2000*g*, 20 min, 20 °C) to generate platelet-poor plasma, and stored at −80 °C. Ang2 and sRAGE were measured in duplicate using an enzyme-linked immunosorbent assay (ELISA; R&D Systems, Minneapolis, MN, USA). Interassay coefficients of variation were 5.3 % for Ang2 and 3.3 % for sRAGE.

Equations and definitions

Metrics of oxygenation used were PaO_2/FIO_2 and OI [[mean airway pressure (mPaw) × FIO_2 × 100]/PaO_2]. The vasopressor score [23] is dopamine (μg/kg/min) × 1 + dobutamine (μg/kg/min) × 1 + epinephrine (μg/kg/min) × 100 + norepinephrine (μg/kg/min) × 100 + phenylephrine (μg/kg/min) × 100 + milrinone (μg/kg/min) × 10 + vasopressin (U/kg/min) × 10,000. Severity of illness score used was the Pediatric Risk of Mortality (PRISM) III at 12 h. Non-pulmonary organ failures at PARDS diagnosis were identified using accepted pediatric definitions [24]. The designation of "immunocompromised" required presence of an immunocompromising diagnosis (oncologic, immunologic, rheumatologic, transplant) and active immunosuppressive therapy, or congenital immunodeficiency [25]. Risk factors for PARDS were identified [26] and dichotomized to either "direct" or "indirect" as per guidelines [1].

Outcomes

Primary outcome was PICU mortality. We also reported ventilator-free days (VFD) at 28 days and length of mechanical ventilation. All mention of "mechanical ventilation" implied "invasive" ventilation; non-invasive support was not counted toward VFD or ventilator days. For VFD and ventilator days, the first day was initiation of invasive ventilation. Liberation from invasive ventilation for >24 h defined duration of ventilation. Patients requiring re-intubation after 24 h of extubation had additional days counted towards total ventilator days. VFD were determined by subtracting total ventilator days

from 28 in survivors. All patients with total ventilator days ≥28 days and all PICU non-survivors were assigned VFD = 0.

Statistical analysis

The majority of data were non-normally distributed according to the Shapiro–Wilk test, are reported as median [interquartile range, IQR], and differences between groups compared using non-parametric statistics. Categorical data were compared using Fisher's exact test. Cuzick's non-parametric test of trend was used to assess for trends across ordered groups [27]. Survival curves were similarly tested using a trend of log-ranks. To test association of Ang2 and sRAGE with time to death, Cox regression was performed on biomarker levels, with potential confounders (univariate association with mortality at $p \leq 0.2$ [28]) included one at a time in the model. We a priori examined effect modification by type of lung injury (direct or indirect) and by immunocompromised status. The proportional hazard assumption was assessed by a plot of the [−log(survival) versus time]. To assess the discriminative ability for mortality, area under the receiver operating characteristic (AUROC) curve for non-survival was computed. AUROC for different variables were compared on the basis of the methods of Delong et al. [29]. Analyses were performed with Stata/SE 14 (College Station, TX, USA).

Results

Description of the cohort

Eighty-two PARDS patients were enrolled (Supplementary Fig. 1). Blood was collected at median 15 [IQR 7, 21] hours after PARDS onset; 71 of 82 (87 %) samples were collected ≤24 h. Neither Ang2 nor sRAGE correlated with age (Spearman $\rho = 0.05$ and 0.03, $p = 0.625$ and 0.723, respectively) or time of phlebotomy relative to PARDS onset ($\rho = 0.07$ and -0.14, $p = 0.514$ and 0.196). Ang2 and sRAGE levels were modestly correlated ($\rho = 0.24$, $p = 0.027$).

Association with mortality, organ failures, and VFD

Of 82 children with PARDS, 12 died (15 %). Non-survivors died at median 7 [IQR 5, 21; range 2–31] days after phlebotomy, and no patients died within 48 h of phlebotomy. Non-survivors had worse PRISM III and more organ failures, but similar metrics of lung injury at PARDS onset, compared with survivors (Table 1). Both Ang2 and sRAGE levels were higher in non-survivors (Fig. 1). Highest mortality (31 %) was in patients with both Ang2 and sRAGE levels above the median (Fig. 1c). Ang2 and sRAGE, separately and in conjunction, discriminated PICU mortality (Supplementary Table 1), with AUROC between 0.74 and 0.80, better

Table 1 Characteristics of the PARDS cohort ($n = 82$)

Variable	Survivors ($n = 70$)	Non-survivors ($n = 12$)	p value[a]
Age (years)	4.5 [1.3, 13]	1.6 [0.6, 8.2]	0.203
Female/male (%/%)	35/35 (50/50)	6/6 (50/50)	1
Severity of illness at PARDS onset			
PRISM III at 12 h	10 [5, 15]	17 [11, 30]	0.013
Non-pulmonary organ failures	1 [1, 2]	3 [2, 5]	<0.001
Vasopressor score	8 [3, 15]	17 [6, 32]	0.051
Risk factor for PARDS			
Aspiration pneumonia	8 (11)	1 (8)	0.151
Infectious pneumonia	35 (50)	4 (33)	
Non-pulmonary sepsis	20 (29)	4 (33)	
Trauma	4 (6)	0	
Other	3 (4)	3 (25)	
Type of PARDS			
Direct	44 (63)	7 (58)	0.502
Indirect	26 (37)	5 (42)	
Comorbidities (%)			
Prematurity	8 (11)	0	0.464
Genetic syndrome	17 (24)	2 (17)	0.459
Malignancy	10 (14)	3 (25)	0.296
Stem cell transplant	3 (4)	5 (42)	0.001
Immunocompromised	13 (19)	7 (58)	0.007
At PARDS onset			
PaO_2/FIO_2	180 [115, 236]	233 [141, 259]	0.151
OI	9.4 [5.9, 12.3]	7.7 [6.7, 15.3]	0.916
PEEP (cmH_2O)	10 [8, 12]	11 [8, 13]	0.418
PIP (cmH_2O)	31 [26, 35]	31 [26, 34]	0.623
mPaw (cmH_2O)	17 [13, 21]	18.5 [15, 21]	0.375
Tidal volume (mL/kg)	7.5 [6.3, 8.5]	7.6 [6.5, 8.1]	0.906
Berlin categories			
Mild	28 (40)	7 (58)	0.547
Moderate	26 (37)	3 (25)	
Severe	16 (23)	2 (17)	
PALICC categories			
Mild	31 (44)	7 (58)	0.723
Moderate	18 (26)	2 (17)	
Severe	21 (30)	3 (25)	
Ancillary therapies			
Inhaled nitric oxide	22 (31)	6 (50)	0.322
Neuromuscular blockade	29 (41)	7 (58)	0.351
High frequency oscillation	12 (17)	4 (33)	0.174
ECMO	5 (7)	0	0.444

[a] Values are presented as median [IQR] or number (percentage). Continuous variables are compared using rank-sum test, and categorical using Fisher's exact test

than Berlin or PALICC categories (all $p < 0.01$ when comparing AUROC). Increasing Ang2 and sRAGE correlated with worsening survival functions (Fig. 2a, c) and increasing number of organ failures (Fig. 2b, d). Among the 12 non-survivors, two died of refractory hypoxemia, five of multisystem organ failure (MSOF), and five with poor neurologic prognosis. Interestingly, sRAGE was highest in patients who had care withdrawn for poor neurologic prognosis (Supplementary Fig. 2). Increasing Ang2 ($\rho = -0.22$, $p = 0.046$), but not sRAGE ($\rho = -0.14$, $p = 0.224$), correlated with fewer VFD.

Association with severity and type of lung injury

Ang2, but not sRAGE, differed according to PARDS risk factor, and was higher in indirect PARDS (Fig. 3). When stratified by type of lung injury, both Ang2 and sRAGE were elevated only in non-survivors with direct PARDS relative to survivors (Fig. 3c, f). Plasma sRAGE, but not Ang2, correlated with worsening Berlin and PALICC oxygenation categories (Supplementary Fig. 3). Additionally, increasing mPaw at PARDS onset correlated with increased sRAGE (Supplementary Fig. 3F).

Association with immunocompromised status

Twenty patients with PARDS were immunocompromised. Ang2, but not sRAGE, was elevated in immunocompromised children relative to immunocompetent (Supplementary Fig. 4A, C). Plasma sRAGE ($p = 0.001$), but not Ang2 ($p = 0.081$), was higher in immunocompetent non-survivors relative to survivors, whereas both biomarkers were similar in immunocompromised survivors and non-survivors (Supplementary Fig. 4B, D).

Cox regression analysis

Variables associated with mortality at $p \leq 0.2$ (Table 1) were included one at a time in bivariate Cox regression. Both Ang2 and sRAGE were associated with mortality (Table 2) independent of age, vasopressor score, stem cell transplant status, PaO_2/FIO_2, or Berlin severity category. Because of higher Ang2 in indirect lung injury in our cohort and in others [10], we performed analysis stratified by type of lung injury. Ang2 and sRAGE were associated with mortality only in patients with direct PARDS. Similarly, because of higher Ang2 in immunocompromised patients in our cohort and others [16, 30], we tested the association between both biomarkers and mortality stratified by immunocompromised status. Ang2 and sRAGE were associated with mortality only in the immunocompetent subgroup.

Sensitivity analysis

To diminish heterogeneity of biomarker levels potentially attributable to duration of time with PARDS, we performed a sensitivity analysis. When analyzing the 71 patients with blood collected ≤24 h after PARDS onset, both Ang2 [survivors median 5.7 ng/mL (IQR 3.3, 11.4) versus non-survivors 13.4 ng/mL (10.4, 16.6), rank-sum $p = 0.037$] and sRAGE [survivors 1.3 ng/mL (0.8, 2.9) versus non-survivors 5.0 ng/mL (2.6, 7.2), $p = 0.005$] were higher in non-survivors. Repeating the Cox regression in these 71 patients confirmed that Ang2 and sRAGE were associated with mortality only in direct PARDS and in immunocompetent children (Supplementary Table 2).

Comparison with at-risk intubated controls

An additional 22 intubated control patients with PARDS risk factors, but not meeting oxygenation criteria, were enrolled for an exploratory analysis of these biomarkers' potential diagnostic value. PARDS cases had more diagnoses of pneumonia and had worse oxygenation, respiratory mechanics, and outcomes relative to controls (Supplementary Table 3). Ang2 and sRAGE were both elevated in PARDS relative to controls (Supplementary Fig. 5).

Discussion

Plasma Ang2 and sRAGE early in PARDS were higher in non-survivors and correlated with non-pulmonary organ failures. Ang2 was higher in indirect lung injury and in immunocompromised children. Higher sRAGE was associated with worsening PaO_2/FIO_2, OI, and mPaw. When stratified by type of lung injury and by immunocompromised status, Ang2 and sRAGE were associated with mortality only in direct lung injury and in immunocompetent children.

Ang2 destabilizes endothelium by preventing Tie2 phosphorylation [31], and Ang2 is elevated in adult [32] and pediatric [33] sepsis. In adults, Ang2 was higher in indirect ARDS [10] and in non-survivors [8, 9]. An *ANGPT2* polymorphism was associated with development of ARDS in adults with trauma [34]. In a single study, Ang2 levels at presentation predicted subsequent ARDS development [20]. In our cohort, plasma Ang2 was higher in indirect lung injury and was elevated in PARDS non-survivors, consistent with findings in adults. Ang2 was not associated with PARDS severity as measured by Berlin or PALICC classifications. While potentially due to limited sample size, it is possible that the relationship between Ang2 and mortality is mediated via MSOF, rather than severity of lung injury as measured by oxygenation. Our preliminary comparison with intubated controls implicates Ang2 as potentially able to distinguish PARDS from at-risk patients, a finding that bears replication in a larger population.

Immunocompromised status was found to be a modifier of the association between both Ang2 and sRAGE

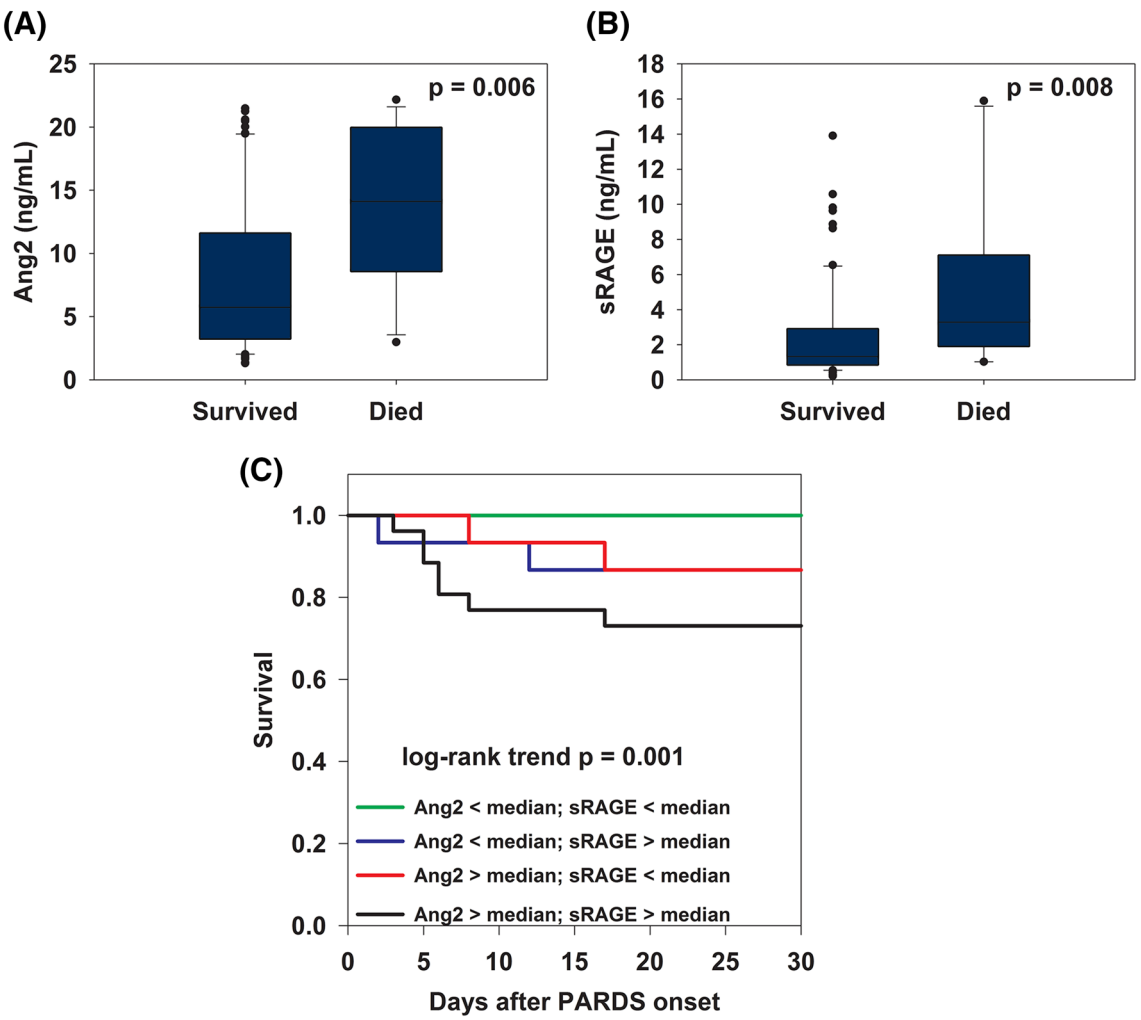

Fig. 1 Plasma **a** Ang2 and **b** sRAGE are higher in non-survivors ($n = 12$) relative to survivors ($n = 70$) with PARDS. *P* values represent the results of rank-sum tests. **c** Survival curves stratified by median plasma Ang2 and sRAGE levels. The worst survival function is seen in those with Ang2 and sRAGE above the median. *P* value represents the result of a test of trend of log-ranks

with mortality, which has not been reported in adults. Similar to our findings, Zinter et al. [16] demonstrated that Ang2 levels within 24 h of PARDS onset were associated with non-survival, with the association only evident in non-stem cell transplant (SCT) patients. In a study of pediatric SCT recipients, elevated Ang2 was associated with increased graft-versus-host disease and mortality [30], suggesting endothelial dysregulation in this population. It is possible that immunocompromised children have elevated Ang2 prior to developing PARDS, and that Ang2 levels measured in this study within 48 h of PARDS onset were reflective of this initial endothelial dysfunction, rather than mortality risk. In immunocompromised children, it may be necessary to measure biomarkers serially and assess whether temporal increases are associated with mortality. Indeed, Zinter et al. [16] demonstrated that increasing Ang2 between day 1 and day 3 of PARDS was associated with non-survival in SCT recipients.

Plasma sRAGE is an alveolar epithelial marker [21, 35, 36], with diagnostic [21] and prognostic [22] significance in adult ARDS. Elevated sRAGE correlates with impaired alveolar fluid clearance in lung injury models [35], as well as adult ARDS [36], suggesting a biologically plausible relationship with ARDS severity. A single pediatric study showed that sRAGE levels following cardiopulmonary bypass (CPB) for congenital heart disease surgery predicted post-CPB lung injury [15]. Similarly, in our larger, heterogeneous PARDS cohort, increased sRAGE correlated with worse oxygenation and respiratory mechanics,

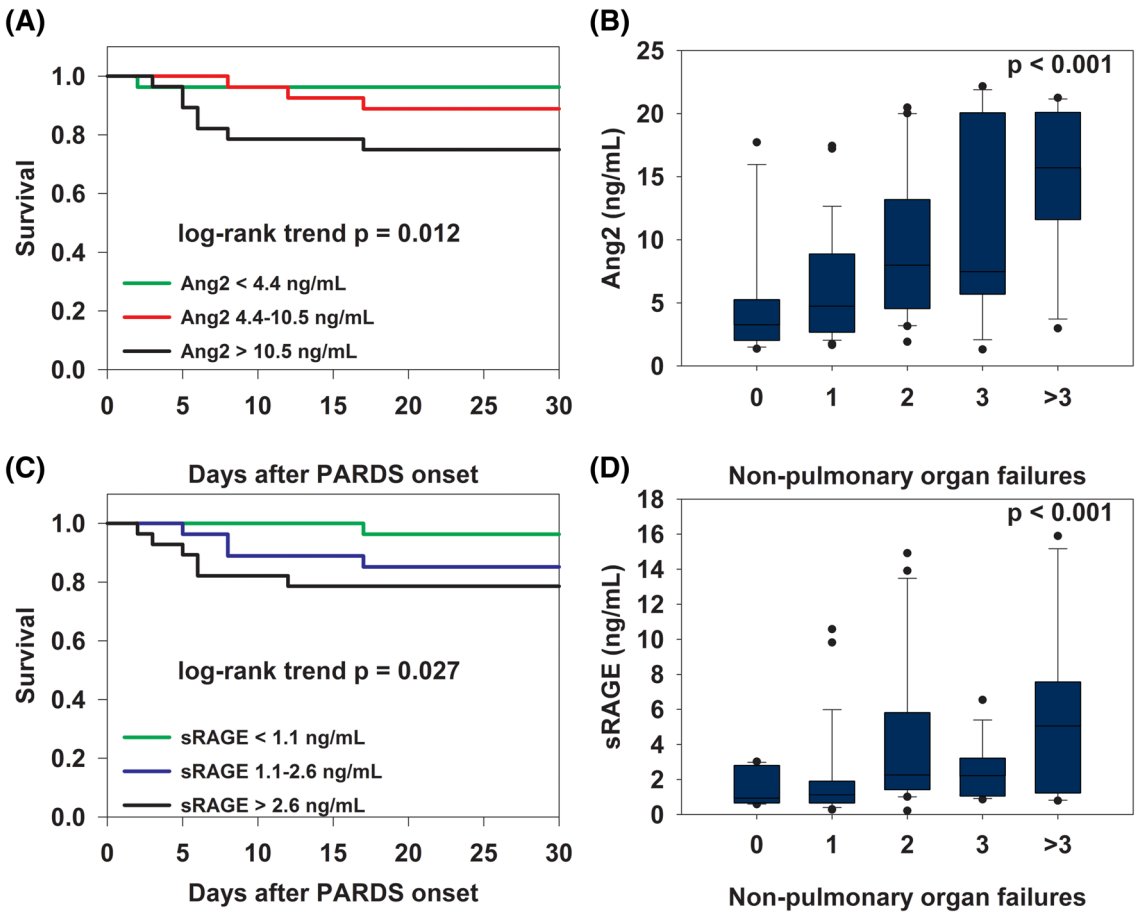

Fig. 2 Tertiles of plasma **a** Ang2 and **c** sRAGE and their associated survival curves. *P* values represent the results of a test of trend of log-ranks. Association of **b** Ang2 and **d** sRAGE with number of non-pulmonary organ failures. *P* values represent the results of a non-parametric test of trend

consistent with impaired fluid clearance leading to greater lung injury [36].

Like Ang2, sRAGE correlated with organ failures, which may partly explain the association with mortality. Despite the highest expression in type I pneumocytes, sRAGE is expressed in multiple tissues, and the elevated levels seen with worsening MSOF are unlikely to be entirely explained by alveolar damage. Studies showing a correlation between organ failures and sRAGE in septic [37] and critically ill adults [38] are consistent with our findings. The relationship between sRAGE and mortality persisted after adjustment for organ failure, suggesting additional utility of this biomarker as an independent predictor of mortality. Notably, sRAGE was highest in patients who ultimately had care withdrawn for poor neurologic prognosis. One potential explanation is that most of these patients had severe MSOF at the time of phlebotomy, most of which had recovered at the time of withdrawal of care, other than the brain dysfunction. However, given the small number of patients who died, this remains an intriguing observation in need of further study.

Both Ang2 and sRAGE were associated with mortality in direct, and not indirect, PARDS. While infection is known to modify the association between Ang2 and mortality [9], type of lung injury is not known to exert any differential effect [10]. Potentially, our study was underpowered to detect an association between Ang2 or sRAGE and mortality in the indirect PARDS cohort. However, the possibility of differential utility of these biomarkers depending on the underlying risk factor is a plausible hypothesis requiring validation in a larger cohort. Here, as with immunocompromised children, it may be necessary to measure biomarkers serially and assess the association between temporal increases and mortality.

Unlike adult ARDS [4], few studies have examined the utility of biomarkers in PARDS [13–18]. We are unaware of any studies examining the role of sRAGE in a general PARDS cohort. Given the distinct epidemiology of

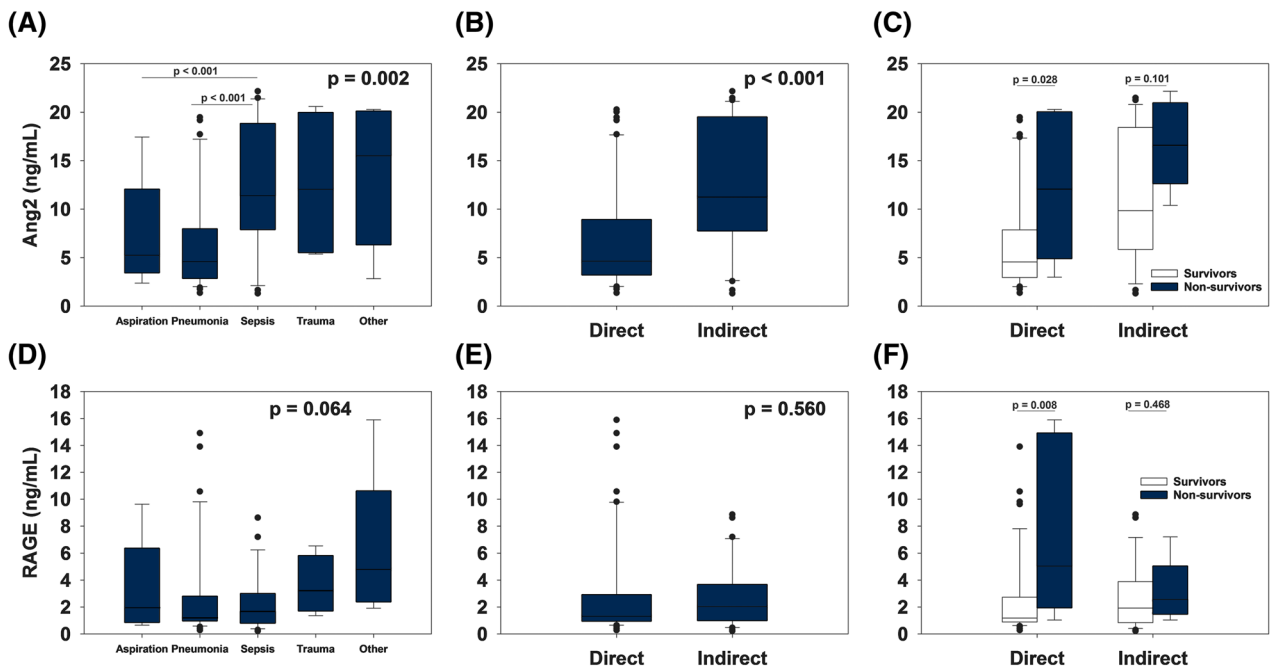

Fig. 3 Comparison of **a** Ang2 and **d** sRAGE with PARDS risk factor, and dichotomized to direct ($n = 51$) and indirect ($n = 31$) lung injury (**b**, **e**). Association of plasma **c** Ang2 and **f** sRAGE and mortality, stratified by type of lung injury status. *P* values represent the results of ANOVA on ranks (**a**, **d**), or of rank-sum tests

Table 2 Association of plasma biomarker levels with mortality

Model[a]	Angiopoietin 2		Soluble RAGE	
	Hazard ratio (95 % CI)	*p* value	Hazard ratio (95 % CI)	*p* value
Whole cohort ($n = 82$)				
Unadjusted	1.12 (1.03–1.22)	0.008	1.17 (1.04–1.33)	0.009
Adjusted for				
Age	1.14 (1.04–1.24)	0.004	1.20 (1.05–1.36)	0.006
Etiology of PARDS	1.14 (1.04–1.24)	0.004	1.16 (1.00–1.33)	0.043
PRISM III	1.08 (0.99–1.19)	0.097	1.09 (0.94–1.26)	0.267
Organ failures	1.04 (0.93–1.15)	0.516	1.15 (1.01–1.31)	0.039
Vasopressor score	1.12 (1.02–1.23)	0.022	1.16 (1.02–1.32)	0.021
Stem cell transplant	1.10 (1.00–1.20)	0.040	1.22 (1.07–1.39)	0.004
Immunocompromised	1.10 (0.98–1.23)	0.096	1.22 (1.07–1.40)	0.004
PaO_2/FIO_2	1.14 (1.04–1.24)	0.004	1.26 (1.11–1.43)	<0.001
Berlin category	1.14 (1.04–1.24)	0.004	1.27 (1.11–1.47)	0.001
Direct lung injury ($n = 51$)				
Unadjusted	1.16 (1.03–1.31)	0.015	1.22 (1.06–1.40)	0.007
Indirect lung injury ($n = 31$)				
Unadjusted	1.12 (0.95–1.33)	0.188	1.04 (0.76–1.42)	0.804
Immunocompetent ($n = 62$)				
Unadjusted	1.20 (1.04–1.39)	0.013	1.33 (1.13–1.57)	0.001
Immunocompromised ($n = 20$)				
Unadjusted	0.97 (0.85–1.12)	0.710	0.90 (0.62–1.31)	0.574

[a] Odds ratios are expressed per 1 ng/mL increase in biomarker levels

PARDS, development of the PALICC definition [3] was a welcome step. However, there are inherent limitations to clinical definitions of syndromes. For PARDS specifically, oxygenation has an inconsistent relationship with outcome [39] and does not reflect the pathophysiology. Ang2 and sRAGE reflect the endothelial and alveolar epithelial dysfunction underlying PARDS, and given correlation with organ failure and mortality, they may serve as alternative metrics for risk stratification or criteria for trial enrollment. Future definitions of PARDS, as well as adult ARDS, may be better served by a combination of clinical and biochemical parameters than by current clinical criteria. Given the lower prevalence of PARDS, relative to adult ARDS, well-designed, multicenter studies measuring multiple markers [13, 14, 16–18] simultaneously, with standardized collection protocols, will be necessary to realize this potential strategy. Additionally, biomarkers can potentially differentiate between endogenous phenotypes of PARDS. Studies in adults have demonstrated ARDS endotypes classified by inflammatory biomarkers, with differing responses to PEEP and differential mortality [40].

Our study has several strengths. It is the first to assess the role of sRAGE in a heterogeneous PARDS cohort and is one of few biomarker studies in pediatrics assessing the utility of multiple biomarkers in the same cohort, allowing direct comparisons. Detailed clinical data were collected prospectively, and multiple correlations made with biomarkers. However, our study has limitations. This was a single-center study and, while PARDS severity and etiologies are similar to others, findings may not be generalizable. Our sample size is small, and associations within subgroups, such as etiology of lung injury (direct or indirect) and immunocompromised status, need to be more thoroughly explored in a larger population. Mortality is low, although comparable to our recently published cohort (13 %) [39], precluding the ability to fit a full multivariate model with all necessary confounders. We measured plasma biomarkers, which may not be the compartment most reflective of PARDS biology. However, given the infrequency of bronchoalveolar lavage in our population, it was not feasible to include biomarkers measured from the alveolar space. A future study with protocolized, minimally invasive lavage could potentially address this. Finally, phlebotomy was allowed up to 48 h, although 87 % samples were collected $\leq$24 h after PARDS onset. Additionally, sensitivity analysis of the 71 patients with plasma collected within 24 h confirmed the association between Ang2 and sRAGE with mortality. It is possible that an earlier time frame for blood collection would yield more reliable results; however, several parameters, such as PICU admission and PARDS onset, are inherently arbitrary, and more acute collection time may not adequately address this variability. Despite these limitations we demonstrated utility of Ang2 and sRAGE in PARDS.

Conclusions

Ang2 and sRAGE were higher in PARDS non-survivors and were correlated with number of non-pulmonary organ failures. In stratified analysis, Ang2 and sRAGE were associated with mortality only in direct lung injury and in immunocompetent children. The utility of these biomarkers for prognostication and risk stratification should be further investigated.

Electronic supplementary material

The online version of this article (doi:10.1007/s00134-016-4352-1) contains supplementary material, which is available to authorized users.

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Compliance with ethical standards

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Conflicts of interest

Dr. Neal J. Thomas reports personal fees from Therabron and CareFusion, and grants from the FDA, all outside of the submitted work. Dr. Nuala J. Meyer reports grants from GlaxoSmithKline, outside of the submitted work. Dr. Jason D. Christie reports grants from GlaxoSmithKline, outside of the submitted work. Dr. Susan S. Margulies reports personal fees from Astrocyte Pharmaceuticals, outside of the submitted work. The remaining authors declare no conflicts of interest.

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