Am J Physiol Lung Cell Mol Physiol 310: L1177–L1184, 2016.
First published April 29, 2016; doi:10.1152/ajplung.00067.2016.

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Circulating nucleosomes are associated with mortality in pediatric acute respiratory distress syndrome

Nadir Yehya,[1] Neal J. Thomas,[2] and Susan S. Margulies[3]

[1]*Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia and University of Pennsylvania, Philadelphia;* [2]*Department of Pediatrics and Public Health Science, Division of Pediatric Critical Care Medicine, Penn State Hershey Children's Hospital, Hershey;* [3]*Department of Bioengineering, University of Pennsylvania, Philadelphia, Pennsylvania*

Submitted 12 February 2016; accepted in final form 21 April 2016

Yehya N, Thomas NJ, Margulies SS. Circulating nucleosomes are associated with mortality in pediatric acute respiratory distress syndrome. *Am J Physiol Lung Cell Mol Physiol* 310: L1177–L1184, 2016. First published April 29, 2016; doi:10.1152/ajplung.00067.2016.—Mechanisms underlying pediatric acute respiratory distress syndrome (PARDS) are poorly understood. The recent implication of circulating nucleosomes as pathogenic in sepsis and trauma-associated ARDS in adults led us to investigate the significance of nucleosomes in PARDS. We conducted a prospective, observational study on children with PARDS at the Children's Hospital of Philadelphia between July 2014 and September 2015. Plasma was collected within 48 h of PARDS onset and nucleosomes quantified by enzyme-linked immunosorbent assay. Samples from 76 children with PARDS (11 deaths, 14%) were collected early [median 15 (IQR 7, 21) h] after PARDS onset. Nucleosome levels were higher in nonsurvivors [0.59 AU (IQR 0.46, 0.84)] relative to survivors [0.21 AU (IQR 0.08, 0.33), rank sum $P < 0.001$]. Nucleosome levels were not associated with either Berlin ($P = 0.845$) or PALICC ($P = 0.886$) oxygenation categories, nor with etiology of PARDS ($P = 0.527$). Nucleosomes were correlated with increasing numbers of nonpulmonary organ failures ($P = 0.009$ for trend), and were higher in patients whose Pa_{O_2}/Fi_{O_2} worsened ($P = 0.012$) over the first 72 h of PARDS. In regression analysis, nucleosome levels were independently associated with mortality after adjusting for either age, severity of illness score, number of nonpulmonary organ failures, vasopressor score, or Pa_{O_2}/Fi_{O_2} (all $P < 0.05$). In conclusion, plasma nucleosome levels in early PARDS were associated with increased mortality, correlated with number of nonpulmonary organ failures, and preceded worsening oxygenation. The potential utility of this biomarker for prognostication, risk stratification, and mechanistic insight should be investigated further.

acute respiratory distress syndrome; ARDS; pediatric acute respiratory distress syndrome; PARDS; nucleosomes

MECHANISMS UNDERLYING pediatricacute respiratory distress syndrome (PARDS) remain elusive. While PARDS was historically defined by adult ARDS criteria (6, 25), it possesses a distinct epidemiologic, comorbidity, and outcome profile, prompting the Pediatric Acute Lung Injury Consensus Conference (PALICC) to develop pediatric-specific definitions in 2015 (23). Among other differences, the PALICC definition of PARDS uses oxygenation index (OI), rather than Pa_{O_2}/Fi_{O_2}, for risk stratification, despite the inconsistent relationship between oxygenation and outcome (1, 31). Common to both adult (35, 36) and pediatric (13, 39) ARDS is the significance of nonpulmonary organ failures as a predictor of poor outcome. While several investigations have focused on the systemic inflammatory response and the associated neutrophil activation and cytokine release (9, 36), the pathogenic mechanisms invoked remain incomplete for explaining the development of lung injury and multisystem organ failure (MSOF).

Recent studies in adult ARDS have implicated circulating nucleosomes, the histone/DNA complexes resulting from nuclear chromatin degradation released after cellular damage, as potentially pathogenic in sepsis (4, 12, 24, 38), aspiration (41), and trauma-related ARDS (2). Normally located within the nucleus, nucleosomes released into the circulation act as damage-associated molecular patterns (DAMP), and have been shown to be toxic to multiple cell types (2, 12, 24), offering a novel mechanism linking diverse inciting insults with subsequent lung injury and organ failure. However, whether nucleosomes are associated with PARDS development or progression is unknown.

Rapid identification of children with PARDS who are most at risk of a poor outcome is essential for accurate risk stratification in clinical trials, as it allows redirection of aggressive interventions toward the sickest cohort. Unlike adult ARDS, few studies address the utility of biomarkers in PARDS. The reduced incidence of PARDS [12.8 per 100,000 person-years; (42)] relative to adults [78.9 per 100,000 person-years; (28)] and the lower mortality in pediatrics (22, 39, 42), complicates the design of studies associating biomarkers to clinically relevant outcomes.

Given recent data implicating circulating nucleosomes in adult ARDS, we sought to determine their relevance in a cohort of children with PARDS. We hypothesized that nucleosome levels would be elevated in in PARDS nonsurvivors relative to survivors.

METHODS

Study design and patient selection. This prospective, observational study was approved by the Children's Hospital of Philadelphia's (CHOP) Institutional Review Board, and written informed consent

Address for reprint requests and other correspondence: N. Yehya, Children's Hospital of Philadelphia, Dept. of Anesthesiology and Critical Care Medicine, Suite 7C-26, 34th St. and Civic Center Boulevard, Philadelphia, PA 19104 (e-mail: yehyan@email.chop.edu).

was obtained from caregivers prior to enrollment. Clinical data were collected prospectively.

Consecutive patients in the pediatric intensive care unit (PICU) were screened daily for Berlin-defined ARDS and eligibility between July 1, 2014, and September 30, 2015. Inclusion criteria were *1*) acute respiratory failure requiring invasive (via endotracheal tube) mechanical ventilation projected to last >24 h, *2*) invasive arterial access, *3*) age > 1 mo (to avoid confounding by neonatal physiology) and < 18 years, *4*) $Pa_{O_2}/Fi_{O_2} \leq 300$ on two consecutive arterial blood gases separated by ≥1 h on positive end-expiratory pressure (PEEP) ≥ 5 cmH_2O, and *5*) bilateral parenchymal infiltrates on radiograph. Exclusion criteria were *1*) respiratory failure primarily from cardiac failure (determined by echocardiography), *2*) exacerbation of underlying chronic respiratory disease, *3*) chronic ventilator dependence, *4*) mixing cyanotic heart disease, *5*) mechanical ventilation for >7 days before $Pa_{O_2}/Fi_{O_2} \leq 300$, *6*) ARDS established outside of the CHOP PICU, and *7*) inability to obtain informed consent.

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

At-risk intubated controls. To facilitate comparisons with intubated patients without PARDS, an additional convenience sample of mechanically ventilated children with risk factors for PARDS who were screened for the study, but who did not meet Berlin (or PALICC) oxygenation criteria ($Pa_{O_2}/Fi_{O_2} > 300$), 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), centrifuged within 30 min of collection (2,000 *g*, 20 min, 20°C) to generate platelet-poor plasma, aliquoted to prevent freeze/thaw cycles, and stored at −80°C. Nucleosomes were measured in duplicate using an enzyme-linked immunosorbent assay (Cell Death detection $ELISA^{PLUS}$, Roche, Basel, Switzerland). Replicates all had variation < 5%. As a standard curve was not provided, nucleosome concentrations are reported as arbitrary units (AU), which are values obtained for each sample normalized to the positive control included in the kit. Interassay coefficient of variation was 5.2%, with a lower limit of detection of 0.01 AU.

Equations and definitions. Metrics of oxygenation utilized were Pa_{O_2}/Fi_{O_2} and OI {[mean airway pressure (mPaw) × Fi_{O_2} × 100]/Pa_{O_2}} at PARDS onset, and 24 and 72 h later. The vasopressor score (15, 37) is dopamine dose ($\mu g \cdot kg^{-1} \cdot min^{-1}$) × 1 + dobutamine ($\mu g \cdot kg^{-1} \cdot min^{-1}$) × 1 + epinephrine ($\mu g \cdot kg^{-1} \cdot min^{-1}$) × 100 + norepinephrine ($\mu g \cdot kg^{-1} \cdot min^{-1}$) × 100 + phenylephrine ($\mu g \cdot kg^{-1} \cdot min^{-1}$) × 100 + milrinone ($\mu g \cdot kg^{-1} \cdot min^{-1}$) × 10 + vasopressin ($U \cdot kg^{-1} \cdot min^{-1}$) × 10,000. Nonpulmonary organ failures at time of ARDS diagnosis were identified by accepted definitions in children (16). Severity of illness score used is the Pediatric Risk of Mortality (PRISM) III at 12 h.

The primary outcome reported was PICU mortality, with the "cause of death" determined by the treating physician. We also reported ventilator-free days (VFD) at 28 days and length of mechanical ventilation. All mention of "mechanical ventilation" in this study implied "invasive" ventilation, and noninvasive support was not counted toward VFD or total ventilator days. For VFD and duration of mechanical ventilation, the first day was initiation of invasive ventilation. Liberation from invasive ventilation for >24 h defined duration of mechanical ventilation. Patients requiring reinitiation of invasive ventilation after 24 h of extubation had the extra days counted toward 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 nonsurvivors were assigned VFD = 0.

Statistical analysis. The majority of data were nonnormally distributed as measured by Shapiro-Wilks, and are reported as median [interquartile range (IQR)], and differences between groups compared using nonparametric statistics. Cuzick's nonparametric test of trend was used to assess for monotonic trends across ordered groups (10). Categorical data were compared by Fisher exact test. To assess predictive ability of nucleosomes for mortality, the area under the receiver operating characteristic (AUROC) curve was computed. AUROC for different predictors were compared based on the methods of DeLong et al. (11). To test association of nucleosomes with mortality, bivariate logistic regression was performed retaining nucleosome levels, with potential confounders included one at a time in the model. The confounders tested (age, PRISM III, organ failures, vasopressor score, and Pa_{O_2}/Fi_{O_2}) were chosen for univariate association with mortality at $P \leq 0.2$. Malignancy was tested given the known association of malignancy with elevated nucleosome levels. As this was a pilot study, we did not perform a sample size estimation a priori. Analysis was performed with Stata/SE 14 (College Station, TX).

RESULTS

Description of the cohort. During the study period, 327 patients were screened, 102 children met criteria, and 76 cases with PARDS were enrolled (Fig. 1). The most common etiologies for PARDS were pneumonia (47%) and nonpulmonary sepsis (29%). Blood was drawn at a median 15 (IQR 7, 21) h after PARDS onset; 67 of 76 (88%) samples were collected ≤24 h after PARDS onset. Nucleosome levels were not correlated with either patient age (Spearman ρ 0.01, $P = 0.922$) or time of blood draw relative to PARDS onset ($\rho = -0.04$, $P = 0.705$).

Association of nucleosomes with mortality. Of the 76 PARDS cases, there were 11 nonsurvivors (14%). Nonsurvivors died at a median of 6 (IQR 4, 16; range 2 to 35) days after

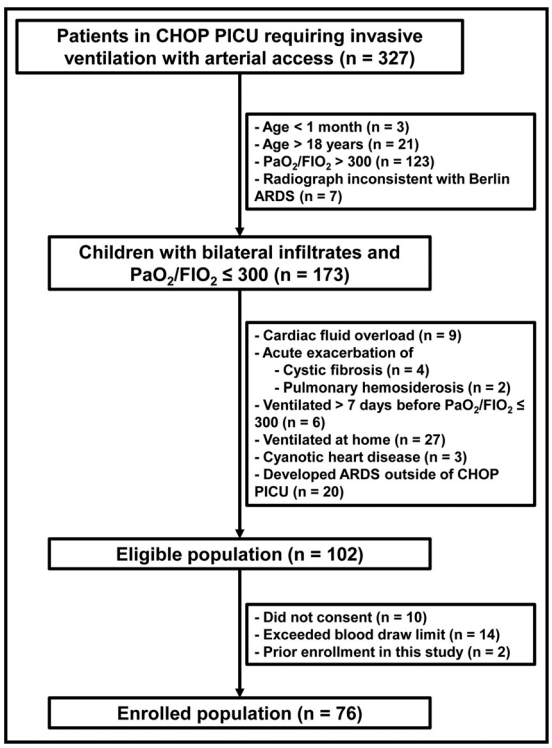

Fig. 1. Flow diagram of study screening and eligibility.

Table 1. *Characteristics of the PARDS cohort**

Variable	Survivors (65)†	Nonsurvivors (11)†	*P* Value
Age, yr	4.3 [1.5, 12.6]	1.3 [0.5, 8.6]	0.165
Female/male, %/%	31/34 (48/52)	6/5 (55/45)	0.518
PRISM III at 12 h	10 [6, 16]	17 [11, 33]	0.025
Malignancy	10 (15)	2 (18)	0.553
Cause of PARDS			0.450
Aspiration pneumonia	8 (12)	1 (9)	
Infectious pneumonia	32 (49)	4 (36)	
Nonpulmonary sepsis	18 (28)	4 (36)	
Trauma	4 (6)	0	
Other	3 (5)	2 (18)	
Nonpulmonary organ failures at PARDS onset	1 [1, 3]	4 [2, 5]	<0.001
Vasopressor score	9 [2, 15]	15 [5, 32]	0.112
At PARDS onset			
Pa_{O_2}/Fi_{O_2}	165 [103, 235]	225 [130, 262]	0.144
OI	10.4 [6, 17.9]	7.7 [7.1, 16.9]	0.965
PEEP, cmH_2O	10 [7, 12]	12 [9, 13]	0.233
Peak pressure, cmH_2O	31 [26, 35]	31 [27, 34]	0.971
Tidal volume, ml/kg	7.6 [6.3, 8.8]	7.5 [6.3, 8]	0.670
Ancillary therapies			
Inhaled nitric oxide	22 (34)	5 (45)	0.506
Prone positioning	3 (5)	0	1
Neuromuscular blockade	29 (45)	7 (64)	0.332
Alternative ventilator modes	20 (31)	5 (45)	0.489
ECMO	5 (8)	0	1

*Values are presented as median IQR in brackets or percentage in parentheses. Continuous variables are compared with a rank sum test and categorical with a Fisher exact test. †Number of subjects.

plasma collection, and no patients died within 48 h of plasma collection. Nonsurvivors had worse PRISM III scores and more nonpulmonary organ failures, but similar measures of lung injury at PARDS onset, compared with survivors (Table 1). Nucleosome levels were higher in nonsurvivors [0.59 AU (IQR 0.46, 0.84)] relative to survivors [0.21 AU (IQR 0.08, 0.33), rank sum $P < 0.001$, Fig. 2*A*]. No association was seen between nucleosome levels and cause of death (ANOVA on ranks $P = 0.866$, Fig. 2*B*).

Confounders were included one at a time in bivariate logistic regression. Nucleosomes remained independently associated with mortality after adjustment for either age, PRISM III, presence of malignancy, number of nonpulmonary organ failures, vasopressor score, or Pa_{O_2}/Fi_{O_2} (Table 2). In a sensitivity analysis restricted to patients without malignancy ($n = 64$, 9 deaths), nucleosomes retained association with mortality (odds ratio 1.73, 95% CI 1.24 to 2.40, $P = 0.001$). Nucleosomes demonstrated good discriminative ability for mortality, with an AUROC of 0.82 (95% CI 0.66 to 0.97), outperforming both

Table 2. *Association between plasma nucleosomes and mortality in bivariate analyses*

Variable*	OR (95% CI)	*P* Value
Nucleosomes	1.52 (1.19 to 1.94)	0.001
Nucleosomes + age, yr	1.52 (1.19 to 1.95)	0.001
Nucleosomes + PRISM III	1.42 (1.10 to 1.84)	0.008
Nucleosomes + malignancy	1.59 (1.21 to 2.10)	0.001
Nucleosomes + nonpulmonary organ failures	1.36 (1.05 to 1.75)	0.041
Nucleosomes + vasopressor score	1.49 (1.16 to 1.92)	0.002
Nucleosomes + Pa_{O_2}/Fi_{O_2}	1.52 (1.18 to 1.95)	0.001

*For all models, per increase of plasma nucleosome concentrations by 0.1 AU.

Berlin and PALICC oxygenation categories ($P < 0.01$ when comparing AUROC, Table 3).

Association with organ failures and lung injury. Nucleosome levels demonstrated no association between oxygenation severity categories at PARDS onset defined by either Berlin (Fig. 3*A*) or PALICC (Fig. 3*B*) criteria. There was no association with etiology of PARDS (Fig. 3*C*), nor when comparing infectious (pneumonia and sepsis) and noninfectious (aspiration, trauma, and others) etiologies (rank sum $P = 0.678$). There was a strong correlation between nucleosome levels and increasing number of nonpulmonary organ failures ($P = 0.009$ for trend, Fig. 3*D*). There was no association between nucleosome levels and the initial peak inflating pressure (PIP) (Spearman $\rho = 0.190$, $P = 0.121$), PEEP ($\rho = 0.124$, $P = 0.316$), ΔP (PIP minus PEEP, $\rho = 0.155$, $P = 0.208$), or tidal volume ($\rho = 0.067$, $P = 0.590$).

Patients who had lower Pa_{O_2}/Fi_{O_2} 72 h after PARDS onset (relative to initial Pa_{O_2}/Fi_{O_2}) had higher nucleosome levels, demonstrating an association between worsening oxygenation over time and nucleosome levels (Fig. 4*A*). A similar trend was seen for patients with worse OI at 72 h (Fig. 4*B*). Elevated nucleosomes had modest predictive ability for worsened Pa_{O_2}/Fi_{O_2} at 72 h (Fig. 4*C*), with a similar trend seen for worsened OI at 72 h (Fig. 4*D*). The association between nucleosome levels and worse oxygenation persisted after adjustment for potential confounders (Table 4).

Comparison with at-risk intubated controls. An additional 23 intubated control patients at risk for PARDS, but not meeting oxygenation criteria, were also enrolled. As expected, PARDS cases had significantly worse nonpulmonary organ failures, vasopressor use, lung injury, and outcomes relative to controls (Table 5). Plasma nucleosome levels

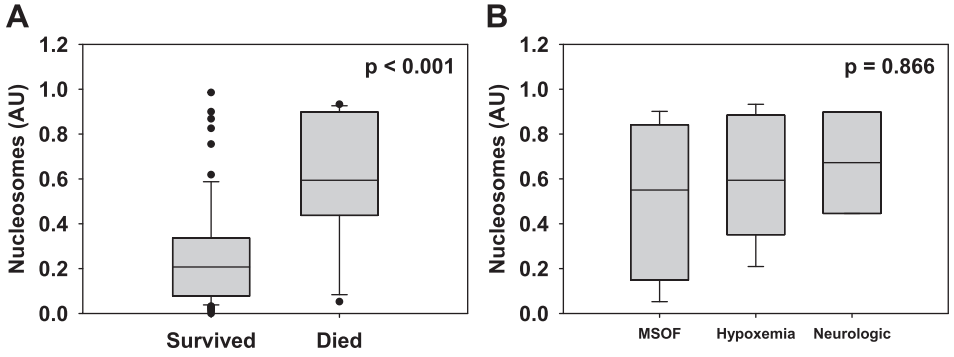

Fig. 2. Plasma nucleosome levels in patients with PARDS ($n = 76$). *A*: levels between survivors ($n = 65$) and nonsurvivors ($n = 11$); *P* value represents the result of a rank sum test. *B*: plasma nucleosomes in nonsurvivors stratified by cause of death; multisystem organ failure (MSOF; $n = 4$), refractory hypoxemia ($n = 5$), or poor neurologic prognosis ($n = 2$). *P* value represents the results of a Kruskal-Wallis ANOVA on ranks.

Table 3. *Predictive characteristics for mortality*

Variable	AUROC (95% CI)	*P* Value
Berlin (Pa_{O_2}/Fi_{O_2}) categories	0.41 (0.23 to 0.59)	1
PALICC (OI) categories	0.44 (0.25 to 0.62)	1
PRISM III at 12 h*	0.71 (0.54 to 0.89)	0.024
Nonpulmonary organ failures*	0.80 (0.70 to 0.89)	0.002
Nucleosomes*	0.82 (0.66 to 0.97)	<0.001

*AUROC for these variables is significantly different than AUROC compared with either Berlin or PALICC oxygenation categories ($P < 0.01$ for all comparisons).

were higher in PARDS patients [median 0.25 AU (IQR 0.08, 0.44)] compared with intubated at-risk controls [0.09 AU (IQR 0.06, 0.15), rank sum $P < 0.001$, Fig. 5]. Confounders with a univariate association with a PARDS diagnosis at $P \leq 0.2$ (Table 5) were included one at a time in bivariate logistic regression. The association between nucleosome levels and PARDS diagnosis persisted after adjustment for confounders (Table 6).

DISCUSSION

This is the first study examining the clinical utility of plasma nucleosomes in PARDS. Plasma nucleosomes were higher in nonsurvivors, correlated with nonpulmonary organ failures, and were associated with subsequent worsening oxygenation. Nucleosomes demonstrated modest predictive ability for PICU mortality and remained independently associated with nonsurvival after adjustment for potential confounders.

We demonstrated a strong correlation between nucleosomes and increasing number of organ failures, which may mediate the association with nonsurvival. In the setting of cell death, nuclear proteins, including the DNA and histone components of chromatin, can be released into circulation. Chromatin is degraded to mono- and oligonucleosomes, and subsequently down to component histone and free DNA (18), where they function as DAMPs. Initial studies focused on the role of nucleosomes in autoimmune disorders (21) and oncologic processes (17). More recently, circulating histones have been implicated as mediators of endothelial and organ dysfunction in sepsis (38), and several clinical studies have demonstrated their relevance to critical illness. Histones and their parent nucleosomes have been implicated in cardiac dysfunction (4, 5), renal injury (12), and dendritic cell necrosis (24). In adults with trauma, nucleosome levels correlated with trauma severity scores, with histone levels correlating with Sequential Organ Failure Assessment scores and subsequent lung injury (2), consistent with our findings in this PARDS cohort.

To our knowledge, this is the first study demonstrating an association between nucleosome levels and mortality in a heterogeneous PARDS cohort. In five studies (3 adult sepsis, 1 adult aspiration ARDS, and 1 pediatric sepsis), nucleosome or histone levels have been demonstrated to be higher in nonsurvivors (4, 12, 24, 40, 41). In a cohort of septic adults ($n = 19$), elevated histone H4 on *day 1* predicted increased mortality at *days 28* and *90* (12). Two separate studies demonstrated elevated total circulating histones [$n = 65$, (4)] and elevated nucleosomes [$n = 49$, (24)] in nonsurvivors of adult septic shock. In a fourth study, adults with aspiration ARDS ($n = 21$) had elevated plasma nucleosomes and H4 above control patients, with higher levels seen in nonsurvivors compared with survivors (41). The single pediatric study (40) described a cohort of meningococcal sepsis ($n = 35$) in which all nonsurvivors died within 48 h of presentation, with nucleosome levels elevated at multiple time points in nonsurvivors relative to survivors.

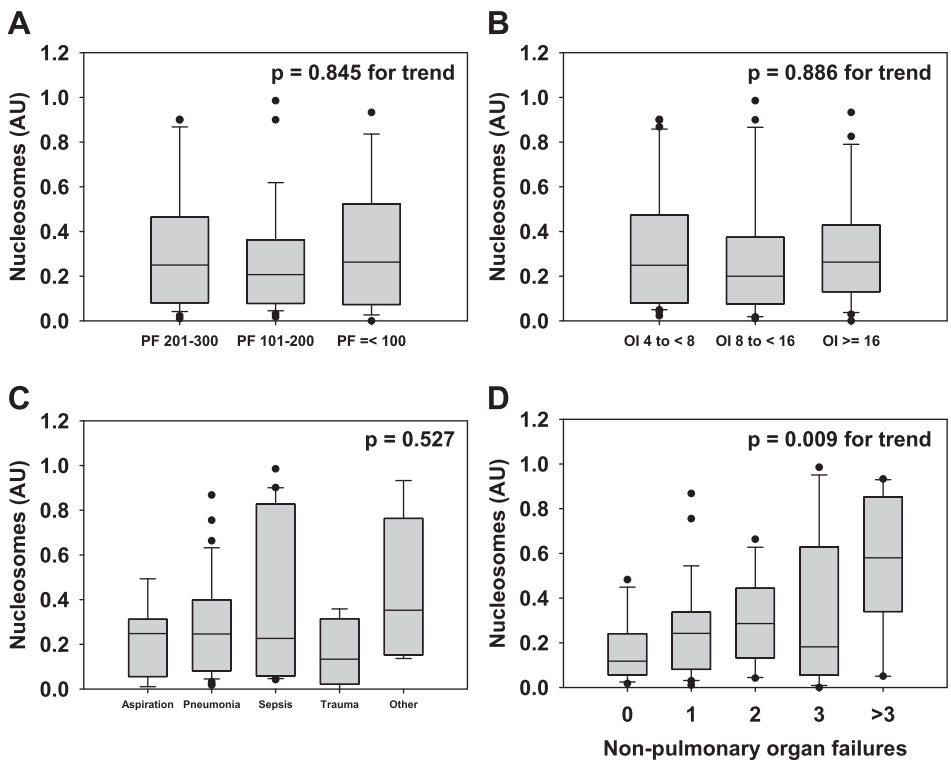

Fig. 3. Relationship between plasma nucleosome levels and Berlin (*A*) or PALICC (*B*) oxygenation categories, PARDS etiologic factor (*C*), and number of nonpulmonary organ failures (*D*). *P* values represent results of either a Kruskal-Wallis or of a nonparametric test of trend.

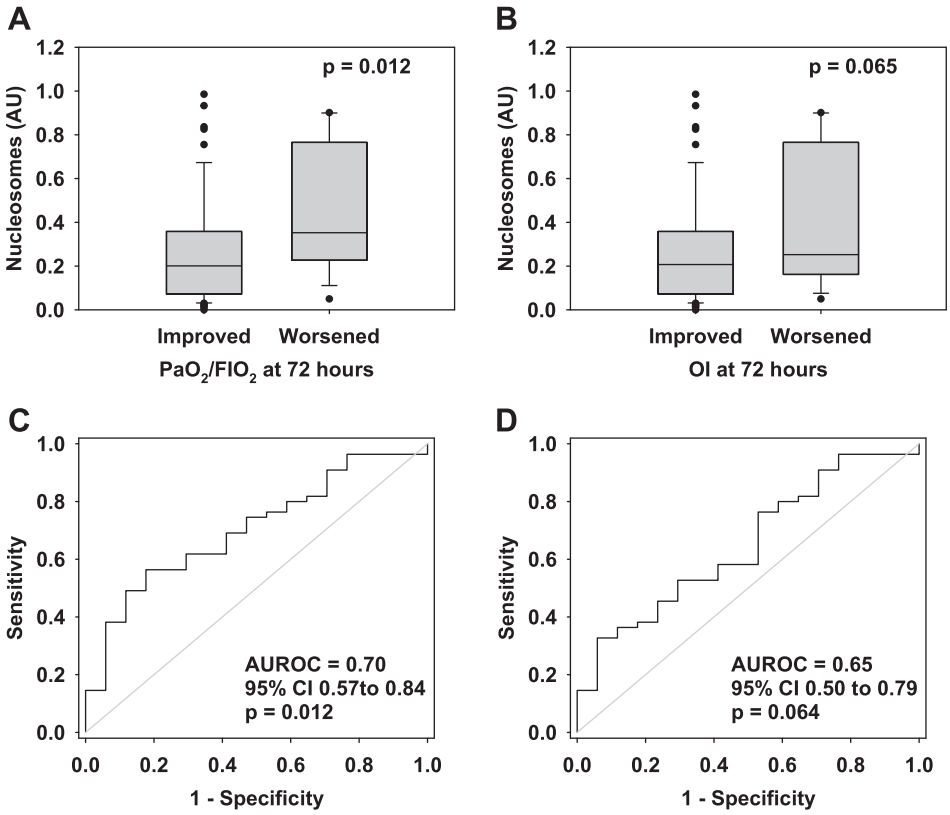

Fig. 4. Plasma nucleosome levels stratified by whether Pa_{O_2}/Fi_{O_2} (*A*) or OI (*B*) at 72 h after PARDS onset was improved or worsened relative to corresponding values at PARDS onset. *P* values represent the results of rank sum tests. AUROC curves were constructed to assess the ability of nucleosomes to predict worsening of Pa_{O_2}/Fi_{O_2} (*C*) or OI (*D*) at 72 h relative to values at PARDS onset.

Few studies exist examining the clinical utility of circulating biomarkers in PARDS, in contrast with the more extensive investigations in adult ARDS (reviewed in Ref. 32). No studies have investigated the role of nucleosomes in PARDS. The lower mortality rate of PARDS (relative to adults), which appears to be decreasing over time (39, 42), makes the selection of clinically relevant outcomes challenging for biomarker studies. Flori et al. have demonstrated the prognostic utility of brain-type natriuretic peptide (27), plasminogen activator inhibitor-1 (30), and soluble intracellular adhesion molecule-1 [sICAM-1, (14)]. Others have validated the association between increased sICAM-1 and nonsurvival in PARDS (3, 29). Elevations in the inflammatory markers C-reactive protein (8) and matrix metalloproteins (20) have also been associated with poor outcomes in PARDS. A single small study suggested utility of the lung epithelial derived Krebs von den Lungen-6 in discriminating nonsurvival in PARDS (7). Our study introduces a new class of circulating biomarker, a DAMP, which has potential therapeutic utility in addition to prognostic value. Nucleosomes and their constituent histones are cytotoxic across multiple cell and organ types. A hypothetically central role for nucleosomes in mediating organ dysfunction (including lung injury) after a variety of disparate types of inciting insults make it an attractive target for inhibition. Prior therapies known to ameliorate histone toxicity, such as heparin (41) and recombinant human activated protein C (38), which do not demonstrate benefit in unselected sepsis (19, 26), may find renewed interest in a targeted, sicker population defined by excess nucleosomes. In pediatrics, the lower overall mortality rate makes survival endpoints for clinical trials impractical. However, stratifying by a validated biomarker may enrich for a sicker cohort with higher mortality. In our cohort, elevated nucleosomes at a median of 15 h after PARDS onset predicted worsening Pa_{O_2}/Fi_{O_2} at 72 h, suggesting that a study stratifying by elevated nucleosome levels could potentially enrich for a persistent PARDS phenotype with a higher mortality. Notably, nucleosome levels did not differ by cause of death. One potential explanation is that most of these patients had severe hypoxemia and MSOF at time of phlebotomy, most of which had recovered at the time of withdrawal of care, other than the

Table 4. *Association between plasma nucleosomes and worsening oxygenation in bivariate analyses*

Variable*	OR (95% CI)	*P* Value
Worse Pa_{O_2}/Fi_{O_2} at 72 h		
Nucleosomes	1.25 (1.03 to 1.52)	0.024
Nucleosomes + age, yr	1.25 (1.03 to 1.52)	0.025
Nucleosomes + PRISM III	1.51 (1.15 to 1.98)	0.003
Nucleosomes + malignancy	1.24 (1.01 to 1.51)	0.039
Nucleosomes + nonpulmonary organ failures	1.39 (1.08 to 1.78)	0.011
Nucleosomes + vasopressor score	1.38 (1.08 to 1.74)	0.008
Nucleosomes + initial Pa_{O_2}/Fi_{O_2}	1.27 (1.03 to 1.57)	0.024
Worse OI at 72 h		
Nucleosomes	1.20 (0.99 to 1.45)	0.066
Nucleosomes + age, yr	1.20 (0.99 to 1.45)	0.070
Nucleosomes + PRISM III	1.41 (1.09 to 1.82)	0.009
Nucleosomes + malignancy	1.18 (0.97 to 1.44)	0.104
Nucleosomes + nonpulmonary organ failures	1.34 (1.05 to 1.72)	0.020
Nucleosomes + vasopressor score	1.32 (1.05 to 1.66)	0.019
Nucleosomes + initial OI	1.26 (1.02 to 1.55)	0.034

*For all models, per increase of plasma nucleosome concentrations by 0.1 AU.

Table 5. *Demographics of control and PARDS patients*

Variable	Control (23)†	PARDS (76)†	*P* Value*
Age, yr	8.3 [2.3, 14.1]	4.1 [1.2, 11.2]	0.215
Female/male, %/%	8/15 (35/65)	37/39 (49/51)	0.350
PRISM III at 12 h	9 [5, 12]	11 [6, 17]	0.065
Malignancy, %	7 (30)	12 (16)	0.101
Risk factor for intubation, %			<0.001
Aspiration pneumonia	0	9 (12)	
Infectious pneumonia	3 (13)	36 (47)	
Nonpulmonary sepsis	10 (43)	22 (29)	
Trauma	6 (26)	4 (5)	
Other	4 (17)	5 (7)	
Nonpulmonary organ failures	1 [0, 2]	2 [1, 3]	0.037
Vasopressor score	0 [0, 15]	10 [3, 16]	0.048
Initial settings			
Pa_{O_2}/Fi_{O_2}	441 [365, 499]	178 [110, 243]	<0.001
OI	2.5 [1.6, 3.7]	9.6 [6.4, 17.4]	<0.001
PEEP, cmH_2O	6 [5, 10]	10 [8, 12]	<0.001
Peak pressure, cmH_2O	21 [15, 27]	31 [26, 35]	<0.001
Tidal volume, ml/kg	7.5 [6.9, 8.3]	7.6 [6.3, 8.5]	0.925
Outcomes			
Ventilator days, all	4 [1, 7]	10 [6, 16]	<0.001
Ventilator days, survivors	4 [1, 7]	11 [7, 16]	<0.001
VFD at 28 days	24 [22, 27]	16 [1, 21]	<0.001
PICU mortality, %	0	11 (14)	0.040

*Values are presented as median IQR in brackets or percentage in parentheses. Continuous variables are compared with a rank sum test and categorical with a Fisher exact test. †Number of subjects.

Table 6. *Association between plasma nucleosomes and having a PARDS diagnosis*

Variable*	OR (95% CI)	*P* Value
Nucleosomes	2.06 (1.28 to 3.31)	0.003
Nucleosomes + PRISM III	2.13 (1.28 to 3.54)	0.003
Nucleosomes + malignancy	1.98 (1.24 to 3.15)	0.004
Nucleosomes + diagnosis	1.91 (1.16 to 3.13)	0.010
Nucleosomes + nonpulmonary organ failures	2.04 (1.24 to 3.35)	0.005
Nucleosomes + vasopressor score	2.09 (1.27 to 3.41)	0.003

*For all models, per increase of plasma nucleosome concentrations by 0.1 AU.

brain dysfunction. However, given the small number of patients who died, this remains an intriguing observation in need of further study.

The relationship to mortality in our cohort may be mediated via MSOF. While this current study cannot establish causality between nucleosomes and mortality, existing translational literature suggests a plausible causal relationship. Furthermore, nucleosome levels remain associated with mortality after adjustment for number of organ failures, suggesting additional risk imparted by the presence of nucleosomes. However, the reduced odds ratio after adjustment for organ failure suggests some measure of confounding of the relationship between nucleosomes and mortality, an issue which may be best clarified by animal models of lung injury. Nonpulmonary organ failure has been consistently demonstrated to be a strong risk factor for nonsurvival in other PARDS cohorts (13, 39). Additionally, several animal and cell studies have demonstrated endothelial (2, 12, 38) and organ dysfunction with exogenous histone treatment, including cardiac toxicities (4) and renal failure (38). The mechanism of endothelial and organ injury remains incompletely defined, but in multiple cell types, histone-mediated calcium influx led to increased cell death (2, 5), which may explain the correlation between nucleosomes and organ failure in our cohort.

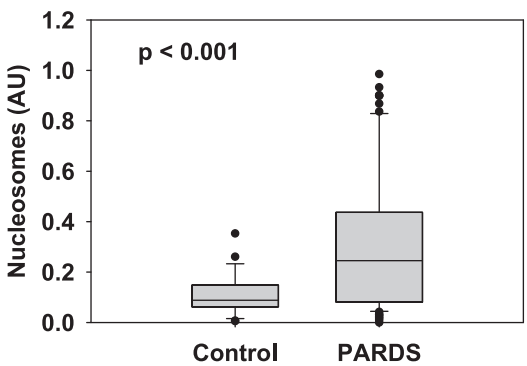

Fig. 5. Plasma nucleosome levels in PARDS cases ($n = 76$) and intubated, at-risk controls without PARDS ($n = 23$). *P* value represents the result of a rank sum test.

We did not find an association with pulmonary-specific metrics of injury, such as Berlin or PALICC oxygenation categories at PARDS onset. This is in contrast with a single study of adults with aspiration ARDS, which showed a stepwise increase in plasma nucleosomes across worsening Berlin severity categories (41). It is possible that the elevated nucleosome levels do not mediate their effects through worsening lung injury, or that severity of lung injury (as measured by initial Pa_{O_2}/Fi_{O_2} or OI) is not reflected by increased nucleosome levels. We are reluctant to conclude this from this study alone, especially given the heterogeneity of PARDS etiologies and the prior laboratory data suggesting direct pulmonary toxicity with exogenous histone treatment (2, 38). Furthermore, in patients with worsening Pa_{O_2}/Fi_{O_2} 72 h after PARDS onset, nucleosomes were elevated relative to those with improving Pa_{O_2}/Fi_{O_2}, suggesting an association between increased nucleosome levels and subsequent worsening lung injury. We also confirm, as in prior cohorts, that initial oxygenation is a poor predictor of outcome in PARDS (22, 33, 34, 39).

Our study has limitations. This was conducted at a single center, and while severity of illness and etiologies of PARDS are similar to other cohorts, these findings may not generalize, and independent validation is a necessity. The mortality rate is low, although comparable to the 13% mortality seen in our recently published cohort defined by similar eligibility criteria (39), which limits the ability to simultaneously adjust for multiple potential confounders. Heterogeneous etiologies of PARDS were included, and future studies could benefit from more homogenous populations to study the prognostic utility within subphenotypes of PARDS. The at-risk intubated control population was significantly less ill than the PARDS cohort, precluding the ability to directly compare these groups. However, after adjustment for confounders, nucleosomes remained independently associated with a diagnosis of PARDS, suggesting potential additional utility of nucleosomes in differentiating PARDS from similarly ill conditions. Bronchoalveolar lavage was not performed, and the presence of nucleosomes in the alveolar compartment could not be assessed. Finally, plasma collection was allowed up to 48 h, although 88% samples were collected ≤24 h after PARDS onset, similar to other studies. It is possible that an earlier timeframe for blood collection would yield more reproducible results; however, several variables,

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 the independent association of circulating nucleosomes with mortality in PARDS.

In conclusion, plasma nucleosomes were associated with increased mortality in PARDS, correlated with the number of nonpulmonary organ failures, and preceded subsequent worsening of oxygenation. The potential utility of this biomarker for prognostication, risk stratification, and mechanistic insight require further investigation.

GRANTS

N. Yehya was supported by the Russell C. Raphaely Endowed Chair in Critical Care Medicine, Department of Anesthesiology and Critical Care Medicine, Children's Hospital of Philadelphia, and by National Heart, Lung, and Blood Institute Grant K12 HL-109009, Emerging Medical Research Growth in Emergency Medicine.

DISCLOSURES

Dr. Neal J. Thomas reports personal fees from Therabron and CareFusion, and grants from the FDA, all outside of the submitted work. Dr. Susan S. Margulies reports personal fees from Astrocyte Pharmaceuticals, outside of the submitted work. The remaining author declares no conflicts of interest.

AUTHOR CONTRIBUTIONS

N.Y., N.J.T., and S.S.M. conception and design of research; N.Y. performed experiments; N.Y. analyzed data; N.Y., N.J.T., and S.S.M. interpreted results of experiments; N.Y. prepared figures; N.Y. drafted manuscript; N.Y., N.J.T., and S.S.M. edited and revised manuscript; N.Y., N.J.T., and S.S.M. approved final version of manuscript.

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