OPEN

Characterization of Platelet Biologic Markers in the Early Pathogenesis of Postoperative Acute Respiratory Distress Syndrome

Hemang Yadav, MBBS[1]
Laurie A. Meade, RN[2]
Rickey E. Carter, PhD[3]
Keith Knutson, PhD[4]
Ognjen Gajic , MD[1]
Daryl J. Kor, MD[4]

IMPORTANCE: Animal models and limited human studies have suggested a plausible role for platelets in the pathogenesis and resolution of acute respiratory distress syndrome (ARDS). However, there are little data regarding the role of platelets in ARDS development.

OBJECTIVES: The objective of this study was to characterize the role of platelets in a postoperative ARDS model through an analysis of two platelet-specific biologic markers: thromboxane A_2 (TxA_2) and soluble CD-40-ligand (sCD40L).

DESIGN, SETTING, AND PARTICIPANTS: This was a nested case-control study of ARDS cases matched to non-ARDS controls. Blood samples were collected from a cohort of 500 patients undergoing thoracic, aortic vascular, or cardiac surgery that placed them at high-risk of developing postoperative ARDS.

MAIN OUTCOMES AND MEASURES: TxA_2 and sCD40L were analyzed at baseline (prior to surgical incision) as well as 2 hours and 6 hours after the key intraoperative events believed to be associated with increased risk of postoperative ARDS.

RESULTS: Of 500 patients enrolled, 20 ARDS cases were matched 1:2 to non-ARDS controls based on age, sex, surgical procedure, and surgical lung injury prediction score. Those who developed ARDS had longer surgeries, greater fluid administration, and higher peak inspiratory pressures. There were no significant differences in levels of TxA_2 or sCD40L at baseline, at 2 hours, or at 6 hours. There was also no difference in the change in biomarker concentration between baseline and 2 hours or baseline and 6 hours.

CONCLUSIONS: Two novel platelet-associated biologic markers (TxA_2 and sCD40L) were not elevated in patients who developed ARDS in a postoperative ARDS model. Although limited by the relatively small study size, these results do not support a clear role for platelets in the early pathogenesis of postoperative ARDS.

KEY WORDS: acute respiratory distress syndrome; biomarker; platelet; postoperative complications; prevention; respiratory failure

Acute respiratory distress syndrome (ARDS) is a life-threatening syndrome of respiratory failure characterized by the acute development of diffuse lung injury in the setting of a known insult such trauma, sepsis, pneumonia, transfusion, or aspiration (1). ARDS is a complex disease process characterized by several pathophysiologic processes including endothelial injury (2, 3), oxidative injury (4, 5), dysregulated inflammation (6), and epithelial injury (7, 8), ultimately leading to alveolar flooding and hypoxemia. ARDS-associated morbidity and mortality remain substantial (9), and there is an urgent need for better understanding of ARDS pathophysiology, as well as tools to better predict who will develop ARDS.

DOI: 10.1097/CCE.0000000000000728

Platelets have an emerging and incompletely understood role in modulating the inflammatory response, extending well beyond their established role of regulating thrombosis (10, 11). Prior studies have indicated a potential role for platelets in the pathogenesis of ARDS, including in COVID-19–related ARDS (12–20). Although the precise mechanism by which platelets are associated with the development of ARDS remains unclear, thromboxane A_2 (TxA_2) and soluble cluster of differentiation-40 ligand (sCD40L) are two biologically active substances released from activated platelets that represent biologic markers of platelet activity (12, 21–24). However, the role of these novel biologic markers in human ARDS remains poorly defined, and studies detailing their potential to improve prediction of ARDS development are lacking.

Postoperative ARDS represents a promising ARDS model that allows us to characterize the early biology of ARDS development. Most patients who develop ARDS typically present well after the inciting event (e.g. COVID-19, pneumonia) has already occurred, often far into the disease course. In contrast, ARDS occurring after high-risk elective surgery is a unique model of ARDS where the clinical insult is known ahead of time. This allows us to characterize the change in the levels of biologic markers from a healthy preoperative state to the fully established ARDS. We have previously used this experimental model to characterize the change in several well-established biologic markers involved in ARDS development (25). Of eight previously validated biomarkers evaluated, ARDS patients were more likely to have higher levels of plasminogen activator inhibitor-1, interleukin-8, and surfactant protein-D in the immediate postoperative period. This study was a preplanned exploratory analysis of platelet-specific biomarkers in the early pathogenesis of ARDS (National Heart, Lung and Blood Initiative [NHLBI] grant K23HL112855: Risk Prediction and Mechanistic Evaluation of Postoperative Acute Lung Injury). Our central hypothesis was that platelets are involved in the early pathogenesis of postoperative ARDS and that biomarkers of platelet activity will be higher in those who develop ARDS compared with those who do not.

METHODS

This study was an observational single-center nested case-control study. This study was a preplanned analysis of platelet-specific biomarkers from a previously published nested case-control study (25). The Mayo Clinic Institutional Review Board (IRB) ('Mayo Clinic Office for Human Subject Protection: Institutional Review Board') approved this study (IRB ID: 11-004138, IRB title: Mechanistic Evaluation of Postoperative Acute Lung Injury, approval date: 08/01/2011). Written informed consent was obtained from all study participants. The Strengthening the Reporting of Observational Studies in Epidemiology guidelines were followed in the design and reporting of this observational study (26). All procedures followed were in accordance with the ethical standards of the institutional committee on human experimentation and with the Helsinki Declaration of 1975.

Study Participants

The study population consisted of consecutive, consenting adult (≥ 18 yr old) patients undergoing elective cardiac, aortic vascular, or thoracic (lung or esophageal resection) surgery with an estimated risk of postoperative ARDS greater than 10 % (Surgical Lung Injury Prediction [SLIP] score ≥ 27) (**Supplemental Table 1**, http://links.lww.com/CCX/B28) (27). These specific surgical populations were chosen due to their known elevated risk for developing postoperative ARDS. Patients were excluded if they had emergency surgery, preexisting bilateral pulmonary infiltrates, recent (within 30 d) high-risk surgery, or mechanical ventilation within 30 days of surgery.

Data Collection and Analysis

The primary outcome of interest was postoperative ARDS. The methods for identifying ARDS cases, ARDS adjudication, and clinical data retrieval have been previously described (25). The primary biomarker predictor variables were plasma levels of TxA_2 and sCD40L. These were quantitatively analyzed in plasma samples collected at three distinct time points. The first sample was obtained following the induction of anesthesia (baseline level). The timing of the second sample varied depending on the surgical procedure. For patients undergoing cardiac surgery, the second sample was obtained 2 hours following separation from cardiopulmonary bypass. For patients undergoing aortic vascular surgery, the second sample was obtained 2 hours after aortic cross clamp removal. For

patient undergoing thoracic (non-cardiac) surgery, the second sample was obtained 2 hours after return to two-lung ventilation. The second sample acquisition time was timed to immediately follow the major intraoperative insult believed associated with development of ARDS. The third sample was collected at 4 hours following the acquisition of the second sample (i.e. 6 hr following the intraoperative insult believed to be associated with ARDS). At each time point, 6 cc of blood were obtained from the patient's arterial (or central venous) catheter (following a 5 cc waste). The samples were collected and placed into EDTA anti-coagulated tubes. Samples were immediately centrifuged twice to ensure they were platelet-poor and were subsequently stored at in cryogenic freezing tubes at –70° to –80°C. Sample analysis occurred simultaneously and in duplicate using customized enzyme-linked immunosorbent assays developed and carried out at the Mayo Clinic Immunology Core.

Statistical Analysis

Sample size calculations assumed an ARDS frequency of 10%, based on a SLIP score of greater than or equal to 27 (27). Using two controls for each case was estimated to provide 80% power to detect an effect size of 0.49 ($\alpha = 0.05$, two-sided) or more specifically a 0.49 SD separation in mean values in the participants who develop ARDS versus those who do not. Patients with ARDS were matched to non-ARDS controls 1:2 based on SLIP score, decile of age, sex, and type of surgical procedure. Categorical variables were summarized as frequency (%) and compared in the univariate analyses using chi-square test or Fisher exact test, as appropriate. Continuous variables were expressed as mean ± SD or median with interquartile range (IQR) as appropriate. Continuous variables were compared using *t* tests for data that were normally distributed and with a Wilcoxon analysis for all other data. Conditional logistic regression models were fit for each biomarker in univariate analyses at three time points as well as change from baseline to 2 hours and baseline to 6 hours. Values for each biomarker were displayed using box plots. Statistical analysis was performed using SPSS (IBM Corporation, Armonk, NY). All *p* values reported are two-sided and were not adjusted for multiple comparisons given the hypothesis-generating nature of this study.

RESULTS

A total of 500 patients were enrolled in the study. Of these, 33 were excluded (reasons outlined in **Fig. 1**). Twenty-six patients developed ARDS (5.6%). Of these, six ARDS cases were excluded due to the blood samples not being immediately centrifuged twice to ensure they were platelet-poor (required for TxA_2 and sCD40L analysis). Of the 441 patients who did not develop ARDS, 39 controls were randomly selected matched 1:2 for age, sex, surgical procedure, and SLIP score. One case was matched 1:1 since there were insufficient control patients with a matching surgery (pericardiectomy).

Clinical Variables

The baseline characteristics of the cohort, and the breakdown of different surgeries performed, are outlined in **Table 1**. The preoperative characteristics of the 20 ARDS cases and the 39 non-ARDS matched controls are outlined in **Supplemental Table 2** (http://links.lww.com/CCX/B28). The ARDS cases had longer surgeries (470.4 ± 166 vs 373.5 ± 153 min; $p = 0.04$), lower preoperative albumins (3.5 ± 0.5 vs 4.1 ± 0.3 g/dL; $p = 0.03$), and lower preoperative bicarbonate levels (24.5 ± 2.7 vs 27.1 ± 3.1 mEq/L; $p = 0.03$). The ARDS cohort were also more likely to be on preoperative statin therapy and preoperative inhaled steroids.

The intraoperative characteristics of the ARDS and non-ARDS matched controls are outlined in **Table 2**. Notably, the ARDS cohort had greater fluid administration (10,640 ± 6,523 vs 8,380 ± 3,791 mL; $p = 0.04$) and higher peak inspiratory pressures (22.9 ± 6.8 vs 18.4 ± 4.7 cm H_2O; $p = 0.01$). The ARDS cohort had a shorter aortic cross-clamp time (88.2 ± 41.3 vs 137.6 ± 79 min; $p = 0.03$). There were no differences in the frequency of other major intraoperative events thought to be related to the development of postoperative ARDS (one lung ventilation and cardiopulmonary bypass).

Outcomes of the ARDS and non-ARDS controls are outlined in **Table 3**. Admission severity of illness as assessed by Acute Physiology and Chronic Health Evaluation III and Sequential Organ Failure Assessment was similar between groups. Those with ARDS had longer hospital stay (15.7 [IQR: 10.2–23] vs 6.5 d [IQR: 5.4–9.8 d]; $p < 0.001$) and fewer

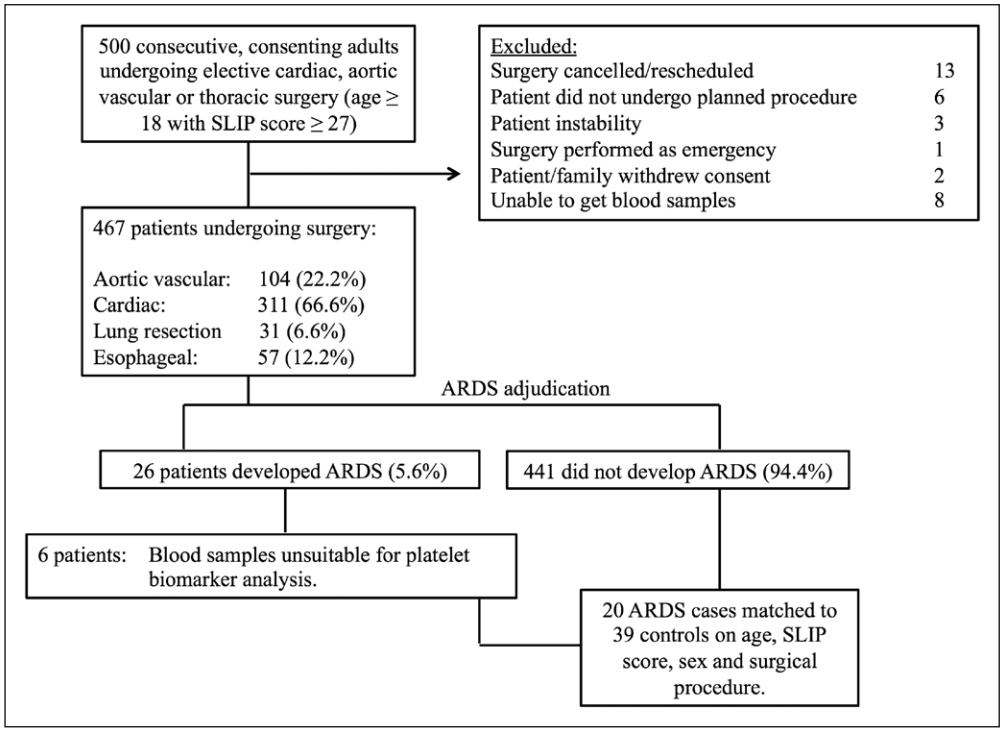

Figure 1. Study participant flow diagram. Patient instability reflects planned elective surgeries that were expedited as emergency surgeries due to patient need. ARDS = acute respiratory distress syndrome, SLIP = Surgical Lung Injury Prediction.

TABLE 1.
Baseline Characteristics and Surgery Descriptions of Enrolled Patients

Variables	ARDS	Non-ARDS Controls	*p*
Number of patients	20	39	
Male, *n* (%)	15 (75)	31 (79.5)	0.69
Age, mean ± SD	62.1 ± 14.3	62.1 ± 14.0	1.00
Surgical Lung Injury Prediction score, mean ± SD	36.8 ± 7.4	36.7 ± 6.4	0.97
Type of surgery, *n* (%)			
Aortic vascular only	8 (40)	16 (41)	
CABG only	2 (10)	4 (10.3)	
Valve only	1 (5)	2 (5.1)	
CABG and valve	1 (5)	2 (5.1)	
CABG and aortic vascular	0 (0)	0 (0)	
Valve and aortic vascular	0 (0)	0 (0)	
CABG, aortic vascular and valve	1 (5)	2 (5.1)	1.00
Pericardiectomy	1 (5)	1 (2.6)	
Esophageal	5 (25)	10 (25.6)	
Lobectomy	1 (5)	2 (5.1)	
Pneumonectomy	0 (0)	0 (0)	
Wedge resection	0 (0)	0 (0)	
Mediastinoscopy	0 (0)	0 (0)	

ARDS = acute respiratory distress syndrome, CABG = coronary artery bypass grafting.

TABLE 2.
Intraoperative Characteristics of Acute Respiratory Distress Syndrome Patients and Nonacute Respiratory Distress Syndrome Matched Controls

Intraoperative Variables		ARDS (*N* = 20)	Non-ARDS Control (*N* = 39)	*p*
Fluid administration (mL), mean ± SD	Crystalloid	4,477 ± 2,053	3,420 ± 1,539	0.05
	Colloid	4,071 ± 4,558	2,711 ± 2,400	0.22
	Total fluid in	10,640 ± 6,523	8,380 ± 3,791	0.04[a]
	Total fluid out	4,271 ± 3,842	4,440 ± 3,326	0.54
Blood product administration (mL), mean ± SD	RBCs	1,036 ± 1,590	683 ± 765	0.36
	Cell saver	729 ± 906	568 ± 632	0.48
	Fresh frozen plasma	744 ± 1,330	402 ± 781	0.30
	Platelets	308 ± 438	150 ± 249	0.15
	Cryoprecipitate	77 ± 147	40 ± 124	0.34
	Total allogeneic blood products	2,163 ± 3,371	1,275 ± 1,766	0.28
Vital signs, mean ± SD	Mean arterial pressure (mm Hg)	74.6 ± 5.9	73.3 ± 6.2	0.43
	Tidal volume (mL/kg ideal body weight)	6.4 ± 1.3	6.8 ± 1	0.29
	Peak inspiratory pressure (cm H_2O)	22.9 ± 6.8	18.4 ± 4.7	0.01[a]
	Oxygen saturation	98.7 ± 1.9	99.3 ± 1.1	0.19
	Positive end-expiratory pressure (cm H_2O)	5 ± 2.4	4 ± 1.9	0.12
Intraoperative events	One lung ventilation (*n*), *n* (%)	11 (55)	13 (33.3)	0.11
	Aortic cross clamp (total), *n* (%)	11 (55)	23 (59)	0.77
	Aortic cross clamp time (min), mean ± SD	88.2 ± 41.3	137.6 ± 79	0.03[a]
	Cardiopulmonary bypass total (*n*), *n* (%)	13 (65)	26 (66.7)	0.90
	Cardiopulmonary bypass time (min), mean ± SD	186.6 ± 67	192.3 ± 77.7	0.82
Intraoperative vasoactive infusions, *n* (%)	Dobutamine	0 (0)	0 (0)	1.00
	Dopamine	2 (10)	0 (0)	0.11
	Epinephrine	10 (50)	19 (48.7)	0.93
	Milrinone	4 (20)	7 (17.9)	0.85
	Norepinephrine	4 (20)	5 (12.8)	0.47
	Vasopressin	7 (35)	12 (30.8)	0.74
	Phenylephrine	1 (5)	1 (2.6)	1.00

ARDS = acute respiratory distress syndrome.
[a]Significant difference, $p < 0.05$.

TABLE 3.
Outcomes of Patients Who Developed Acute Respiratory Distress Syndrome Versus Matched Controls Who Did Not

Outcome Measures	ARDS (*N* = 20)	No ARDS Control (*N* = 39)	*p*
Admission Acute Physiology and Chronic Health Evaluation III score, mean ± SD	69.6 ± 24.6	68.9 ± 27.4	0.93
Admission Sequential Organ Failure Assessment score, mean ± SD	7.1 ± 3	7.4 ± 2.7	0.84
ICU admission, *n* (%)	20 (100)	28 (71.8)	0.01[a]
ICU readmission after floor transfer, *n* (%)	5 (25)	3 (7.7)	0.11
ICU length of stay, median (interquartile range)	3 (1.1–5.9)	1.1 (0.9–2.1)	0.17
Hospital length of stay, median (interquartile range)	15.7 (10.2–23)	6.5 (5.4–9.8)	< 0.001[a]
Death in ICU, *n* (%)	2 (10)	0 (0)	0.33
Death in hospital, *n* (%)	3 (15)	0 (0)	0.03[a]
Death at 28 d, *n* (%)	3 (15)	0 (0)	0.03[a]
Ventilator free days, median (interquartile range)	26.5 (24.7–27.8)	27.7 (27.5–28)	< 0.001[a]

ARDS = acute respiratory distress syndrome.

ventilator-free days (26.5 [IQR: 24.7–27.8] vs 27.7 d [27.5–28 d]; $p < 0.001$). Mortality was also higher in the ARDS group (28-d mortality: 15.0% vs 0.0%; $p = 0.03$).

Biomarker Analyses

The results from the biomarker analyses are shown in **Figure 2** and summarized numerically in **Table 4**. There were no significant differences in baseline, 2-hour, or 6-hour levels of TxA_2 or sCD40L. Similarly, there were no differences in the change in either biomarker between baseline and 2 hours and baseline to 6 hours.

DISCUSSION

In this investigation, we performed the first study of two novel biomarkers of platelet function in the early development of ARDS. Neither TxA_2 nor sCD40L was associated with ARDS development. These results do not support a role for neither TxA_2 nor sCD40L in the *early* development of ARDS. Furthermore, these platelet-specific biomarkers do not appear to be viable candidates for ARDS risk prediction.

In addition to their primary role of facilitating homeostasis, platelets are hypothesized to have an important supportive role in the host immune response. The mechanism by which platelets modulate the host immune response is thought to be primarily through interactions with other host immune cells through platelet cell surface receptors (such as p-selectin, Toll-like receptors, and cluster of differentiation-40) and the release of immunomodulatory mediators (such as TxA_2, chemokine (C-X-C motif) ligand 4, C-C Motif Chemokine Ligand 5, and von Willebrand Factor) (10). Both abnormal coagulation and a dysregulated inflammatory response are implicated in the pathogenesis of ARDS (28). Consequently, there has been substantial interest in the role of platelets in ARDS development. Preclinical studies supported a role for platelet-neutrophil interactions in ARDS development (12). In animal models, platelet depletion and disrupting neutrophil-platelet interaction substantially reduced vascular permeability and neutrophil lung infiltration (13, 15). Human studies have been more limited. In case series, patients with ARDS had elevated levels of platelet-specific proteins in the bronchoalveolar lavage (14). Several observational studies of prehospital aspirin use suggested a possible protective effect of antiplatelet therapy for ARDS prevention (29, 30). However, a recent phase IIb double-blind, placebo-controlled randomized controlled trial of aspirin in ARDS prevention did not demonstrate any clear benefit of aspirin use in reducing ARDS frequency (31).

Our study sought to improve our mechanistic understanding regarding the role of platelets in the early pathogenesis of ARDS using a nested case-control study design. sCD40L is a biologically active mediator released almost exclusively by activated platelets, with

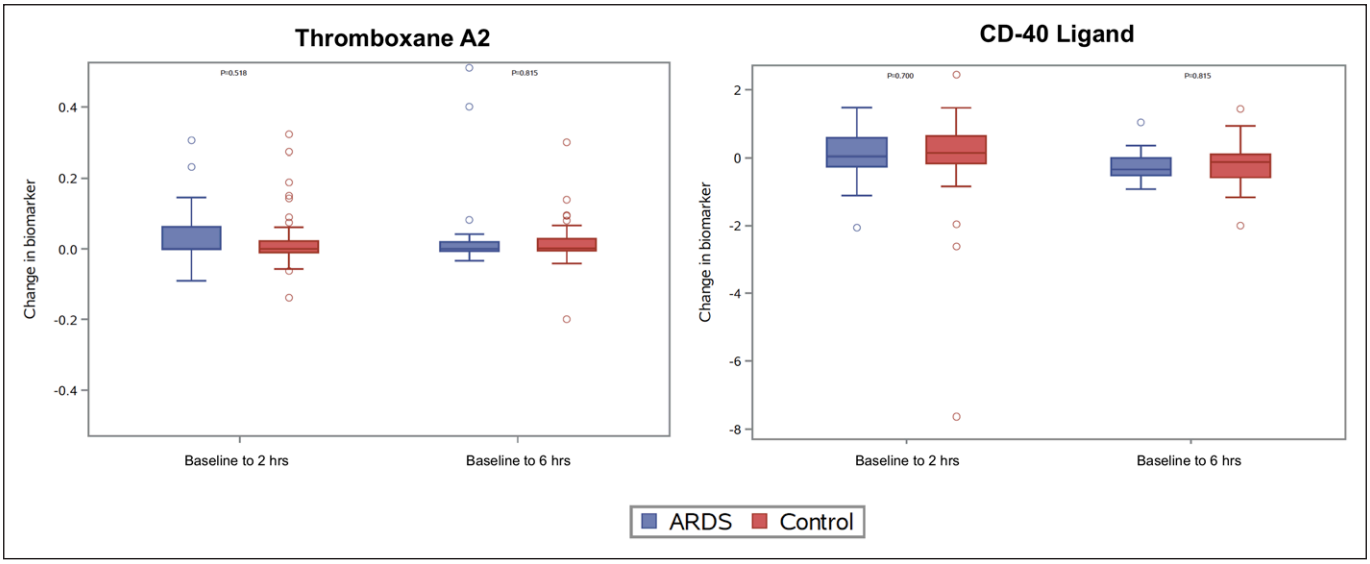

Figure 2. Biologic markers at baseline to 2 hr and baseline to 6 hr. Box plots represent median and interquartile range. Upper and lower limits represent the highest or lowest measured value within 1.5× the interquartile range. *Circles* outside the box and *whisker* plot represent individual outliers. All levels are in ng/mL.

TABLE 4.

Biologic Markers at Baseline, 2-Hours and 6-Hours in Acute Respiratory Distress Syndrome Patients and Matched Nonacute Respiratory Distress Syndrome Controls

Biomarker	Time Point	ARDS (*N* = 20)	No ARDS Control (*N* = 39)	OR (95% CI)	*P*
Thromboxane A_2	Baseline	0.010 (0.000–0.103)	0.012 (0.000–0.088)	1.25 (0.67–2.32)	0.48
	2 hr	0.031 (0.005–0.277)	0.022 (0.006–0.178)	1.16 (0.66–2.05)	0.60
	6 hr	0.073 (0.008–0.324)	0.082 (0.005–0.190)	1.07 (0.57–1.99)	0.83
	Baseline to 2 hr	0.000 (–0.002 to 0.063)	0.000 (–0.010 to 0.022)	0.81 (0.42–1.55)	0.52
	Baseline to 6 hr	0.000 (–0.007 to 0.019)	0.001 (–0.006 to 0.028)	0.92 (0.47–1.8)	0.81
CD40 ligand	Baseline	0.775 (0.000–2.123)	0.633 (0.000–1.935)	0.93 (0.51–1.69)	0.82
	2 hr	1.161 (0.374–1.869)	0.943 (0.373–1.900)	0.96 (0.55–1.67)	0.87
	6 hr	0.688 (0.086–1.548)	0.650 (0.118–1.363)	0.95 (0.53–1.69)	0.85
	Baseline to 2 hr	0.041 (–0.255 to 0.578)	0.128 (–0.170 to 0.635)	1.14 (0.59–2.19)	0.70
	Baseline to 6 hr	–0.341 (–0.518 to 0.000)	–0.125 (–0.578 to 0.098)	0.92 (0.48–1.78)	0.81

OR = odds ratio.

All values are reported as median (interquartile range). All biomarker levels are in pg/mL.

known proinflammatory properties (32). Elevated sCD40L in stored platelets has been associated with increased frequency of transfusion-related acute lung injury (33). TxA_2 is a proinflammatory chemokine released exclusively by platelets and platelet-neutrophil aggregates. Inhibition of platelet function with aspirin reduced TxA_2 in preclinical models and was associated with reduced ARDS development (12). These two biologic markers were felt to represent appropriate markers of platelet function and activation in order to better understand early ARDS pathophysiology.

Our study showed no significant difference between ARDS cases and non-ARDS controls in TxA_2 and sCD40L. These findings suggest that the early pathophysiology of postoperative ARDS is not dependent on platelet activation and function, at least as measured by sCD40L and TxA2. Since preventative strategies preferentially target early pathophysiologic processes,

these results appear to support the findings of the Lung Injury Prevention Study-A study that found that platelet inhibition with aspirin administration did not reduce ARDS development (31).

Importantly, our study only analyzed biomarkers in the first 6 hours following the primary insult believed to portent increased risk of postoperative ARDS. The lack of association between platelet-specific biomarkers and ARDS seen in our study does not preclude a role for platelets later in ARDS pathogenesis or in ARDS resolution (34). Furthermore, this study does not preclude a role for platelet activation in ARDS resulting from other major ARDS risk factors such as pneumonia, sepsis, or aspiration. Although biologic markers can theoretically improve clinical ARDS risk prediction (35), in order to be effective they need to reproducible, sensitive, specific, and elevated in a timely manner so as to be operationalized into the framework of an ARDS prevention clinical trial (36). The lack of early elevation of TxA_2 and sCD40L would appear to preclude them from any meaningful role as an ARDS prediction biomarker in the perioperative environment.

This study has several strengths that deserve mention. This is the first study that has evaluated the temporal profile of platelet-specific biologic markers in ARDS. Second, since blood samples were obtained prior to the insult responsible for ARDS development, this study allows the unique opportunity for characterization of biomarker levels very early in the disease course. In other ARDS biomarker studies, measurements have been taken when a patient presents to the emergency department or hospital, typically far into the disease course. Third, several effects were made to standardize ARDS cases and non-ARDS controls to ensure a fair comparison. We recruited subjects with similar baseline comorbidities and presurgical risk of postoperative ARDS as measured by the preoperative SLIP score. We also matched closely on surgical procedure, again limiting heterogeneity between cases and controls. Our study also employed a robust multistep ARDS adjudication procedure to ensure accuracy of the primary outcome of interest. Last, all biomarker analyses were performed simultaneously, and in duplicate, to reduce measurement bias.

This study also has several notable limitations. Most notably, the rate of ARDS development was lower than expected (5.6% vs 10% expected), reducing our overall power to detect differences in biomarker patterns. The reasons for the unexpectedly low rate of ARDS are unclear, but possible reasons include suboptimal performance of the SLIP score in predicting postoperative ARDS and a decline in the overall rate of ARDS from the time of study planning and SLIP score derivation to the time of study conduct. Further, a small number of ARDS cases were lost since the process for collecting platelet-poor samples was only implemented after the study had already begun. The relatively low ARDS rate and small number of ARDS cases consequently raise concern for a possible type I error. In addition to a low rate of ARDS, the short duration of mechanical ventilation and low mortality suggest that the enrolled study population may have had a more modest overall severity of illness than what was expected. This, in addition to the single-center nature of this study, may limit generalizability. Our assumption is that the baseline blood draw, drawn immediately after induction of anesthesia, represents a true baseline for biomarkers studied. This is in line with the parent study where baseline levels of markers such as interleukin (IL)–6 and IL-8 were in the normal range at the postinduction laboratory draw. However, it is possible that platelet biomarkers behave differently and are affected by the induction medications, and this initial laboratory draw may not necessarily be their true baseline. In that setting, a laboratory value drawn at a preoperative visit may would have been helpful to give another reference baseline value. Finally, the observational nature of this study also creates potential for confounding and bias. We attempted to mitigate these concerns by matching each case to two controls and matching on key characteristics such as age, surgical procedure, and baseline risk for developing ARDS. As highlighted above, there were relatively few differences between cases and controls in preoperative and perioperative characteristics. However, some baseline differences did exist, and these differences may affect our ability to detect changes in between-group biomarker patterns.

CONCLUSIONS

Platelet-derived biologic markers (TxA_2 and sCD40L) were not elevated in patients who developed postoperative ARDS when compared with non-ARDS controls. This study does not support a role for these

biomarkers in the early pathogenesis of postoperative ARDS. Platelet-specific biomarkers do not appear to be suitable candidates for ARDS risk prediction in the perioperative setting. However, given the relatively small number of ARDS cases and the study of just two biomarkers of platelet function, further study of platelet function in ARDS pathogenesis should be considered.

ACKNOWLEDGMENTS

We would additionally like to thank Mr. Adam Bartley for his help with statistical analysis.

1 *Division of Pulmonary and Critical Care Medicine, Mayo Clinic, Rochester, MN.*

2 *Department of Anesthesiology and Perioperative Medicine, Mayo Clinic, Rochester, MN.*

3 *Department of Health Sciences Research, Mayo Clinic, Rochester, MN.*

4 *Department of Immunology, Mayo Clinic, Jacksonville, FL.*

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website (http://journals.lww.com/ccejournal).

Dr. Yadav contributed to conceptualization, data curation, formal analysis, investigation, methodology, project administration, validation, visualization, writing (original draft), and writing (review and editing). Dr. Meade contributed to conceptualization, data curation, investigation, methodology, project administration, resources, software, and writing (review and editing). Dr. Carter contributed to conceptualization, formal analysis, funding acquisition, methodology, resources, supervision, validation, and writing (review and editing). Dr. Knudson contributed to conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, resources, validation, visualization, and writing (review and editing). Dr. Gajic contributed to conceptualization, funding acquisition, methodology, resources, project administration, supervision, validation, and writing (review and editing). Dr. Kor contributed to conceptualization, data curation, formal analysis, funding acquisition, methodology, resources, project administration, supervision, validation, and writing (review and editing).

Supported, in part, by National Center for Advancing Translational Sciences Grant Number: UL1 TR000135; National Heart, Lung and Blood Institute (NHLBI) Grant Number: K23 HL112855 (to Dr. Kor); and NHLBI Grant Number K23HL151671 (to Dr. Yadav).

The authors have disclosed that they do not have any potential conflicts of interest.

For information regarding this article, E-mail: yadav.hemang@mayo.edu

Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Institutes of Health.

REFERENCES

1. Ferguson ND, Fan E, Camporota L, et al: The Berlin definition of ARDS: An expanded rationale, justification, and supplementary material. *Intensive Care Med* 2012; 38:1573–1582
2. Ware LB, Eisner MD, Thompson BT, et al: Significance of von Willebrand factor in septic and nonseptic patients with acute lung injury. *Am J Respir Crit Care Med* 2004; 170: 766–772
3. Ong T, McClintock DE, Kallet RH, et al: Ratio of angiopoietin-2 to angiopoietin-1 as a predictor of mortality in acute lung injury patients. *Crit Care Med* 2010; 38:1845–1851
4. Chow CW, Herrera Abreu MT, Suzuki T, et al: Oxidative stress and acute lung injury. *Am J Respir Cell Mol Biol* 2003; 29:427–431
5. Imai Y, Kuba K, Neely GG, et al: Identification of oxidative stress and Toll-like receptor 4 signaling as a key pathway of acute lung injury. *Cell* 2008; 133:235–249
6. Parsons PE, Eisner MD, Thompson BT, et al: Lower tidal volume ventilation and plasma cytokine markers of inflammation in patients with acute lung injury. *Crit Care Med* 2005; 33:1–6
7. Eisner MD, Parsons P, Matthay MA, et al; Acute Respiratory Distress Syndrome Network: Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. *Thorax* 2003; 58:983–988
8. Calfee CS, Ware LB, Eisner MD, et al; NHLBI ARDS Network: Plasma receptor for advanced glycation end products and clinical outcomes in acute lung injury. *Thorax* 2008; 63:1083–1089
9. Rubenfeld GD, Caldwell E, Peabody E, et al: Incidence and outcomes of acute lung injury. *N Engl J Med* 2005; 353:1685–1693
10. Yadav H, Kor DJ: Platelets in the pathogenesis of acute respiratory distress syndrome. *Am J Physiol Lung Cell Mol Physiol* 2015; 309:L915–L923
11. Washington AV, Esponda O, Gibson A: Platelet biology of the rapidly failing lung. *Br J Haematol* 2020; 188:641–651
12. Zarbock A, Singbartl K, Ley K: Complete reversal of acid-induced acute lung injury by blocking of platelet-neutrophil aggregation. *J Clin Invest* 2006; 116:3211–3219
13. Grommes J, Alard JE, Drechsler M, et al: Disruption of platelet-derived chemokine heteromers prevents neutrophil extravasation in acute lung injury. *Am J Respir Crit Care Med* 2012; 185:628–636
14. Idell S, Maunder R, Fein AM, et al: Platelet-specific alpha-granule proteins and thrombospondin in bronchoalveolar lavage in the adult respiratory distress syndrome. *Chest* 1989; 96:1125–1132
15. Yiming MT, Lederer DJ, Sun L, et al: Platelets enhance endothelial adhesiveness in high tidal volume ventilation. *Am J Respir Cell Mol Biol* 2008; 39:569–575
16. Carvalho AC, Quinn DA, DeMarinis SM, et al: Platelet function in acute respiratory failure. *Am J Hematol* 1987; 25:377–388
17. Wei Y, Wang Z, Su L, et al: Platelet count mediates the contribution of a genetic variant in LRRC16A to ARDS risk. *Chest* 2015; 147:607–617

18. Zaid Y, Guessous F, Puhm F, et al: Platelet reactivity to thrombin differs between patients with COVID-19 and those with ARDS unrelated to COVID-19. *Blood Adv* 2021; 5:635–639
19. Tejera P, Christiani DC: Deconstructing ARDS variability: Platelet count, an ARDS intermediate phenotype and novel mediator of genetic effects in ARDS. *Semin Respir Crit Care Med* 2019; 40:12–18
20. Morales-Ortíz J, Deal V, Reyes F, et al: Platelet-derived TLT-1 is a prognostic indicator in ALI/ARDS and prevents tissue damage in the lungs in a mouse model. *Blood* 2018; 132:2495–2505
21. Ruggeri ZM: Platelets in atherothrombosis. *Nat Med* 2002; 8:1227–1234
22. Hashimoto N, Kawabe T, Imaizumi K, et al: CD40 plays a crucial role in lipopolysaccharide-induced acute lung injury. *Am J Respir Cell Mol Biol* 2004; 30:808–815
23. Wakamoto S, Fujihara M, Takahashi D, et al: Enhancement of endothelial permeability by coculture with peripheral blood mononuclear cells in the presence of HLA class II antibody that was associated with transfusion-related acute lung injury. *Transfusion* 2011; 51:993–1001
24. Miotla JM, Jeffery PK, Hellewell PG: Platelet-activating factor plays a pivotal role in the induction of experimental lung injury. *Am J Respir Cell Mol Biol* 1998; 18:197–204
25. Yadav H, Bartley A, Keating S, et al: Evolution of validated biomarkers and intraoperative parameters in the development of postoperative ARDS. *Respir Care* 2018; 63:1331–1340
26. von Elm E, Altman DG, Egger M, et al; STROBE Initiative: The strengthening the reporting of observational studies in epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Int J Surg* 2014; 12:1495–1499
27. Kor DJ, Warner DO, Alsara A, et al: Derivation and diagnostic accuracy of the surgical lung injury prediction model. *Anesthesiology* 2011; 115:117–128
28. Williams AE, Chambers RC: The mercurial nature of neutrophils: Still an enigma in ARDS? *Am J Physiol Lung Cell Mol Physiol* 2014; 306:L217–L230
29. Erlich JM, Talmor DS, Cartin-Ceba R, et al: Prehospitalization antiplatelet therapy is associated with a reduced incidence of acute lung injury: A population-based cohort study. *Chest* 2011; 139:289–295
30. Chen W, Janz DR, Bastarache JA, et al: Prehospital aspirin use is associated with reduced risk of acute respiratory distress syndrome in critically ill patients: A propensity-adjusted analysis. *Crit Care Med* 2015; 43:801–807
31. Kor DJ, Carter RE, Park PK, et al; US Critical Illness and Injury Trials Group: Lung Injury Prevention with Aspirin Study Group (USCIITG: LIPS-A): Effect of aspirin on development of ARDS in at-risk patients presenting to the emergency department: The LIPS-A randomized clinical trial. *JAMA* 2016; 315:2406–2414
32. Blumberg N, Spinelli SL, Francis CW, et al: The platelet as an immune cell-CD40 ligand and transfusion immunomodulation. *Immunol Res* 2009; 45:251–260
33. Khan SY, Kelher MR, Heal JM, et al: Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. *Blood* 2006; 108:2455–2462
34. Levy BD, Serhan CN: Resolution of acute inflammation in the lung. *Annu Rev Physiol* 2014; 76:467–492
35. Agrawal A, Matthay MA, Kangelaris KN, et al: Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients. *Am J Respir Crit Care Med* 2013; 187:736–742
36. Yadav H, Thompson BT, Gajic O: Fifty years of research in ARDS. Is acute respiratory distress syndrome a preventable disease? *Am J Respir Crit Care Med* 2017; 195: 725–736