OBJECTIVES: Cardiopulmonary bypass triggers systemic inflammation, resulting in lung injury, and frequently leads to prolonged mechanical ventilation. Biomarkers of systemic inflammation are required to predict the risk of such complications. We hypothesize that specific serum proteins can be used as biomarkers to predict the severity of lung injury following cardiac surgery.

DESIGN: Retrospective chart review study.

SETTING: Clinical variables were collected and used in conjunction with unbiased proteomic analysis using mass spectrometry that was performed on frozen plasma samples from a study group (patients with mechanical ventilation > 48 hr post surgery) and a control group (patients with mechanical ventilation < 48 hr post surgery).

SUBJECTS: Subjects included were infants who underwent cardiac surgery with similar complexity (Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery 3 or 4) using cardiopulmonary bypass. Patients in both groups were matched for their weight, age, and duration of cardiopulmonary bypass.

INTERVENTION: None.

MEASUREMENTS AND MAIN RESULTS: Four-hundred eighty-three proteins were identified (99% minimum confidence and two peptides minimum, protein false discovery rate 0.1%) on proteomic analysis of four control and four study patients at precardiopulmonary bypass, 0, and 48 hours postcardiopulmonary bypass samples. Thirty-six of 178 proteins were significantly different (≥ 1.5-fold; \( p < 0.05 \)) at precardiopulmonary bypass (top increased: tenascin; top decreased: tetranectin), 18 of 140 proteins at 0 hour (top increased: hemoglobin beta; top decreased: C8 beta), and 25 of 166 proteins at 48 hours post surgery (top increased: proteoglycan 4; top decreased: galectin-3–binding protein). The top pathway involved cytoskeleton remodeling. Other pathways involved immune response and blood coagulation. Proteoglycan 4 was validated by enzyme-linked immunosorbent assay in a different set of samples (\( n = 20 / \text{group; mean ± sd: } 128 \pm 67 \text{ vs } 195 \pm 160 \text{ ng/mL} \) \( p = 0.037 \)).

CONCLUSIONS: Multiple proteomic biomarkers were associated with worse respiratory outcomes. Precardiopulmonary bypass biomarkers might indicate risk factors (e.g., abnormalities of coagulation), whereas those identified at 0 hour and post cardiopulmonary bypass may reflect mechanisms of ongoing pathobiology.

KEY WORDS: biomarkers; cardiopulmonary bypass; congenital cardiac; lung injury; pediatrics; proteomics
injury manifestations vary in terms of severity and may significantly impact clinical management. Post-CPB lung injury can initially present with hypoxemia and abnormal ventilation and may occasionally lead to acute respiratory distress syndrome (ARDS) and acute respiratory failure, requiring invasive mechanical ventilation (8). Hammermeister et al (9) reported that up to 20% of patients who had cardiac surgery using CPB required mechanical ventilation for longer than 48 hours post surgery. In a recent report using the Pediatric Cardiac Critical Care Consortium data, 25% of pediatric patients with cardiac surgery required mechanical ventilation for longer than 44 hours (10). Furthermore, more prolonged ventilation has been associated with ARDS, hospital-acquired infections, sepsis, and mortality (11).

Identifying specific biological molecules as a diagnostic or prognostic biomarker is critical to earlier detection of diagnosis and prognosis of diseases or adverse effects. Several biomarkers have been explored, including proteins that are variable, diverse, and directly related to cellular pathology (12, 13). Unbiased mass spectrometry (MS) is an evolving technology that enables proteomic analysis at a large scale. All detectable proteins in a sample can be identified and quantified using targeted data analysis (14, 15).

We hypothesized that pediatric patients who underwent CPB surgery might have variable serum levels of specific proteins that correlate with clinical outcomes. These proteins can be used as biomarkers that may have a prognostic value of prolonged mechanical ventilation after cardiac surgery. In our study, we performed unbiased proteomic analysis on a limited number of plasma samples. Proteomic analysis identified several biomarker candidates associated with prolonged mechanical ventilation, and we validated one of the top candidates as a proof-of-concept.

**MATERIALS AND METHODS**

**Patient Selection**

At Children’s of Alabama, under separate institutional review board approval, all patients younger than 8 years who are having cardiac surgery are approached for participation in the congenital heart center biorepository. From this repository, subjects were selected. This study was reviewed and approved by the University of Alabama at Birmingham’s Institutional Review Board (IRB) approval number IRB-300003366. Informed consent was waived. Of these subjects, children younger than 1 year old requiring cardiac surgery with Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery (STAT) (16) categorizations 3 and 4 were selected for our study. Patients who required mechanical ventilation before surgery were excluded. We had a total of 458 patients who met our selection criteria over 7-year period (January 2012 to May 2019). The previous literature suggested the length of mechanical ventilation following CPB to be less than 44 hours in 75% of the cases (10). Our blood sampling was done before and at 0 and 48 hours after CPB.

Consequently, we selected the 48 hours cutoff of mechanical ventilation to classify our subjects into two groups. The study group included patients who required invasive mechanical ventilation for longer than 48 hours post surgery. The control group included patients who needed either no mechanical ventilation or mechanical ventilation for less than 48 hours post surgery. We had a pool of 40 patients in each group who were matched based on age (± 3 mo), weight (± 0.5 kg), STAT categories, and CPB time (± 20 min) length.

**Plasma Samples**

Blood samples before and after CPB were collected by bedside nurses in heparinized tubes. Subsequently, blood plasma was isolated by centrifugation and then stored in the heart center biorepository –80°C freezer. Plasma samples were studied at three time points, pre CPB and 0 and 48 hours post CPB.

**The Discovery Phase**

We randomly selected four subjects from the control group and four patients from the study group. For each subject, we obtained the pre-CPB and 48 hours post-CPB plasma samples and did the unbiased proteomic analysis. The characteristics of this group of patients are presented in Table 1.

**Proteomics Analysis.** To improve proteomic coverage, each sample was individually depleted of the most abundant proteins using a depletion spin column kit as per the manufacturer’s instructions (Thermo Scientific Pierce Protein Biology, catalog number: A36369, Abundant Protein Depletion Spin Columns;
depletes: albumin, immunoglobulin G, a1-acid glycoprotein, a1-antitrypsin, a2-macroglobulin, apolipoprotein A-I, apolipoprotein A-II, fibrinogen, haptoglobin, immunoglobulin A, immunoglobulin M, transferrin). The flow-through fractions obtained after the immuno-depletion step were collected, exchanged into 100 mM triethylammonium-bicarbonate, and concentrated to approximately 20–30 μL using molecular weight cutoff centrifugal filter devices (Amicon Ultra—0.5 mL 3k, Millipore, Billerica, MA). The protein fractions were quantified; 40 µg of protein per sample were reduced with dithiothreitol and denatured at 70°C for 35 minutes before loading onto 10% bis-tris protein gels and separated. The gels were stained overnight with colloidal coomassie for visualization purposes, the entire gel lane was cut into 6-molecular weight fractions, and each plug was equilibrated in 100 mM ammonium bicarbonate and digested overnight with Trypsin Gold, MS Grade (Promega, Cat. number V5280, WI) following manufacturer’s instruction. Peptide extracts were reconstituted in 0.1% formic acid/double distilled water at 0.1 µg/µL. MS was carried out, and the data were processed, searched, filtered, grouped, and quantified, as previously reported in detail (17). Following protein identification and relative quantification by normalized spectral counting (NSC), the most statistically significant changed proteins from each pairwise comparison were further analyzed using gene ontology assignments and pathway analysis (carried out in full detail as previously reported in Ludwig et al [17], under section 2.5 LLC-ESI-MS2 and Protein IDs for GeLC).

**Statistical and Multivariate Analysis.**

Nonparametric statistical analysis was performed for the proteomic data generated between each pairwise comparison, *t* test (single tail, unequal variance, cutoff of *p* < 0.05). For protein abundance ratios determined with NSCs, we set a 1.5-fold change as the threshold for significance, determined empirically by analyzing the inner-quartile data from the control experiments using ln-ln plots, where the Pearson’s correlation coefficient (*R*) is 0.98, and greater than 95–99% of the normalized intensities fell between the set fold change. In each case, both tests (*t* test and fold change) have to pass to be considered significant. All multivariate analysis, including 2D hierarchical clustering analysis HeatMaps, principal component analysis plots, etc., were carried out using Quicore Omics Explorer (Quicore, Lund, Sweden).

**Systems Analysis.** Gene ontology (GO) assignments and pathway analysis were carried out using MetaCore (GeneGO, St. Joseph, MI). Interactions identified within MetaCore were manually correlated using full-text articles. Detailed algorithms have been described previously (18, 19).

**The Validation Phase**

**Proteoglycan 4 Enzyme-Linked Immunosorbent Assay Analysis.** We selected serum samples from 20 patients in the control group and 20 patients in the study group to be included in our validation phase. Samples were analyzed for proteoglycan 4
concentration by enzyme-linked immunosorbent assay (ELISA) (Cusabio Proteoglycan 4 ELISA kit, Wuhan, P.R. China, Catalog Number. CSB-E14124h). Samples were diluted 10-fold and analyzed following the manufacturer’s protocol.

Statistical Analysis. Tests were applied at two time points: right before surgery (pre CPB) and 48 hours post surgery (post CPB). Descriptive analyses (means, sds, medians, and interquartile ranges) of the study subject’s demographics and characteristics were performed. One-way analysis of variance (ANOVA) described the differences among groups as appropriate. Groups means were compared using all pairwise multiple comparison procedures (Holm-Sidak method).

RESULTS

Results of the Proteomic Analysis

1) Before CPB: Table 2 shows the comparisons of the mean levels of the proteins before CPB. Zinc-alpha-2-glycoprotein, alpha-2-Heremans Schmid-glycoprotein, angiotensinogen, and beta-2-glycoprotein 1 were approximately two-fold higher in the study than in the control group. Vitamin K–dependent protein S, coagulation factor V, apolipoprotein A-I, and tetranectin were lower in the study group compared with the control group. Proteoglycan 4 levels trended higher, although not statistically significant, in the study group (p = 0.1).

2) 0 hour after CPB: Table 3 shows the comparisons of the mean levels of the proteins after CPB at 0 hour. Tyrosine-protein kinase was increased three-fold in the study group. Angiotensinogen, transmembrane protease serine 13, thyroxine-binding globulin, zinc-alpha-2-glycoprotein, and alpha-antichymotrypsin were around 1.5–1.8-fold higher in the study group, whereas corticosteroid-binding globulin, zinc-alpha-2-glycoprotein, and complement component C8 beta chain were two- to four-fold decreased.

3) 48 hours post CPB: Table 4 shows the comparisons of the mean levels of the proteins 48 hours after CPB. Proteoglycan 4, keratin type I, and keratin type II were 2.6-fold higher in the study group, whereas selenoprotein P and galectin-3–binding protein were lower than the control group.

Pathways Analysis. Pathway analysis is used to identify highly associated and well-characterized molecular physiologic cellular processes. This approach was applied to the most significantly changed proteins stemming from the combined 0- and 48-hour post-CPB study plasma specimens. There were seven significantly associated networks (Table S2, http://links.lww.com/CCX/A781) that included number 2 cytokine signaling and number 6 regulation of blood coagulation (illustrated in Fig. S1, http://links.lww.com/CCX/A777 and Fig. S2, http://links.lww.com/CCX/A778) due to 27 and 11 significant protein changes (also called “seed nodes”), respectively. The total node number is always higher than the seed nodes, which stem from the top hit protein list identified from our datasets due to the fact that second-degree associations are also common, and these additional nodes will connect the dots between proteins that are not directly but somewhat indirectly connected by a separate protein variable. Of note, for our illustrations and to gain a higher level of confidence, we only explore those networks that contain a high degree of connectivity without the need for more than a few second-degree nodes required for connectivity. As a whole, proteins identified within these datasets appear to point toward response to an endogenous hormone, proliferation, cytokine-mediated response, exocytosis/secretion, blood coagulation, and complement activation. Focusing on any one of these areas of apparent response will likely yield additional information regarding the pathology of the disease process. Table S3 (http://links.lww.com/CCX/A782) shows the main GO processes of the discovered proteins in the combined 0- and 48-hour post-CPB study plasma specimens. Again, the GO processes involve cytoskeletal remodeling, cell adhesion, blood coagulation, and immune response processes.
**TABLE 2.**
The Top Hit Proteins of the Proteomic Analysis of the Pre cardiopulmonary Bypass Plasma Samples

| Protein Name Pre Cardiopulmonary Bypass, Normal Spectrum Counts | Control Mean ± sd | Study Mean ± sd | Significance Analysis of Microarrays | t test | Folds |
|---------------------------------------------------------------|-------------------|----------------|-------------------------------------|--------|------|
| Tenascin                                                      | 4.36 ± 2.60       | 19.23 ± 18.59  | 0.70                                | 0.1038 | 4.4  |
| Corticosteroid-binding globulin                              | 8.29 ± 6.08       | 24.05 ± 19.62  | 0.61                                | 0.1041 | 2.9  |
| Complement factor I                                          | 6.78 ± 5.54       | 19.06 ± 11.17  | 0.74                                | 0.0569 | 2.8  |
| Coagulation factor XIII A chain                              | 1.65 ± 0.69       | 3.94 ± 2.30    | 0.77                                | 0.1112 | 2.4  |
| Gamma-glutamyl hydrolase                                     | 3.69 ± 1.23       | 7.86 ± 3.76    | 0.84                                | 0.0548 | 2.1  |
| Zinc-alpha-2-glycoprotein                                    | 17.35 ± 11.22     | 35.54 ± 5.68   | 1.08                                | 0.0196 | 2.0  |
| Alpha-2-Heremans Schmid-glycoprotein                         | 31.73 ± 6.15      | 62.92 ± 16.42  | 1.38                                | 0.0127 | 2.0  |
| L-selectin                                                    | 1.66 ± 0.19       | 3.28 ± 1.32    | 1.07                                | 0.0828 | 2.0  |
| Transthyretin                                                 | 43.05 ± 21.72     | 84.32 ± 44.17  | 0.63                                | 0.0814 | 2.0  |
| Angiotensinogen                                               | 35.24 ± 15.12     | 68.83 ± 21.24  | 0.92                                | 0.0230 | 2.0  |
| Beta-2-glycoprotein 1                                        | 14.63 ± 9.36      | 28.05 ± 3.28   | 1.06                                | 0.0289 | 1.9  |
| Fibrinogen beta chain                                        | 42.88 ± 41.65     | 81.88 ± 19.17  | 0.64                                | 0.0803 | 1.9  |
| Thyroxine-binding globulin                                   | 15.82 ± 6.26      | 30.14 ± 14.22  | 0.70                                | 0.0684 | 1.9  |
| Plexin domain-containing protein 2                           | 2.31 ± 0.62       | 4.10 ± 1.61    | 0.80                                | 0.0552 | 1.8  |
| Carboxypeptidase N catalytic chain                           | 7.52 ± 2.07       | 13.27 ± 5.02   | 0.81                                | 0.0880 | 1.9  |
| Plasma kallikrein                                            | 7.51 ± 3.72       | 12.77 ± 4.19   | 0.67                                | 0.0548 | 1.7  |
| Plasma protease C1 inhibitor                                  | 65.3 ± 39.42      | 107.05 ± 21.03 | 0.69                                | 0.0630 | 1.6  |
| Pregnancy zone protein                                       | 57.58 ± 21.39     | 86.64 ± 4.65   | 1.12                                | 0.0686 | 1.5  |
| Ficolin-3                                                    | 11.12 ± 3.42      | 6.75 ± 2.79    | 0.70                                | 0.0482 | –1.6 |
| Ig heavy chain V-III region BRO (extracellular)               | 5.29 ± 2.21       | 2.96 ± 1.34    | 0.65                                | 0.0736 | –1.8 |
| Vitamin K–dependent protein S                                 | 8.71 ± 2.09       | 4.57 ± 1.38    | 1.19                                | 0.0101 | –1.9 |
| Ig kappa chain V-I region AG (plasma membrane)               | 8.88 ± 3.76       | 4.37 ± 1.69    | 0.83                                | 0.0455 | –2.0 |
| Beta-2-microglobulin                                         | 7.66 ± 4.36       | 3.73 ± 1.59    | 0.66                                | 0.0860 | –2.1 |
| Coagulation factor V                                         | 21.07 ± 6.63      | 9.88 ± 5.39    | 0.93                                | 0.0388 | –2.1 |
| Fibulin-1                                                    | 4.50 ± 2.89       | 2.07 ± 0.98    | 0.63                                | 0.1403 | –2.2 |
| Ig kappa chain V-I region WEA                                 | 5.48 ± 2.97       | 2.46 ± 1.49    | 0.68                                | 0.0684 | –2.2 |
| Apolipoprotein A-I                                           | 156.29 ± 47.47    | 67.33 ± 52.49  | 0.89                                | 0.0230 | –2.3 |
| Cadherin-5                                                   | 5.02 ± 3.40       | 1.91 ± 0.71    | 0.76                                | 0.0814 | –2.6 |
| Ig kappa chain V-IV region Len                               | 6.47 ± 4.34       | 2.37 ± 1.27    | 0.73                                | 0.0773 | –2.7 |
| Keratin, type II cytoskeletal 6A                              | 31.34 ± 18.05     | 10.41 ± 8.06   | 0.80                                | 0.0859 | –3.0 |
| Ig kappa chain V-I region EU                                 | 4.90 ± 4.31       | 1.53 ± 0.58    | 0.69                                | 0.1079 | –3.2 |
| Keratin, type I cytoskeletal 16                               | 30.97 ± 21.8      | 9.36 ± 6.89    | 0.75                                | 0.0703 | –3.3 |
| Apolipoprotein D                                             | 11.42 ± 8.16      | 3.38 ± 1.10    | 0.87                                | 0.0714 | –3.4 |
| Apolipoprotein A-II                                          | 9.35 ± 6.5        | 2.62 ± 1.20    | 0.87                                | 0.0643 | –3.6 |
| Tetraneectin                                                 | 10.22 ± 5.02      | 1.97 ± 1.11    | 1.34                                | 0.0215 | –5.2 |

Ig = immunoglobulin. Top hits for the "pre cardiopulmonary bypass" specimens were generated by applying the stats and fold change to the normalized spectral counts as indicated in the methods provided (significance analysis of microarrays > 0.6 or p < 0.05, with fold < or > 1.5).
Results of the Validation Phase

**Patient Characteristics.** There were no statistical differences among the groups in terms of age, weight, or STAT category. The mean length of mechanical ventilation (hr) was 30 hours for the control group and 110 hours for the study group (p < 0.01). Although longer in the study group, differences in ICU and hospital length of stay did not reach statistical significance (p = 0.08, p = 0.07, respectively) (Table 5).

**Proteoglycan 4 Plasma Levels.** No differences in proteoglycan 4 levels at the pre-CPB time point were seen (p = 0.48). The study group (128 ± 67 ng/mL) had lower levels of proteoglycan 4 compared with the control group (195 ± 160 ng/mL) in the 48-hour post-CPB sampling (p = 0.03) (Table 6).

**DISCUSSION**

Lung dysfunction is one of the most common consequences after cardiac surgery (1–3). The clinical significance of pulmonary dysfunction can be variable, but it can lead to prolonged mechanical ventilation support for a significant percentage of patients following CPB (8, 9). Our study did not aim to identify patients with lung dysfunction. Instead, we assumed that all patients have some degree of lung dysfunction after cardiac surgery. Some patients have lung complications requiring prolonged mechanical ventilation. Given the clinical importance of postoperative lung injury, we aimed to discover serum biomarkers that can provide a prognosis of the severity of lung injury and suggest potential therapeutic targets for future study.
To identify biomarkers for the lung injury following CPB, we obtained unbiased proteomic analysis before and after CPB in a limited number of patients and identified a large number of proteins that are involved in multiple overlapped cellular processes. Consequently, we performed pathway analysis to determine the molecular and physiologic processes that these proteins are involved with, revealing six significant pathways.

The cytoskeleton remodeling pathway was one of the most significant pathways as multiple keratin isoforms were increased in the study samples (Fig. S3, http://links.lww.com/CCX/A779). Other discovered pathways involved blood coagulation, cell adhesions,

| Protein Name Post, Normal Spectrum Counts | Control | Study | Statistical Analysis |
|------------------------------------------|---------|-------|---------------------|
| **Proteoglycan 4**                      | 4.62 ± 2.44 | 12.33 ± 4.33 | 1.14 | 0.0144 | 2.7 |
| **Keratin, type I cytoskeletal 14**      | 7.78 ± 6.78 | 20.36 ± 7.16 | 0.90 | 0.0218 | 2.6 |
| **Keratin, type II cytoskeletal 5**      | 8.16 ± 7.74 | 21.18 ± 7.51 | 0.85 | 0.0261 | 2.6 |
| **Tenascin**                             | 7.59 ± 7.44 | 17.35 ± 8.37 | 0.62 | 0.0663 | 2.3 |
| **Attractin**                            | 6.97 ± 4.84 | 13.66 ± 2.4 | 0.93 | 0.0314 | 2.0 |
| **Thyroxine-binding globulin**           | 9.23 ± 6.56 | 17.48 ± 4.93 | 0.72 | 0.0475 | 1.9 |
| **Angiotensinogen**                      | 32.35 ± 5.11 | 58.18 ± 18.44 | 1.10 | 0.0316 | 1.8 |
| **Cholinesterase**                       | 5.15 ± 3.78 | 8.73 ± 2.18 | 0.60 | 0.0821 | 1.7 |
| **Leucine-rich alpha-2-glycoprotein**    | 18.11 ± 10.0 | 30.59 ± 8.3 | 0.68 | 0.0524 | 1.7 |
| **Alpha-2-Heremans Schmid-glycoprotein** | 23.14 ± 6.72 | 37.96 ± 13.16 | 0.75 | 0.0539 | 1.6 |
| **Transforming growth factor-beta–induced protein ig-h3** | 3.56 ± 1.13 | 5.85 ± 2.12 | 0.70 | 0.0609 | 1.6 |
| **Vasorin**                              | 3.92 ± 2.22 | 6.40 ± 1.08 | 0.75 | 0.0541 | 1.6 |
| **Zinc-alpha-2-glycoprotein**            | 20.33 ± 7.35 | 33.0 ± 1.69 | 1.40 | 0.0189 | 1.6 |
| **Alpha-1-antichymotrypsin**             | 143.84 ± 26.2 | 221.76 ± 79.84 | 0.73 | 0.0722 | 1.5 |
| **Pigment epithelium-derived factor**    | 7.97 ± 2.51 | 12.23 ± 1.02 | 1.21 | 0.0175 | 1.5 |
| **Beta-2-glycoprotein 1**                | 14.93 ± 5.05 | 22.75 ± 5.37 | 0.75 | 0.0392 | 1.5 |
| **Epididymis luminal protein 214**       | 97.37 ± 20.63 | 55.02 ± 35.33 | 0.76 | 0.0476 | -1.8 |
| **Trypsin-3**                            | 6.11 ± 3.19 | 3.33 ± 1.23 | 0.63 | 0.0904 | -1.8 |
| **Ig gamma-4 chain C region**            | 48.18 ± 18.44 | 23.44 ± 20.3 | 0.64 | 0.0608 | -2.1 |
| **Ig lambda chain V-I region HA**        | 2.93 ± 1.79 | 1.23 ± 0.83 | 0.65 | 0.0783 | -2.4 |
| **Apolipoprotein A-II**                  | 10.83 ± 5.66 | 4.19 ± 2.62 | 0.80 | 0.0482 | -2.6 |
| **Selenoprotein P**                      | 3.07 ± 0.71 | 1.14 ± 0.93 | 1.18 | 0.0091 | -2.7 |
| **Immunoglobulin J chain**               | 5.09 ± 2.87 | 1.82 ± 1.34 | 0.77 | 0.0524 | -2.8 |
| **Galectin-3–binding protein**           | 5.54 ± 2.34 | 1.95 ± 1.38 | 0.97 | 0.0235 | -2.8 |

Ig = immunoglobulin.
Top hits for the “post” specimens were generated by applying the stats and fold change to the normalized spectral counts as indicated in the methods provided (significance analysis of microarrays > 0.6 or \( p < 0.05 \), with fold < 1.5 or > 1.5).
Asfari et al

and immune response, which were further investigated by using network analysis. Based on significant protein changes, both cytokine signaling and regulation of blood coagulation were among the most important networks.

As a result of the detailed molecular and biological processes analysis, the identified proteins are involved in multiple pathophysiologic processes that could be responsible for the lung disease and coagulopathy that are seen after cardiac surgery. Validating some of these proteins could lead to a better understanding of the disease process and could open the door for future diagnostic and therapeutic targets.

Proteoglycan 4 is one of the proteoglycans found in many different organs, including the lungs. Proteoglycan 4 is found to have an essential role in the inflammatory and innate immunologic response in the lung and be part of the cytoskeletal structure of the extracellular matrix, cellular plasma membrane, and intracellular lung structures (20). Recent evidence

TABLE 5.
Patient Characteristics of the Validation Phase of the Study

| Variable | Control (n = 20) | Study (n = 20) | p |
|----------|-----------------|--------------|---|
| Age (d)  | 56 ± 49         | 32.2 ± 45    | 0.12 |
| Weight (kg) | 4.06 ± 1.13    | 3.56 ± 1.09  | 0.16 |
| Gestational age (wk) | 37.6 ± 1.7   | 37.35 ± 1.8   | 0.6 |
| Society of Thoracic Surgeons-European Association for Cardiothoracic Surgery risk of mortality category | 3.8 ± 0.4   | 3.8 ± 0.4   | 1.0 |
| Cardiopulmonary bypass time (min) | 97 ± 32 | 101 ± 27 | 0.7 |
| Length of mechanical ventilation (hr) | 29.72 ± 16 | 109.74 ± 93 | < 0.01 |
| ICU LOS (d) | 17.45 ± 10 | 54.62 ± 93 | 0.08 |
| Hospital LOS (d) | 20.9 ± 10 | 59.35 ± 92 | 0.07 |

LOS = length of stay.
Data presented as mean ± sd.

TABLE 6.
Descriptive Analysis of the Control and Study Proteoglycan 4 Plasma Concentration Pre and Post Cardiopulmonary Bypass

| Timepoint            | Mean ± sd | SE | Median (Quartile 1, Quartile 3) |
|----------------------|-----------|----|---------------------------------|
| Pre-CPB study        | 146 ± 82  | 18 | 131 (71, 225)                   |
| Post-CPB study       | 128 ± 67  | 15 | 127 (74, 172)                   |
| Pre-CPB control      | 168 ± 49  | 11 | 156 (136, 203)                  |
| Post-CPB control     | 195 ± 160 | 36 | 147 (97, 250)                   |

| Comparison           | Difference of Means | t    | p    |
|----------------------|---------------------|------|------|
| Pre study vs post study | 17.680              | 0.566| 0.573|
| Pre control vs post control | 26.400              | 0.845| 0.401|
| Pre study vs pre control | 22.275              | 0.713| 0.478|
| Post study vs post control | 66.355              | 2.125| 0.037|

CPB = cardiopulmonary bypass.
Comparison of the proteoglycan 4 concentrations (ng/mL) among and within the groups.
suggests the important role of proteoglycan 4 in regulating the inflammatory response by binding to some surface receptors in multiple organs, including the lungs (21–26). Proteoglycan levels and structures are altered significantly in response to lung injury related to inflammation or infection, as exemplified in ARDS, asthma, and chronic obstructive pulmonary disease (27–30). Our initial proteomic analysis showed the difference in proteoglycan levels before and after CPB for the study group compared with the control. In the validation study, we confirmed that lower plasma levels of proteoglycan 4 were associated with mechanical ventilation longer than 2 days for pediatric patients following CPB. The prospect of a biomarker, or a panel of biomarkers, that would predict lung injury would be very useful for postoperative clinical management. It could provide a potential avenue for future therapies to mitigate lung injury associated with CPB.

CONCLUSIONS

A limitation of this study is the small sample size. In addition, several other factors, such as hemodynamic instability, can impact mechanical ventilation usage after cardiac surgery. We did not factor the indication for mechanical ventilation in our classification of the included patients into the study versus control. Further complicating our study is the variable definition of prolonged ventilation after congenital heart surgery that has been previously described (31). To address this, we carefully matched the patient characteristics, including the STAT category, during both the discovery and validation phases, as demonstrated in Tables 3 and 5. We assumed that patients with a matched length of CPB time, weight, age, and surgical complexity have lung dysfunction as the indication for mechanical ventilation. On the other hand, mechanical ventilation is a cause of lung injury by itself, and this cannot be ruled out that as a reason for alterations of proteoglycan 4 levels.

Our proteomic analysis discovered multiple proteins involved in cytoskeleton remodeling, blood coagulation, and complement and immune response activation. These findings indicate multiple pathways and possible therapeutic targets that can be further investigated. The validation phase of the study is the first to identify the potential prognostic value of plasma levels of proteoglycan 4 in lung injury and prolonged mechanical ventilation following cardiac surgery using CPB. The precise role and behavior of proteoglycan 4 in lung injury after cardiac surgery will be investigated in future studies. Future studies evaluating proteoglycan 4 and other biomarkers may have significant diagnostic and possible therapeutic implications.

1. Clark SC: Lung injury after cardiopulmonary bypass. Perfusion 2006; 21:225–228
2. Kirklin JK: Prospects for understanding and eliminating the deleterious effects of cardiopulmonary bypass. Ann Thorac Surg 1991; 51:529–531
3. Ng CS, Wan S, Yim AP, et al: Pulmonary dysfunction after cardiac surgery. Chest 2002; 121:1269–1277
4. Westaby S: Organ dysfunction after cardiopulmonary bypass. A systemic inflammatory reaction initiated by the extracorporeal circuit. Intensive Care Med 1987; 13:89–95
5. Apostolakis E, Filos KS, Koletsis E, et al: Lung dysfunction following cardiopulmonary bypass. J Card Surg 2010; 25:47–55, 2010
6. Vohra HA, Whistance R, Modi A, et al: The inflammatory response to miniaturised extracorporeal circulation: A review of the literature. Mediators Inflamm 2009; 2009:707042
7. Giacinto O, Satriano U, Nenna A, et al: Inflammatory response and endothelial dysfunction following cardiopulmonary bypass: Pathophysiology and pharmacological targets. Recent Pat Inflamm Allergy Drug Discov 2019; 13:158–173
8. Huffmyer JL, Groves DS: Pulmonary complications of cardiopulmonary bypass. Best Pract Res Clin Anaesthesiol 2015; 29:163–175
9. Hammermeister KE, Burchfiel C, Johnson R, et al: Identification of patients at greatest risk for developing major complications at cardiac surgery. *Circulation* 1990; 84:IV380–389

10. Gaies M, Tabbutt S, Schwartz SM, et al: Clinical epidemiology of extubation failure in the pediatric cardiac ICU: A report from the pediatric cardiac critical care consortium. *Pediatr Crit Care Med* 2015; 16:837–845

11. Monteverde E, Fernández A, Poterala R, et al: Characterization of pediatric patients receiving prolonged mechanical ventilation. *Pediatr Crit Care Med* 2011; 12:e287–e291

12. Li XJ, Yi EC, Kemp CJ, et al: A software suite for the generation and comparison of peptide arrays from sets of data collected by liquid chromatography–mass spectrometry. *Mol Cell Proteomics* 2005; 4:1328–1340

13. Nesvizhskii AI, Aebersold R: Interpretation of shotgun proteomic data: The protein inference problem. *Mol Cell Proteomics* 2005; 4:1419–1440

14. Domon B, Aebersold R: Options and considerations when selecting a quantitative proteomics strategy. *Nat Biotechnol* 2010; 28:710–721

15. Gillet LC, Navarro P, Tate S, et al: Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: A new concept for consistent and accurate proteome analysis. *Mol Cell Proteomics* 2012; 11:O111.016717

16. Jacobs ML, O’Brien SM, Jacobs JP, et al: An empirically based tool for analyzing morbidity associated with operations for congenital heart disease. *J Thorac Cardiovasc Surg* 2013; 145:1046–1057.e1

17. Ludwig MR, Kojima K, Bowersock GJ, et al: Surveying the serologic proteome in a tissue-specific Kras(G12D) knockin mouse model of pancreatic cancer. *Proteomics* 2016; 16:516–531

18. Bhatia VN, Perlman DH, Costello CE, et al: Software tool for researching annotations of proteins: Open-source protein annotation software with data visualization. *Anal Chem* 2009; 81:9819–9823

19. Ekins S, Bugrim A, Brovold L, et al: Algorithms for network analysis in systems-ADME/Tox using the MetaCore and MetaDrug platforms. *Xenobiotica* 2006; 36:877–901

20. Frevert C, Wight TN: Matrix proteoglycans. *In: The Encyclopedia of Respiratory Medicine*. Laurent GJ (Eds). London, United Kingdom, Elsevier, 2006, pp 184–187

21. Das N, Schmidt TA, Krawetz RJ, et al: Proteoglycan 4: From mere lubricant to regulator of tissue homeostasis and inflammation: Does proteoglycan 4 have the ability to buffer the inflammatory response? *Bioessays* 2019; 41:e1800166

22. Iqbal SM, Leonard C, Regmi SC, et al: Lubricin/proteoglycan 4 binds to and regulates the activity of Toll-like receptors *in vitro*. *Sci Rep* 2016; 6:18910

23. Alquraini A, Jamal M, Zhang L, et al: The autocrine role of proteoglycan-4 (PRG4) in modulating osteoarthritic synoviocyte proliferation and expression of matrix degrading enzymes. *Arthritis Res Ther* 2017; 19:89

24. Alquraini A, Garguilo S, D’Souza G, et al: The interaction of lubricin/proteoglycan 4 (PRG4) with toll-like receptors 2 and 4: An anti-inflammatory role of PRG4 in synovial fluid. *Arthritis Res Ther* 2015; 17:353

25. Nahon JE, Hoekstra M, Havik SR, et al: Proteoglycan 4 regulates macrophage function without altering atherosclerotic lesion formation in a murine bone marrow-specific deletion model. *Atherosclerosis* 2018; 274:120–127

26. Parish CR: The role of heparan sulphate in inflammation. *Nat Rev Immunol* 2006; 6:633–643

27. Bensadoun ES, Burke AK, Hogg JC, et al: Proteoglycan deposition in pulmonary fibrosis. *Am J Respir Crit Care Med* 1996; 154:1819–1828

28. Merrilees MJ, Ching PS, Beaumont B, et al: Changes in elastin, elastin binding protein and versican in alveoli in chronic obstructive pulmonary disease. *Respir Res* 2008; 9:41

29. Araujo BB, Dolnikoff M, Silva LF, et al: Extracellular matrix components and regulators in the airway smooth muscle in asthma. *Eur Respir J* 2008; 32:61–69

30. Lee KY, Chuang HC, Chen TT, et al: Proteoglycan 4 is a diagnostic biomarker for COPD. *Int J Chron Obstruct Pulmon Dis* 2015; 10:1999–2007

31. Rose L, McGinlay M, Amin R, et al: Variation in definition of prolonged mechanical ventilation. *Respir Care* 2017; 62:1324–1332