Inflammatory Response After Neonatal Cardiac Surgery and Its Relationship to Clinical Outcomes

William T. Mahle, MD, Emilia Matthews, MS, Kirk R. Kanter, MD, Brian E. Kogon, MD, Shannon E. G. Hamrick, MD, and Matthew J. Strickland, PhD

Children's Healthcare of Atlanta, Emory University School of Medicine, Atlanta, Georgia

Background. **Cardiac surgery in infants results in a profound inflammatory response secondary to cardiopulmonary bypass (CPB) and the need for blood products. It is not clear how this inflammatory response modulates postoperative course or whether quantification of proinflammatory cytokines can aid with risk stratification. In this study, we prospectively assessed a panel of candidate markers to determine the time course for inflammation and the association of specific markers with clinical outcomes defined as intensive care unit length of stay (LOS).**

Methods. **We obtained preoperative blood samples from 92 neonates undergoing surgery with CPB and then serially for 5 days after surgery. Numerous interleukins were assayed along with tumor necrosis factor (TNF)-alpha and interferon (INF)-gamma. The most common surgical procedures were arterial switch procedure (n = 35) and Norwood operation (n = 34). Multivariate analysis was performed to determine if inflammatory mediators could independently predict prolonged intensive care unit LOS.**

Results. **Compared with the presurgery level, there were statistically significant increases ($p < 0.005$) for 8 out of 11 inflammatory markers: INF-gamma, interleukin (IL)-10, IL-13, IL-2, IL-5, IL-8, TNF-alpha, and IL-6 after surgery. The only cytokine on the first postoperative day that was independently associated with prolonged length of stay was IL-8 ($p = 0.002$). Cytokine values measured on postoperative day 3 were most valuable in predicting prolonged LOS. A model that included use of circulatory arrest, and day 3 measures of IL-6 and IL-8 yielded an area under of the curve of 0.88 (95% confidence interval 0.79 to 0.96) for predicting a prolonged LOS.**

Conclusions. **In summary, neonatal heart surgery for complex lesions elicits a broad inflammatory response. This early inflammatory response appears nonspecific and did not predict clinical course. Persistence of specific inflammatory mediators on the third day after surgery, however, provided important prognostic information. As such, select cytokines may serve as valuable biomarkers in this population. Whether strategies targeting specific cytokines can alter clinical course is not known.**

(Ann Thorac Surg 2014;97:950–6)

Outcomes for neonatal cardiac surgery have improved dramatically over the last 4 decades. Nonetheless, cardiac operations performed in the neonate are still associated with substantial morbidity and mortality. The immediate postoperative period is characterized by low cardiac output and transient end-organ dysfunction owing, at least in part, to the need for cardiopulmonary bypass (CPB). Numerous studies in animals and humans have suggested that there is a significant inflammatory response that occurs after surgery with CPB, and investigators have proposed that interventions to diminish the inflammatory response might improve the outcome after surgery [1]. Such interventions might include systemic corticosteroids, ultrafiltration, or agents specifically administered to blunt the inflammatory cascade [2].

A number of studies examining the inflammatory response after cardiac surgery in neonates have demonstrated an abrupt increase in inflammatory markers in the first 24 to 48 hours after surgery that corresponds to myocardial function or renal function [3, 4]. However, the inflammatory response immediately after surgery may represent transient red cell injury and not reflect a generalized inflammatory response in the patient. We hypothesized that persistent elevation in inflammation beyond the first 48 hours might be a more powerful predictor of clinical outcome. This inflammatory signature might serve both as a biomarker for neonates at increased risk and may offer insights to possible therapeutic avenues. As such, we conducted a prospective study of inflammatory response after neonatal surgery using CPB.

Material and Methods

A prospective study was conducted between August 2009 and April 2013 examining the relationship of inflammatory response to clinical outcomes. The study was approved by the Institutional Review Board of Emory University School of Medicine.

Inclusion Criteria

Term or near-term (≥34 weeks gestation) neonates with congenital heart disease presenting for cardiac

Accepted for publication Oct 25, 2013.

Address correspondence to Dr Mahle, Children's Healthcare of Atlanta, Emory University School of Medicine, 1405 Clifton Rd NE, Atlanta, GA 30322-1062; e-mail: wmahle@emory.edu.

Published by Elsevier Inc

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2013.10.069

surgery within the first 30 days of life were eligible for the study. The study only included subjects undergoing surgery requiring CPB.

Exclusion Criteria

Newborns with multiple organ abnormalities in addition to their heart defect, such as diaphragmatic hernia, tracheoesophageal fistula, and congenital syndromes, were excluded from participation. Newborns with either known genetic syndromes or congenital infections that are associated with developmental delay were also excluded. Newborns with perinatal depression as defined by a cord blood gas pH less than 7.0 or a 5-minute Apgar score 5 or less, were excluded.

Operative Support

At our institution, preoperative corticosteroids (30 mg/kg of methylprednisolone [Solu-Medrol; Pfizer, Inc, New York, NY]), are routinely administered approximately 1 hour prior to sternal incision. Packed red blood cells were administered during the procedure to maintain a hematocrit at or above 30% during CPB. After weaning from CPB, modified venovenous ultrafiltration was performed for as long as 10 minutes at the discretion of the operating surgeon. The remaining CPB pump volume was passed through a cell-washing system and transfused within 2 hours of the patient's arrival at the intensive care unit (ICU).

Cytokine Analysis

Serial measurements of 11 candidate markers were obtained immediately preoperatively (prior to sternal incision), immediately after completion of CPB, and on postoperative days 1, 2, 3, 4, and 5. Cytokine standards were provided for each individual assay. Based on previous experiments, the most accurate measurement of cytokine levels in blood spots was obtained by using standard curves prepared in the same matrix (ie, blood spots). Whole blood from enrolled subjects was applied to blood cards. Blood spots were air dried. Blood spots were excised from patient samples and standard curve samples using 5-mm biopsy punches. For both standards and patient samples it was assumed that the elution protocol represents a tenfold dilution of eluted cytokines relative to the original blood sample.

Protein Assay Kit

Twenty-five mL of each eluted sample was analyzed in duplicate on either single spot or multiplexed cytokine analysis plates using the Meso Scale Discovery (Rockville, MD) protocol. Standard curve data were used after subtraction of background cytokine levels. Fitted data (both individual pipetting duplicates and averages) were compiled in Excel (Microsoft Corporation, Redmond, WA).

Statistics

Eleven inflammatory markers were analyzed, including interferon (INF)-gamma, interleukin (IL)-1 beta, IL-10, IL-12, IL-13, IL-2, IL-4, IL-5, IL-8, tumor necrosis factor (TNF)-alpha, and IL-6. Due to skewness in the distributions of the inflammatory marker concentrations, all analyses were performed on natural log transformed concentrations. Data were collected for numerous covariates including demographic data, operative and perfusion data, and postoperative clinical outcomes.

Statistical Analysis

Natural log-transformed inflammatory marker concentrations at 6 time points (immediately after completion of CPB and postoperative days 1 through 5) were compared with preoperative baseline concentrations using paired *t* tests.

Spearman rank correlation coefficients were used to assess associations between individual inflammatory marker concentrations at 3 time points (preoperatively, postoperatively, and at postoperative day 1) with selected clinical variables, including birth weight, length of time on bypass, duration of circulatory arrest, ischemic minutes, admit lowest pH, postoperative ICU lowest pH, admit highest lactate, and postoperative ICU highest lactate. Spearman rank correlation coefficients were also used to describe associations among the 11 inflammatory markers.

Cox proportional hazards regression was used to estimate the relationship between log-transformed inflammatory marker concentrations and cardiac ICU (CICU) length of stay (LOS), controlling for length of time on bypass. Patients who died during their hospital stay were censored at the time of death. A sensitivity analysis was conducted to examine whether censoring patients who died violated the assumption of independent censoring. To assess this, survival time for those who died was recoded to 170 days so that length of stay for these patients exceeded the longest survival time in the data set. To generate receiver operating characteristic curves we created logistic regression models with ICU length of stay dichotomized at 14 days as the outcome and examined both clinical variables and cytokine values as predictors. Statistical analyses were performed using SAS software, version 9.3 (SAS Institute Inc, Cary, NC).

Results

There were 92 subjects enrolled in the study. The demographics of the enrolled subjects are shown in Table 1. The most common surgical procedures were arterial switch (n = 35) and the Norwood procedure (n = 34). The median age at surgery was 5 days (interquartile range [IQR], 4 to 7 days). Nine subjects (9.6%) died after surgery. Postoperative extracorporeal membrane oxygenation support was used in 7 subjects and postoperative cardiac arrest occurred in 5. The median interval from surgery to ICU discharge was 7 days (interquartile range, 4 to 11 days). Thirty subjects had a postoperative ICU LOS of greater than 14 days.

There were reasonable correlations among cytokine markers on postoperative day 1 with correlation coefficients varying from 0.021 to 0.561 (Table 2). The strongest correlation was between IL-13 and TNF-alpha (r = 0.70). Compared with the presurgery level, there