

Endothelial-Dependent Vasomotor Dysfunction in Infants After Cardiopulmonary Bypass

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Objectives: Cardiopulmonary bypass–induced endothelial dysfunction has been inferred by changes in pulmonary vascular resistance, alterations in circulating biomarkers, and postoperative capillary leak. Endothelial-dependent vasomotor dysfunction of the systemic vasculature has never been quantified in this setting. The objective of the present study was to quantify acute effects of cardiopulmonary bypass on endothelial vasomotor control and

attempt to correlate these effects with postoperative cytokines, tissue edema, and clinical outcomes in infants.

Design: Single-center prospective observational cohort pilot study.

Setting: Pediatric cardiac ICU at a tertiary children's hospital.

Patients: Children less than 1 year old requiring cardiopulmonary bypass for repair of a congenital heart lesion.

Intervention: None.

Measurements and Main Results: Laser Doppler perfusion monitoring was coupled with local iontophoresis of acetylcholine (endothelium-dependent vasodilator) or sodium nitroprusside (endothelium-independent vasodilator) to quantify endothelial-dependent vasomotor function in the cutaneous microcirculation. Measurements were obtained preoperatively, 2–4 hours, and 24 hours after separation from cardiopulmonary bypass. Fifteen patients completed all laser Doppler perfusion monitor (Perimed, Järfälla, Sweden) measurements. Comparing prebypass with 2–4 hours postbypass responses, there was a decrease in both peak perfusion ($p = 0.0006$) and area under the dose-response curve ($p = 0.005$) following acetylcholine, but no change in responses to sodium nitroprusside. Twenty-four hours after bypass responsiveness to acetylcholine improved, but typically remained depressed from baseline. Conserved endothelial function was associated with higher urine output during the first 48 postoperative hours ($R^2 = 0.43$; $p = 0.008$).

Conclusions: Cutaneous endothelial dysfunction is present in infants immediately following cardiopulmonary bypass and recovers significantly in some patients within 24 hours postoperatively. Confirmation of an association between persistent endothelial-dependent vasomotor dysfunction and decreased urine output could have important clinical implications. Ongoing research will explore the pattern of endothelial-dependent vasomotor dysfunction after cardiopulmonary bypass and its relationship with biochemical markers of inflammation and clinical outcomes. (*Pediatr Crit Care Med* 2020; 21:42–49)

Key Words: capillary leak syndrome; cardiopulmonary bypass; endothelium; inflammation; microcirculation; nitric oxide

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Cardiopulmonary bypass (CPB) is essential for the repair of congenital heart defects, but the ensuing systemic inflammatory response complicates postoperative

management. CPB increases serum levels of inflammatory mediators (cytokines, activated complement, and reactive oxygen species), and results in activation of transcription factors such as nuclear factor- κ B in the cells of the vascular wall. Up-regulation of adhesion molecules on leukocytes, platelets, and endothelial cells promotes leukocyte extravasation and capillary leak (1). It has been proposed that this inflammatory milieu causes derangement in endothelial-dependent vasomotor function leading to alterations in vasomotor tone that can impact regional blood flow (2).

CPB-induced endothelial dysfunction has been inferred by changes in circulating levels of endothelial adhesion molecules, nitric oxide (NO[•]) byproducts, and endothelium-derived procoagulation and fibrinolytic factors (3–6). Although there have been studies that explored the degree of endothelial vasomotor dysfunction in response to CPB in adults, it is unclear if similar dysfunction occurs in children who lack a preexisting burden of vascular pathology (7). Furthermore, the specific contribution of this microcirculatory derangement to organ dysfunction and its relationship to other CPB-induced changes in endothelial function, such as increased capillary leak, are unknown.

Laser Doppler perfusion monitoring (LDPM; Perimed, Järfälla, Sweden) allows noninvasive quantitative assessment of microvascular perfusion. When coupled with transdermal iontophoresis of vasoactive agents, it provides an objective assessment of the functional status of the microvascular endothelium. Iontophoresis of acetylcholine evaluates endothelial function since the largely NO[•]-dependent vasodilatory response to acetylcholine is endothelium dependent (8, 9). In contrast, sodium nitroprusside (SNP) is an NO[•] donor whose vasodilatory properties are endothelium independent. LDPM coupled with iontophoresis of acetylcholine and SNP has been used to assess vasomotor dysfunction in adults, where it has been shown to correlate with defects in vasomotor function in coronary and cerebrovascular disease and hypertension (10). This methodology has also recently been employed to detect the presence of endothelial dysfunction, which is inferred by an impaired response to acetylcholine and an intact response to SNP, in adults 7 days after CPB (7).

In children, LDPM has been used to evaluate vasomotor function in diabetes, Kawasaki disease, and small for gestational age infants (11–14). However, LDPM has not been used in children to directly assess for the presence endothelial dysfunction in children after CPM nor whether endothelial dysfunction correlates with circulating inflammatory markers or clinical endpoints. To this end, we hypothesized that microcirculatory endothelial vasomotor dysfunction would be detectable by LDPM in infants after CPB and would correlate with serum cytokine levels, capillary leak, and severity of illness during the immediate 48-hour postoperative period.

METHODS

Study Design

This was a prospective cohort, pilot study conducted at the Pediatric Heart Institute and Pediatric Cardiac Critical Care Unit

at the Monroe Carrel Jr. Children's Hospital at Vanderbilt University in Nashville, TN. Patients were enrolled from November 2014 to February 2016. The study protocol was designed by the principal investigators and approved by the Institutional Review Board at Vanderbilt University Medical Center. Written consent was obtained from parents or legal guardians of all patients prior to participation in the study.

Patients

Patients less than 1 year old undergoing heart surgery requiring the use of CPB were eligible for the study. Parents or legal guardians were approached during the preoperative clinic visit for potential enrollment. Patients were excluded or removed from the study if one of the following criteria were present: febrile illness within 4 weeks of surgery, need for surgical reexploration or mechanical circulatory support (extracorporeal membrane oxygenation) within 24 hours postoperatively, or the presence of internal cardiac pacemaker. If a patient was removed from the study on the basis of the postoperative clinical course, data obtained prior to exclusion were included in results. Postoperative management was at the discretion of the ICU team and was not modified based on inclusion of a patient in this study.

Operative and Postoperative Management

CPB was achieved through a median sternotomy for a majority of procedures. Arterial cannulation was established at the ascending or transverse aorta, venous cannulation at superior and/or inferior vena cava. After the administration of heparin, CPB was initiated with roller pump extracorporeal circulation and an interposed membrane oxygenator. Mechanical ventilation was ceased when full CPB was achieved. The desired degree of hypothermia was induced by active circuit cooling (18–34°C, depending on the nature of the procedure); cardiac arrest was induced for intracardiac repairs by the application of an aortic cross-clamp and infusion of high potassium del Nido cardioplegic solution into the excluded aortic root. At the completion of repair, the heart was evacuated of any air, the cross-clamp was removed to restore coronary circulation, mechanical ventilation was resumed, the patient was returned to normothermia, modified ultrafiltration was performed, and circuit support was withdrawn. Patient was transferred directly to the ICU, and postoperative management was provided as directed by standardized procedural-based protocols and modified at the discretion of the ICU team. Management was not modified based on the inclusion of a patient in this study.

Timing of Measurements and Data Collection

Patients underwent serial assessments of endothelial-dependent vasomotor function at three time points: preoperative (within 7 d of surgical date), 2–4 hours after removal from bypass, and 24 hours postbypass. Additional data collected at all time points included blood pressure before acetylcholine and SNP iontophoresis, estimation of total body water by bioelectrical impedance, and blood sampling for biomarker analysis. Hematocrit levels were recorded preoperatively and first

postoperative reading. A baseline creatinine level was recorded preoperatively, and the peak value was recorded for the 48-hour postoperative period. Clinical outcome data collected over the first 48 postoperative hours included peak lactate, total IV fluid requirement, maximum Vasoactive-Inotropic Score (VIS), mean VIS, and total urine output (UOP). The VIS was adapted from Gaies et al (15), and milrinone dosing was not included due to its standard use in the immediate postoperative period in our cardiac ICU, regardless of severity of illness.

Endothelial-Dependent Vasomotor Function

Assessment of vasomotor function was accomplished by utilizing LDPM (PeriFlux 5010; Perimed, Stockholm, Sweden) with iontophoresis (PeriIont 382b; Perimed, Stockholm, Sweden) of vasoactive compounds. The probe for the LDPM was equipped with a well housing a drug delivery electrode for iontophoresis, allowing for perfusion readings at the site of drug administration. During each reading, probe temperature was standardized at 33°C to avoid fluctuations in skin temperature. To assess endothelium-dependent vasomotor function, the drug delivery was performed using parameters previously established for use in children (13, 14). The delivery electrode was soaked with 180 μ L of 2% acetylcholine (Sigma-Aldrich, St. Louis, MO) and secured to the forearm of the patient. The dispersive electrode was attached at least 15 cm proximal, typically on the shoulder. Prior to the start of iontophoresis, basal perfusion was recorded for 2.5 minutes. Acetylcholine was then delivered with a 0.1 mA anodal current for 20 seconds for a total of five doses separated by 60 seconds. Perfusion was monitored for a total of 10 minutes in order to standardize area under the curve

(AUC) measurements. Following a 10-minute washout period, the protocol was repeated on the same arm at a separate site using 180 μ L of 1% SNP (Sigma) and a 0.2 mA cathodal current. The order of acetylcholine iontophoresis followed by SNP iontophoresis was maintained throughout the study for consistency. All readings were performed by the primary author who was a pediatric critical care fellow at the time of this study.

Perfusion output data from the LPDM system are provided in perfusion units (PUs), an arbitrary unit of measurement. To measure the response to iontophoresis, PeriSoft Version 2.5 for Windows (Perimed, Stockholm, Sweden) was used to calculate the peak perfusion value and the AUC for the 10-minute monitoring period. **Figure 1** provides an example of a complete set of perfusion recordings for a single patient. The peak perfusion value was defined as the highest perfusion reading achieved during the iontophoresis period, which was then normalized by subtracting the baseline perfusion value to correct for variability in baseline perfusion values between patients. Prior to calculating the AUC and peak perfusion values, the perfusion curve was zeroed to the baseline mean perfusion value. Changes in these measurements of vasomotor function after bypass were expressed as fraction of the prebypass values.

Bioelectric Impedance

To estimate changes in total body water, a portable bioelectric impedance analysis (BIA) device (Vanderbilt University, Nashville, TN) was used to take readings with each assessment of vasomotor function (16). Four electrodes were placed on the hands and ipsilateral feet of the patient. Data output from BIA device included resistance (ohms, Ω), reactance (Ω), and z scores for both resistance and reactance measurements, which were normalized for the patient's height. Three consecutive readings were taken while the patient was lying motionless and supine and results were averaged. Resistance values and z scores were used to assess changes in total body water (17).

Plasma Biomarkers

When possible, 0.5 mL/kg of blood was collected in an EDTA-treated vacutainer at the time of assessment of vasomotor function. Samples were placed on ice, centrifuged at 2,200 revolutions per minute for 5 minutes to obtain plasma, and then stored at -80°C . BioPlex assays (Bio-Rad, Hercules, CA) were used to obtain levels of platelet endothelial cell adhesion molecule (PECAM)-1,

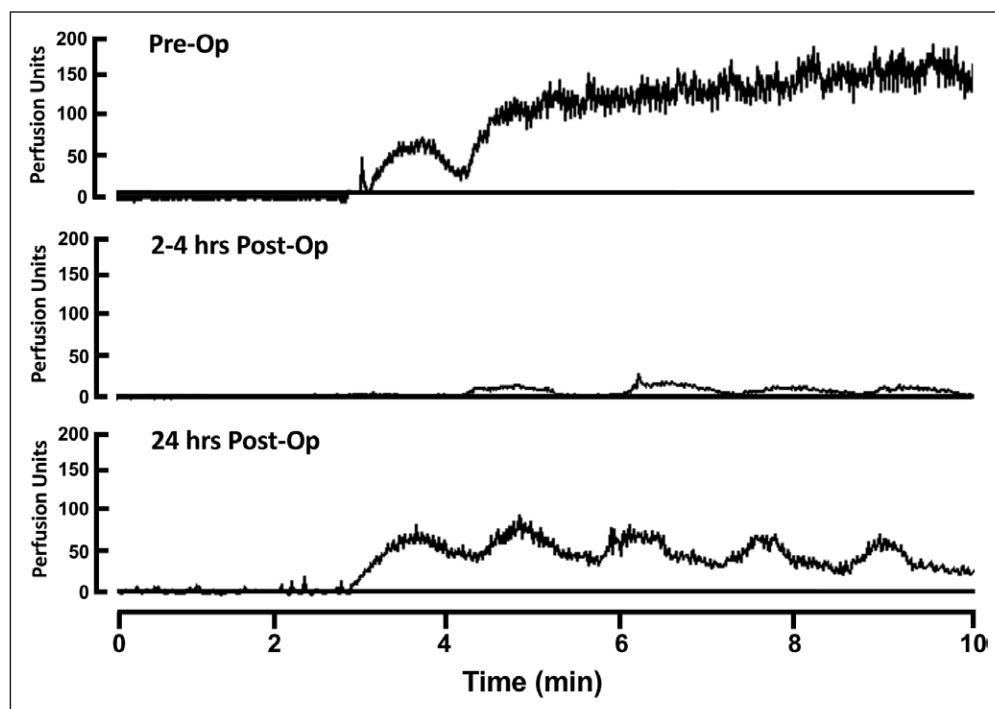


Figure 1. Preoperative (Pre-Op) and postoperative (Post-Op) laser Doppler perfusion monitoring output readings from a single patient during five sequential pulses of acetylcholine iontophoresis. Upward deflection indicates increasing perfusion.

soluble Tie2 receptor, soluble vascular endothelial growth factor receptor (sVEGFR)-1, sVEGFR-2, angiotensin-2, interleukin-6, interleukin-8, interleukin-18, and tumor necrosis factor- α . Assays were performed according to manufacturer's recommendations.

Statistical Analysis

All data are reported as medians with the 25–75th interquartile range (IQR). Wilcoxon rank sum test was used to compare pre- and post-CPB results of vasomotor function, blood pressure, and laboratory values. In order to normalize and stratify endothelial-dependent vasomotor dysfunction, a relative endothelial function index (EFI) was created for both peak and AUC values. The index was calculated by dividing the postoperative values by the preoperative value for each patient. Therefore, a value of less than 1 indicates vasomotor dysfunction. EFI values were used as a continuous variable in linear regression models with clinical and biochemical outcomes. GraphPad Prism version 5.0 (GraphPad Software, San Diego, CA) was used for all statistical analysis. *P* values of less than 0.05 were considered significant.

RESULTS

Patients

From November 2014 to February 2016, 23 patients were enrolled. Reliable preoperative LDPM readings could not be obtained for four patients, and four patients did not complete postoperative readings secondary to surgical delay or cancellation. Therefore, a total of 15 patients completed the entire study and provided data for paired analysis in linear regression models. Patient characteristics and Risk-Adjusted Classification for Congenital Heart Surgery classifications are provided in Table 1.

Vasomotor Function

Examples of LDPM data from a single patient at all three time points obtained during iontophoresis of acetylcholine are provided in Figure 1. In the preoperative state, maximal vasodilation is achieved during the second 20 seconds epoch of acetylcholine iontophoresis and was maintained throughout the remainder of the measurement. In the immediate postoperative example, each epoch of iontophoresis was evident but maximal vasodilation was reduced compared with preoperative levels. The results were qualitatively similar at 24 hours, but the magnitude of vasodilation was improved. Baseline cutaneous perfusion, which may also in part reflect endothelial function, tended to be decreased immediately following CPB for both sets of measurements (Fig. 2, A and D), and this effect reached statistical significance for the SNP group.

Comparison of preoperative responses to acetylcholine with responses 2–4 hours after separation from CPB revealed a significant decrease in both median peak values (44 [IQR, 32.2–89.1] vs 10.1 [3.6–30.5] PUs; *p* = 0.005) and median AUC (13,143 [6,889–29,079] vs 2,915 [507–11,979] PU/10 min; *p* = 0.005), indicating the presence of significant

TABLE 1. Patient Clinical and Surgical Characteristics

Characteristic	Result (n = 17)
Age, mo, median (IQR)	5.00 (0.25–6.00)
Estimated gestational age, wk, median (IQR)	38.9 (36–39)
Birth weight, kg, median (IQR)	3.04 (2.27–3.66)
Surgical weight, kg, median (IQR)	5.37 (3.9–5.95)
Cardiopulmonary bypass time, min, median (IQR)	139 (100–183)
Aortic cross-clamp time, min, median (IQR)	75 (44–86)
RACHS category, n (%)	
RACHS 1	0 (0)
RACHS 2	5 (29.4)
Atrial septal defect/VSD closure	3 (17.6)
Bidirectional cavopulmonary anastomosis (Glenn)	1 (5.9)
Tetralogy of Fallot repair	1 (5.9)
RACHS 3	7 (41.2)
Transitional or complete atrioventricular septal defect repair	4 (23.5)
Double-outlet right ventricle repair	1 (5.9)
Mitral valve repair	1 (5.9)
Tricuspid valve repair	1 (5.9)
RACHS 4	4 (23.5)
Interrupted aortic arch repair	2 (11.7)
Arterial switch operation	1 (5.9)
VSD repair with right ventricle to pulmonary artery conduit (Rastelli operation)	1 (5.9)
RACHS 5	0 (0)
RACHS 6	1 (5.9)
Norwood operation	1 (5.9)

IQR = interquartile range, RACHS = Risk Adjustment for Congenital Heart Surgery, VSD = ventricular septal defect.

Values for continuous variables are expressed as median with IQR. Values for categorical variable are expressed as a number with percentage of patient population.

endothelial-dependent vasomotor dysfunction (Fig. 2A-C). After 24 hours, peak values (21.8 [9–50.8] PUs; *p* = 0.07) and AUC (8,608 [2,806–17,570] PUs; *p* = 0.1 compared with preoperative) improved from immediately post-CPB but still tended to be depressed compared with baseline; however, this change was not statistically significant. The difference between the preoperative and 24-hour data was impacted by the fact that in four patients, endothelial-dependent vasomotor function actually improved between the immediate and 24-hour postoperative measurements and actually exceeded preoperative responses. This is reflected by the large discrepancy between the third quartile and maximum values at 24 hours

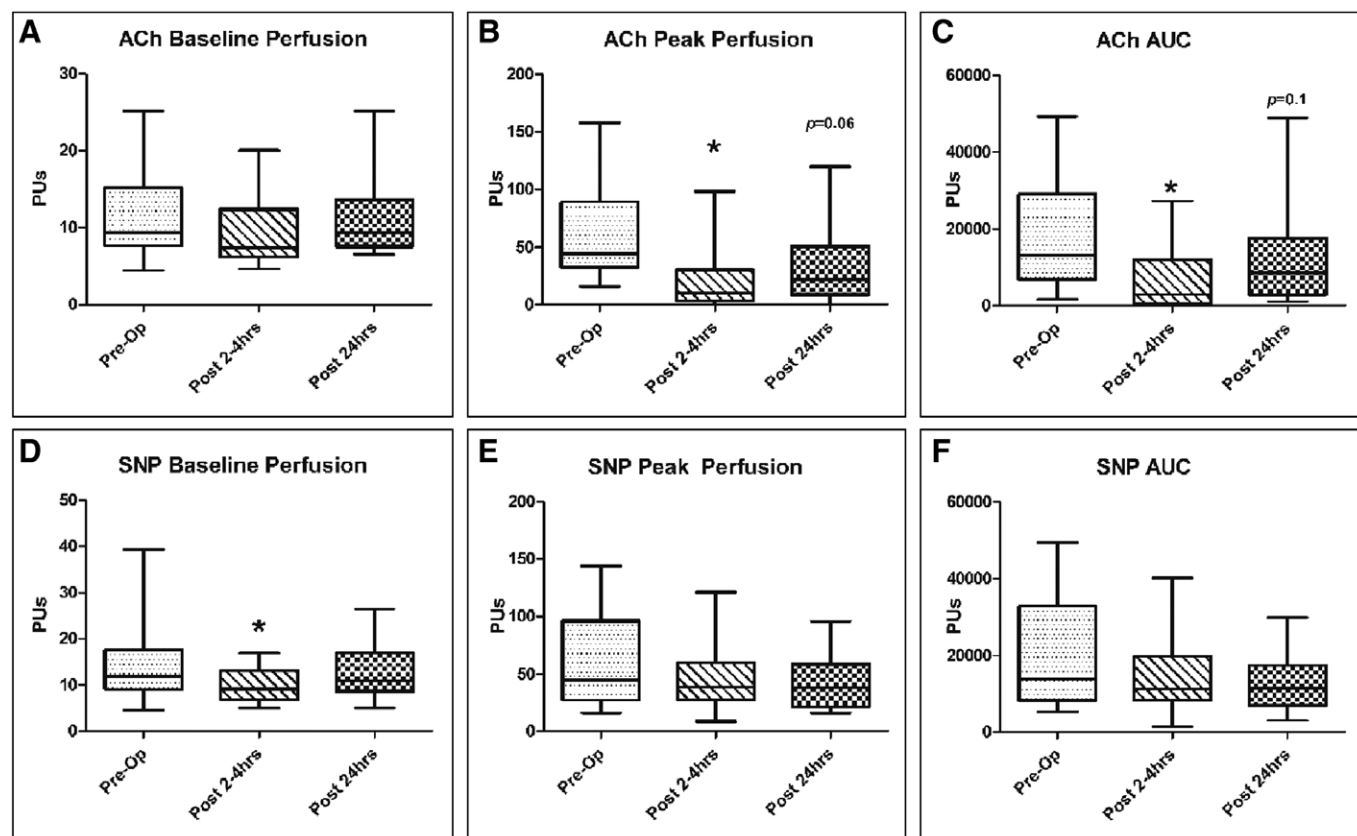


Figure 2. Iontophoresis results. **A–C**, Results for acetylcholine (ACh) iontophoresis, comparing preoperative (Pre-Op) values with postcardiopulmonary bypass values. **D–F**, Results for sodium nitroprusside (SNP) iontophoresis. All data are expressed in perfusion units (PUs). Area under the curve (AUC) for 10-min measurement period after being zeroed to baseline values. Catheters within shaded area represent medians, edges of shaded area represent first and third quartiles, and whiskers represent maximum and minimum values. Wilcoxon rank sum test was used to compare postoperative values to Pre-Op values. * $p < 0.05$.

post-CPB in Figure 2, B and C. Endothelial-independent vasomotor responses to SNP iontophoresis did not significantly differ between preoperative and either 2–4 hours (peak: 44.8 [26.9–96.4] vs 38.6 [27.1–59.9] PUs, $p = 0.25$; AUC: 13,839 [8,134–32,821] vs 11,249 [8,280–19,836] PUs, $p = 0.23$) or 24 hours post-CPB (peak: 37.9 [21.5–58.7] PUs, $p = 0.28$; AUC: 11,398 [7,024–29,845] PU/10 min, $p = 0.12$) (Fig. 2, D–F). Together, these data demonstrate significant impairment of microvascular dilation to acetylcholine, but not SNP after CPB. This suggests the presence of impaired endothelial vasodilator production, but normal responsiveness of the vascular smooth muscle to NO.

We also estimated the prevalence of endothelial dysfunction in the population by examining the change in response to vasodilators at 2 and 24 hours relative to each patient's respective baseline response. All but two had a decrease in acetylcholine responsiveness at 2 hours; thus, the prevalence of endothelial-dependent vasomotor dysfunction at 2 hours postbypass is 13/15 or 86.6%, and at 24 hours, it is 11/15 or 73.3%. This is compared with the estimated prevalence of endothelial-independent vasomotor dysfunction (SNP response) at 2 hours being 10/15 or 66.6% and at 24 hours also being 10/15 or 66.6%.

Because LDPM perfusion measurements are dependent on the concentration of RBCs, preoperative hematocrit levels

were compared with those measured immediately after surgery and no significant difference was detected (38 mg/dL [IQR, 36.5–43.5] vs 39 [35–41.5]; $p = 0.8$). There were also no differences between systolic and diastolic blood pressures during the pre- and postoperative (both 2–4 and 24 hr post-CPB) perfusion measurements.

Clinical Outcomes

Clinical markers assessed during the first 48 hours post-CPB are listed in Table 2. Serum creatinine measurements were also analyzed preoperatively and again around 48 hours post-CPB. There was a small but significant rise in creatinine (0.42 mg/dL [IQR, 0.4–0.47] vs 0.50 [0.43–0.57]; $p = 0.006$). Although the rise in creatinine was significant, it did not cross threshold to meet criteria for stage 1 pediatric acute kidney injury (AKI) making this change of questionable clinical significance. There were no deaths during the study period.

Postoperative increases in total body water as a reflection of capillary leak were added subsequent to initiation of the study, resulting in data being obtained from only 11 patients. There was a significant decrease in resistance values from baseline (571 Ω [435.5–614.2]) to 2–4 hours post-CPB (423.5 Ω [362.4–493.3]; $p = 0.01$), indicating an increase in total body water immediately after bypass. There was an additional significant decrease in resistance values from 2 to 4 hours (423.5 Ω

TABLE 2. Clinical Outcome Markers During Initial 48 Hours Compared With Endothelial Function Index

Outcome	Result (IQR) (n = 17)	R ² Peak EFI at 2–4 hr	p	R ² AUC EFI at 2–4 hr	p	R ² Peak EFI at 24 hr	p	R ² AUC EFI at 24 hr	p
Lactate, mg/dL	1.8 (1.5–3.2)	0.033	0.52	0.036	0.5	0.024	0.58	0.02	0.67
IV fluids, mL/kg	262 (146–332.3)	0.16	0.14	0.153	0.15	0.004	0.82	0.001	0.93
Urine output, mL/kg	163.8 (129–202.3)	0.024	0.58	0.075	0.32	0.384	0.01	0.433	0.008
Maximum VIS	3 (0–6.2)	0.033	0.57	0.019	0.62	0 ^a	0.98	0 ^a	0.99
Mean VIS	1.2 (0–1.9)	0.004	0.81	0.018	0.63	0.056	0.39	0.057	0.39
Total time intubated, hr	35.5 (15.5–91)	0.007	0.77	0.003	0.84	0.025	0.57	0.039	0.48

AUC = area under the curve, EFI = endothelial function index, IQR = interquartile range, VIS = Vasoactive-Inotropic Score.

^aResult < 10 e⁻³.

Total time intubated was collected beyond 48 hr if necessary until patient was successfully extubated for 24 hr. VIS = dopamine dose (μg/kg/min) + dobutamine (μg/kg/min) + 100 × epinephrine dose (μg/kg/min) + 166 × vasopressin dose (U/kg/hr) + 100 × norepinephrine (μg/kg/min).

Boldface values indicate statistical significance.

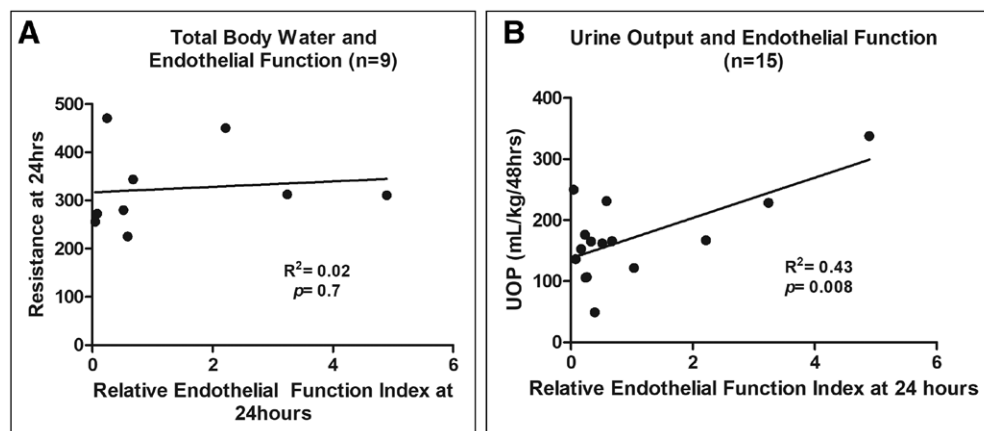


Figure 3. Relative endothelial function index = postoperative acetylcholine (ACh) response/preoperative ACh response. All relative endothelial function index values are for area under the curve results. Results for peak perfusion responses followed the same pattern. UOP = urine output.

[362.4–493.3] to 24 hours post-CPB (311.9 Ω [268.6–384.1]; $p = 0.002$). However, there was no association between resistance and peak or AUC EFI values (Fig. 3A).

Univariate linear regression was used to compare clinical markers with EFI at 2–4 and 24 hours post-CPB. Better endothelium-dependent vasomotor function at 24 hours post-CPB was associated with higher UOP over the first 48 hours postoperatively ($R^2 = 0.43$; $p = 0.008$) (Fig. 3B). Although this is an intriguing and further hypothesis-generating preliminary association, it should be noted that given the small patient population, a single patient with high UOP and better endothelium-dependent vasomotor function could greatly influence the strength of this association. It was also not possible to control for factors that could influence UOP such as diuretics. There were no patients who received renal replacement therapy or peritoneal dialysis during the study period. EFI did not show a significant relationship with any other clinical parameter.

Biomarker Data

Plasma samples were obtained from 13 patients for biomarker analysis (Supplemental Table 1, Supplemental Digital Content

1, <http://links.lww.com/PCC/B37>). There were significant changes at one or both time points in all biomarkers post-CPB except for PECAM-1. Univariate linear regression was used to compare relative endothelial function indices with changes in biomarker levels at 2–4 and 24 hours post-CPB in patients who had paired endothelial-dependent vasomotor function data to look for preliminary associations. No statistically significant relationships were observed.

DISCUSSION

CPB induces a systemic inflammatory response that has been postulated to cause endothelial injury. Utilizing LDPM coupled with drug iontophoresis to measure the endothelium-dependent response to acetylcholine, we now confirm for the first time the presence of profound endothelium-dependent vasomotor dysfunction in infants after CPB. We also confirm the presence of capillary leak and a systemic inflammatory response after CPB through assessment of tissue resistance and inflammatory biomarkers. In our limited patient population, initial regression modeling suggests a relationship between better endothelial responsiveness to acetylcholine at 24 hours postoperatively and higher UOP during the initial 48-hour post-CPB period. Confirmation of this relationship will require further investigation with a larger patient cohort. In addition, despite a significant increase in cytokine levels, that are thought to be associated with worse clinical outcomes, we observed no evidence of a relationship between vasomotor function and cytokine levels (18–20). This will also be important to better delineate with a larger study population because it could have a role in determining whether the mechanism of

endothelial injury is linked to the inflammatory milieu following CPB.

Understanding mechanisms of endothelial dysfunction following CPB may have important clinical implications if the observed association with postoperative UOP can be confirmed. Fluid overload and the ability to achieve adequate UOP after aggressive fluid resuscitation have been strongly linked to clinical outcomes and mortality in multiple realms of the PICU (21, 22). In addition, CPB has been linked with AKI and postoperative outcomes in adults (23). However, a recent study using statins as anti-inflammatory therapy failed to ameliorate AKI after CPB, suggesting a non-inflammatory etiology of renal injury (24). Methods to prevent endothelial injury or recover endothelial vasomotor function after CPB may have the potential to improve postsurgical outcomes.

In healthy adults, acetylcholine-induced cutaneous vasodilation is mediated by a combination of factors, primarily NO[•] and prostaglandins (25). Proposed causes of impaired endothelial NO[•] production fall into two general categories: inadequate substrate (L-arginine) for NO[•] synthesis and endothelial NO[•] synthase (eNOS) dysfunction. The latter can be associated with eNOS “uncoupling,” in which the enzyme transitions from a dimeric, NO[•]-producing enzyme, to a monomeric state that produces superoxide rather than NO[•] (26). In regards to possible therapeutic targets, L-arginine can be regenerated from L-citrulline within caveolae, where eNOS is located, by a two-step enzymatic process (27). Therefore, supplementation of either L-arginine or L-citrulline, both of which are depleted during CPB, may protect against pulmonary endothelial dysfunction (28). However, there are no studies looking at the effect of substrate supplementation on peripheral vasomotor dysfunction or other clinical outcomes. eNOS requires tetrahydrobiopterin (BH4) as a cofactor, and depletion of BH4 promotes uncoupling. Oxidative stress causes oxidation of BH4 and promotes uncoupling of eNOS (29). BH4 supplementation has shown promise for improving endothelial dysfunction after CPB in animal models, but no clinical trials have been attempted (30, 31). Another potential mechanism of eNOS uncoupling is S-glutathionylation of the enzyme by reactive oxygen species. Reversal of S-glutathionylation can restore eNOS function in adults after CPB (32). The primary cause of oxidative stress following CPB is presumably reperfusion injury, but other modifiable stressors such as free hemoglobin leading to toll-like receptor 4 activation may also play a role (33, 34). Last, the rapid recovery of endothelial function in some patients raises the possibility of a genetic component to recovery of eNOS function, and there is some evidence that eNOS and other polymorphisms can affect capacity for vasodilation (35, 36).

There are significant limitations to the current observational pilot study. The principal limitation was the relatively small sample size. This limited our ability to fully define the change in endothelial-dependent vasomotor dysfunction at 24 hours postoperatively and to more definitively correlate clinical and biochemical outcomes with endothelial-dependent vasomotor dysfunction. Our population was resource limited primarily by

personnel availability for recruitment and a desire for single-operator consistency with vasomotor reactivity assessments. Despite relatively low numbers, the data suggest that CPB can have a profound effect on endothelial-dependent vasomotor function in infants with presumably normal baseline endothelial function. In addition, it demonstrated a potentially important association between endothelial dysfunction and UOP during the early postoperative period that will need to be more fully defined by examining a larger population. This would also allow correction for diuretic dosing and other confounders that influence UOP. An additional confounder was that two infants enrolled in this study were small for gestational age at birth (< 10th percentile), which has been associated with reduced endothelial function (13). We were unable to correct for this or do a subgroup analysis; however, data were paired for comparison of preoperative versus postoperative vasomotor function, which should mitigate bias related to their inclusion in the analysis. Last, our study did not assess reproducibility of iontophoresis results using our study equipment and protocol to calculate a coefficient of variability. Performing repeat measurements was not practical due to time constraints, particular with respect to preoperative measurements due to the need for prolonged cooperation in our patient population of infants. In other studies, variability in peak perfusion and AUC after acetylcholine iontophoresis range from 15% to 20% in healthy adult volunteers (7, 12, 13) but has been confirmed in infants. Design of a future larger scale study would benefit from the use of a healthy control population to establish reproducibility and assist with properly powering patient enrollment.

CONCLUSIONS

Endothelial-dependent vasomotor dysfunction is present in infants immediately after CPB and recovers in some patients within 24 hours post-CPB. Our data demonstrate a relationship between better endothelial-dependent vasomotor function at 24 hours post-CPB and postoperative UOP over the first 48 hours. This relationship will require a larger patient cohort to fully determine significance of the finding. Future research is needed to further understand the mechanism of endothelial injury and delineate the relationships between endothelial-dependent vasomotor dysfunction and clinical outcomes after CPB.

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