Published in final edited form as:

Intensive Care Med. 2008 October ; 34(10): 1851–1857. doi:10.1007/s00134-008-1174-9.

Plasma Angiopoietin-2 Levels Increase in Children Following Cardiopulmonary Bypass

John S Giuliano Jr¹, Patrick M Lahni^{1,2}, Michael T Bigham¹, Peter B Manning³, David P. Nelson⁴, Hector R Wong^{1,2}, and Derek S Wheeler^{1,2}

¹ Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center; Department of Pediatrics, University of Cincinnati College of Medicine

² The Kindervelt Laboratory for Critical Care Medicine Research, Cincinnati Children's Research Foundation

³ Division of Cardiothoracic Surgery, Cincinnati Children's Hospital Medical Center, Department of Surgery, University of Cincinnati College of Medicine

⁴ Division of Cardiology, Texas Children's Hospital, Department of Pediatrics, Baylor College of Medicine

Abstract

Objective—To investigate the effects of cardiopulmonary bypass (CPB) on plasma levels of the vascular growth factors, angiopoietin (angpt)-1, angpt-2, and vascular endothelial growth factor (VEGF)

Design—Prospective, clinical investigation

Setting—12-bed pediatric cardiac intensive care unit of a tertiary children's medical center

Patients—48 children (median age, 5 months) undergoing surgical correction or palliation of congenital heart disease were prospectively enrolled following informed consent

Interventions—None

Measurements and Results—Plasma samples were obtained at baseline and at 0, 6, and 24h following CPB. Angpt-1, angpt-2, and VEGF levels were measured via commercial ELISA. Angpt-2 levels increased by 6h (0.95, IQR 0.43 – 2.08 ng mL⁻¹ vs. 4.62, IQR 1.16–6.93 ng mL⁻¹, p<0.05) and remained significantly elevated at 24h after CPB (1.85, IQR 0.70–2.76 ng mL⁻¹; p<0.05). Angpt-1 levels remained unchanged immediately after CPB, but were significantly decreased at 24h after CPB (0.64, IQR 0.40–1.62 ng mL⁻¹ vs. 1.99, IQR 1.23–2.63 ng mL⁻¹, p<0.05). Angpt-2 levels correlated significantly with CICU length of stay (LOS) and were an independent predictor for CICU LOS on subsequent multivariate analysis.

Conclusions—Angpt-2 appears to be an important biomarker of adverse outcome following CPB in children.

Keywords

Vascular growth factor; cardiopulmonary bypass; inflammation; pediatrics; congenital heart disease; systemic inflammatory response syndrome (SIRS)

Address for Correspondence: Derek S. Wheeler, MD, Division of Critical Care Medicine, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Cincinnati, OH 45229-3039, (513) 636-4259 TEL, (513) 636-4267 FAX, derek.wheeler@cchmc.org.

Disclosures
None

Introduction

The capillary leak syndrome is a serious, potentially life-threatening manifestation of the systemic inflammatory response syndrome (SIRS) that occurs in critically ill children following the use of cardiopulmonary bypass (CPB) for surgical palliation or correction of congenital heart disease [1–3]. Currently, a consensus definition of the capillary leak syndrome following CPB is lacking, and the true incidence of this complication is therefore not known. However, we [4] and others [5,6] have shown that capillary leak increases the cardiac intensive care unit (CICU) length of stay (LOS), which has been associated with worse long-term cognitive outcome in infants with d-transposition of the great arteries [7]. The vascular endothelium is particularly sensitive to the adverse effects of CPB, which include complement activation, ischemia-reperfusion injury, and the release of a cascade of proinflammatory cytokines, resulting in a subsequent change in endothelial cell phenotype from a quiescent, anticoagulant state to an activated, procoagulant state. Leukocyte adhesion leads to further endothelial cell injury and the subsequent disruption of endothelial cell barrier function, all of which characterize the capillary leak syndrome. While the capillary leak syndrome has not been adequately defined, clinical manifestations are easily recognized and include tissue edema, fluid overload (manifest as a positive postoperative fluid balance), and subsequent organ dysfunction [8,9].

Several investigators have previously demonstrated an increase in vascular growth factors, such as the vascular endothelial growth factor (VEGF) in children with congenital heart disease [9–12]. The angiopoietins are a family of vascular growth factors that are necessary for both developmental and pathologic angiogenesis. To date, four angiopoietins have been described, with angiopoietin-1 (angpt-1) and angiopoietin-2 (angpt-2) being the best characterized in humans to date. Both angpt-1 and angpt-2 appear to have opposing effects on the Tie-2 tyrosine kinase receptor located primarily on the luminal surface of endothelial cells. Angpt-1 inhibits inflammation, prevents capillary leak, and inhibits endothelial apoptosis [13,14], whereas angpt-2 promotes increased vascular permeability and inflammation [15,16]. Plasma angpt-2 levels are increased in adults with congestive heart failure [17] and the acute coronary syndrome [18]. However, to our knowledge, the effect of CPB on systemic angiopoietin expression has not been previously characterized. Accordingly, we hypothesized that angpt-2 levels will increase following CPB in children and act as a potential biomarker of adverse outcome in this population. The results of this study have been previously published in abstract form [19].

Methods

Patient Population

The study was approved by the Cincinnati Children's Hospital Medical Center Institutional Review Board. Forty-eight consecutive children under the age of 17 years undergoing surgical correction or palliation of congenital heart disease requiring cardiopulmonary bypass were enrolled in the study following written informed consent. Exclusion criteria included the presence of sepsis, acute or chronic lung disease, immunodeficiency, corticosteroid use within 1 week preceding surgery, or cardiac arrest within 1 week preceding surgery. Patient demographics, including age at surgery, diagnosis, RACHS-1 score [20], duration of CPB, aortic cross-clamp time, and CICU LOS were collected. The postoperative fluid balance at 24h following CPB was calculated by dividing the fluid balance (the total fluids in – the total fluids out) by the patient weight in kilograms. Additionally, an inotrope score was calculated at 24h following CPB by adding the doses of dopamine and dobutamine in micrograms per kilogram per minute and assigning an arbitrary equivalent value of 10 micrograms per kilogram per minute for each 0.1 microgram per kilogram per minute epinephrine [21].

Intraoperative management

Balanced general anesthesia was attained with fentanyl, neuromuscular blockade, and isoflurane. Aprotinin was administered to infants <2 months old. Full-flow bypass with moderate hypothermia (28°C) was used for circulatory support. Regional low-flow cerebral perfusion with brief deep hypothermic circulatory arrest (18°C) was used in 7 children during reconstruction of the aortic arch. Intraoperative corticosteroids were administered at a dose of 30 mg/kg methylprednisolone at the initiation of CPB to all patients. Our practice has since evolved to include an additional dose of corticosteroids 4h prior to initiation of CPB – none of the children in the current cohort received this preoperative dose regimen [22]. Cold-blood cardioplegia with additional dosing at 20- to 30-minute intervals was given during aortic cross-clamping. Cardioplegia was delivered antegrade except in arterial switch operations, in which retrograde cardioplegia was used after the initial dose. Ultrafiltration (UF) was used in all cases; both conventional and modified UF (after bypass termination) was used, except in infants < 2 months of age, in which conventional UF alone was used during rewarming.

Sampling

Arterial blood samples were obtained at baseline (prior to the initiation of CPB), at hour 0 (after the termination of CPB), and at hours 6 and 24 following cessation of CPB. Samples were collected in tubes containing sodium citrate and were centrifuged immediately at $4,000 \times g$ for 10 minutes in order to separate plasma from the cellular components. Samples were stored in 50 μ L aliquots in order to avoid multiple freeze-thaw cycles at -70°C until later analysis.

Enzyme-linked immunosorbent assay (ELISA)

Expression of angpt-1, angpt-2, VEGF, and soluble Tie-2 receptor was measured using commercially available sandwich ELISA kits (R & D Systems, Minneapolis, MN). Briefly, 96-well microtiter plates were coated with the appropriate capture antibodies (100 μ L at $4 \mu\text{g mL}^{-1}$) in PBS overnight at room temperature. Plates were washed with PBS containing 0.05% Tween 20 three times and non-specific binding sites were blocked by incubation with 300 μ L of 1% bovine serum albumin (BSA) in PBS for 1h at room temperature. After washing, 100 μ L of standard (recombinant human antibodies) or samples (angiopoietins diluted 2:1 with 1% BSA in PBS, soluble Tie-2 and VEGF undiluted) were added and incubated for 2h at room temperature. Plates were subsequently washed and detection antibody was added. After two hours at room temperature, plates were washed and incubated with streptavidin-HRP (horse radish peroxidase, 1:200) in 1% BSA in PBS for 20 minutes at room temperature. Plates were washed and 100 μ L of substrate solution (1:1 mixture of hydrogen peroxide and tetramethylbenzidine) was added. This reaction was stopped after 20 minutes with an acidic stop solution. The optical density was measured at 450 nm with a wavelength correction of 540 nm.

Statistical analysis

Data was analyzed using SigmaStat for Windows Version 3.11 software (Systat Software, Inc, San Jose, CA). Continuous data are expressed as median and interquartile ranges and were compared using Kruskal-Wallis ANOVA with Dunn's post hoc test, as indicated. To detect correlations between continuous data, we used the Spearman correlation coefficient. Univariate analyses were performed to determine which factors were associated with prolonged postoperative CICU length of stay. For purposes of model building, variables which were associated with postoperative CICU LOS at a p-value ≤ 0.20 were then included in a list of potential independent risk factors in stepwise linear regression analysis, with LOS the dependent variable. A p-value < 0.05 was considered statistically significant.

Results

Patient characteristics, types of surgical repair, and intraoperative data for the entire cohort are summarized in Tables 1 and 2. Plasma samples from 48 consecutive children (median age 5.1 mos, IQR 1.7–34.2 mos) were obtained and analyzed. All of the children survived to hospital discharge. CPB resulted in a significant increase in expression of angpt-2, as shown in Figure 1. These levels significantly increased by 6 h post-CPB when compared to baseline (4.62, IQR 1.16–6.93 ng mL⁻¹ vs. 0.95, IQR 0.43–2.08 ng mL⁻¹, respectively; $p < 0.05$) and remained significantly increased at 24h post-CPB (1.85, IQR 0.70–2.76 ng mL⁻¹; $p < 0.05$). In contrast, CPB resulted in a decrease over time in angpt-1 levels, as shown in Figure 2. Angpt-1 levels were significantly decreased at 24h after CPB when compared to immediately post-CPB (0.64, IQR 0.40–1.62 ng mL⁻¹ vs. 1.99, IQR 1.23–2.63 ng mL⁻¹, respectively; $p < 0.05$). Not surprisingly then, the ratio of angpt-2/-1, which may be a more important marker of endothelial injury, significantly increased by 6h post-CPB (0.59, IQR 0.34–1.52 vs. 0.20, 0.35–1.17, respectively; $p < 0.005$) and remained significantly increased at 24h post-CPB (2.07, 0.44–3.52; $p < 0.05$) (Table 2). We then measured the soluble Tie-2 receptor (sTie-2) and found a significant increase immediately post-CPB (6.40, IQR 4.35–8.86 pg mL⁻¹ vs. 2.86, IQR 1.79–4.39 ng mL⁻¹; $p < 0.05$) which returned to baseline by 24h post-CPB (3.28, IQR 2.09–4.38 ng mL⁻¹; $p < 0.05$) (Table 2). Previous studies have suggested that CPB results in a transient increase in the expression of VEGF, an important marker of endothelial injury [9–12]. However, VEGF levels in our cohort were unchanged from baseline (Table 3).

Our practice is to routinely administer aprotinin to all children less than 2 months of age undergoing cardiopulmonary bypass. Thirteen children (28%) received aprotinin in the current cohort. Aprotinin may modulate the systemic inflammatory response to CPB [23], and for this reason, we also analyzed our results excluding these children. Plasma angpt-2 levels remained significantly increased at 24 h post-CPB compared to baseline in these children (1.44, IQR 0.40–2.17 ng mL⁻¹ vs. 0.32, IQR 0.04–0.60 ng mL⁻¹, $p < 0.05$), though the increase at 6 h post-CPB (0.57, IQR 0.28–1.22 ng mL⁻¹) was no longer significant. Similarly, plasma angpt-1 levels remained significantly decreased at 6 h (0.76, IQR 0.44–1.34 ng mL⁻¹) and 24 h post-CPB (0.54, IQR 0.40–1.47 ng mL⁻¹) compared to immediately after CPB (1.89, IQR 1.28–2.50 ng mL⁻¹, $p < 0.05$). There were no significant differences between plasma angpt-1 levels measured at baseline (1.12, IQR 0.52–2.36 ng mL⁻¹) compared to any of these other time-points.

There were significant correlations between angpt-2 levels measured at 6h following CPB and the duration of CPB ($r = 0.42$, $p = 0.02$), as well as the complexity of surgery, as noted by the RACHS-1 score ($r = 0.43$, $p = 0.03$). Importantly, there was a significant negative correlation between age and the 6h angpt-2 level ($r = -0.58$, $p < 0.001$). Angpt-2 levels at 6h significantly correlated with the inotrope score at 24h ($r = 0.68$, $p < 0.001$), post-operative fluid balance ($r = 0.50$, $p = 0.009$), and CICU LOS ($r = 0.50$, $p = 0.007$). These associations remained significant at 24h angpt-2 levels for age ($r = -0.48$, $p = 0.008$), inotrope score ($r = 0.47$, $p = 0.009$), duration of CPB ($r = 0.4$, $p = 0.03$), postoperative fluid balance ($r = 0.41$, $p = 0.04$), and CICU LOS ($r = 0.43$, $p = 0.02$).

In contrast, there were no significant correlations between plasma angpt-1 levels and any of these clinical indices. Consistent with our hypothesis that the ratio between the Tie-2 agonist/antagonist pair may be a more important marker of endothelial injury, there was a significant correlation between the angpt-2/-1 ratio at 6h and inotrope score ($r = 0.41$, $p = 0.02$), positive postoperative fluid balance ($r = 0.47$, $p = 0.01$), and CICU LOS ($r = 0.33$, $p = 0.05$). These correlations remained significant at 24h for inotrope score ($r = 0.40$, $p = 0.02$), positive postoperative fluid balance ($r = 0.44$, $p = 0.02$), and CICU LOS ($r = 0.48$, $p = 0.004$).

Finally, we performed univariate and multivariate regression analysis to determine factors which were independently associated with increased CICU LOS. We included all variables with a $p \leq 0.20$ on univariate regression (Table 4) on subsequent multivariate regression analysis. Baseline angpt-2/-1 ratio ($p=0.004$), 24h post-CPB angpt-2/-1 ratio ($p=0.05$), 24h post-CPB angpt-2 ($p=0.006$), and positive postoperative fluid balance ($p<0.001$) remained significant independent predictors of prolonged CICU LOS.

Discussion

Herein we demonstrate for the first time that CPB results in a relatively rapid and significant increase in plasma angpt-2 levels in children with congenital heart disease. Angpt-2 is stored in the Weibel-Palade bodies and rapidly released in response to activation of the vascular endothelium by hypoxia, thrombin, VEGF, or shear stress [24]. We were unable to demonstrate any significant increase in VEGF levels, though levels of the soluble Tie-2 receptor (sTie-2) were significantly increased following CPB. The significance of this response has not been completely investigated, but it is tempting to speculate that sTie-2 is released by the activated endothelial cell to bind excess circulating angpt-2 in an attempt to return back to a quiescent state [25]. Perhaps more important, the levels of angpt-1 decreased over time following CPB. Given the opposing effects of angpt-1 and angpt-2 on the vascular endothelium, we suggest that the ratio between angpt-1 and angpt-2 may be more relevant than the absolute level of each individual factor. To this end, the angpt-2/-1 ratio at 24h following CPB was an independent predictor of increased CICU LOS in our cohort.

Cardiopulmonary bypass elicits a complex host response characterized, at least in part, by the activation of the vascular endothelium [26]. Plasma VEGF levels have been shown to increase significantly following CPB and appear to correlate with the severity of the postoperative capillary leak syndrome [9]. Plasma VEGF is highest in neonates and children with cyanotic heart lesions beginning at 24h following CPB [9]. Our cohort differed slightly and VEGF levels were not assessed beyond 24 h, which may have accounted for our different results.

VEGF appears to increase angpt-2 expression in vascular endothelial cells and has been shown to interact with angpt-1 and angpt-2 during blood vessel formation [27]. Together, these growth factors tightly control the complex process of angiogenesis by signaling pericyte migration, microvessel branching, and microvessel stabilization. Angpt-1 and angpt-2 bind with equal affinity, but have opposing effects on the Tie-2 receptor kinase located predominantly on the luminal surface of vascular endothelial cells [13]. Angpt-1 appears to have anti-inflammatory effects through the inhibition of the transcription factor, nuclear factor (NF)- κ B and the subsequent down regulation of leukocyte adhesion molecules on the vascular endothelium [28]. Conversely, angpt-2 increases capillary permeability via phosphorylation of the myosin light chain (MLC) kinase [15,29]. MLC kinase increases contractile forces within the endothelial cell, leading to an increase in capillary permeability. In addition, angpt-2 accentuates inflammation by sensitizing the vascular endothelium to the proinflammatory cytokine, TNF- α [16].

We and others have recently shown that angpt-2 levels are associated with increased pulmonary capillary leak and increased mortality in critically ill children and adults with septic shock [15,30,31]. It is important to note that the levels of angpt-2 in our cohort were dramatically lower compared to the levels reported in both critically ill children and adults with septic shock. This likely reflects a greater severity of endothelial injury in patients with septic shock compared to patients following CPB. To this end, the levels of angpt-2 reported in patients with SIRS were more comparable to the levels reported in our cohort.

Our study does have several limitations which deserve mention. First, our cohort was small and non-uniform with respect to the complexity of surgeries performed. We understand that children under 2 months received aprotinin and conventional ultrafiltration during rewarming which may have skewed the data, but the small cohort size precluded any significant sub-group analysis. The temporal kinetics of angpt-1 and angpt-2 did not change when children receiving aprotinin were excluded from analysis. We can therefore speculate that aprotinin did not have a significant effect on angiopoietin expression following CPB. In addition, the effects of ultrafiltration are unknown. The benefits of ultrafiltration on clinical indices of capillary leak, inflammation, and cardiorespiratory function following CPB are well known [32], and the effects of ultrafiltration on angpt-2 expression remains to be elucidated. Second, we only collected blood samples at 6 and 24 h following CPB. We were therefore unable to determine the temporal kinetics of this response beyond 24 h. The half-life of angpt-2 *in vivo* has not been studied, though studies performed *in vitro* suggest that angpt-2 is rapidly released from the Weibel-Palade bodies of vascular endothelial cells immediately upon stimulation, with levels persisting for as long as 16 hours after stimulation [24]. Finally, the clinical correlates of endothelial injury and capillary leak are difficult to define. We chose the 24h inotrope score, postoperative fluid balance, and CICU LOS as correlates of endothelial injury and the capillary leak syndrome. Despite these limitations, angpt-2 appears to be an important biomarker of adverse outcome following CPB. Further studies pertaining to the role of angpt-2 in the pathophysiology of capillary leak syndrome following CPB are warranted.

Acknowledgements

We would like to thank Tracey VanVliet and Lois Bogenschutz in the Cardiology research department at Cincinnati Children's Hospital for their assistance with this project.

Funding Sources

The study was supported by the National Institutes of Health KO8 GM077432 (DSW).

References

1. Tarnok A, Schneider P. Pediatric cardiac surgery with cardiopulmonary bypass: pathways contributing to transient systemic immune suppression. *Shock* 2001;16:24–32. [PubMed: 11770029]
2. Wan S, LeClerc JL, Vincent JL. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. *Chest* 1997;112:676–92. [PubMed: 9315800]
3. Zhang S, Wang S, Li Q, et al. Capillary leak syndrome in children with C4A-deficiency undergoing cardiac surgery with cardiopulmonary bypass: A double-blind, randomised controlled study. *Lancet* 2005;366:556–562. [PubMed: 16099291]
4. Wheeler DS, Dent CL, Manning PB, et al. Factors prolonging length of stay in the cardiac intensive care unit following the arterial switch operation. *Cardiol Young* 2008;18:41–50. [PubMed: 18093360]
5. Brown KL, Ridout DA, Goldman AP, et al. Risk factors for long intensive care unit stay after cardiopulmonary bypass in children. *Crit Care Med* 2003;31:28–33. [PubMed: 12544989]
6. van Dongen EI, Glandsorp AG, Mildner RJ, et al. The influence of perioperative factors on outcomes in children aged less than 18 months after repair of tetralogy of Fallot. *J Thorac Cardiovasc Surg* 2003;126:703–710. [PubMed: 14502142]
7. Newburger JW, Wypij D, Bellinger DC, et al. Length of stay after infant heart surgery is related to cognitive outcome at age 8 years. *J Pediatr* 2003;143:67–73. [PubMed: 12915826]
8. Seghaye MC, Grabitz RG, Duchateau J, et al. Inflammatory reaction and capillary leak syndrome related to cardiopulmonary bypass in neonates undergoing cardiac operations. *J Thorac Cardiovasc Surg* 1996;112:687–697. [PubMed: 8800157]
9. Abrahamov D, Erez E, Tamariz M, et al. Plasma vascular endothelial growth factor level is a predictor of the severity of postoperative capillary leak syndrome in neonates undergoing cardiopulmonary bypass. *Pediatr Surg Int* 2002;18:54–59. [PubMed: 11793065]

10. Starnes SL, Duncan BW, Kneebone JM, et al. Vascular endothelial growth factor and basic fibroblast growth factor in children with cyanotic congenital heart disease. *J Thorac Cardiovasc Surg* 2000;119:534–539. [PubMed: 10694614]
11. Himeno WH, Akagi T, Furui J, et al. Increased angiogenic growth factor in cyanotic congenital heart disease. *Pediatr Cardiol* 2003;24:127–132. [PubMed: 12360391]
12. Ootaki Y, Yamaguchi M, Yoshimura N, et al. Vascular endothelial growth factor in children with congenital heart disease. *Ann Thorac Surg* 2003;75:1523–1526. [PubMed: 12735573]
13. Brindle NPJ, Saharinen P, Alitalo K. Signaling and functions of angiopoietin-1 in vascular protection. *Circ Res* 2006;98:1014–1023. [PubMed: 16645151]
14. Witzembichler B, Westermann D, Kneuppel S, et al. Protective role of angiopoietin-1 in endotoxic shock. *Circulation* 2005;111:97–105. [PubMed: 15611372]
15. Parikh SMMT, Schultz A, Yuan HT, Christiani D, Karumanchi SA, Sukhatme VP. Excess Circulating Angiopoietin-2 May Contribute to Pulmonary Vascular Leak in Sepsis in Humans. *PLoS Med* 2006;3
16. Fiedler U, Reiss Y, Scharpfenecker M, et al. Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. *Nat Med* 2006;12:235–9. [PubMed: 16462802]
17. Chong AY, Caine GJ, Freestone B, et al. Plasma angiopoietin-1, angiopoietin-2, and angiopoietin receptor Tie-2 levels in congestive heart failure. *J Am Coll Cardiol* 2004;43:423–428. [PubMed: 15013125]
18. Lee KW, Lip GYH, Blann AD. Plasma angiopoietin-1, angiopoietin-2, angiopoietin receptor Tie-2, and vascular endothelial growth factor levels in acute coronary syndromes. *Circulation* 2004;110:2355–2360. [PubMed: 15302795]
19. Giuliano JS Jr, Lahni PM, Bigham MT, et al. Angiopoietin expression in children following cardiopulmonary bypass. *Circulation* 2007;116:II-515.
20. Jenkins KJ, Gauvreau K, Newburger JW, et al. Consensus-based method for risk adjustment for surgery for congenital heart disease. *J Thorac Cardiovasc Surg* 2002;123:110–118. [PubMed: 11782764]
21. Wernovsky G, Wypij D, Jonas RA, et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants. A comparison of low-flow cardiopulmonary bypass and circulatory arrest. *Circulation* 1995;92:2226–2235. [PubMed: 7554206]
22. Schroeder VA, Pearl JM, Schwartz SM, et al. Combined steroid treatment for congenital heart surgery improves oxygen delivery and reduces postbypass inflammatory mediator expression. *Circulation* 2003;107:2823–2828. [PubMed: 12756159]
23. Kozik DJ, Tweddell JS. Characterizing the inflammatory response to cardiopulmonary bypass in children. *Ann Thorac Surg* 2006;81:S2347–S2354. [PubMed: 16731102]
24. Fiedler U, Scharpfenecker M, Koidl S, et al. The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood* 2004;103:4150–6. [PubMed: 14976056]
25. Lin P, Polverini P, Dewhirst M, et al. Inhibition of tumor angiogenesis using a soluble receptor establishes a role for Tie2 in pathologic vascular growth. *J Clin Invest* 1997;100:2072–2078. [PubMed: 9329972]
26. Verrier ED, Morgan EN. Endothelial response to cardiopulmonary bypass surgery. *Ann Thorac Surg* 1998;66:S17–S19. [PubMed: 9869436]
27. Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. *Proc Natl Acad Sci U S A* 2002;99:11205–10. [PubMed: 12163646]
28. Kim I, Moon SO, Park SK, et al. Angiopoietin-1 reduces VEGF-stimulated leukocyte adhesion to endothelial cells by reducing ICAM-1, VCAM-1, and E-selectin expression. *Circ Res* 2001;89:477–9. [PubMed: 11557733]
29. Roviezzo F, Tsigkos S, Kotanidou A, et al. Angiopoietin-2 causes inflammation in vivo by promoting vascular leakage. *J Pharmacol Exp Therap* 2005;314:738–744. [PubMed: 15870388]
30. Orfanos SE, Kotanidou A, Glynos C, et al. Angiopoietin-2 is increased in severe sepsis: correlation with inflammatory mediators. *Crit Care Med* 2007;35:199–206. [PubMed: 17110873]

31. Giuliano JS Jr, Lahni PM, Harmon K, et al. Admission angiopoietin levels in children with septic shock. *Shock* 2007;28:650–654. [PubMed: 18092380]
32. Thompson LD, McElhinney DB, Findlay P, et al. A prospective, randomized study comparing volume-standardized modified and conventional ultrafiltration in pediatric cardiac surgery. *J Thorac Cardiovasc Surg* 2001;122:220–228. [PubMed: 11479493]

Figure 1.
Box-and-whisker plot of plasma angpt-2 levels following cardiopulmonary bypass.

Figure 2.
Box-and-whisker plot of plasma angpt-1 levels following cardiopulmonary bypass.

Table 1

Patient Demographics, n=48 (CICU=cardiac intensive care unit; LOS=length of stay)

Characteristics	Results
Age (months), median (IQR)	5.1 (1.7–34.2)
Gender, M:F	26:23
Weight (kg), median (IQR)	5.95 (4.9–12.2)
CPB time (minutes), median (IQR)	114 (76–150.5)
Aortic cross-clamp time (minutes), median (IQR)	69.5 (43.5–92)
24-h Post-operative Fluid Balance, median (IQR)	40 (5–70)
24-h Inotrope Score, median (IQR)	11.25 (0–18)
CICU LOS (days), median (IQR)	4 d (2–35)

Note: The 24-h post-operative fluid balance is indexed to patient weight (kg).

Table 2

Procedures performed and corresponding complexity of surgery (VSD=ventricular septal defect; AVC=atrioventricular canal; ASD=atrial septal defect; TOF=tetralogy of Fallot; RACHS-1=Risk adjusted classification for congenital heart surgery)

Procedure	No. (%) [†]
VSD repair	10 (21)
AVC repair	8 (17)
Norwood procedure	7 (15)
ASD repair	7 (15)
TOF repair	6 (13)
Ross procedure	2 (4)
Arterial switch	2 (4)
Interrupted aortic arch repair	1 (2)
Aortic coarctation repair	1 (2)
Aortic stenosis repair	1 (2)
Truncus arteriosus repair	1 (2)
Rastelli procedure	1 (2)
Anomalous origin of the coronary artery repair	1 (2)
Slide tracheoplasty	1 (2)
RACHS-1	No. (%) [‡]
Risk category 1	7 (15)
Risk category 2	17 (36)
Risk category 3	12 (26)
Risk category 4	3 (6)
Risk category 5/6	8 (17)

[†] Percentages do not add up to 100% as some patients underwent multiple procedures.

[‡] Percentages are based upon 47 patients, since one patient (slide tracheoplasty) was not scored.

Table 3
Angiopoietin Expression Following Cardiopulmonary Bypass

Marker	Pre-CPB	Post-CPB	6h Post-CPB	24h Post-CPB
Angpt-2/-1	0.20 (0.35, 1.17)	0.29 (0.13, 0.58)	0.59 (0.34, 1.52) ^{†‡}	2.07 (0.44, 3.52) ^{†‡§}
VEGF	0.06 (0.005, 0.20)	0.16 (0.06, 0.26)	0.19 (0.06, 0.31)	0.12 (0.02, 0.27)
sTie-2	2.86 (1.79, 4.39)	6.40 (4.35, 8.86) ^{*†}	3.71 (2.91, 5.26)	3.28 (2.09, 4.38)

Values expressed as median ng mL⁻¹ followed by interquartile ranges.

* p<0.05 when compared to the 24h post-CPB;

[†] p<0.05 when compared to pre-CPB;

[‡] p<0.05 when compared to post-CPB;

[§] p<0.05 when compared to 6h post-CPB.

CPB = cardiopulmonary bypass; VEGF = Vascular endothelial growth factor; Angpt = angiopoietin; sTie-2 = soluble Tie-2 receptor

Table 4

Factors associated with increased CICU LOS by univariate analysis

Characteristics	p-value
Age (months)	0.02
RACH-1 Score	<0.001
Weight	0.04
CPB time	0.10
Aortic cross-clamp time	0.20
DHCA time	<0.001
Baseline Angpt-1	0.10
Post-CPB Angpt-1	0.58
6 h CPB Angpt-1	0.83
24 h CPB Angpt-1	0.82
Baseline Angpt-2	0.04
Post-CPB Angpt-2	0.74
6 h CPB Angpt-2	0.65
24 h CPB Angpt-2	0.17
Baseline Angpt Ratio	<0.001
Post-CPB Angpt Ratio	0.03
6 h CPB Angpt Ratio	0.96
24 h CPB Angpt Ratio	0.05
Inotrope Score at 24 h	0.002
Post-operative fluid balance at 24 h	<0.001