Published in final edited form as:

J Pediatr. 2017 November ; 190: 49–55.e2. doi:10.1016/j.jpeds.2017.07.035.

Alkaline Phosphatase in Infant Cardiopulmonary Bypass: Kinetics and Relationship to Organ Injury and Major Cardiovascular Events

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Abstract

Objectives—To determine the kinetics of alkaline phosphatase (AP) activity and concentration after infant cardiopulmonary bypass including isoform-specific changes, and to measure the association between post-operative AP activity and major post-operative cardiovascular events, organ injury/dysfunction, and post-operative support requirements

Study design—Prospective cohort study of 120 infants 120 days of age undergoing cardiopulmonary bypass. AP total and isoform-specific activity was assessed at 6 time-points (pre-operation, rewarming, 6, 24, 48, and 72h post-operation). Low AP activity was defined as <80 U/L. AP concentrations and biomarkers of organ injury/dysfunction were collected through 24h post-operation. Major cardiovascular events were defined as cardiac arrest, mechanical circulatory support, or death.

Results—AP activity loss occurred primarily during the operation (median decrease 89 U/L; $P < .0001$) secondary to decreased bone and liver 2 isoforms. Activity declined through 24h in 27% of patients. AP activity strongly correlated with serum concentration ($r = 0.87-0.91$; $P < .0001$).

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The authors declare no conflicts of interest.

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Persistent low AP activity at 72h was independently associated with occurrence of a major cardiac event (OR 5.6; $p<0.05$). Early AP activity was independently associated with subsequent vasoactive-inotropic score ($p<0.001$), peak lactate ($p<0.0001$), peak creatinine ($p<0.0005$), NT-proBNP ($p<0.05$), and intestinal fatty acid binding protein ($p<0.005$).

Conclusions—AP activity decreases during infant cardiopulmonary bypass and may continue to drop for 24h. Activity loss is secondary to decreased bone and liver 2 isoform concentrations. Early low AP activity is independently associated with subsequent post-operative support and organ injury/dysfunction, and persistence of AP activity ≥ 80 U/L at 72h is independently associated with increased odds of major cardiovascular events.

Keywords

thoracic surgery; reperfusion injury; postoperative care; adenosine; biomarkers; intensive care units

Congenital cardiovascular defects are one of the most common birth defects (4-10 per 1000 live births).¹ Surgical repair is often required during infancy and despite improving mortality, risk of death or transplant in the first year of life remains $>25\%$ for complex surgeries.² Post-operative morbidity is also a significant concern. Cardiopulmonary bypass (CPB), selective cerebral perfusion (SCP), and deep hypothermic circulatory arrest (DHCA) are often necessary for successful repair, however these techniques independently result in global ischemia/reperfusion, inflammation, and organ injury/dysfunction.³⁻¹⁸ Our understanding of these injury pathways is incomplete, and therapies to reduce post-surgical injury are largely limited to supportive care.

Alkaline phosphatase (AP), an endogenous metalloenzyme with multiple isoforms, has recently demonstrated beneficial physiologic activities *in vitro*. These activities include dephosphorylation of endotoxin to a less toxic monophosphoryl byproduct and conversion of harmful extracellular adenine nucleotides to adenosine.¹⁹⁻²¹ Preclinical and phase 2 adult studies of AP therapy for sepsis and inflammatory colitis have shown a reduction in inflammation and organ injury.²²⁻²⁴

AP may also be important after cardiothoracic surgery. AP activity decreases after cardiothoracic surgery in adults and children.²⁵⁻²⁸ In addition, low post-operative serum AP activity is independently associated with increased post-operative support requirements in infants.²⁸ However, significant gaps exist in our understanding of AP after infant cardiothoracic surgery, including the timing and persistence of decreased AP activity, isoform-specific changes, the balance of enzyme loss versus deactivation, and the association with major cardiovascular events as well as organ injury.

This study was designed to address these knowledge gaps. We hypothesized that loss of AP activity would begin during surgery and continue through the initial post-operative period. Activity of all AP isoforms would be equally affected and reflect decreased serum concentration rather than enzyme deactivation. We further hypothesized that early low AP activity would be associated with subsequent increased odds of a major cardiovascular event,

increased post-operative support requirements, and increased evidence of renal, intestinal, and cardiac injury/dysfunction.

Methods

Our study was a prospective, observational cohort study of infants <120 days of age undergoing cardiothoracic surgery with CPB at Children's Hospital Colorado between September 2013 and February 2016. Exclusion criteria were weight <2kg (limited blood volume) and adjusted gestational age <34 weeks (altered AP production).²⁹ The protocol was approved by the Colorado Multiple Institution Review Board and informed consent from subject parents was obtained prior to enrollment. The study held to all ethical standards as dictated by Children's Hospital Colorado, the University of Colorado, Denver, the Colorado Multiple Institution Review Board, and the Code of Ethics of the World Medical Association.

The primary study aims were to define the post-operative kinetics of AP, assess the correlation of AP activity to AP concentration, and determine if low post-operative AP activity is associated with an increased risk of cardiac arrest, mechanical circulatory support, or death. Low post-operative AP was defined *a priori* as <80U/L based on a potential threshold effect seen in our previously published data.²⁸ Secondary aims included assessment of isoform-specific AP changes, validation of the association between AP activity and post-operative support requirements, and measurement of the association between AP activity and organ injury/dysfunction.

We obtained baseline clinical information from all subjects (Table 1). Serum samples were obtained pre-operatively, with rewarming from CPB, and at 6, 24, 48, and 72h after arrival in the cardiac intensive care unit (CICU). The 48h sample was not performed in subjects <3kg to limit blood draw volumes. Also, to minimize discomfort the protocol did not allow venipuncture. Therefore, no additional samples were obtained after removal of indwelling catheters.

Samples for AP activity were frozen to -70°C; total and isoform specific AP activity were analyzed using commercially available assays (Mayo Laboratories, Rochester, MN). Total AP activity was measured using a standard photometric p-nitrophenol phosphate cleavage assay. AP cleaves p-nitrophenol phosphate to yield phosphate and n-nitrophenol. The rate of p-nitrophenol production (determined photometrically at 450 nm) is directly proportional to AP activity. Isoforms were separated using electrophoresis with additional isoform sialation to achieve separation between liver and bone isoforms. Isoform activity was then determined using the specific chromogenic substrate, 5-bromo-4chloro-3-indolyl phosphate/nitro blue tetrazolium in combination with densitometric quantification.

AP concentration and organ-specific injury/functional biomarkers were measured through 24h using multiplex immunoassays (Meso Scale Diagnostics, Gaithersburg, MD). Biomarkers included N-terminal pro b-type natriuretic peptide (NT-proBNP-cardiac function), intestinal fatty acid binding protein (iFABP-enterocyte injury), and neutrophil

gelatinase-associated lipocalin (NGAL-proximal renal tubule injury). We also recorded peak creatinine and lactate from post-operative measurements obtained as standard of care.

The primary clinical outcome was occurrence of any of the following major cardiovascular events: 1) cardiac arrest, 2) unplanned post-operative mechanical circulatory support, or 3) death (within 30 days or during the primary hospitalization). Secondary clinical outcomes included vasoactive inotropic score (VIS)³¹ at 6, 24, 48, and 72h post-operation, time to first extubation, and CICU/hospital length of stay (LOS).

Statistical Analyses

Subjects' baseline characteristics were summarized using descriptive statistics. Correlation between AP activity and concentration was assessed by Spearman correlation test. Spearman correlation test was performed to select candidate predictors for multivariable modeling. Distributions of continuous outcomes were inspected prior to modeling; natural log transformation was applied as indicated. General linear models were used for log transformed outcomes and logistic regression models for dichotomized outcomes. Initial potential covariates were selected based on clinical or biological significance. Covariates that were significantly associated with both the primary explanatory variable and the outcome on univariate analysis were included in the initial model. Backwards stepwise regression was used to arrive at the final model. P value < 0.05 was considered statistically significant. SAS V9.4 (SAS Institute, Cary, North Carolina) was used for data management and analyses; GraphPad Prism 6 was used for graphics.

Results

One hundred and twenty-two subjects were enrolled in the study. Two subjects were converted to non-bypass surgery after enrollment and excluded. Subjects 1-100 were enrolled consecutively from the first 120 eligible patients (83% enrollment rate; Table 2; available at www.jpeds.com). The final 22 subjects were enrolled from eligible morning surgeries only to allow processing time for pilot laboratory tests. Baseline clinical and surgical data are presented in Table 1.

AP activity was obtained in 98.8% of possible time points through 24h post-operation (Table 3; available at www.jpeds.com). Median AP activity through 72h post-operation is shown in Table 4. AP activity decreased substantially between the pre-operative and rewarming samples (median decrease 89U/L; $p < 0.0001$). AP activity continued to fall in 61% of subjects between rewarming and 6h, and 27% demonstrated a further decline between 6h and 24h. Low AP activity ($< 80\text{U/L}$) was found in 5% of infants pre-operation, 30% at rewarming, 40% at 6h, and 34% at 24h. A comparison of baseline characteristics for infants with AP activity $< 80\text{U/L}$ vs. $> 80\text{U/L}$ at the rewarming time point is presented in Table 1. After 24h, AP activity rose consistently with only rare exceptions. The mean increase between 24 and 72h was 26.9 U/L ($p < 0.0001$). This change likely underestimates the rise for the whole cohort as we did not obtain 72h levels in a subset of healthier infants who no longer had indwelling catheters (Table 5; available at www.jpeds.com). Sixteen infants demonstrated AP activity $< 80\text{U/L}$ at 72h. Only a minority of infants (9%) regained pre-operative activity levels by 72h.

Isoform-specific activity is shown in Figure 1. Bone and liver 2 isoforms represented the clear majority of AP prior to surgery. Bone and liver 2 AP activities fell between pre-operation and rewarming, were stable to slightly decreased by 6h, and started slowly rising by 24h. Intestinal AP activity doubled between pre-operative and rewarming samples but remained a small portion of total AP activity. The liver 1 isoform increased markedly and constituted 17% of total AP activity at rewarming. Liver 1 activity was maintained through 6h then subsequently declined.

Serum AP activity and concentration were compared to assess if changes in AP activity could be attributed primarily to decreased AP concentration. AP concentrations declined from pre-operation to rewarming, and then gradually increased after 24h. Activity and concentration were strongly correlated ($r=0.87-0.91$; $p<0.0001$), suggesting protein loss rather than enzyme dysfunction as the primary cause of diminished AP activity.

Our primary clinical outcome was the odds of a major cardiovascular event in subjects with AP activity ≥ 80 U/L. Eleven subjects experienced a major cardiovascular event (9%). Eight subjects experienced early cardiac arrest or need for mechanical circulatory support and 3 died after progressive decline in hospital weeks to months after surgery. Early major cardiac events occurred exclusively in subjects with an AP activity of ≥ 80 U/L. Conversely, the three subjects with late post-operative death maintained AP levels above 80U/L at least through the 72h time point.

On univariate analysis, subjects with low AP activity at 6, 24, or 72h demonstrated increased odds of experiencing a major event (6h Odds Ratio [OR] 4.4, $p<0.05$; 24h OR 3.8, $p<0.05$; 72h OR 8.7, $p<0.005$). On multivariable analysis, low AP activity at 72h remained statistically significant (OR 5.6; $p<0.05$), with the other time points demonstrating an OR of 2 but with confidence intervals crossing 1. The absolute risk of a major event in subjects with a 72h AP activity ≥ 80 U/L was 30%. Univariate assessment of potential confounders and final multivariable models for all clinical outcomes are shown in Table 6 and Table 7 (tables available at www.jpeds.com).

Secondary clinical outcomes included post-operative VIS, length of intubation, CICU/hospital LOS (Table 8; available at www.jpeds.com). AP activity demonstrated an independent negative association with VIS at all time points on multivariable modeling. AP was most strongly associated with concurrent VIS. In our models, each 10U/L decrease in AP at the 6hr time point was associated with a 6% increase in VIS 6hr ($p<0.0001$), and for each 10U/L decrease in AP at the 24hr time point, VIS at 24hr increased by 7% ($p<0.0001$). Of note, AP activity at the rewarming time point (prior to the initiation of inotropic and vasoactive support) was also independently associated with subsequent VIS at 6 and 24hrs. AP activity at 24h was independently associated with length of intubation (4% increase in length of intubation for each 10 U/L decrease in AP; $p<0.05$), with a similar trend for AP activity at 6h that did not reach statistical significance. AP activity was not independently associated with CICU or hospital LOS.

Next, we assessed if early AP activity (rewarming and 6h) was associated with biomarkers of organ perfusion, injury, and dysfunction. AP activity at 6h was associated with higher

subsequent (24h) NT-proBNP and iFABP levels but not concurrent (6h) levels of these markers (Table 9). AP activity at 6h also demonstrated a strong independent association with peak creatinine and peak lactate (Table 9). Looking earlier, AP activity at rewarming also showed a strong, independent negative association with peak creatinine and peak lactate, but not NT-proBNP or iFABP (Table 9). Surprisingly, we did not find an association between AP activity and NGAL. However, in our cohort, NGAL also showed no association with peak creatinine or change in creatinine, so it is unclear how well the biomarker performed in our specific population.

Discussion

Our key findings center on a significant early loss of AP activity that began prior to separation from bypass and was attributable to decreased concentrations of bone and liver 2 isoforms. Most subjects showed ongoing AP loss through 6h post-operation and nearly a third experienced continued decline through 24h. Early low AP activity demonstrated a strong, independent association with subsequent cardiovascular support requirements, peak lactate, and peak creatinine. Early low AP activity was also associated with subsequent biomarkers of enterocyte injury and cardiac dysfunction. Recovery of AP activity typically began within 72h, however only a small minority of subjects regained their pre-operative AP activity by that time. Failure to increase AP activity above 80U/L by 72h was an ominous sign, with this subset of subjects demonstrating increased odds of a major cardiovascular event.

AP activity after CPB was first evaluated in 1974 by Neutze et al, who described ~50% decrease in AP activity in 9 children between pre-operation and post-operative day one.²⁷ These findings were subsequently replicated by two adult studies^{25,26} and by our group in a larger cohort of infants.²⁸ Less is known about the timing of AP activity loss during this first 24h or its recovery following surgery. Kats et al described decreasing AP activity over the first 2h of coronary artery bypass grafting followed by a recovery to baseline within 24h.²⁶ In our cohort, most AP activity loss also occurred early. However, we found ongoing decreases through 6 and even 24h post-operation, indicating the process is not isolated to the operation. Also different from prior adult cohorts, AP activity in our infants remained well below pre-operative levels through 72h, demonstrating a more profound deficiency and potentially slower resolution of the underlying mechanism.

The exact etiology of AP activity loss after cardiac surgery has yet to be defined. In the only study to directly assess this etiology, Lum et al explored if hypomagnesemia could explain the post-bypass decrease in AP activity because magnesium is a key cofactor for AP.²⁵ Although serum AP activity and magnesium decreased simultaneously, *ex-vivo* addition of magnesium failed to increase AP activity. Our study provides the first evidence that decreased AP activity is secondary to decreased serum concentration. Several possible reasons exist to explain this decrease in AP concentration. Lum et al examined the role of hemodilution but did not identify any correlation between AP activity and total protein levels,²⁵ a finding we have previously replicated.²⁸ Filtration is also unlikely as AP is larger than the filtration size of our circuit and AP activity decreases in some cases of off-bypass infant cardiac surgery.²⁸ Decreased production of AP has been proposed, however patients

receiving a systemic infusion of bovine intestinal AP during CPB demonstrated preservation of native AP activity, a finding more consistent with consumption than decreased production.²⁶ A consumptive process is also supported by animal models suggesting AP targets endotoxin released during intestinal ischemia-reperfusion, forming a conjugate that is subsequently cleared by Kupffer cells in the liver.³²

Isoform-specific changes lend additional insight. Previous studies documented decreases in bone AP in multiple settings including neonatal infection,³³ low cardiac output in Fontan patients,³⁴ and coronary artery bypass grafting.²⁶ These changes were thought to reflect decreased osteoblast activity, although other AP isoforms were not measured. Our findings demonstrate a proportional decrease in bone and liver 2 isoforms during infant cardiac surgery. This proportional decrease suggests either decreased production of both isoforms or more likely a consumptive process. In contrast, liver 1 and intestinal isoforms were not detectable pre-operation but rose post-operatively. The increase in serum intestinal AP is not surprising; previous studies have identified intestinal brush border proteins in the serum after cardiac surgery.³⁵ The reason for the immediate post-operative increase in liver 1 AP is less clear. Muraoka et al reported a similar finding in healthy liver donors.³⁶ They hypothesized these changes were related to liver regeneration and it is possible that a similar physiology occurs following ischemia-reperfusion of the liver in our population.

From a clinical standpoint, AP fell prior to separation from bypass, temporally preceding key short term clinical outcomes. Early low AP activity was strongly associated with subsequent cardiovascular instability and poor oxygen delivery as evidenced by higher VIS and peak lactate levels. The worst cases of cardiovascular instability (post-operative cardiac arrest or unplanned mechanical circulatory support requirement) were uniformly preceded by low AP activity, and persistently low AP activity at 72h was associated with increased odds of experiencing a major cardiovascular event. Low AP activity also associated with and temporally preceded biomarker evidence of renal, enterocyte, and cardiac injury/dysfunction. Interestingly, low AP activity was not associated with CICU LOS, hospital LOS, or late death in hospital, indicating that the associations we observed were limited to the early postoperative period.

Our study supports at minimum the potential clinical utility of AP as a novel biomarker of post-operative perfusion, renal dysfunction, intestinal injury, and cardiac arrest/need for mechanical circulatory support. The finding that low AP activity is already present prior separation from bypass increases its potential impact, as it may provide very early risk stratification both for clinical and research purposes. The strong association with post-operative renal dysfunction was particularly interesting given the interest in development of early biomarkers for acute kidney injury; AP significantly outperformed NGAL for this purpose in our cohort. In addition to possible use as a prognostic biomarker, our findings provide preliminary evidence for the use of AP as a biomarker of response to therapy in the early post-operative period. Low AP demonstrated a progressively stronger association with cardiovascular support requirements and length of mechanical ventilation through the first 24h post-operation, and persistence of low AP activity at 72h was associated with markedly increased odds and absolute risk of cardiac arrest or need for mechanical circulatory support.

Further study is warranted to assess if AP trend could be used to monitor recovery and serve as an adjunct biomarker to identify high risk patients requiring escalation of therapy.

Although development of AP as a biomarker is intriguing, its greater potential may be as a therapeutic target. It has been proposed that AP could be protective after ischemia-reperfusion injury and other inflammatory processes through the dephosphorylation of toxic phosphate-containing molecules in a number of pathologies.²²⁻²⁴ Two proposed targets of AP, endotoxin^{18,32,37} and extracellular adenine nucleotides^{20,38,39}, are released following ischemia-reperfusion injury and can lead to cardiovascular instability, tissue injury, and inflammation. We previously identified a decrease in serum capacity to convert extracellular adenosine monophosphate (AMP) to adenosine in the immediate post-operative period that is directly related to serum AP activity.²¹ *Ex-vivo* addition of liver AP markedly improved this capacity. It is possible that decreased AP activity could result in poor clearance of endotoxin and/or extracellular adenine nucleotides leading to subsequent inflammation and cardiovascular instability. Two phase 2 trials in adult sepsis^{22,23} and 1 in adult ulcerative colitis²⁴ have used AP therapy with promising early results on kidney function and intestinal inflammation respectively. The fact that cardiac surgery provides a predictably timed physiologic insult and AP activity declines early in the perioperative course makes therapeutic intervention particularly attractive in this setting. Recently a phase 2 study of bovine intestinal AP infusion during adult coronary artery bypass grafting demonstrated decreased inflammation in this low risk patient population, supporting the idea that this treatment is feasible and may have clinical efficacy for cardiac surgery patients.⁴⁰

Our study has several limitations. This cohort was recruited from a single institution. Although multiple studies have demonstrated a decrease in AP activity after cardiac surgery, specific findings from our study may not be generalizable to other centers, particularly those using different perfusion and filtration techniques. As with all observational studies we cannot differentiate between associations and causal relationships. Animal models and human clinical trials will ultimately be needed to assess any direct role of AP in modulating post-cardiac surgery cardiovascular stability, organ injury, and outcomes. Our study focuses on soluble alkaline phosphatase. Alkaline phosphatase is also present in many different organs at the cellular/tissue level⁴¹, and future studies, including animal models, should assess the role of tissue-based alkaline phosphatase in organ protection after infant cardiac surgery. Finally, due to the restrictions of blood volume we were limited in the number of tests we could analyze. Additional analysis of intraoperative time points could improve our understanding of AP loss in this population.

AP activity decreases markedly during infant cardiac surgery with CPB and may continue to drop through 24h post-operation. Activity loss is secondary to decreased bone and liver 2 isoform concentrations. Early low AP activity is independently associated with subsequent increased post-operative support and organ injury/dysfunction, and persistence of AP

80U/L at 72h is independently associated with increased odds of cardiac arrest, mechanical circulatory support, or death.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to thank our subjects and their parents for participating in this study and also acknowledge the tremendous contributions of the nurses in the cardiac intensive care unit and the CTRC. We extend our thanks to Ellen Blatt, MD for her support of this project.

Supported by NIH/NHLBI 1K23HL123634 (to J.D.), AHA 13CRP17300016 (to J.D.), and NIH/NCATS Colorado CTSA Grant Number UL1 TR001082 (University of Colorado). P.W. is a consultant for Aboott Inc, Fresenius Inc, and Baxter Inc, and has received research support from Fresenius Inc and Medtronics.

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List of Abbreviations

AMP	adenosine monophosphate
AP	alkaline phosphatase
CICU	cardiac intensive care unit
CPB	cardiopulmonary bypass
DHCA	deep hypothermic circulatory arrest
iFABP	intestinal fatty acid binding protein
LOS	length of stay
NGAL	neutrophil gelatinase associated lipocalin
NT-proBNP	n-terminal pro-brain natriuretic peptide
OR	odds ratio
SCP	selective cerebral perfusion

Figure 1. Changes in isoform-specific alkaline phosphatase activity (pre-operative through 72 hours)

0=pre-operative; R=rewarming; 6=6 hour; 24=24 hour; 48=48 hour; 72=72 hour. Solid line=isoform-specific activity; dashed line=percent of total activity.

Table 1
Demographics, baseline clinical characteristics, and comparison between infants with AP activity 80U/L vs. >80U/L at rewarming from CPB

Baseline and Surgical Characteristics	Full Cohort	Rewarming AP activity 80 U/L	Rewarming AP Activity >80 U/L	p-value 80 vs. >80
Male (%)	68 (55.7%)	21 (58.3%)	44 (53.7%)	NS
Age at surgery, days; median[range]	15 (1, 120)	5.5 (1, 120)	45 (2, 119)	<0.0001
Preterm (%)	16 (13.2%)	4 (11.1%)	12 (14.8%)	NS
Weight; median[range]	3.5 (2.1, 7.9)	3.2 (2.2, 4.9)	3.8 (2.1, 7.9)	<0.005
Pre-operative AP activity, median[range]	184 (55, 618)	127 (55, 275)	252.5 (94, 618)	<0.0001
Pre-operative mechanical ventilation (%)	39 (32.2%)	14 (38.9%)	24 (29.3%)	NS
Pre-operative inotropic support (%)	15 (12.3%)	3 (8.3%)	12 (14.6%)	NS
Aristotle Score ³⁰ -Comprehensive; median[range]	10 (3, 19.5)	11.8 (3, 16)	9 (3, 19.5)	<0.005
Aristotle Score ³⁰ -Basic; Median[range]	9 (3, 15)	10.5 (3, 15)	9 (3, 14.5)	<0.005
CPB time, minutes; median[range]	137 (0, 399)	161.5 (75, 399)	126.5 (54, 323)	<0.005
Cross-clamp time, minutes; median[range]	74 (0, 241)	82 (0, 241)	68.5 (0, 188)	<0.05
Deep hypothermic circulatory arrest, minutes; median[range]	0 (0, 154)	6 (0, 77)	0 (0, 154)	<0.0001
Selective cerebral perfusion, minutes; media[range]	0 (0, 115)	0 (0, 82)	0 (0, 115)	<0.005
Single ventricle physiology (%)	38 (31.7%)	17 (48.6%)	20 (24.4%)	<0.05
Perioperative steroid administration (%)	65 (55.6%)	29 (80.6%)	36 (44.4%)	<0.005

Table 2**Non-enrolled patients**

Number	Reason for not enrolling
1	Urgent/Emergent
2	Competing study
3	Declined
4	Competing study
5	Competing study
6	Urgent/Emergent
7	Urgent Transplant
8	No available study personnel
9	Urgent Transplant
10	No available study personnel
11	Declined
12	Parents not available for consent
13	Urgent/Emergent
14	Declined
15	Declined
16	Declined
17	Parents not available for consent
18	Ward of the State
19	Emergent Surgery
20	Emergent Surgery

Table 3
Missed alkaline phosphatase samples

Study ID Number	Time Point	Reason for Missed Sample
6	6 hour	Quantity not sufficient
9	6 hour	Quantity not sufficient
15	24 hour	Indwelling catheters removed
20	Rewarming	Gross hemolysis
57	Pre-operative	Gross hemolysis
73	Rewarming	Separation from bypass prior to sample collection

Table 4
Alkaline phosphatase activity

Time Point	n	Median AP Activity (U/L)	IQR	Range
Pre-operation	120	184	128, 273	55, 618
Rewarming	118	97	75, 133	34, 315
6 Hour	118	92	65, 121	32, 302
24 Hour	119	99	70, 147	32, 310
72 Hour	95	124	97, 171	42, 293

Table 5
Comparison of baseline and surgical characteristics of subjects with and without 72h blood draw for alkaline phosphatase activity

Baseline and Surgical Characteristics	Full cohort	72 Hour AP activity	
		No	Yes
Male (%)	16 (55.7%)	16 (59.3%)	52 (54.7%)
Age at surgery, days; median[range]	15 (1, 120)	68 (2, 119)	9 (1, 120)
Preterm %	16 (13.2%)	4 (14.8%)	12 (12.8%)
Weight	3.5 (2.1, 7.9)	4.3 (2.1, 7.2)	3.5 (2.1, 7.9)
Pre-operative intubation	39 (32.2%)	4 (15.4%)	35 (36.8%)
Pre-operative inotropic support	15 (12.3%)	1 (3.7%)	14 (14.7%)
Aristotle Score-Comprehensive; median[range]	10 (3, 19.5)	8 (3, 14.5)	10.2 (3, 19.5)
Cardiopulmonary bypass time, minutes; median[range]	137 (0, 399)	99.5 (0, 274)	147 (54, 399)
Cross-clamp time, minutes; median[range]	74 (0, 241)	60 (0, 147)	75 (0, 241)
Deep hypothermic circulatory arrest, minutes; median[range]	0 (0, 154)	0 (0, 38)	1 (0, 154)
Selective cerebral perfusion, minutes; media[range]	0 (0, 115)	0 (0, 115)	0 (0, 82)
Single ventricle physiology (%)	38 (31.7%)	3 (11.5%)	35 (37.2%)
Steroid	65 (55.6%)	9 (34.6%)	58 (61.7%)
VIS 6 hour; median[range]	9 (0, 27)	5 (0, 25)	9 (2.5, 27.0)
VIS 24 hour; median[range]	7 (0, 30)	3 (0, 30)	8 (0, 26)
Intubation, hours; median[range]	44.4 (0.1, 762.6)	23 (0.1, 453.1)	50.9 (3.2, 762.5)
ICU length of stay, days; median[range]	6 (1, 42)	2 (1, 28)	7 (1, 42)

Table 6
Univariate Analysis of AP Time Points and Potential Confounders

	Combined Primary Outcome	VIS 6H	VIS 24H	VIS 48H	Log Intubation Time	Log ICU Length of Stay	Log Hospital Length of Stay
AP PRE-OP	-0.06 0.5223	-0.29 0.0013	-0.34 0.0002	-0.29 0.0012	-0.37 <.0001	-0.27 0.0038	-0.36 <.0001
AP POST-OP	-0.13 0.1498	-0.33 0.0003	-0.43 <.0001	-0.38 <.0001	-0.49 <.0001	-0.38 <.0001	-0.45 <.0001
AP 6H	-0.18 0.0494	-0.46 <.0001	-0.52 <.0001	-0.49 <.0001	-0.55 <.0001	-0.40 <.0001	-0.47 <.0001
AP 24H	-0.21 0.0238		-0.56 <.0001	-0.50 <.0001	-0.57 <.0001	-0.35 0.0001	-0.44 <.0001
AP 48H				-0.38 0.0006	-0.51 <.0001	-0.32 0.0048	-0.41 0.0002
AP 72H	-0.20 0.0492						
AGE	-0.10 0.2823	-0.37 <.0001	-0.45 <.0001	-0.40 <.0001	-0.38 <.0001	-0.35 0.0001	-0.40 <.0001
SEX	-0.19 0.042	-0.04 0.6797	0.01 0.9557	-0.01 0.9232	0.00 0.9893	0.01 0.9348	0.02 0.8417
PRETERM	0.04 0.6416	-0.20 0.03	-0.12 0.1951	-0.06 0.5045	0.07 0.43	0.12 0.2068	0.06 0.5564
WEIGHT	-0.22 0.014	-0.31 0.0006	-0.44 <.0001	-0.41 <.0001	-0.52 <.0001	-0.45 <.0001	-0.49 <.0001
COMPREHENSIVE SCORE	0.13 0.1556	0.31 0.0006	0.43 <.0001	0.52 <.0001	0.43 <.0001	0.38 <.0001	0.35 0.0001
BASIC SCORE	0.14 0.1273	0.36 <.0001	0.48 <.0001	0.55 <.0001	0.48 <.0001	0.38 <.0001	0.36 <.0001
CPB TIME	0.14 0.1171	0.43 <.0001	0.53 <.0001	0.59 <.0001	0.46 <.0001	0.36 <.0001	0.36 <.0001

	Combined Primary Outcome	VIS 6H	VIS 24H	VIS 48H	Log Intubation Time	Log ICU Length of Stay	Log Hospital Length of Stay
AORTIC CROSS CLAMP TIME	0.18	0.31	0.38	0.37	0.23	0.20	0.15
	0.0465	0.0006	<.0001	<.0001	0.011	0.032	0.1051
CIRCULATORY ARREST TIME	0.17	0.33	0.38	0.39	0.41	0.43	0.42
	0.067	0.0002	<.0001	<.0001	<.0001	<.0001	<.0001
SELECTIVE CEREBRAL PERFUSION TIME	0.20	0.34	0.50	0.51	0.51	0.40	0.47
	0.0331	0.0001	<.0001	<.0001	<.0001	<.0001	<.0001

Table 7
Combined Outcome Final Model

Effect	Odds Ratio Estimates			P Value
	Point Estimate	95% Wald Confidence Limits		
WEIGHT	0.456	0.16	1.297	0.1409
AP 72HR 80 vs >80	5.638	1.415	22.471	0.0142

Table 8
Multivariable analysis of post-operative alkaline phosphatase activity and post-operative support

AP Time Point	Dependent Variable (Outcome)	Increase in Dependent Variable per 10U/L Decrease in AP Activity	p-value
Rewarming	VIS 6h	3%	<0.05
	VIS 24h	4%	<0.05
	Length of Intubation	2%	NS
	ICU Length of Stay	2%	NS
	Hospital Length of Stay	3%	NS
6 hour	VIS 6h	6%	<0.0001
	VIS 24h	4%	<0.05
	Length of Intubation	4%	NS
	ICU Length of Stay	1%	NS
	Hospital Length of Stay	3%	NS
24 hour	VIS 24h	7%	<0.0001
	Length of Intubation	4%	<0.05
	ICU Length of Stay	1%	NS
	Hospital Length of Stay	2%	NS

Table 9
Multivariable analysis of post-operative alkaline phosphatase activity and organ injury/
dysfunction

AP Time Point	Dependent Variable	Increase in Dependent Variable per 10U/L Decrease in AP Activity	p-value
Rewarming	iFABP 6h	1%	NS
	NT-proBNP 6h	0%	NS
	Peak Creatinine	2%	<0.0005
	Peak Lactate	7%	<0.0005
	NGAL 6h	2%	NS
6 hour	iFABP 6h	10%	NS
	iFABP 24h	12%	<0.005
	NT-proBNP 6h	-1%	NS
	NT-proBNP 24h	6%	<0.05
	Peak Creatinine	4%	<0.005
	Peak Lactate	9%	<0.0001
	NGAL 6h	0%	NS