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

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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 postoperative AP activity and major postoperative cardiovascular events, organ injury/dysfunction, and postoperative 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 (preoperation, rewarming, 6, 24, 48, and 72 hours postoperation). Low AP activity was defined as ≤80 U/L. AP concentrations and biomarkers of organ injury/dysfunction were collected through 24 hours postoperation. 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 24 hours in 27% of patients. AP activity strongly correlated with serum concentration (r = 0.87-0.91; $P < .0001$). Persistent low AP activity at 72 hours was associated independently with occurrence of a major cardiac event (OR 5.6; $P < .05$). Early AP activity was associated independently with subsequent vasoactive-inotropic score ($P < .001$), peak lactate ($P < .0001$), peak creatinine ($P < .0005$), N-terminal pro-brain natriuretic peptide ($P < .05$), and intestinal fatty acid binding protein ($P < .005$).

Conclusions AP activity decreases during infant cardiopulmonary bypass and may continue to decrease for 24 hours. Activity loss is secondary to decreased bone and liver 2 isoform concentrations. Early low AP activity is associated independently with subsequent postoperative support and organ injury/dysfunction, and persistence of AP activity ≤80 U/L at 72 hours is associated independently with increased odds of major cardiovascular events. *(J Pediatr 2017;190:49-55).*

Congenital cardiovascular defects are one of the most common birth defects (4-10 per 1000 live births).[1] Surgical repair often is required during infancy and despite improving mortality, the risk of death or transplant in the first year of life remains >25% for complex surgeries.[2] Postoperative morbidity is also a significant concern. Cardiopulmonary bypass (CPB), selective cerebral perfusion, and deep hypothermic circulatory arrest often are 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 postsurgical injury largely are limited to supportive care.

Alkaline phosphatase (AP), an endogenous metalloenzyme with multiple isoforms, recently has 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 also may be important after cardiothoracic surgery. AP activity decreases after cardiothoracic surgery in adults and children.[25-28] In addition, low postoperative serum AP activity is associated independently with increased postoperative 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

AP	Alkaline phosphatase
CICU	Cardiac intensive care unit
CPB	Cardiopulmonary bypass
iFABP	Intestinal fatty acid binding protein
LOS	Length of stay
NGAL	Neutrophil gelatinase associated lipocalin
NT-proBNP	N-terminal pro-brain natriuretic peptide

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loss vs 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 postoperative period. Activity of all AP isoforms would be affected equally 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 postoperative 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 (Aurora, Colorado) between September 2013 and February 2016. Exclusion criteria were weight <2 kg (limited blood volume) and adjusted gestational age <34 weeks (altered AP production).[29] The protocol was approved by the Colorado Multiple Institutional Review Board, and informed consent from subject parents was obtained before enrollment. The study held to all ethical standards as dictated by Children's Hospital Colorado, the University of Colorado, Denver, the Colorado Multiple Institutional Review Board, and the Code of Ethics of the World Medical Association.

The primary study aims were to define the postoperative kinetics of AP, assess the correlation of AP activity to AP concentration, and determine whether low postoperative AP activity is associated with an increased risk of cardiac arrest, mechanical circulatory support, or death. Low postoperative AP was defined a priori as ≤80 U/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 postoperative support requirements, and measurement of the association between AP activity and organ injury/dysfunction.

We obtained baseline clinical information from all subjects (**Table I**). Serum samples were obtained preoperatively, with rewarming from CPB, and at 6, 24, 48, and 72 hours after arrival in the cardiac intensive care unit (CICU). The 48-hour sample was not performed in subjects ≤3 kg 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 with commercially available assays (Mayo Laboratories, Rochester, Minnesota). Total AP activity was measured with 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 with the use of electrophoresis with additional isoform sialylation to achieve separation between liver and bone isoforms. Isoform activity was then determined with the specific chromogenic substrate, 5-bromo-4 chloro-3-indolyl phosphate/nitro blue tetrazolium in combination with densitometric quantification.

AP concentration and organ-specific injury/functional biomarkers were measured through 24 hours by the use of multiplex immunoassays (Meso Scale Diagnostics, Gaithersburg, Maryland). 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 postoperative 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 postoperative mechanical circulatory support, or

Table I. Demographics, baseline clinical characteristics, and comparison between infants with AP activity ≤80 U/L vs >80 U/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, n (%)	68 (55.7)	21 (58.3)	44 (53.7)	NS
Age at surgery, d, median (range)	15 (1, 120)	5.5 (1, 120)	45 (2, 119)	<.0001
Preterm, n (%)	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)	<.005
Preoperative AP activity, median (range)	184 (55, 618)	127 (55, 275)	252.5 (94, 618)	<.0001
Preoperative mechanical ventilation, n (%)	39 (32.2)	14 (38.9)	24 (29.3)	NS
Preoperative inotropic support, n (%)	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)	<.005
Aristotle score[30]-Basic, median (range)	9 (3, 15)	10.5 (3, 15)	9 (3, 14.5)	<.005
CPB time, min, median (range)	137 (0, 399)	161.5 (75, 399)	126.5 (54, 323)	<.005
Crossclamp time, min, median (range)	74 (0, 241)	82 (0, 241)	68.5 (0, 188)	<.05
Deep hypothermic circulatory arrest, min, median (range)	0 (0, 154)	6 (0, 77)	0 (0, 154)	<.0001
Selective cerebral perfusion, min, median (range)	0 (0, 115)	0 (0, 82)	0 (0, 115)	<.005
Single ventricle physiology, n (%)	38 (31.7)	17 (48.6)	20 (24.4)	<.05
Perioperative steroid administration, n (%)	65 (55.6)	29 (80.6)	36 (44.4)	<.005

NS, not significant.