OBSERVATIONAL STUDY

OPEN

Prothrombotic Microvesicle Generation in Pediatric Cardiopulmonary Bypass: A Pilot Observational Study

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IMPORTANCE: Over 10% of children develop thrombosis after cardiac surgery for congenital heart disease. Children with a single ventricle physiology have the highest risk of thrombosis associated with increased length of the postoperative stay, neurologic complications, and mortality. To decrease these complications, research is needed to understand the mechanisms that promote cardiopulmonary bypass (CPB) surgery-induced thrombin generation and clot formation.

OBJECTIVES: The objective of this pilot observational study was to measure the generation of prothrombotic microvesicles (MVs) and thrombin generation in 21 children collected 5 minutes after initiation of CPB, at the end of CPB, upon arrival in the pediatric congenital cardiac unit (PCCU), and 20 to 24 hours after arrival in the PCCU.

DESIGN, SETTING, AND PARTICIPANTS: An observational pilot study measured platelet and leukocyte MV, platelet aggregation, coagulation, and thrombin generation in 21 children undergoing CPB surgery. The study setting was a tertiary pediatric hospital. Inclusion criteria included age between birth to 5 years and weight on the day of surgery greater than three kilograms.

MAIN OUTCOMES AND MEASURES: Bleeding outcomes were measured by chest tube output and thrombotic outcomes were measured by surveillance ultrasound. Laboratory outcomes of prothrombotic MVs and thrombin generation were measured by high-resolution flow cytometry and calibrated automated thrombogram, respectively.

RESULTS: Time on CPB correlated with a significant increase in WBCs and phosphatidylserine-expressing MVs. Children with single ventricle physiology had increased levels of prothrombotic MVs ($p = 0.017$), platelet aggregation, peak thrombin ($p = 0.019$), and D-dimer ($p = 0.029$) upon arrival to the ICU compared with children with a dual ventricle. Only single ventricle children had a positive correlation between generation of platelet MV with peak thrombin ($p = 0.010$).

CONCLUSIONS AND RELEVANCE: Larger prospective studies are needed to determine if prothrombotic MVs can predict children with congenital heart disease at risk for thrombotic events.

KEYWORDS: blood coagulation; cardiopulmonary bypass; congenital heart defects; extracellular vesicles; perioperative period; thrombosis

An estimated 20,000 children a year require cardiopulmonary bypass (CPB), also known as the heart-lung machine, for surgical repair of congenital heart defects (CHD) (1). Although life prolonging, postoperative CPB complications of hemorrhage and thrombosis result in significant mortality and neurologic morbidity (1). CPB-related hemorrhage can be attenuated by the maintenance of platelet levels and antifibrinolytic therapy. However, current CPB anticoagulation therapies fail to prevent devastating

DOI: 10.1097/CCE.0000000000001236

KEYPOINTS

Question: Does time on cardiopulmonary bypass (CPB) in congenital heart surgery increase the generation of prothrombotic microvesicles (MVs), thrombin generation, and clot formation?

Findings: Our prospective pilot observation cohort study of 21 children undergoing CPB documented that time on CPB correlated with a significant increase in WBCs and phosphatidylserine expressing MVs. Only single ventricle children had a significant positive correlation between generation of platelet MV with peak thrombin.

Meaning: Prothrombotic MVs may be a therapeutic target to decrease thrombotic events in single ventricle physiology patients after CPB.

thromboembolism (2, 3). Postoperative CPB thrombosis is associated with longer hospital stays, repeat operations, stroke, and increased mortality (4, 5). This predominantly affects children undergoing palliative repair for single ventricle physiology, with postoperative thrombosis contributing to as much as 25% of deaths (6). Thus, there is a compelling need to understand the mechanisms that promote pump-related thrombotic complications in patients with single ventricle physiology (7).

Pediatric and adult studies have shown that prolonged CPB time leads to an increased incidence of arterial and venous thrombosis (3–5). Prolonged circulation time on CPB results in increased duration of shear stress within the extracorporeal circuit. This increased applied shear stress causes circulating leukocyte and platelet activation (8) that leads to thrombin generation and clot formation (9). Less well-studied is the enhancement of thrombosis by CPB generation of cell-derived extracellular microvesicles (MVs) (10). MVs are a class of extracellular vesicles with sizes between 0.5 to 1 micron and express cell-surface markers, unlike exosomes or apoptotic bodies (11, 12). MVs generated by sheer stress can contribute to clot formation by the initiation and propagation of clotting even without the exposure to an artificial surface (13, 14). Several studies have identified that plasma concentration of MVs correlates with thromboembolic events in patients supported with heart assist devices (15, 16). However, studies have not defined the generation of MVs or their correlation with coagulation, thrombin generation, and aggregation in children during and after congenital CPB surgery.

Routine coagulation tests such as prothrombin time (PT), partial thromboplastin time, platelet count, fibrinogen level, activated clotting time, or heparin dose are poor predictors of postoperative clotting complications (17). Our studies in an in vitro pediatric CPB model using human whole-blood document the release of prothrombotic platelet and leukocyte-derived MVs (14, 18). Children with single ventricle CHD are known to have more platelet-derived MV concentrations at 19,380 ± 18,360 μL^{-1} ($n = 19$) than children with dual ventricle physiology were 5,304 ± 2,448 μL^{-1} ($n = 21$) (19). Our hypothesis is that time on CPB increases the generation of prothrombotic MV more in single ventricle than dual ventricle correlating with an increase in thrombin generation and platelet activation. Further understanding of the effect of the CPB system on platelets and tissue factor expressing MV may lead to therapies to decrease thrombotic complications.

MATERIALS AND METHODS

Prospective observational study procedures were followed in accordance with the ethical standards of the University of Texas Health Sciences Center at San Antonio Institutional Review Board (UTHSCSA IRB#20140224H) and with the Helsinki Declaration of 1975. For 2 years from November 2015 to October 2017, newborns to 5-year-olds with congenital heart disease that were scheduled for moderate to severe surgical complexity CPB surgery (20) were screened to be part of the study. Participants' cardiac surgical complexity was determined by the RACHS Version 1 (Risk Adjustment for Congenital Heart Surgery-1) to be greater or equal to 2, a validated measure of surgical complexity and mortality (21). Patients admitted to our ICU before surgery were approached by the study team for consent after they completed their surgical consent. Exclusion criteria were weight under 3 kg, mild surgical complexity, extracorporeal membrane oxygenation/mechanical circulatory support before or after the procedure, receiving two runs of CPB support during surgery, and/or underlying bleeding or clotting disorder.

Blood Sampling

Samples were collected in 3.2% sodium citrate and sodium heparin tubes for each patient at four time points: within 5 minutes after initiation of CPB, within 5 minutes at the end of CPB, within 5 minutes upon arrival in the pediatric congenital cardiac unit (PCCU), and 20 to 24 hours after arrival in the PCCU. All samples were obtained from indwelling catheters placed as part of routine operative care.

Clinical

Two surgeons performed most of the repairs and the same three anesthesiologists performed all the procedures each with a consistent anticoagulation strategy and transfusion thresholds. The pediatric CPB circuit consisted of a Sorin roller pump and KIDS Dideco oxygenator (D100 or D101). Circuit was primed with a 1:1 ratio of washed RBCs and fresh frozen plasma. All patients received ultrafiltration at the end of the procedure as per our institutions protocol. All patients returned to the operating room (OR) with heparin drip at 10 U/kg/hr, which was maintained for the first 24 hours post-operatively. For study patients, each received a screening upper and lower vein ultrasound in the first 24 hours post-operatively to screen for non-clinically relevant thrombosis. Hourly volume of chest tube bleeding, IV fluid, and urine output divided by weight (kg) were recorded to compare bleeding, hypovolemia, or hypervolemia outcomes, respectively.

Laboratory Analysis

Analysis done at each of the four time points included a complete blood count, Multiple Electrode Impedance Aggregometry, Calibrated Automated Thrombogram, and coagulation assays. The University Hospital Laboratory completed the blood count as per hospital guidelines. Whole-blood platelet aggregation was determined using an impedance aggregometer (Multiplate, Dynabyte Medical GmbH, Germany). After 30 minutes of resting time, whole blood from citrated tubes was mixed with adenosine diphosphate (ADP), arachidonic acid (ASPI), ristocetin, and collagen following the manufacturer's directions running for 12 minutes. On a TEG 5000 system (Haemonetics, Boston, MA), blood samples from citrated tubes were analyzed using heparinase cups to avoid the effects of circulating heparin on results. After 15 minutes of resting time, blood was mixed with Kaolin and $CaCl_2$ to start clotting, and then using the manufacturer's directions each test was completed recording the R (clot initiation time), k (clot formation time), angle (clot propagation), and MA (clot strength).

Platelet-free plasma (PFP) was prepared by sequential centrifugation at 2500× *g* for 15 minutes at room temperature ×2 and then aliquoted into 1.5-mL Nunc Cryotubes (Sigma) for storage at –80°C. Using manufacturer's directions, the STAGO STA-R Evolution measured PT, activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, anti-thrombin III, von Willebrand antigen, anti-Xa activity, and Procoag-PPL clot time. INR (International Normalization Ratio) was calculated by determining the ratio of sample PT to a reference PT from 30 healthy adults. For all assays except Anti-Xa, samples were treated before analysis with 1mL of Dade hepzyme per 100mL sample at 37°C for 1 minute to reverse residual heparin from the circuit. Thrombin generation was initiated without a trigger (HBSS/BSA only) by automatically dispensing fluorogenic substrate (Z-Gly-Gly-Arg-AMC at 16 mM) and $CaCl_2$ (416 μM). Thrombin generation was calibrated against wells holding 20 μL α–2 macroglobulin/thrombin complex and 80 μL of PFP. The thrombogram curve was analyzed with Thrombinoscope software v3.0.0.29 (Thrombinoscope BV, Maastricht, The Netherlands) (12).

Microvesicle Analysis

To detect and quantify MVs, the International Society of Thrombosis and Hemostasis guidelines were followed, and our protocol procedures were submitted to the EV-TRACK knowledgebase (EVTRACK ID: EV170038) (22). Previous studies helped to define optimal reproducible preanalytical conditions (e.g., centrifugation and storage) for EV analysis (23, 24). Separate aliquots (25 μL) of PFP were diluted with 75 μL of phosphate-buffered saline containing 1% bovine serum albumin and incubated for 15 min in the dark at 37°C. MV size (500-1,000 nm) was determined by flow cytometer using a representative gate derived from a standard side scatter MegaMix beads (STAGO Catalog#01078) on the side scatter of BD FACS Celesta. Prothrombotic platelet MVs (PMVs) were identified with antibodies for CD41a and PS.

Leukocyte MVs (LMVs) expressing tissue factors were identified with CD142.

Statistical Analysis

Simple comparisons between groups were completed with unpaired *t* test with Welch correction. Equal distribution of covariates between groups was tested using standardized mean differences (SMD) (see **Supplementary Table 1**, http://links.lww.com/CCX/B490). Following established guidelines, an SMD of less than 0.2 was considered negligible, and values between 0.2 and 0.5 were considered small. In this analysis, most SMD values were less than 0.4, indicating generally small differences between groups (**Table 1**). Comparisons of continuous variables between single and dual ventricle groups were conducted using a Mixed-Methods model (REML) approach using the Geisser-Greenhouse (e) correction. This was performed considering the interaction within terms of single vs. dual ventricle; within subjects, and start time vs. end time. Multiple comparisons at each time point were also completed using a Fisher least significant difference (LSD) test between subjects to account for missing values. Correlations between variables such as PMV, CPB, and thrombin generation were completed with simple linear regression. All statistical analyses were performed using GraphPad Prism 10, v10.0.0 (131) (GraphPad Software LLC, San Diego, CA).

RESULTS

Twenty-four children were approached for consent and three were excluded for withdrawal after consent (1) or plasma samples were lost (2). Twenty-one children were included in this analysis (Table 1) with a median age of 14.7±3.45 months (mean ± SD), 86% male, and an average RACHS at 3.0±0.14. There was no difference in age or RACHS score between the single and dual ventricle group (Table 1)

TABLE 1.
Demographics

	Single Ventricle			Dual Ventricle			
	Mean	SD	*N*	Mean	SD	*N*	*p*
Age (mo)	17.2	21.3	11	12.3	18.6	10	0.582
Weight (kg)	8.5	4.79	11	6.8	4.9	10	0.451
Body surface area (m^2)	0.4	0.17	11	0.3	0.18	10	0.484
Risk Adjustment for Congenital Heart Surgery-1	3.1	1.14	11	2.9	1.1	10	0.700
CPB (min)	75	26.8	11	77.5	34.4	10	0.871
Cross-clamp (min)	29	21.6	10	58	24.8	9	0.016
Lowest temp during CPB (°C)	30.8	4.7	11	32.0	2.58	10	0.224
Final activating clot time in OR (s)	133	18	11	153	41	10	0.089
OR packed RBCs (mL/kg)[a]	101	63.5	6	66	51.3	5	0.174
OR platelet (mL/kg)	29	20	6	32	17.5	3	0.405
OR fresh frozen plasma (mL/kg)	51	21.8	6	44	24.4	6	0.328
OR cryoprecipitate (mL/kg)	13	10.4	6	11	7.0	3	0.956
Chest tube bleeding over first 4 hr (mL/kg/hr)	3.1	1.8	11	3.4	2.32	10	0.354
Inputs (mL/kg/d)	107	25	10	121	25	10	0.106
Urine output (mL/kg/hr)	1.6	0.9	10	1.4	0.8	10	0.342
Highest postoperative lactate	4.7	3.1	8	4.4	4.1	9	0.428
Highest postoperative vasoactive inotropic score	93	48	10	92	162	10	0.490
Hours on mechanical ventilation	46	74	6	25	41	7	0.281

CPB = cardiopulmonary bypass, OR = operating room.
[a]Packed RBCs or cell-saver.

and a comprehensive list of surgeries performed for each group is in **Table 2**. Overall cohort weight was 7.6 ± 1.2 kg and body surface area was 0.36±0.04 m^2 with no difference in the single and dual ventricle group (Table 1). The cohort's average CPB time was 76±1.6 min with no differences between groups. The average aortic cross-clamp time (AXC) for the entire cohort was 44 ± 21 min with one patient in each group having no cross-clamp time. The dual ventricle group had a significantly longer cross-clamp time than single ventricle group at 58±25 minutes compared with 29 ± 22 minutes, respectively ($p = 0.018$). There was no difference in the lowest temperature reached on CPB between the single and dual ventricle group. There was no difference in last recorded activating clot time in the OR before going to the ICU.

Clinical Events

None of the twenty-one patients needed surgical exploration for bleeding after returning to the ICU. Out of the 21 patients enrolled, there was no significant difference in blood transfusion products during CPB or in the ICU (Table 1). Four patients (three single ventricles and one dual ventricle) developed a thrombotic event including one ischemic cerebral stroke, one internal mammary artery, and two deep vein thrombosis. The ischemic stroke and the internal mammary were discovered due to clinical complications of neurologic changes and cardiac dysfunction, respectively. Both deep vein thrombosis was diagnosed with ultrasound and thought to be line-associated and considered clinically insignificant, as in our practice it is not routine to do ultrasounds on postoperative day #1. There was no difference in chest tube bleeding per weight in the first 4 hours. Lastly, there was no evidence of difference in hypovolemia or low cardiac output syndrome between groups as measured by the average ins and outs, highest post-op lactate and vasoactive inotropic score.

TABLE 2.
Surgical Procedure

Tetralogy of fallot		2
Bi-directional glenn	6	
Fontan procedure	2	
Atrioventricular canal defect		1
Truncus arteriosus		2
Ventricular septal defect		2
Transposition of great arteries		1
Norwood	2	
Other	PA band	Ross, TAPVR
Thrombotic event	3	1

PA-band = pulmonary artery band, TAPVR = total anomalous pulmonary venous return.

Complete Blood Count and Routine Coagulation

At the start of CPB, the levels of WBC, hematocrit (%) and platelets were below normal (**Supplementary Table 2**, http://links.lww.com/CCX/B490, mean ± SD). WBC and hematocrit then significantly rose in the single ventricle group from the start of CPB until arrival to the ICU. Platelet count (**Fig. 1**) only rose in the single ventricle group from start to arrival in the ICU. Coagulation values (mean ± SD) are in Supplementary Table 1 (http://links.lww.com/CCX/B490). INR (Fig. 1) remained elevated above normal range of 1.2 for all time points with no difference between single and dual ventricle groups. There was no difference in anti-Xa levels at the start or end of CPB between single vs. dual ventricle groups, average anti-Xa at end of bypass was 0.41±0.05. D-Dimer (Fig. 1), a significant marker of thrombosis, remained elevated in the single ventricle group at all time points, significantly higher than dual ventricle upon arrival to the ICU, 0.51 ± 0.19 vs. 0.29 ± 0.12 mg/dL, respectively Fisher LSD 95% CI (0.0268–0.4161) $p = 0.0293$. No Anti-thrombin III transfusions were given to any of the patients, activity remained low during CPB rising upon arrival to ICU with no difference between groups. Procoagulant phospholipid clot (PPL) time (Fig. 1), a measure of MVs expressing procoagulant phosphatidylserine contribution to thrombosis, documented a significant difference at the end of CPB consistent with the increase in MVs in the single ventricle patients. At the end of CPB, single vs. dual ventricle PPL time was 50 ± 4.8 vs. 56 ± 3.5 s, respectively, Fisher LSD 95% CI (–10.67 to –1.681) $p = 0.0106$.

Thromboelastography

Before and during CPB surgery, thromboelastographic variables of clot formation R, k, angle, and MA did not

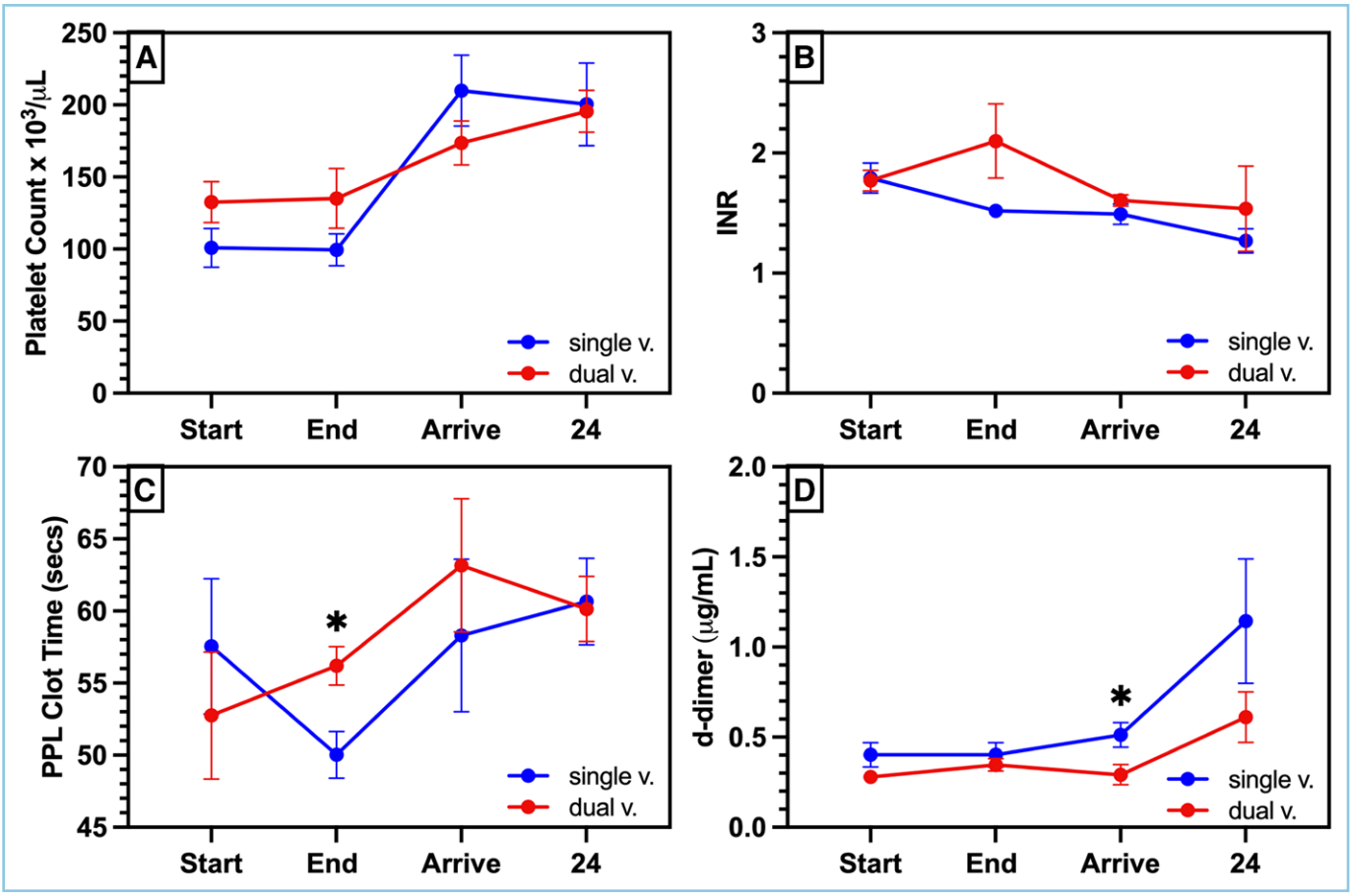

Figure 1. Platelet count and coagulation during and after cardiopulmonary bypass (CPB) surgery: Results are mean ± SEM and significance is $p < 0.05$. **A**, Despite no difference in platelet transfusions, platelet count rose more in single compared with dual ventricles upon arrival to the ICU. **B**, International normalization ratio, a measure of tissue factor-initiated coagulation, remained lower for single ventricle than dual ventricles during and after CPB. **C**, ProcoagPPL clot time, a measure of phospholipid driven coagulation was significantly less in single ventricle compared with dual ventricle at the end of CPB. **D**, D-dimer, a measure of clot formation, was significantly greater in single compared dual ventricle upon arrival to the ICU.

change. After CPB, surgery single ventricle patients trended toward increased clot formation, propagation, and clot strength. The trend to increase clot formation in single ventricle patients crossed lines with dual ventricle patients as documented in **Figure 2** and in **Supplementary Table 3** (http://links.lww.com/CCX/B490). Clot propagation (alpha angle), a marker of speed of clot formation rose faster in single ventricle patients than dual ventricles to be significantly higher upon arrival to the ICU, 65 ± 6.7 vs. 58° ± 3.1°, Fisher LSD 95% CI (1.76–11.34) $p = 0.011$.

Platelet Aggregation

Due to dilution from fresh frozen plasma and washed packed RBC circuit prime, platelet aggregation values began significantly less than reference values for ADP, ASPI, collagen, and ristocetin as documented in **Supplementary Table 4** (http://links.lww.com/CCX/B490). After arrival at the ICU, platelet aggregation response to ADP, ASPI, collagen, and ristocetin increased significantly both in single and dual ventricle groups (**Supplementary Fig.**, http://links.lww.com/CCX/B490). Platelet aggregation response to ristocetin, a marker of shear-mediated von-Willebrand function, rose faster in single ventricle patients than dual ventricle to be significantly higher upon arrival to the ICU, 103±49.4 vs. 60±49.4, respectively, Fisher LSD 95% CI (2.432–83.18) $p = 0.040$.

Thrombin Generation

Thrombin generation before and during CPB was low as documented by prolonged time to thrombin initiation (lag time) and decreased rate of thrombin generation (peak) documented in **Supplementary Table 5** (http://links.lww.com/CCX/B490). After arrival to

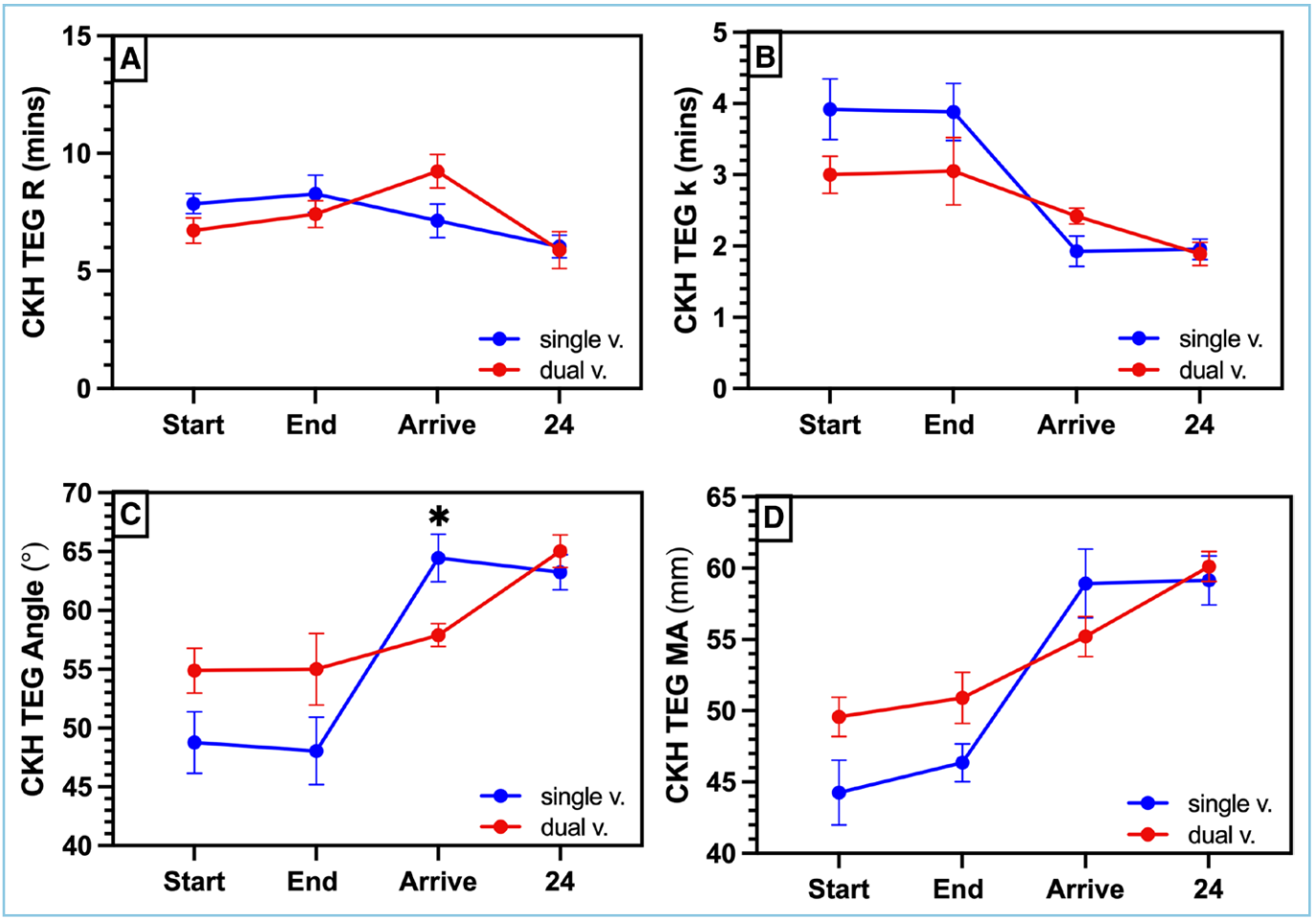

Figure 2. Thromboelastograph (TEG) with Hepzyme before, during, and after cardiopulmonary bypass surgery: results are mean ± SEM and significance is $p < 0.05$. **A**, TEG measured clot initiation time (R) was lower in single compared with dual ventricle patients on arrival to the ICU. **B**, TEG measured clot formation time (k) decreased faster in single ventricle compared dual ventricle patients on arrival to the ICU. **C**, TEG measured clot propagation (alpha angle) was significantly higher in single compared with dual ventricle patients on arrival to the ICU. **D**, TEG measured clot propagation (alpha angle) was significantly higher in single compared with dual ventricle patients on arrival to the ICU. TEG measured clot strength rose faster in single ventricle compared with dual ventricle on arrival to the ICU.

ICU, single ventricle patients decreased the time to thrombin initiation and increased the rate of thrombin generation. Upon arrival to the ICU, time to peak thrombin decreased significantly more in single compared with dual ventricle patient's (**Fig. 3**), 14.2 ± 4.6 vs. 22.0 ± 6.6, respectively, Fisher LSD 95% CI (–13.13 to –2.53) $p = 0.007$. Peak thrombin generation rose significantly more in the single compared with the dual ventricle upon arrival (**Fig. 4**), 180 ± 92 vs. 92 ± 62, respectively, Fisher LSD 95% CI (16.6–159.8) $p = 0.019$.

Prothrombotic Microvesicles

Platelet expressing prothrombotic phosphatidylserine MVs increased more from start to arrival in single ventricle, Fischer LSD 95% CI (–5.4 to –0.29) $p = 0.029$ than dual ventricles, Fischer LSD 95% CI (–3.9 to 1.03) $p = 0.249$ (Fig. 4). There was no difference between groups at ICU arrival. There was a significant although weak correlation between CPB time and generation of platelet MVs determined by linear regression ($R^2 = 0.26$, F (1,19) = 6.706, $p = 0.018$). Analyzing samples from the end of CPB and arrival to the ICU, there was a significant although weak correlation between generation of PMVs and peak thrombin for single ventricle ($R^2 = 0.29$, F (1,20) = 8.214, $p = 0.010$) compared with dual ventricle ($R^2 = 0.12$, F (1,17) = 2.34, $p = 0.144$). Furthermore, there was a significant although weak correlation between generation of PMVs with ADP and ASPI-induced platelet aggregation. PMV correlation with ADP-induced platelet aggregation in a single ventricle ($R^2 = 0.23$, F (1,20) = 5.91, $p = 0.025$)

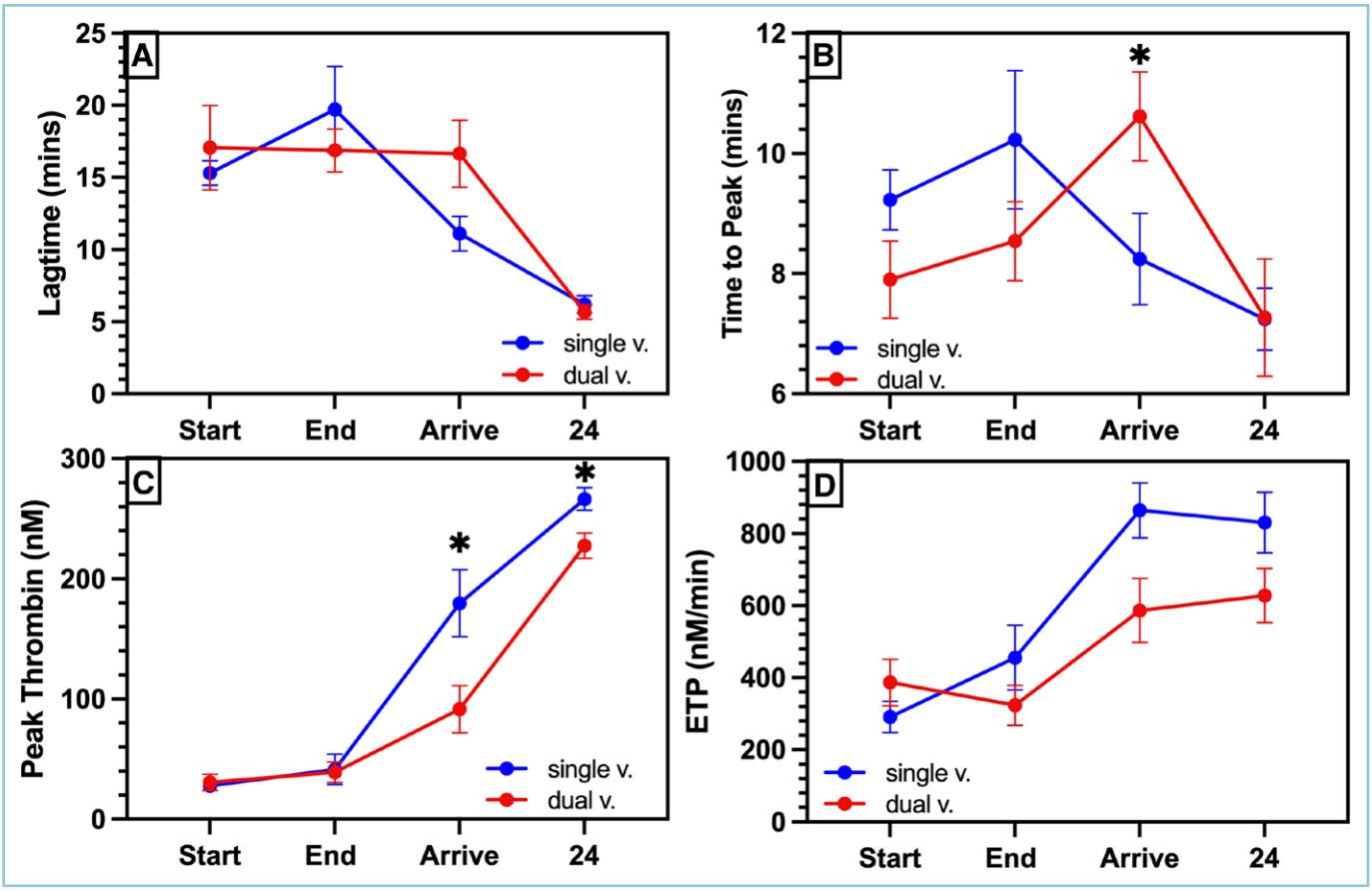

Figure 3. Thrombin generation before, during, and after cardiopulmonary bypass (CPB): Results are mean ± SEM and significance is $p <$ 0.05. **A**, Lag time, time to thrombin initiation, decrease more in single than dual ventricle patients. **B**, Time to peak thrombin generation decreases significantly more in single than dual ventricle. **C**, Peak thrombin generation increased significantly more in single than dual ventricle upon arrival and 24 hr in the ICU. **D**, Endogenous thrombin potential increased more in single than dual ventricle at the end of CPB, arrival in the ICU, and 24 hr in the ICU.

compared with dual ventricle ($R^2 < 0.0001$, F (1,18) = 0.001, $p = 0.975$). PMV correlation with ASPI-induced platelet aggregation for a single ventricle ($R^2 = 0.23$, F (1,18) = 5.24, $p = 0.034$) compared with dual ventricle ($R^2 = 0.01$, F (1,18) = 0.151, $p = 0.702$). Tissue factor positive MVs increased more from start to arrival in single ventricle, Fisher LSD 95% CI (–7.5 to –0.73) $p = 0.011$ than dual ventricle patient's, Fisher LSD 95% CI (–3.4 to 3.5) $p = 0.999$. Tissue factor positive MVs increased in single ventricle patient's more than dual ventricle, Fisher LSD 95% CI (0.9–8.7) $p = 0.017$ but did not correlate with markers of thrombin generation or platelet aggregation.

DISCUSSION

Children with CHD have over twice the incidence of postoperative thrombotic complications from CPB surgery than adults (6, 25). Interestingly, studies document that single ventricle physiology does not affect the severity of bleeding outcomes in patients during or after CPB surgery (26, 27). In contrast, children with single ventricle CHD physiology have an increased risk of thrombotic events compared with children with dual ventricle physiology (25). Thus, an essential need exists to identify the mechanisms that increase post-thrombotic outcomes in single ventricle patients. This compelled us to perform an observational cohort to understand the effects of CPB surgery on single-ventricle and dual-ventricle patient's coagulation, platelet activation, thrombin generation, and MV generation.

Pediatric and adult studies document that prolonged CPB time leads to an increased incidence of arterial and venous thrombosis (3–5). Rizza et al (28) documented that CPB surgery-induced thrombin generation can persist up to 12 hours after surgery. Our results document that children with single ventricle

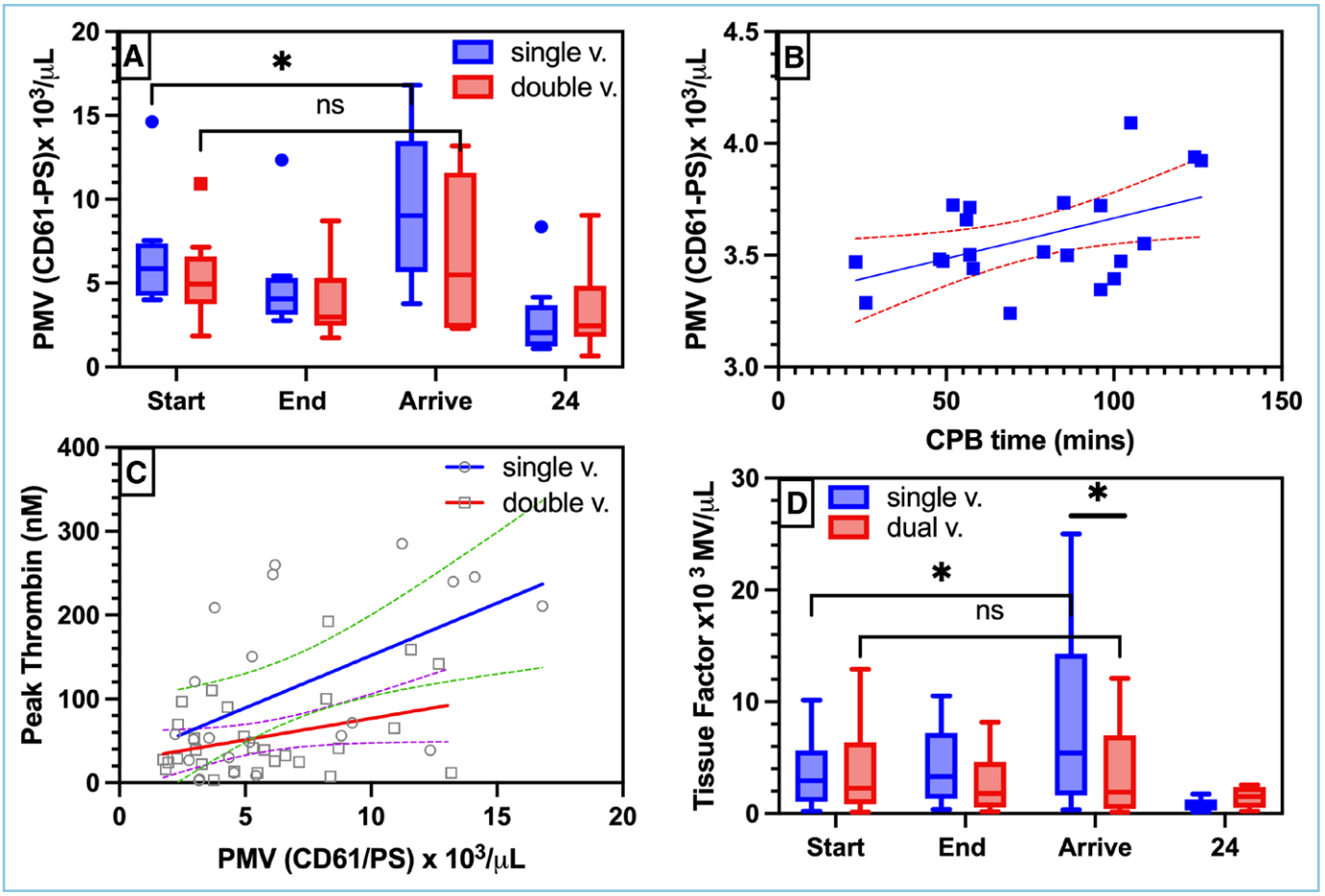

Figure 4. Cell-derived microvesicles (MVs) before, during, and after cardiopulmonary bypass (CPB) surgery: Results are mean ± SEM and significance is $p < 0.05$. **A**, Platelet MVs expressing phosphatidylserine (CD61/Lactadherin) increased more in single compared with dual ventricles. **B**, Generation of platelet MV significantly correlated with increased time of CPB support. **C**, Single ventricle patient's platelet MV correlated with peak thrombin generation more than dual ventricle patients. **D**, Tissue factor expressing MVs (CD142) increased more in single than dual ventricle patients.

physiology were prothrombotic upon arrival to the ICU with increased levels of MV, platelet aggregation, tissue factor, and D-dimer compared with children with a dual ventricle. Furthermore, children with single ventricles had decreased ProcoagPPL clot time, a marker of MV contribution to thrombosis, then dual ventricle patients at the end of CPB. Earlier small studies in adults document an association between increased postoperative concentrations of pump produced PMVs with adverse thrombotic events (8). Although our study was not large enough to associate MV generation with clinical events, it did establish MV as a biomarker and therapeutic target that may reduce common postoperative complications in patients with single ventricle.

Within the past decade, researchers have explored the contribution of isolated platelet-derived MVs to thrombosis and clot formation (12, 18, 29) and performed clinical studies to define their role in coagulation. Early studies were minimal, examining one time point in adult patients in comparison to healthy controls (11, 29). Overall, studies infrequently examined the effect of CPB time, an independent predictor of mortality (30). Related laboratory studies document that MV generation is dependent on the length of time that elevated levels of shear stress affect blood (31). Two small studies ($n < 6$), in adults found a significant correlation between the generation of microparticles during surgery and an increase in the potential for thrombosis and clot formation (11, 29). Our study confirms these earlier findings and extends them to document the effect of CPB time and single ventricle physiology on the generation of prothrombotic MVs. Although our study adds to earlier literature, it is unclear if the causation of the single ventricle physiology, cyanosis, or increased hematocrit (that was increased

at 24 hours postoperative) were the source of the increase in PMV. Future laboratory and animal studies that examine removal or inhibition of PMV will be needed.

Our study strengths include the assessment of MVs, coagulation, platelet aggregation, and thrombin generation in a young population of single and dual ventricle patients before, during, and after CPB surgery. Earlier studies of thrombin generation in children documented either arrival to the ICU (32) or comparisons of before and after surgery (3, 28) yet none looked at MVs or the change at the start, after surgery, and arrival in the ICU. Our study had significant limitations including a small sample size and the inability to match populations for age, gender, or cross-clamp time. Although there was a positive association between PMV and single ventricle physiology, it does not exclude other causes such as size of the CPB circuit, content of transfusion products, temperature change, and/or pressure resistance of arterial catheters. A article by Hanson *et al* (33) concluded that multiple blood components may have MVs and are transfused intraoperatively in pediatric congenital heart disease surgery at seemingly normal/low-normal pretransfusion values. It is unclear how these transfusions may affect MV level or function. We did not find any significant association with those considerations. Please note other limitations including no screening prior to enrollment with ultrasound for thrombosis and that all patient's had a postoperative right internal jugular central line, which could be the source of a clinical thrombotic event. Lastly, our study had missing values because insufficient samples drawn, or clinical issues prevented samples from being obtained. Therefore, we used a mixture of two-way ANOVA or mixed-effects models with simple comparison tests to compensate for these missing values and lack of normalization in the data.

Our study is the first to document a prothrombotic MV imbalance between single and dual ventricles patient's during and after CPB. Further development of these biomarkers could lead to risk stratification of groups for anticoagulation therapy and decrease the widespread use of postoperative heparin, which has not been shown to be effective in preventing complications (34). New therapeutics may decrease MV-driven thrombosis (e.g., cilostazol [35]) increasing the safety of CPB surgery. Our pilot study suggests that single ventricle patients may generate MV-induced thrombosis and platelet activation during and after CPB surgery prompting the need for larger studies to confirm these findings.

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Dr. Meyer contributed to the concept/design, derived the models and protocol, obtained grant support for the study, consented and enrolled patients, obtained regulatory approval, trained the operators of the model, analyzed the data, drafted the article, critically revised the article, and approved the article. Ms. Rishmawi consented and enrolled patients, performed the measurements, processed the experimental data, and approved the article. Ms. Elkhalili consented and enrolled patients, performed the measurements, processed the experimental data, and approved the article. Mr. Rupert consented and enrolled patients, entered clinical data, built and supported the REDCap database, and approved the article. Mr. Walker contributed to the concept/design, developed the blood draw protocol during surgery, and approved the article. Dr. Calhoon supplied departmental support as Chair of Cardiothoracic Surgery, reviewed the data, and approved the article. Dr. Cap contributed to the concept/design, analyzed the data, critically revised the article, and approved the article. Dr. Kane contributed to concept/design, obtained regulatory approval, obtained grant support for the study, critical revision of article, and approval of article.

This project was supported by the National Institutes of Health through Grant UL1 TR001120 and Grant K23 HL124336. This study was also supported by a Grant in Aid Award from the American Heart Association and Children's Heart Foundation (16GRNT30980009).

The authors have disclosed that they do not have any potential conflicts of interest.

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Informed consent was obtained from all patients for being included in the study as part of UT Health Institutional Review Board Approval: IRB#20140224H.

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