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Incidence and Timing of Thromboembolic Events after the Norwood Procedure in the Single Ventricle Reconstruction Clinical Trial of the Pediatric Heart Network

By

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Master of Science
in Clinical Research

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Incidence and Timing of Thromboembolic Events after the Norwood Procedure in the Single Ventricle Reconstruction Clinical Trial of the Pediatric Heart Network

By

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B.S., Harding University, 2008
M.D., University of Texas Southwestern Medical School, 2013

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An abstract of
A thesis submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Master of Science
in Clinical Research
2019

Abstract

Incidence and Timing of Thromboembolic Events after the Norwood Procedure in the Single Ventricle Reconstruction Clinical Trial of the Pediatric Heart Network

By Michael H. White, M.D.

Background: Thromboembolic events lead to increased morbidity and mortality in infants with single ventricle congenital heart disease undergoing staged surgical reconstruction. The reported incidence and timing of thrombosis varies widely, making it difficult to understand the burden of thrombosis and target prevention.

Objectives: Determine the cumulative incidence and timing of thrombosis following the Stage I Norwood, and determine the association between thrombosis and baseline, surgical, and clinical characteristics, and hospital length of stay.

Methods: The Pediatric Heart Network Single Ventricle Reconstruction trial dataset was used to perform a retrospective cohort study, which includes infants with hypoplastic left heart physiology from 2005 to 2009 who underwent randomization to the Stage I Norwood with modified Blalock-Taussig shunt or right ventricle to pulmonary artery shunt. Cumulative incidence of thrombosis was determined and thrombosis-free survival was evaluated using the Kaplan-Meier method and cumulative incidence function. Patient characteristics and hospital length of stay were compared between thrombosis and non-thrombosis groups.

Results: There were 549 infants included in the trial and the cumulative incidence of thrombosis was 6.4% during Stage I. Through Stage II discharge, thrombosis-free survival was lower in males (85 vs 93%; 95% CI, 80-89% vs 88-96%; $p < 0.001$ by log-rank test) and infants with non-HLHS anatomy (80 vs 89%; 95% CI, 86-92% vs 68-89%; $p = 0.01$ by log-rank test) but did not different by shunt type. Using multivariable logistic regression, male sex (OR 3.01, 95% CI, 1.4-6.6, $p = 0.01$), longer cardiopulmonary bypass time (per 10 min increase, OR 1.01, 95% CI, 1.0-1.01, $p = 0.05$), non-HLHS anatomy (OR 2.5, CI: 1.1-5.7, P=0.02), and lower oxygen saturation (per 1% increase, OR 0.9, 95% CI, 0.9-0.98, $p = 0.02$) were associated with thrombosis. Thrombosis was associated with prolonged Norwood hospital length of stay; median 36 vs. 23 days (IQR: 26-58 vs. 15-38 days, $p < 0.001$).

Conclusion: The cumulative incidence of thrombosis was highest during Stage I (6.4%), with most events occurring within 15 days of surgery. Thrombosis was associated with longer cardiopulmonary bypass time and lower oxygen saturation, and thrombosis-free survival was lower for males and those with non-HLHS anatomy.

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Acknowledgements

I would like to express gratitude and thanks to my mentors: Lazaros Kochilas, Kavita Patel, Robert Sidonio, Wilbur Lam, and Sharon Castellino. Without their guidance, expertise, and tireless support this project would not have been achievable. I am also thankful for the Pediatric Hematology and Oncology Department at Children's Healthcare of Atlanta for providing me with crucial project assistance and feedback. I sincerely thank my thesis readers, Amita Manatunga and Beau Bruce, as well as Mitch Klein for help in the preparation of my research project and thesis, along with their instruction in the MSCR program where I have gained valuable education. I am thankful for the financial support I've received through the National Institute of Health TL1 Award and the National Hemophilia Foundation - Shire Clinical Fellowship Award. Lastly, I give thanks to the families of the children who selflessly participated in the Pediatric Heart Network Single Ventricle Reconstruction Trial with the hope of providing us with data that will help to improve the health of children everywhere. Their experience has provided vital insight and will result in advancements in care for future children.

Support

Scholarship and research supported by the National Center for Advancing Translational Sciences of the National Institutes of Health under Award Number UL1 TR002378-01 and TL1 TR002382-01. Indirect support also provided by the National Hemophilia Foundation – Shire Clinical Fellowship Program. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health, the National Hemophilia Foundation, or Shire.

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A. INTRODUCTION

Infants with complex forms of cyanotic congenital heart disease (CHD) such as hypoplastic left heart syndrome (HLHS) and other morphologically related single right ventricles have historically had dismal survival, with up to 95% of infants with HLHS dying before one month of age [1, 2]. In recent decades, with the development of staged surgical reconstructions and improvements in surgical and medical care, survival has dramatically improved such that many infants survive until adulthood. With increases in survival, increasing morbidity from complications such as thrombosis are now emerging.

Pediatric congenital heart disease (CHD) is associated with a higher risk for thrombosis than in the general pediatric population [3-5], and the rates of thrombosis in these patients with CHD is on the rise [6]. Infants with single ventricle physiology CHD who undergo staged surgical reconstruction are among the pediatric patients at highest risk for thrombotic complications, with prior reports estimating that up to 50% of patients will have a thrombotic complication during the course of their disease [7-9]. Thrombotic events can be potentially life, limb, or organ threatening, and often cause long-term vascular morbidity. Thrombotic events in children with single ventricle CHD are associated with increased morbidity, including cardioembolic stroke, pulmonary embolism, superior vena cava (SVC) syndrome, cardiopulmonary arrest, and post-thrombotic syndrome [8]. Additionally, thrombosis leads to exposure to anticoagulation (with its associated risks of bleeding complications, training to administer injectable medications, frequent monitoring/lab tests), possible need for thrombectomy or thrombolysis, increased intensive care unit (ICU) and hospital length of stay, increased healthcare costs, and long-

term sequelae like post-thrombotic syndrome [3, 8, 10-12]. Reasons for this increase in thrombosis include alterations in blood flow, hypercoagulable state, the presence and central lines, foreign vascular material, and surgery [5, 8].

Prevention of thrombosis in these patients, an approach termed thromboprophylaxis, is not standardized and often varies by institution or provider due to a lack of high-level evidence regarding the timing and risk factors for thrombosis [7, 13]. Prior studies have limitations due to small sample size, retrospective study design, heterogeneity of patient population, and single center experience. In order to understand the large burden of thrombosis in the single right ventricle population, better characterization of the timing of thrombotic complications and the associated factors is needed in order to help guide the approach to targeted thromboprophylaxis [13].

To address the wide variation in reported incidence of thromboembolic events in infants with single right ventricle congenital heart disease, I studied the Pediatric Heart Network Single Ventricle Reconstruction randomized clinical trial data set in order to determine the incidence and timing of thrombosis following the Stage I and Stage II surgical reconstructions, as well as identify characteristics associated with thromboembolic events.

B. BACKGROUND

Outcomes in Infants and Children with Congenital Heart Disease

Congenital heart disease (CHD) affects up to 1% of all births in the US, resulting in 40,000 infants born with congenital heart disease every year [14, 15]. These infants have complex medical needs and often require hospitalizations, surgeries, additional medications, and long-term pediatric subspecialty care. These infants also experience a higher rate of morbidity and mortality than the general population [3, 4]. Significant advances in pediatric cardiovascular surgery and medical care have led to the drastic improvement in survival in infants with CHD. For example, the average age of survival in infants with severe forms of CHD was 2 years of age in 1987 to 1993, but increased to an average of 23 years of age in 1999 to 2005 [16]. There are now an estimated 1.4 million adults living with CHD in the United States [11, 17]. With prolonged survival of this cohort of patients comes an increase in awareness of secondary complications, such as thrombosis.

Thrombosis in Single Right Ventricle Congenital Heart Disease

Single right ventricle CHD includes hypoplastic left heart syndrome (HLHS) and other conditions with a single functioning right ventricle and similar physiology. The cumulative incidence of single right ventricle lesions is reaching 3% but represent a disproportionally significant societal burden due to their due to their high morbidity and mortality [14]. Prior to the development of the Stage I Norwood procedure, infants with single right ventricle CHD such as hypoplastic left heart syndrome (HLHS) and had dismal survival, with up to 95% of infants with HLHS dying before one month of age [1, 2]. But now, with the development of the Norwood surgical procedure, in which blood flow is restored to the

aorta and blood is shunted to the lungs by a systemic-to-pulmonary-artery shunt, these patients are able to survive into childhood and even adulthood. After the initial Stage I Norwood surgical procedure, these patients subsequently undergo two more stages of surgical reconstruction in order to survive [11, 16, 18]. The Stage I Norwood surgery is performed at approximately 2 weeks of life and allows the functioning single ventricle to pump blood to the body. Additionally, a cardiac shunt is required in order for blood to be directed to the lungs for oxygenation. The two types of shunts utilized are the modified Blalock-Taussig shunt (MBTS) and the right ventricle to pulmonary artery shunt (RVPAS). It is reported that infants who receive the RVPAS have improved one-year survival compared to those who receive the MBTS [19]. The Stage II procedure, which is performed at approximately 6 months of age, divides the prior shunt and allows for systemic blood to return to both the heart and lungs, respectively. The Stage III Fontan surgery restores systemic blood flow back to the heart while the functioning single ventricle supplies blood only to the body and is performed between 18-48 months.

Among children with CHD, those with single ventricle anatomy and physiology are among those at highest risk for thrombosis. However, reports in the literature vary widely in regard to the incidence and timing of thrombosis. Manlhiot et al in 2012 showed that 51% of children with single right ventricle CHD had a thrombotic complication across the three stages of surgical reconstruction [7, 8]. This high incidence of thrombosis have been attributed to alterations in blood flow, hypercoagulable state, the presence and central lines, foreign vascular material, platelet activation, coagulation factor abnormalities, and surgery [5, 8, 20, 21]. Furthermore, the rates of thrombosis in pediatric patients with CHD is on the rise, increasing by over 250% in the past decade [6].

Thrombosis-Related Morbidity and Mortality in Single Ventricle CHD

Thrombosis-related morbidity in the single ventricle CHD population includes stroke, superior vena cava (SVC) syndrome, exposure to prolonged anticoagulation (with its associated risks of bleeding complications, training to administer injectable medications, frequent monitoring/lab tests), possible need for thrombectomy or thrombolysis (catheter directed or surgical), increased intensive care unit (ICU) and hospital length of stay, increased healthcare costs, difficult vascular access, and long-term sequelae like post-thrombotic syndrome [3, 8, 10-12]. Mortality from thrombosis has been described to be 21%, with thrombosis as a secondary cause in an additional 24% [9]. In another study, hospitalized children with CHD and venous thromboembolism (VTE) had a mortality rate of 10.8% compared to 1.3% in those without VTE [22]. Additionally, shunt thrombosis is known to be a leading cause of mortality in this population, with shunt thrombosis mortality reported up to 40% [23].

Thromboprophylaxis and the Knowledge Gap

The reported incidence and timing of thrombosis in infants with single right ventricle widely varies in the literature, and the risk factors associated with thrombosis are not clear. Due to this knowledge gap, prevention of thrombosis with medication, a strategy called pharmacologic thromboprophylaxis, has not been standardized, leading to inadequate prevention of thrombosis [7, 13]. Much of the data in the literature regarding prevention, diagnosis, and treatment of pediatric thrombosis is derived from adults or small studies in children. Prior clinical trials in children have had difficulties demonstrating significant difference, also likely due to small sample sizes [24]. To my knowledge, no

studies have been able to clearly identify which subset of infants are at highest risk for developing thrombosis across the first two stages of surgical reconstruction. More so, none have described the timing of thrombosis in detail. In order to understand the burden of thrombosis that will eventually help guide targeted thromboprophylaxis, better characterization of thrombotic complications and associated factors is needed [13].

C. METHODS

Research Objectives

1) Determine the cumulative incidence of thrombosis from Stage I through Stage II reconstruction in infants with single right ventricle congenital heart disease and estimate the risk of thrombosis during these clinical time points (Stage I, Interstage I, Stage II).
2) Determine the association between the incidence of thrombosis and specific baseline, surgical, and clinical characteristics.
3) Determine the association between risk of thrombosis and hospital length of stay.

Study Design

I used publicly available data from the Pediatric Heart Network (PHN) Single Ventricle Reconstruction (SVR) trial to perform a retrospective cohort study. The details of the SVR trial have been described in detail in prior publications [25, 26]. Access to this data and permission for this study was granted by the Pediatric Heart Network and the public use datasets are available at http://www.pediatricheartnetwork.org/ForResearchers/PHNPublicUseDatasets.aspx. The public use dataset does not contain private health information that is personally identifiable. The Institutional Review Board at each of the participating Pediatric Heart Network centers approved the protocol for the SVR trial, and informed consent was provided for each patient at the participating institution by a parent or guardian. Additionally, Institutional Review Board approval was obtained at Emory University for the purposes of this retrospective study.

Cohort Criteria / Study Participants

The Pediatric Heart Network (PHN) is a collaborative group of hospitals in North America that participate in clinical research studies in children with CHD. The SVR trial spans from 2005 to 2009 and includes data on infants diagnosed with hypoplastic left heart syndrome (HLHS) and other single right ventricle physiology anomalies who were randomized to the modified Blalock-Taussig shunt (mBTS) or right ventricle to pulmonary artery shunt (RVPAS) during the Stage I Norwood procedure. The infants were followed from trial enrollment until 14-month follow-up, when all participants had completed Stage II hospitalization. The primary outcome was the experience of a first-time thrombotic event. A newer iteration of this study (SVRIII) expands the monitoring of this cohort until the Fontan stage at 2-6 years of age, but these data have not yet been released in the public use dataset.

Inclusion criteria for the SVR trial have been published [26], and include infants with single right ventricle physiology conditions such as hypoplastic left heart syndrome (HLHS), conditions with hypoplastic left ventricle such as tricuspid atresia (TA) and pulmonary atresia with intact ventricular septum (PA with IVS), and various forms of single right ventricle anatomy and physiology, such as double inlet ventricle, straddling atrioventricular valve, unbalanced atrioventricular canal and criss-cross heart. All patients included had a planned Stage I Norwood and did not have any other associated genetic anomalies or medical conditions that would be likely to prohibit them from the Stage I Norwood surgery and subsequent surgical reconstructions.

A total of 920 newborns were screened for enrollment; 664 being medically eligible and 549 patients were randomized. No infants who were randomized in the SVR trial were excluded from analysis for the purpose of this study.

Data Collection and Measurements

After IRB approval, SVR trial datasets were downloaded with permission from the Pediatric Heart Network website. Demographic information, baseline characteristics, and cardiac anatomy were obtained at trial enrollment. Medical history and details of the hospital course were obtained during the Stage I Norwood hospitalization, at discharge, prior to Stage II hospitalization, during Stage II hospitalization, at Stage II hospitalization discharge, and at the 14-month end of trial follow-up. Echocardiographic data were obtained at trial enrollment, Stage I Norwood hospitalization discharge, prior to Stage II hospitalization, and the 14-month end of trial follow-up.

Thrombosis was defined as a composite variable comprised of the following complication codes: thrombus/thromboembolism, superior vena cava occlusion, and inferior vena cava occlusion. The presence of thrombosis was recorded at each of the mentioned timepoints were data was collected. The SVR trial protocol did not mandate screening for thrombosis, therefore, the presence of thrombosis was determined by the standard of care at each participating PHN institution. Stroke was not included in the definition of thrombosis. Time zero for the cohort is defined as the day of the Stage I Norwood surgery.

Baseline characteristics include demographic information and gestational age (in weeks), birth weight (in grams), birth weight percentile, and anatomic diagnosis. Surgical characteristics include weight and weight z-score at Stage I Norwood surgery (in grams),

total cardiopulmonary bypass time (in minutes), total deep hypothermic circulatory arrest time (in minutes), cooling time (in minutes), Stage I Norwood shunt type (mBTS or RVPAS), mBTS diameter, RVPAS diameter, lowest hematocrit and temperature (C) during Stage I Norwood hospitalization, aprotinin administration, steroid administration, and extracorporeal membrane oxygenation (ECMO) during the Stage I Norwood surgery. Clinical characteristics include Stage I Norwood nutrition (formula or breast milk), heart catheterization needed before Stage I Norwood surgery, baseline and post-Stage I Norwood right ventricle ejection fraction, Stage I Norwood percent oxygen saturation at discharge, number of medications at Stage I Norwood discharge, number of complications during the Stage I Norwood hospitalization, and Stage II percent oxygen saturation at discharge. Hospital length of stay includes total ICU and hospital lengths of stay (in days) for both the Stage I and Stage II hospitalizations.

Statistical Plan

Descriptive statistical analyses were performed to characterize the study population by calculating means with standard deviations (SD) for normally distributed continuous variables, median with interquartile ranges (IQR) for parametric variables with skewed distribution, or count and proportions (frequencies) for categorical variables. Summary statistics were also calculated for thrombosis and non-thrombosis groups. Less than five percent of data were missing for each variable analyzed and only complete case analysis was performed.

To accomplish the first research objective, the cumulative incidence, or incidence proportion, of thrombosis was determined for each clinically relevant time point using the formula:

$$\text{CI (t)} = \frac{(\#new\ cases\ during\ t)}{(\#at\ risk\ at\ beginning\ of\ t)}$$

where CI is the cumulative incidence and t is the specific time period of interest. Time-to-event data was determined as the time to a patient's first thrombotic event in days. Survival analysis was performed using the Kaplan-Meier method to estimate the thrombosis-free survival when censoring for death during the Stage I Norwood hospitalization and from the Stage I Norwood surgery through Stage II hospital discharge. Thrombosis-free survival was then compared based on stratifications by gender, Stage I Norwood shunt type, and cardiac anatomic diagnosis with the use of log-rank tests. Survival analysis was also performed using the cumulative incidence function to estimate the cumulative incidence of thrombosis with death as a competing risk.

For the second research objective, independent variables (baseline, surgical, and clinical characteristics) were compared between thrombosis and non-thrombosis groups. Chi-square and Fisher's exact test were used for categorical variables and Wilcoxon rank-sum test was used for continuous variables. To estimate measures of association between the independent variables and the dependent variable of interest, thrombosis, univariable logistic regression was performed. Multivariable analysis via logistic regression modeling was then used to determine the relationship between time-independent predictor variables and thrombosis, incorporating statistically significant and/or clinically relevant independent variables on univariable analysis.

To determine the association between risk of thrombosis and hospital length of stay, total length of intensive care unit and hospital stays were compared using the Wilcoxon rank-sum test.

For all tests described, a p value < 0.05 was considered statistically significant. All statistical analyses were performed using SAS® software v9.4 (2012, SAS Institute, Cary, NC, USA).

D. RESULTS

Patient Characteristics

In the SVR trial, 549 infants were randomized to the two Stage I Norwood shunt types. Among the cohort, 274 infants were randomized to receive the RVPAS and 275 were randomized to the mBTS (Figure 1). Median age at randomization was 5 days (IQR: 1-9 days). The majority of infants were white (n=436, 79.4%) males (n=340, 61.9%) born term (n=483, 88%) with a median gestational age of 38 weeks (IQR: 37-39 weeks) and median birth weight of 3.10 kg (IQR: 2.9-3.4 kg). The predominant anatomic diagnosis was hypoplastic left heart syndrome (HLHS) in 86% (n=474) of the infants, with the other cardiac anomalies listed in Table 1.

At 12-month follow-up there was a 100% trial retention rate, and at 14-month follow-up, 11 patients withdrew from the study or were lost to follow-up, yielding a 98% retention rate.

Cumulative Incidence and Risk for Thrombosis

During the study period, from the day of the Stage I Norwood surgery through Stage II hospital discharge (Table 2), 57 first-time thrombotic events occurred with a cumulative incidence of 10.4%. There were 35 thrombotic events during the Stage I hospital period, with a cumulative incidence of 6.4%. Sixty-one percent of all thrombotic events during the study period occurred during this hospitalization period. Seven thrombotic events occurred during Interstage I, with a cumulative incidence from Stage I Norwood surgery through the end of Interstage I of 7.7% (n=42/549). Fifteen patients experienced their first

thrombotic event during the Stage II hospitalization, with a cumulative incidence of 2.7%. There were 2 thrombotic events during Interstage II, which is not included since this is a truncated time period.

Thrombosis-Free Survival and Cumulative Incidence Function

During the Stage I Norwood hospitalization, 549 infants were at risk, 90 infants died and 35 (6.4%) infants experienced a thrombotic event, with 93.3% (95% CI, 90.8-95.2%) of infants free from thrombosis at 60 days following the Stage I Norwood surgery using Kaplan-Meier estimates (Figure 2). The median time to thrombosis was 15 days (IQR: 7-22 days), and 94% (n=33/35) of thrombotic events occurred within 30 days. Thrombosis-free survival at 28 days for those with HLHS anatomy was significantly higher ($p < 0.001$ by the log-rank test) at 95.5% (95% CI, 93.1-97.1%) compared to 83.8% (95% CI, 72.6-90.7%) in those with non-HLHS anatomy (Figure 5). There was no difference in thrombosis-free survival at 28 days by gender (Figure 4, $p = 0.08$ by the log-rank test) or shunt type (Figure 3, $p = 0.85$ by log-rank test).

From the time of the Stage I Norwood surgery through Stage II hospital discharge, 167 infants died and 57 (10.4%) infants experienced a thrombotic event. Kaplan-Meier estimates of thrombosis-free survival at 60, 120, and 300 days were 92.7% (95% CI, 90.1-94.7%), 90.6% (95% CI, 87.7-92.9%), and 87.9% (95% CI, 84.6-90.6%), respectively (Figure 6). The median time to thrombosis was 23 days (IQR: 11-100 days). Overall thrombosis-free survival was again higher in infants with HLHS anatomy compared to those with non-HLHS anatomy (Figure 8, $p = 0.01$ by log rank test). Overall thrombosis-free

survival was higher in females (Figure 7, $p < 0.001$ by log rank test). Again, there was no difference in overall thrombosis-free survival according to shunt type.

The cumulative incidence of thrombosis following the Stage I Norwood surgery was 14.1% at 100 days (95% CI, 9.6-19.5%) when considering censorship for death as a competing event for the primary outcome, thrombosis (Figure 9).

Baseline, Surgical, and Clinical Characteristics Associated with Thrombosis

Univariable analysis was performed to compare independent variables (baseline, surgical, and clinical characteristics) between thrombosis and non-thrombosis groups. Baseline characteristics associated with thrombosis include male sex ($p = 0.005$) and hypoplastic left heart syndrome (HLHS) anatomy ($p = 0.01$). Gestational age, birth weight, birth weight percentile, and race were not associated with thrombosis (Table 3). Surgical characteristics associated with thrombosis include longer cardiopulmonary bypass time (145 vs 137 minutes, $p = 0.045$) and aprotinin administration during surgery ($p = 0.03$). Weight at Norwood surgery, shunt type, shunt diameter, and ECMO use during Stage I Norwood surgery were not associated with thrombosis (Table 4). Clinical characteristics associated with thrombosis include lower Stage I Norwood discharge oxygen saturation (80 vs 83%, $p = 0.02$). Nutrition type, baseline right ventricle ejection fraction, and Stage II discharge oxygen saturation were not associated with thrombosis (Table 5).

Univariable logistic regression was performed to estimate measures of association between the independent variables already described and the outcome of thrombosis. Unadjusted odds ratios are reported in Table 6. Multivariable logistic regression modeling with stepwise selection was then performed, incorporating statistically significant and/or

clinically relevant independent variables on univariable analysis. Males were three times more likely than females to experience thrombosis (OR 3.01, 95% CI, 1.4-6.6, p = 0.01) and infants with non-HLHS cardiac anatomy were 2.5 times more likely than infants with HLHS to experience thrombosis (OR 2.5, 95% CI, 1.1-5.7, p = 0.02). Additionally, Stage I discharge oxygen saturation (per 1% increase, OR 0.9, 95% CI, 0.9-0.98, p = 0.01) and longer cardiopulmonary bypass time (per 10 minute increase, OR 1.01, 95% CI, 1.0-1.01, p = 0.05) were associated with thrombosis and the adjusted odds ratios are reported in Table 7.

Thrombosis and Hospital Length of Stay

Development of thrombosis during the Stage I Norwood hospitalization was associated with prolonged median ICU stay and length of hospital stay; 27 vs. 13 days (IQR: 13-46 days vs. 9-25 days, p < 0.001) and 36 vs. 23 days (IQR: 26-58 days vs. 15-38 days, p < 0.001), respectively (Table 8).

E. DISCUSSION

Pediatric congenital heart disease (CHD) is associated with a higher risk for thrombosis than the general pediatric population [3-5], and the rates of thrombosis in pediatric patients with CHD who undergo surgery is on the rise [6]. Furthermore, infants with single ventricle physiology CHD who undergo staged surgical reconstruction are among the pediatric patients at highest risk for thrombotic complications, but prior reports widely vary and have many limitations [5, 7-9]. In order to understand the large burden of thrombosis in the single right ventricle population, better characterization of the timing of thrombotic complications and the associated factors is needed in order to help guide the approach to targeted thromboprophylaxis [13]. In this largest reported prospective cohort of infants undergoing single ventricle reconstruction, I aimed to: (1) determine the cumulative incidence of thrombosis from Stage I through Stage II reconstruction in infants with single right ventricle congenital heart disease and estimate the risk of thrombosis during these clinical time points (Stage I, Interstage I, Stage II); (2) determine the association between the incidence of thrombosis and specific baseline, surgical, and clinical characteristics; and (3) determine the association between risk of thrombosis and hospital length of stay.

The cumulative incidence of thrombosis was highest during the Stage I Norwood hospitalization (6.4%), with the majority of thrombotic events during the study period occurring during this time period (61%). The median time to thrombosis was 15 days, highlighting the importance of the timing of thromboprophylaxis in this high-risk post-operative period. During this period, the probability of thrombosis was higher for infants

with non-HLHS anatomy compared to those with HLHS anatomy, suggesting that approaches to thromboprophylaxis could be targeted according to risk factor stratifications.

The overall cumulative incidence of thrombosis from the Stage I Norwood surgery to the Stage II hospital discharge was 10.4%, which is notably lower than the incidence of 40-50% reported by Manlhiot et al [9]. Our finding of a lower cumulative incidence of thrombosis may be explained by the lack of screening for asymptomatic thrombosis, larger sample size, differences in surgical approach, and more aggressive thromboprophylaxis. The SVR trial did not mandate screening for asymptomatic thromboses, meaning our reported incidence favors the true incidence of symptomatic and clinically relevant thrombosis. During this period, males and infants with non-HLHS anatomy were more likely to experience thrombosis, again exhibiting the concept that future thromboprophylaxis could be aimed at those with certain risk factors.

Notable characteristics associated with thrombosis include male sex, non-HLHS anatomy, longer cardiopulmonary bypass time, and a lower Stage I Norwood discharge oxygen saturation. While these characteristics are not imminently relevant as modifiable risk factors, they can provide insight into at-risk populations. Additionally, there was no significant difference in thrombosis between the two Stage I Norwood shunt types (mBTS vs. RVPAS). Regarding clinical outcomes, thrombosis is associated a 2-fold increase in Stage I Norwood ICU stay and a 13 day increase in the total hospital length of stay.

A unique aspect of the study is our approach to survival analysis that has not been reported in the context of the timing of thrombosis. Traditional survival analysis using the Kaplan-

Meier method makes use of the product limit approach, assuming that censorship is independent of the outcome. This assumption can bias the survival estimates if the independent censoring assumption is broken. While this assumption cannot be confirmed with our currently available data, historical data in the single ventricle population shows that thrombosis is associated with an increased risk for death. Therefore, the assumption of independent censoring does not necessarily apply to our population, and survival analysis using the cumulative incidence function is more appropriate. The data show that ignoring death as a competing risk (using the KM method) underestimates the cumulative incidence of thrombosis (i.e. overestimating the thrombosis-free survival). The importance of competing risk analysis has been reported in the literature [27].

There are many advantages related to the prospective design and structured approach provided by the original SVR trial, however, limitations of this study include retrospective analysis of the original dataset in which thrombosis was not the primary endpoint of the original trial. Additionally, historical risk factors for thrombosis, such as thrombus location, central line location, and the thromboprophylaxis method for each patient, were not reported. Lastly, analysis of recurrent thrombotic events was not discussed in this study because recurrent events were not clearly measured in the trial.

Future directions for this research include analysis of the Stage III Fontan data, when publicly available through the Pediatric Heart Network, in order to complete the analysis of the incidence and timing of thrombosis across all three stages of surgical reconstruction. In the largest analysis of the single right ventricle population, our findings update the medical literature regarding the incidence of thrombosis in the single right ventricle CHD

population and provide a closer look into the highest-risk periods for thrombosis. This work lays the foundation for future studies focused on time and risk-targeted thromboprophylaxis.

In conclusion, the overall cumulative incidence of thrombosis from Stage I Norwood surgery through Stage II hospital discharge was 10.4%, with the highest risk period for thrombosis during the Stage I Norwood hospitalization. Specifically, most thrombotic events occur within 15 days of the Norwood surgery. Thrombosis-free survival is higher for females and those with HLHS anatomy when compared to males and infants with non-HLHS anatomy, respectively. Thrombosis is associated with male sex, longer cardiopulmonary bypass time, lower Stage I Norwood discharge oxygen saturation, and non-HLHS anatomy. Additionally, thrombosis is associated with longer Stage I Norwood ICU and hospital lengths of stay.

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G. TABLES / FIGURES

TABLE 1: Characteristics of the study population from the Single Ventricle Reconstruction Trial of the Pediatric Heart Network

Characteristic	Enrolled
Number of infants	549
Age at randomization– days	5.0 ± 4.0
Gestational age – weeks	38 (37–39)
Sex	
Male	340 (61.9%)
Female	209 (38.1%)
Race or ethnic group	
White	436 (79.4%)
Black	86 (15.7%)
Other	27 (4.9%)
Birth weight[1] – g	3085 (2860–3420)
Birth weight percentile	
< 30 %	277 (50.5%)
30 - 70%	190 (34.6%)
> 70 %	82 (14.9%)
Anatomic Diagnosis	
Hypoplastic left heart syndrome	474 (86.3%)
Critical aortic stenosis	5 (0.9%)
Single RV with systemic outflow obstruction	23 (4.2%)
Right dominant AV canal with systemic outflow obstruction	31 (5.6%)
Straddling mitral valve with LV hypoplasia and outflow obstruction	1 (0.2%)
Functional single right ventricle	15 (2.7%)
Alive at Norwood discharge	459 (83.6%)

TABLE 2: Timing and number of thromboembolic events from birth through 14-month follow-up in the SVR trial

		549 at risk 35 clots 90 deaths	**424 at risk 7 clots 59 deaths**	**358 at risk 15 clots 18 deaths**	
Complication	Pre-Norwood	Stage I Hospitalization*	Interstage I	Stage II Hospitalization	Interstage II**
SVC Occlusion	0	1	0	0	1
IVC Occlusion	0	1	0	0	1
Thrombus	2	33	7	15	0
Total Events	2	35	7	15	2
		CI: 35/549 = **6.4%**			
			CI: 42/549 = **7.7%**		
				CI: 57/549 = **10.4%**	

TABLE 3: Baseline characteristic associated with thrombosis (values reported as median (IQR 25^{th} – 75^{th}) or n (%))

Characteristic	**Thrombosis (n=57, %)**	**No Thrombosis (n=492, %)**	**p value**
Gestational age (weeks)	38 (38 – 39)	38 (37 – 39)	0.84
Birth weight* (grams)	3.1 (2.9 – 3.4)	3.2 (2.8 – 3.5)	0.96
Birth weight percentile			0.49
< 30 %	33 (57.9 %)	244 (49.6 %)	
30 - 70 %	17 (29.8 %)	173 (35.2 %)	
> 70 %	7 (12.3 %)	75 (15.2 %)	
Sex			**0.005**
Male	45 (78.9 %)	294 (60.0 %)	
Female	12 (21.1 %)	197 (40.0 %)	
Race			0.12
White	50 (89.3 %)	386 (79.1 %)	
Black	6 (10.7 %)	80 (16.4 %)	
Other	0 (0.0 %)	22 (4.5 %)	
Anatomic Diagnosis			**0.01**
Hypoplastic left heart	44 (77.2 %)	430 (87.4 %)	
Other	13 (22.8 %)	62 (12.6 %)	

TABLE 4: Surgical characteristics associated with thrombosis (values reported as median (IQR 25^{th} – 75^{th}) or n (%))

Characteristic	Thrombosis (n=57, %)	No Thrombosis (n=492, %)	p value
Weight at Norwood (kg)	3.1 (2.8 – 3.4)	3.20 (2.8 – 3.5)	0.82
Weight z-score (kg)	-0.73 (-1.2 – 0.02)	-0.51 (-1.3 – 0.20)	0.55
Total bypass time (minutes)	145 (119 – 184)	137 (104 – 169)	**0.045**
Total DHCA time+ (minutes)	36 (15 – 53)	34 (13 – 46)	0.17
Cooling time (minutes)	28 (21 – 40)	30 (20 – 41)	0.65
Norwood shunt type			0.74
mBT Shunt	29 (50.9 %)	239 (48.6 %)	
RV to PA Shunt	28 (49.1 %)	253 (51.4 %)	
mBTS diameter (mm)	3.5 (3.5 – 4.0)	3.5 (3.5 – 3.5)	0.48
RVPAS diameter (mm)	5.0 (5.0 – 6.0)	5.0 (5.0 – 6.0)	0.07
Lowest hematocrit	30 (26 – 33)	29 (27 – 32)	0.99
Lowest temperature (C)	17 (16 – 18)	17 (16 – 18)	0.80
Aprotinin administered	38 (66.7 %)	389 (79.1 %)	**0.03**
Steroids given	53 (93.0 %)	445 (90.4 %)	0.53
ECMO during Norwood	1 (1.8 %)	34 (6.9 %)	0.16

TABLE 5: Clinical characteristic associated with thrombosis (values reported as median (IQR 25^{th} – 75^{th}) or n (%))

Characteristic		**Thrombosis (n=57, %)**		**No Thrombosis (n=492, %)**		**p value**
Stage I nutrition						0.25
	Formula	35	(83.3 %)	297	(75.4 %)	
	Breast milk	18	(42.9 %)	186	(47.2 %)	
Catheterization prior to Stage I		2	(3.5 %)	37	(7.6 %)	0.41
Ejection fraction - Baseline						0.06
	< 40 %	28	(93.3 %)	229	(73.2 %)	
	40.1-49.9 %	1	(3.3 %)	35	(11.2 %)	
	>50.0 %	1	(3.3 %)	49	(15.7 %)	
Stage I oxygen saturation (%)		80	(79 – 84)	83	(80 – 86)	**0.02**
Stage II oxygen saturation (%)		80	(77 – 82)	80	(77 – 84)	0.26

TABLE 6: Univariable logistic regression evaluating association between relevant clinical characteristics and thrombosis

Characteristic		OR*	95% Wald Confidence Limits	p value
Male sex (vs Female)		**2.5**	**1.3 – 4.9**	**0.007**
Other Anatomy (vs HLHS)		**2.0**	**1.05 – 4.02**	**0.04**
Norwood O2 sat (-1 %)		**1.1**	**1.02 – 1.2**	**0.01**
Bypass Time (+10 mins)		1.004	1.0 – 1.01	0.13
Low Birth Weight (<2500 g)		1.0	0.45 – 2.2	0.96
RVPAS (vs mBTS)		1.1	0.7 – 2.0	0.66
MBTS Diameter (mm)				
	3.0 vs 3.5	1.7	0.5 – 6.4	0.44
	4.0 vs 3.5	1.7	0.7 – 3.9	0.22
RVPAS Diameter 5.0 mm (vs 6.0 mm)		**0.4**	**0.2 – 0.96**	**0.04**

*OR: odds ratio

TABLE 7: Multivariable logistic regression evaluating association between thrombosis and relevant clinical characteristics

Characteristic	aOR*	95% Wald Confidence Limits	p value
Male sex (vs Female)	3.02	1.4 – 6.6	0.01
Other Anatomy (vs HLHS)	2.5	1.1 – 5.7	0.02
Norwood O2 sat (+1 %)	0.9	0.9 – 0.98	0.01
Bypass time (+10 mins)	1.01	1.0 – 1.01	0.05

*aOR: adjusted odds ratio

TABLE 8: Clinical outcomes associated with thrombosis (values reported as median (IQR 25^{th} – 75^{th}) or n (%))

Characteristic		Thrombosis (n=57, %)		No Thrombosis (n=492, %)		p value
Ejection fraction - Norwood						0.88
	< 40 %	28	(84.8 %)	209	(80.4 %)	
	40.1-49.9 %	3	(9.1 %)	24	(9.2 %)	
	>50.0 %	2	(6.1 %)	27	(10.4 %)	
Unintended catheterization		2	(3.5 %)	37	(7.6 %)	0.41
CPR during hospitalizations		14	(24.6 %)	83	(17.0 %)	0.16
ECMO during hospitalizations		1	(12.3 %)	81	(16.6 %)	0.40
Stage I ICU length of stay (days)		27	(13 – 46)	13	(9 – 25)	**<0.001**
Stage I length of stay (days)		36	(26 – 58)	23	(15 – 38)	**<0.001**
Number of significant complications after Stage I		5	(2 – 6)	2	(1 – 4)	**<0.001**
Number of Stage I medications		6	(4 – 7)	5	(4 – 6)	**0.03**
Alive at Norwood discharge		50	(84.8 %)	409	(83.9 %)	0.89
Alive at 12 months		38	(6.9 %)	342	(3.5 %)	0.85

FIGURE 1: CONSORT diagram of the Single Ventricle Reconstruction trial

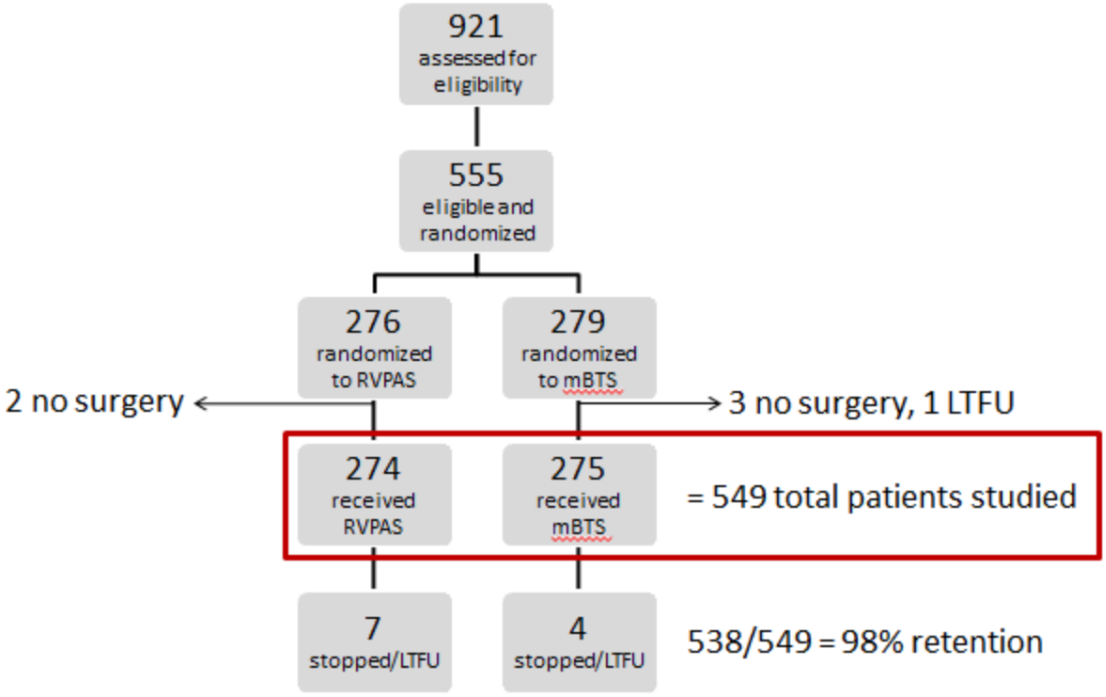

FIGURE 2: Thrombosis-free survival during the Stage I Norwood hospitalization

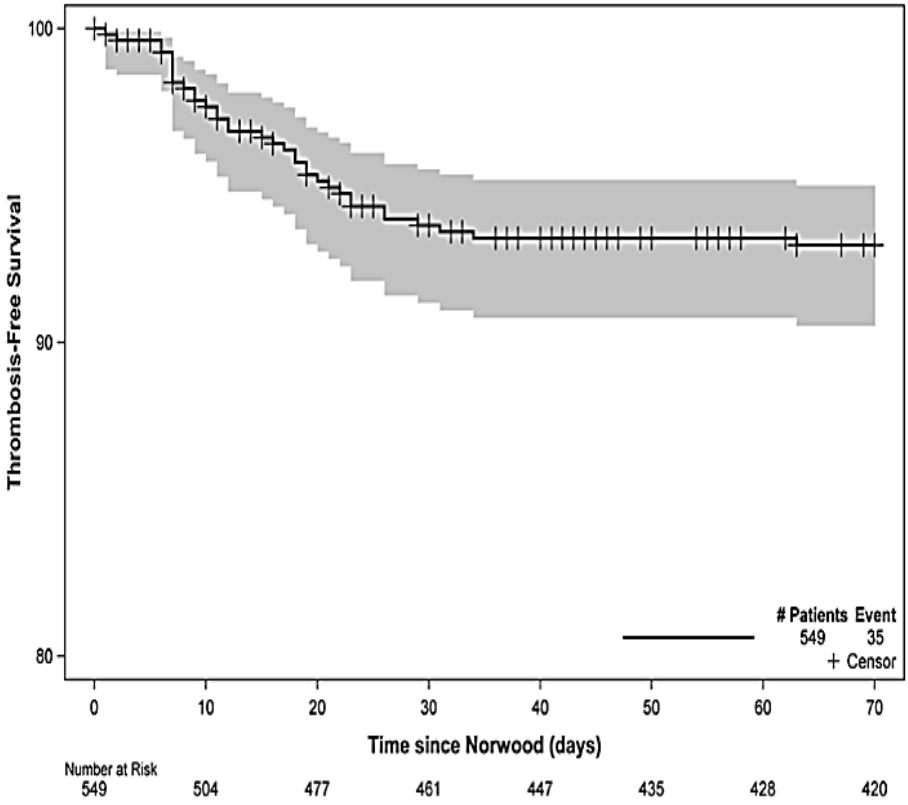

FIGURE 3: Difference in thrombosis-free survival during the Stage I Norwood hospitalization by shunt type

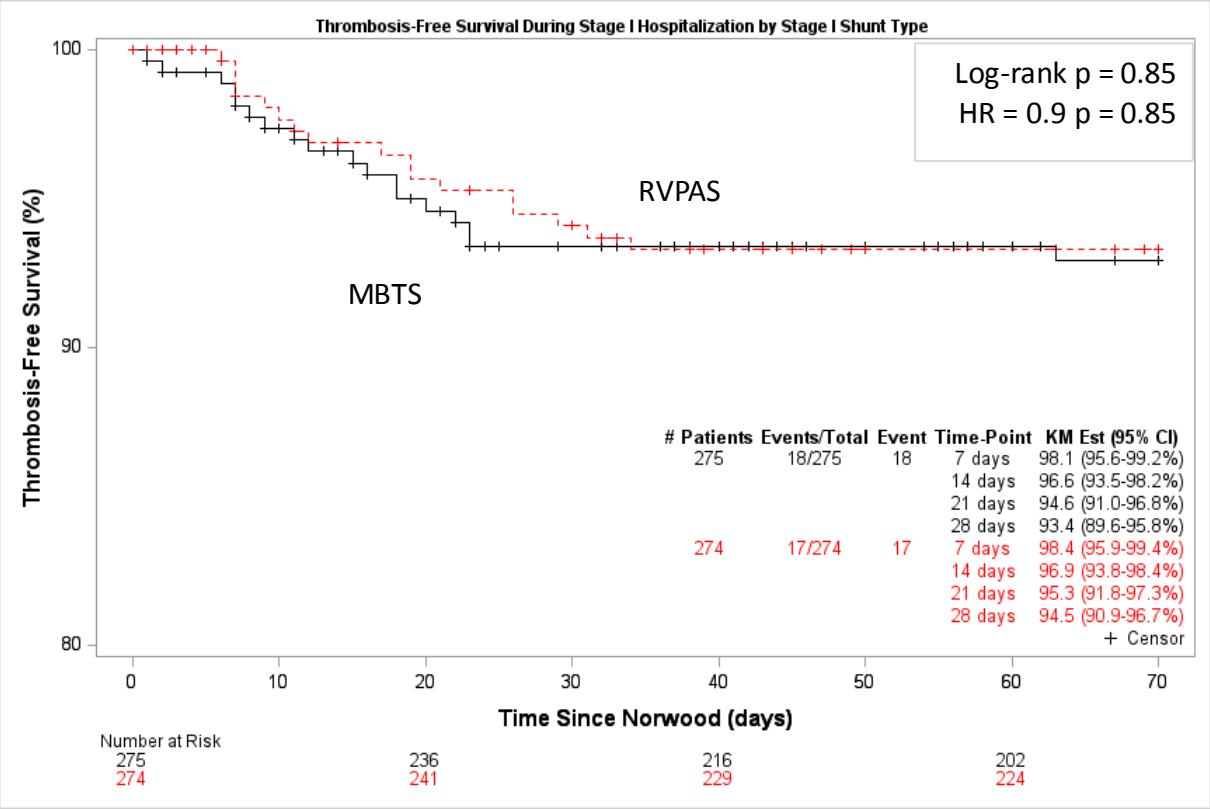

FIGURE 4: Difference in thrombosis-free survival during the Stage I Norwood hospitalization by gender

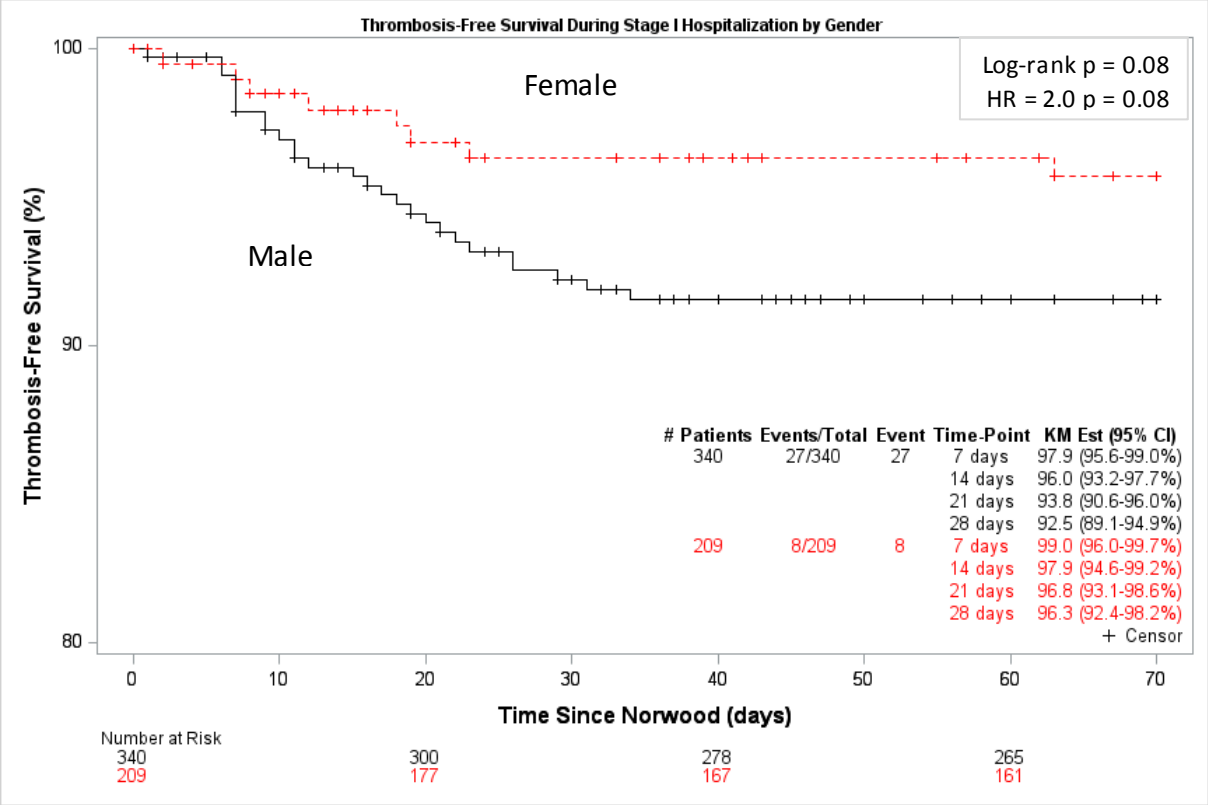

FIGURE 5: Difference in thrombosis-free survival during the Stage I Norwood hospitalization by cardiac anatomic diagnosis

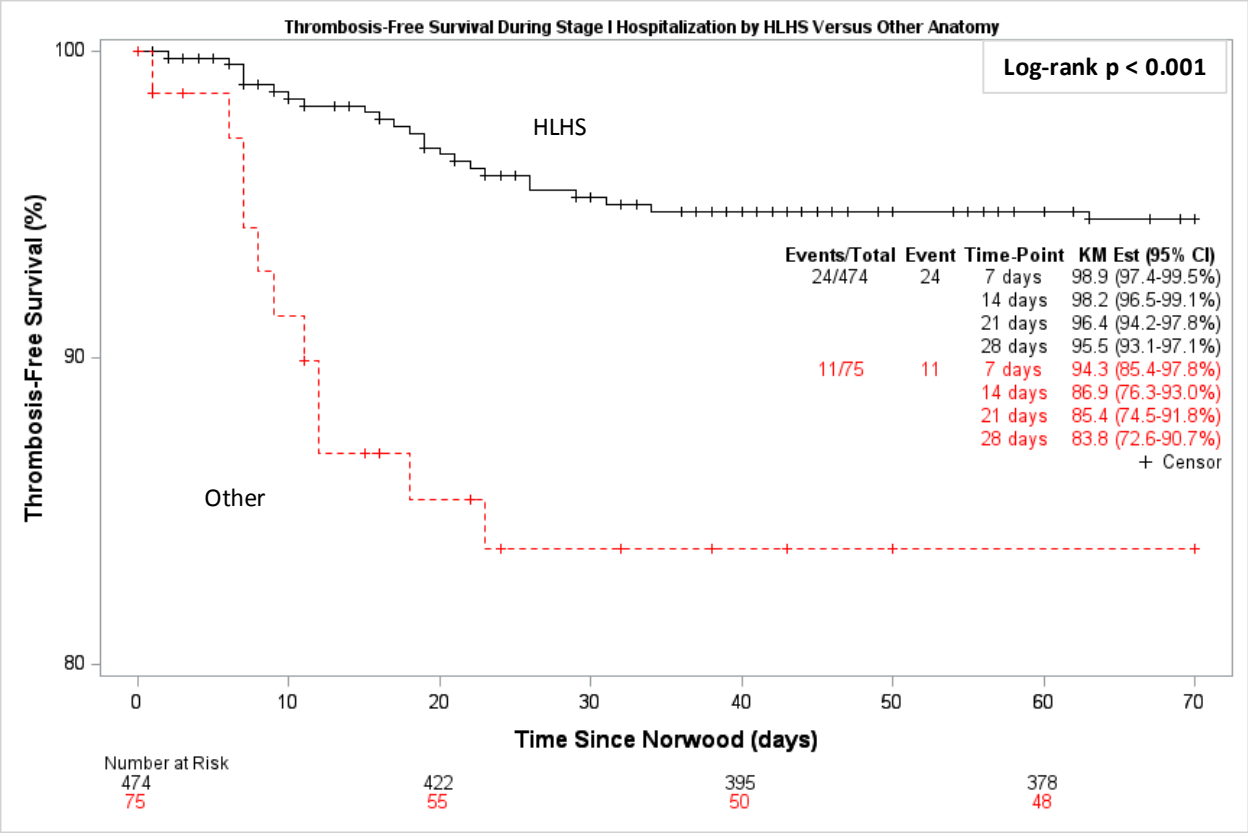

FIGURE 6: Thrombosis-free survival from the Stage I Norwood surgery through Stage II hospital discharge

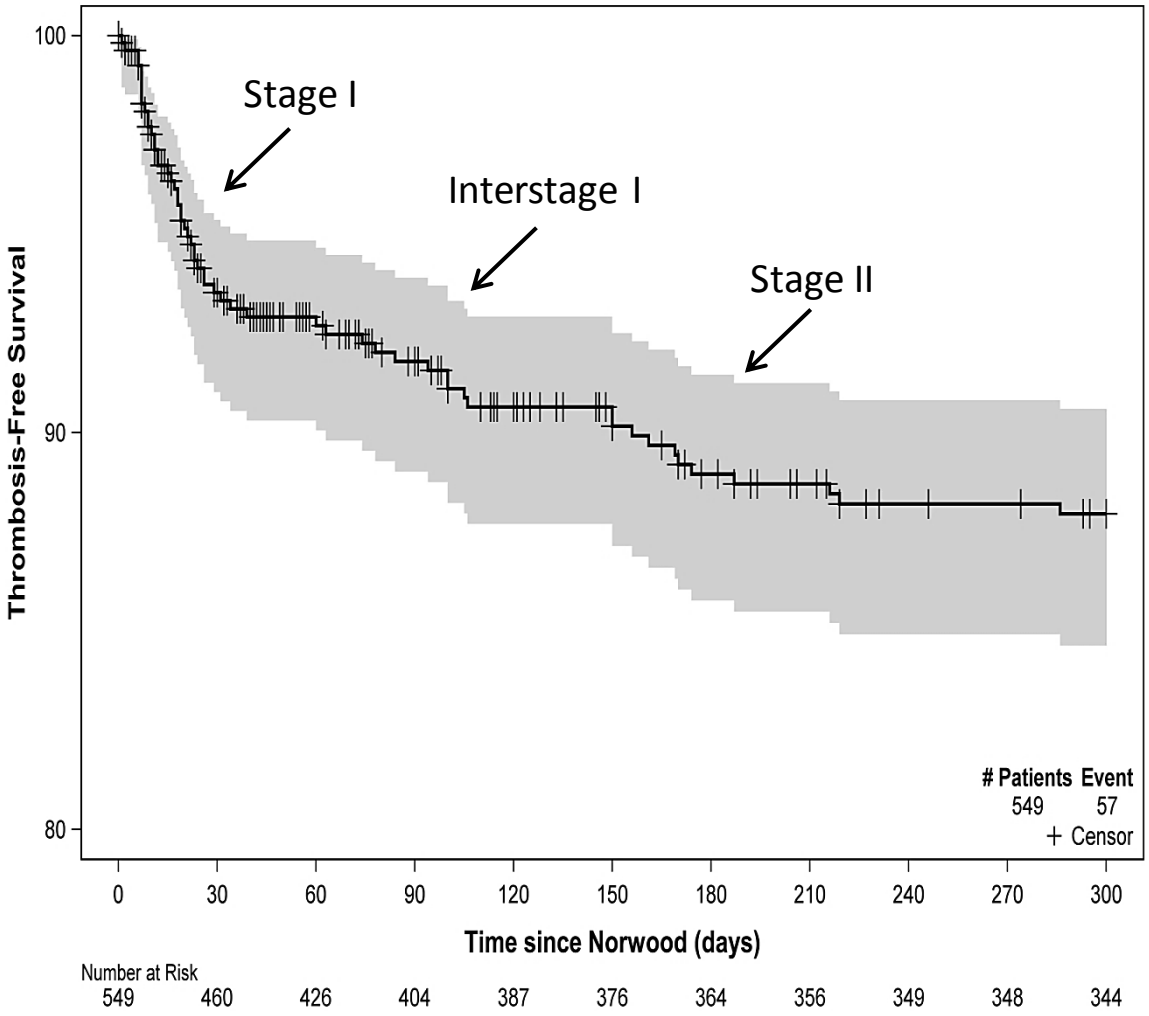

FIGURE 7: Difference in thrombosis-free survival from the Stage I Norwood surgery through Stage II hospital discharge by gender

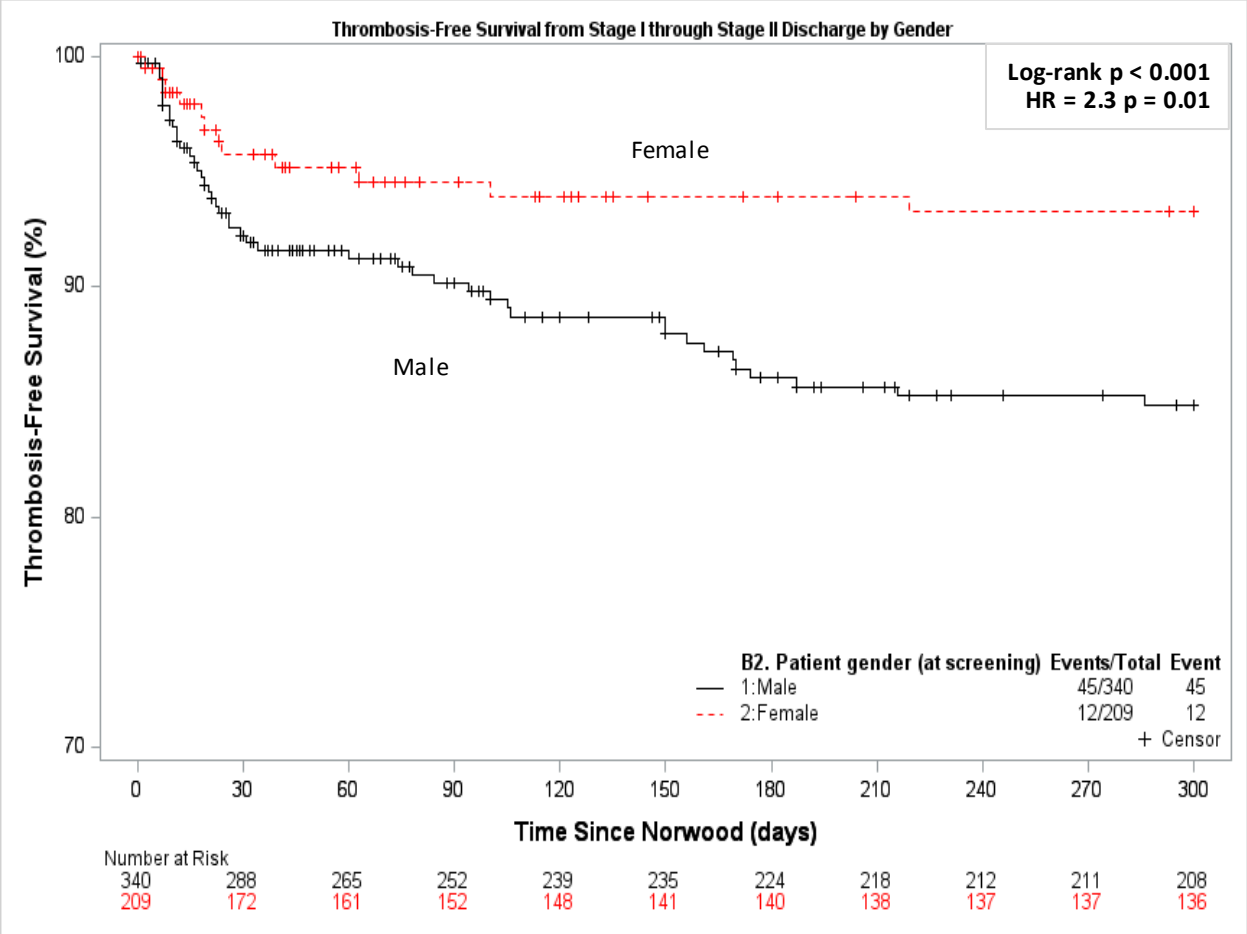

FIGURE 8: Difference in thrombosis-free survival from the Stage I Norwood surgery through Stage II hospital discharge by cardiac anatomic diagnosis

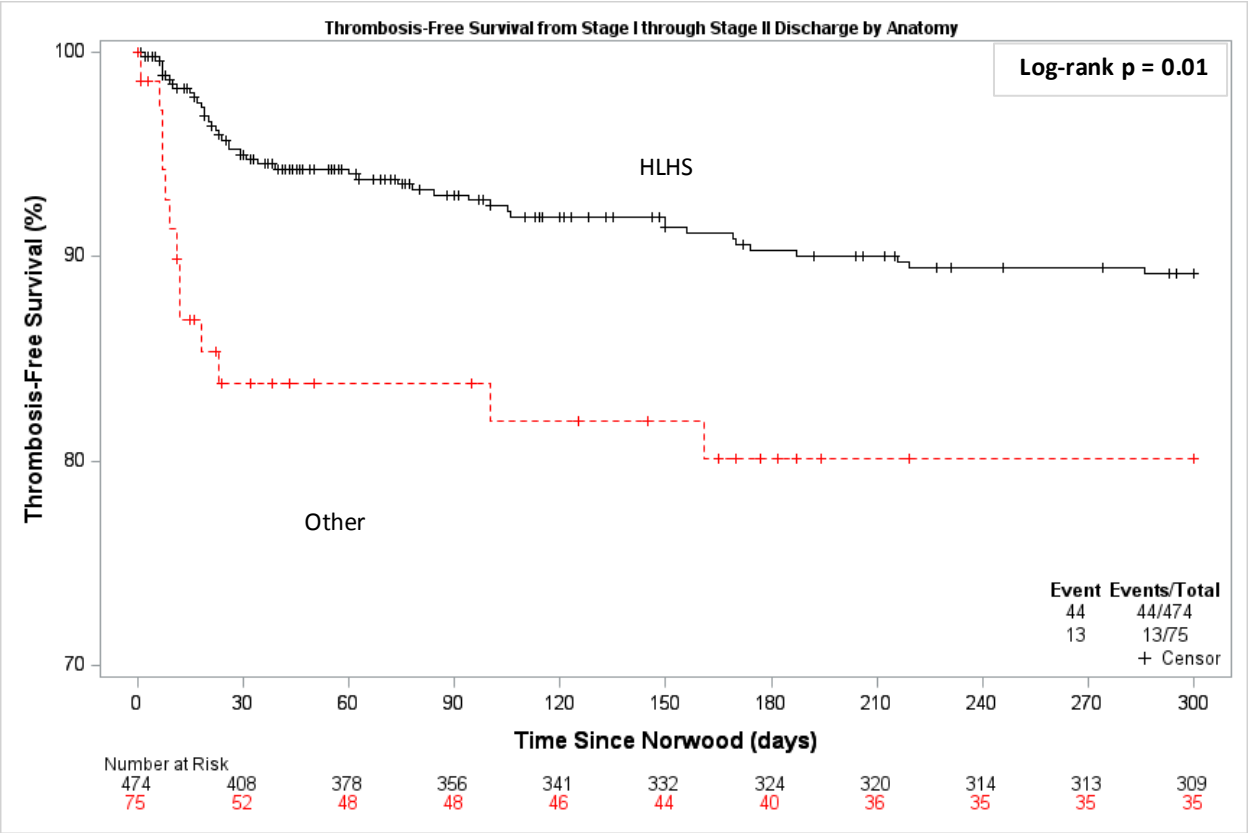

FIGURE 9: Cumulative incidence of thrombosis during Stage I Norwood hospitalization and Interstage I