Non-Surgical Related Bleeding in Pediatric Patients with Single Ventricle Physiology Following Cardiac Surgery

Inauguraldissertation
zur Erlangung des Grades eines Doktors der Medizin
des Fachbereichs Medizin
der Justus-Liebig-Universität Gießen

In Kooperation mit dem Boston Children's Hospital, Harvard Medical School

vorgelegt von Seibold, Carolin
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Aus dem Fachbereich Medizin der Justus-Liebig-Universität Gießen

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A Introduction

A.1 Single Ventricle Physiology

A.1.1 Anatomic Background

Single Ventricle Physiology (SVP) is a term used to describe a variety of severe structural congenital heart defects (CHD). All of these have in common, that either the left or right ventricle is underdeveloped and shows significant hypoplasia with decreased ventricular function. In addition, different vascular and valvular malformations in varying degrees of severity may occur (Jacobs, Jacobs et al. 2008). Different subtypes of single ventricle (SV) diagnoses include: Hypoplastic Left Heart Syndrome (HLHS), Double Outlet Right Ventricle (DORV), Double Inlet Right Ventricle (DIRV), Atrioventricular Canal (AVC) defects, severe Ebstein's Anomaly, Pulmonary and Tricuspid Atresia (Anderson, Becker et al. 1975). In the following, the example of HLHS is used to illustrate the disease, being the most common form with 1 to 2 in 10,000 newborns and accounting for 2-7% of all CHD (Fyler, Buckley et al. 1980, Samanek, Slavik et al. 1989, Morris, Outcalt et al. 1990, Perry, van der Velde et al. 1993, Reller, Strickland et al. 2008).

HLHS as defined by Tchervenkov et al.(2006) is "a spectrum of cardiac malformations with normally aligned great arteries without a common atrioventricular junction, characterized by underdevelopment of the left heart with significant hypoplasia of the left ventricle including atresia, stenosis, or hypoplasia of the aortic or mitral valve, or both valves, and hypoplasia of the ascending aorta and aortic arch".

In HLHS, the left ventricular size may vary from well identifiable in mild to almost non-existent in very severe cases. Right ventricular structures are compensatory enlarged and hypertrophic. Blood flows from the pulmonary veins into the left atrium, then through an atrial septal defect (ASD) into the right atrium and hence the right ventricle. Here oxygenated blood is mixed with desaturated blood coming from the upper and lower vena cava (Left-Right-Shunt). This mixed blood is now flowing from the right ventricle through the pulmonary arteries and the patent ductus arteriosus (PDA) into the lungs as well as the descending aorta. At the same time, there is retrograde blood flow through the PDA into the hypoplastic ascending aorta and therefore into the coronary arteries (Tchervenkov, Jacobs et al. 2006). Compare Figure 1 for anatomic details of HLHS.

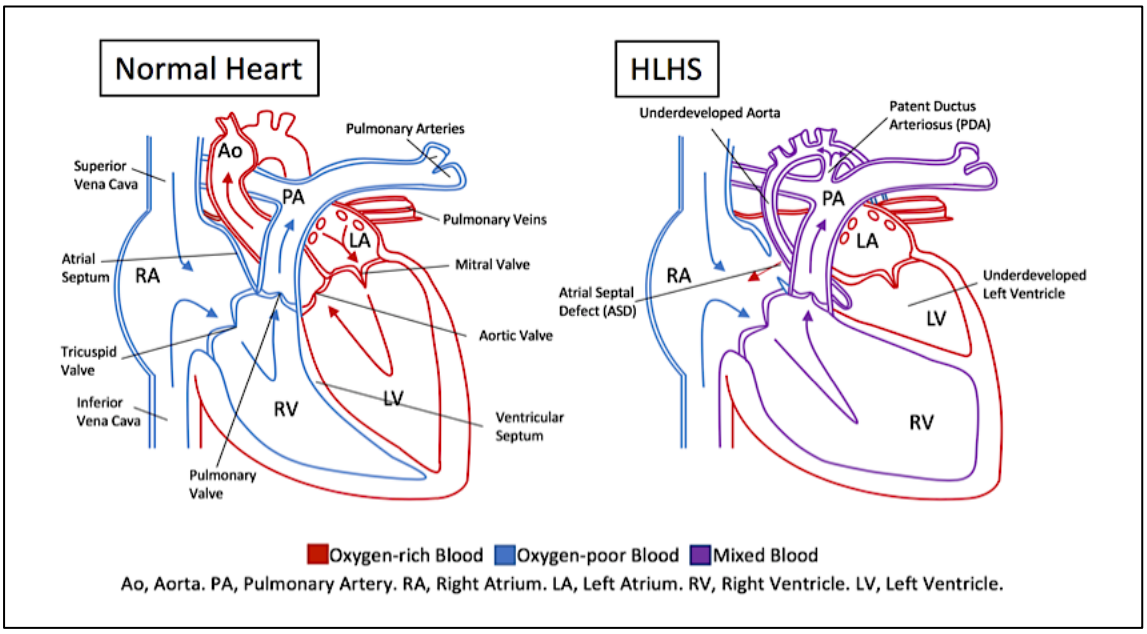

FIGURE 1. Normal Heart and Hypoplastic Left Heart Syndrome (HLHS) in comparison.

A.1.2 Treatment Options for Patients with SVP

While SV used to be a consistently fatal diagnosis, the prognosis of patients born with only one functional ventricle has significantly improved over the last decades: After multiple unsuccessful attempts at surgical treatment, Norwood and colleagues have marked the beginning of a new era with the introduction of the Norwood Procedure in the early 1980s (Norwood, Kirklin et al. 1980, Norwood, Lang et al. 1981, Norwood, Lang et al. 1983). Ductus stenting as a bridge to transplantation (Ruiz, Zhang et al. 1993), neonatal heart transplantation (Bailey, Concepcion et al. 1986) and the Hybrid approach as an alternative to the Norwood procedure (Gibbs, Wren et al. 1993) have since been added as options of the treatment of SV. Prostaglandin infusions to ensure ductus potency until surgery as well as refined surgical techniques and improved peri- and postsurgical care have been important factors contributing to improved survival in this critically ill patient cohort (Tweddell, Hoffman et al. 2002).

In the following, different surgical option in the treatment of HLHS will be described more closely.

A.1.2.1 Norwood-3-Stage Procedure

The aim of this procedure is to ultimately achieve separated pulmonary and systemic circulation through a three-staged surgical approach. Stage I (Norwood Procedure) is performed at the age of approximately one week. A neo aorta originating from the right

ventricle is built using the main pulmonary artery. The reestablishment of pulmonary blood flow is achieved by inserting a shunt (see Figure 2). This can either be the classic modified Blalock-Taussig (MBT) shunt (connection between the subclavian or innominate artery and the pulmonary artery) or alternatively a right ventricle to pulmonary artery (RVPA) shunt (Ohye, Sleeper et al. 2010). At 3-6 months of age, the shunt is removed in a second surgery called Hemi-Fontan or Bidirectional Glenn-Procedure. The superior vena cava is then connected to the right pulmonary artery to reestablish pulmonary blood flow (Norwood and Jacobs 1993). During the final surgery, the Fontan procedure, which is performed at around 18-48 months of age, the inferior vena cava is connected to the pulmonary artery as well, creating two separated forms of circulation (see Figure 2).

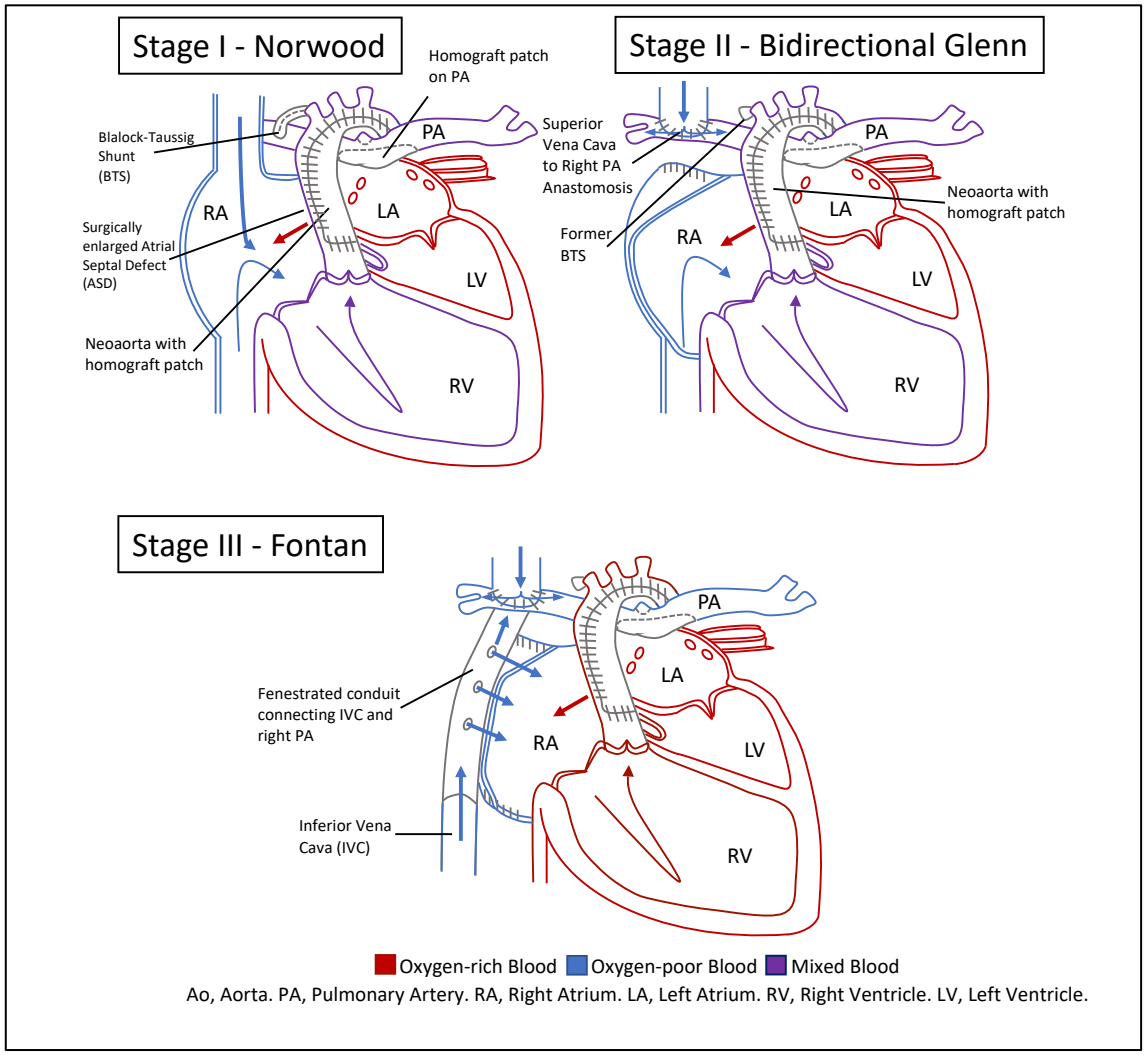

***FIGURE 2**. Three-staged surgical palliation for HLHS. Stage I-Norwood, Stage II-Bidirectional Glenn, Stage III-Fontan.*

A.1.2.2 Hybrid or Giessen Procedure

This procedure was developed in Giessen, Germany and differs from the classical Norwood procedure with regard to the first two stages. Instead of placing a shunt, surgical bilateral pulmonary artery banding (PAB) is performed to reduce pulmonary blood flow, followed by transcatheter PDA stenting. The second surgery, called comprehensive stage II, can be seen as a combination of Norwood stages I and II, with the creation of a neo aorta, Glenn anastomosis, resection of the atrial septum and removal of the PAB. The third stage (Fontan) is performed in the same way as described above (Gibbs, Wren et al. 1993). Benefits that can result from this procedure are the avoidance of ECMO therapy within the first weeks after birth as well as gaining time to decide between biventricular (BiV) repair (compare following paragraph) or SV palliation (Akinturk, Michel-Behnke et al. 2007).

A.1.2.3 BiV Repair

Considering long-term complications (Alsaied, Bokma et al. 2017) after the Fontan procedure/ univentricular palliation, BiV circulation can be a valid therapeutic aim in the treatment of patients with borderline left ventricular hypoplasia. This can either be achieved through primary BiV repair in the neonatal period (Tchervenkov, Tahta et al. 1998) or through secondary BiV conversion after initial stages of SV palliation (Kalish, Banka et al. 2013). Another approach is staged left ventricular recruitment (SLVR). This therapeutic strategy includes initial SV palliation and subsequent procedures to gradually recruit left ventricular structures for ultimate BiV conversion for suitable candidates (Emani, McElhinney et al. 2012).

Due to the advancement in surgical techniques, primary heart transplantation and compassionate care are two remaining therapeutic options that are less pursued today.

A.1.3 Postoperative Challenges

Progress in both surgical and medical treatment has significantly improved prognoses of SV patients. While SVP used to be an almost universally fatal diagnosis with extremely high mortality rates, its prognosis has improved significantly with patients now regularly reaching adolescence and adulthood (Mahle, Spray et al. 2000). However, despite improved surgical outcomes in SV patients, numerous remaining postoperative complications with consequences for morbidity and mortality still exist (Jenkins, Gauvreau et al. 2002).

According to a study conducted by Agarwal et al. at least one complication can be observed in 43% of pediatric patients undergoing open heart surgery with or without the use of cardiopulmonary bypass (CPB). These complications lead to a significantly increased length of stay on the cardiac intensive care unit (CICU LOS), longer mechanical ventilation, longer overall hospital stays and higher mortality (Agarwal, Wolfram et al. 2014). Hornik et al. (2011) examined complications occurring specifically after the Norwood Procedure and observed at least one complication in 75% of patients. Mortality increased with increased numbers of complications, ranging from only 7% in patients without complications over 17% in patients with 1 complication up to 45% in patients with 5 or more complications.

Postoperative complications can be divided into those with an either cardiac or extra-cardiac origin (Hornik, He et al. 2011, Agarwal, Wolfram et al. 2014): Among cardiac complications, low cardiac output, arrhythmia, resuscitation, reoperation, left or right heart failure and extracorporeal membrane oxygenation (ECMO) are just some of the occurring severe complications that need intensive care and treatment. Common extra-cardiac complications include acute renal failure, focal neurologic deficits, respiratory insufficiency, sepsis, thrombosis and postoperative bleeding.

When taking a closer look at postoperative bleeding complications and their consequences, their relevance and severity become clear: Reported bleeding incidence rates for early postoperative bleeding in pediatric patients with CHD range from 1.5% to 24% (Andrew, Marzinotto et al. 1994, Kuhle, Eulmesekian et al. 2007, Schechter, Finkelstein et al. 2012, Emani, Zurakowski et al. 2013, Trucco, Lehmann et al. 2015). Consequences of bleeding include the administration of blood products with consecutive risks, surgical intervention, postoperative renal replacement procedures (dialysis), ECMO, prolonged hospital LOS, longer mechanical ventilation and increased mortality (Wolf, Maher et al. 2014, Guzzetta, Allen et al. 2015). Patients who seem to be particularly at risk for hemorrhage are patients with cyanotic CHD, neonates, patients undergoing complex surgical procedures with long periods of CPB and re-sternotomy (Williams, Bratton et al. 1999, Guzzetta, Allen et al. 2015, Ramírez-Flores, Ibarra-Sarlat et al. 2019). All of these risk factors apply to most SV patients on the CICU and in addition to immediate postoperative bleeding occurring early after surgery, they are also at risk for thrombosis and non-surgical related, spontaneous bleeding as outlined in the following paragraphs (Faraoni and Van der Linden 2014).

A.2 Non-Surgical Related Bleeding as an Important Complication in the CICU

Anticoagulation therapy in patients with SVP is initiated early after surgery due to multiple factors leading to an increased risk of thrombosis in patients with CHD and especially SVP (Manlhiot, Brandao et al. 2012, Giglia, Massicotte et al. 2013): These include arrhythmia, hemodynamic abnormalities such as low cardiac output, permanently implanted foreign materials such as mechanical valves, stents or shunts as well as altered hemostatic function. Factors frequently associated with thrombosis include postoperative central venous catheters (13-40% incidence of thrombosis), shunts (8-12% shunt thrombosis with 20% occurring in the immediate postoperative period) or Fontan circulation (17-33% thrombosis after Fontan) (Al Jubair, Al Fagih et al. 1998, Li, Yow et al. 2007, Anton, Cox et al. 2009). Such complications influence further treatment and outcome of SV patients. If possibilities for intravascular access are restricted, future cardiac catheterizations and the insertion of intravenous or intra-arterial lines can be very challenging and in case of failure may lead to fatal outcome (Lui, Saidi et al. 2017). Additionally, thromboses and embolisms may lead to ischemia of extremities, vascular damage, pulmonary embolism or ischemic stroke.

However, specific considerations regarding anticoagulation therapy in pediatric patients with SVP need to be made, since they are prone to both thrombosis and bleeding (Giglia, Massicotte et al. 2013) due to specific abnormalities as explained in the following paragraph.

A.2.1 Non-Surgical Related Bleeding: Underlying Causes

Cardiopulmonary bypass-associated coagulopathy resulting from activation of coagulation factors, systemic inflammatory response and consumptive coagulopathy increase patients' postoperative bleeding risk especially in neonates and infants (Miller, Mochizuki et al. 1997). With cyanotic CHD associated abnormalities in hemostatic proteins and cells complicate treatment even further. However, dosing and monitoring guidelines are often extrapolated from adult studies and hence limited in their applicability. In the following, hemostatic abnormalities that need to be considered in SV patients as well as available treatment and monitoring options for anticoagulation on the CICU are described.

A.2.1.1 Hemostatic Abnormalities in Pediatric Patients with SVP

In addition to physiological variations in the infantile hemostatic system, children with CHD show additional abnormalities regarding hemostasis. These put them at an increased risk for both bleeding and thrombosis. Cyanotic CHD and especially SVP are associated with an even higher incidence of hemostatic abnormalities and resulting adverse events than noncyanotic and acquired CHD. These aberrancies can be seen in irregular levels and counts of proteins and cells as well as in their function leading to hyper- or hypocoagulable states. Factors that contribute to these abnormalities are not entirely known, however in cyanotic CHD it is hypothesized that they are caused by cyanosis or genetic disposition (Odegard, Zurakowski et al. 2009). Chronic venous hypertension and hepatic congestion or decreased perfusion due to low cardiac output as well as protein losing enteropathy have also been proposed as possible causes for abnormal levels of coagulation proteins (Callegari, Christmann et al. 2019). Irregularities in coagulation and fibrinolysis that have been reported, are found in Table 1 (Cromme-Dijkhuis, Hess et al. 1993, Jahangiri, Shore et al. 1997, van Nieuwenhuizen, Peters et al. 1999, Odegard, McGowan et al. 2002, Odegard, McGowan et al. 2002, Odegard, McGowan et al. 2003, Odegard, Zurakowski et al. 2009):

TABLE 1*. Described Abnormalities in the Hemostatic System of Pediatric Patients with CHD.*

		Decreased	Increased	Increased or Decreased
Level	Coagulation Factors	II, V, VII, IX, X, fibrinogen	VIII	
	Coagulation Inhibitors	Protein C, Protein S, Antithrombin		
	Fibrinolytic Proteins	Plasminogen		
Function	Coagulation	X		
	Fibrinolysis			X
	Platelet Count and Function			X

Tempe and Virmani (2002) found higher hematocrit and hemoglobin levels in patients with cyanotic CHD and hypothesized different factors attributed to cyanotic congenital heart disease as underlying causes: Right-to-left shunts lead to decreased arterial oxygen saturation and therefore lower tissue oxygenation. More erythropoietin is released to increase erythrocytosis in order to reach improved tissue oxygenation. The result is polycythemia and increased blood viscosity which itself leads to hypercoagulability. On the other hand, high hematocrit levels are negatively associated with platelet and fibrinogen function leading to an increased bleeding risk. By increased blood viscosity, tissue oxygenation decreases further due to decreased blood flow in small capillary vessels resulting in a vicious circle.

In summary, these factors place patients with SVP at an increased risk for the development of both thrombosis and bleeding, making adequate anticoagulation extremely difficult (Guzzetta, Allen et al. 2015). Current anticoagulation practices in pediatric cardiac surgery patients are further described in the following paragraphs.

A.2.1.2 Anticoagulation Therapy

Multiple agents are used in the immediate postoperative period to prevent thrombotic events including anticoagulant, antiplatelet and fibrinolytic therapy. The description of these agents in the following will be limited to unfractionated heparin (UFH), low-molecular-weight heparin (LMWH, Lovenox ®), acetylsalicylic acid (ASA, Aspirin ®) and Tirofiban (Aggrastat ®), since they were most often used on the CICU at Boston Children's Hospital (BCH) - the institution in which data collection for the research at hand took place. For most of these medications, dosing has been derived from studies done on adults, since no studies with outcomes specifically for pediatric patients have been made to date. Dosing guidelines were published in *Chest* by Monagle et al. (2012):

A.2.1.2.1 UFH

By binding to Antithrombin III and accelerating its reactions, UFH inhibits coagulation factors IIa, IXa, Xa, XIa and XIIa. Its effect is hence dependent on the presence of Antithrombin III and ineffective in case of deficiency. UFH has to be administered parenterally and continuously due to its poor bioavailability. UFH is metabolized hepatically and its metabolites are cleared renally. It is the chosen agent in case of renal insufficiency, its half-life is dose-dependent. It is completely reversible using protamine, making it ideal for the use during CPB and in high risk patients. Therapeutic dosing is

age-dependent with patients younger than one year receiving 28 units/kilogram/hour (U/kg/h) and older patients receiving 20 U/kg/h. Prophylactic/line heparin usually consists of 10-15 U/kg/h. UFH needs to be frequently monitored using either activated thromboplastin time (aPTT), anti-factor Xa activity (anti-Xa) or a combination of both (Oladunjoye, Sleeper et al. 2018). Target ranges for aPTT are 1.5-3 times baseline aPTT and are defined by each laboratory individually, but usually vary between 60-100s. Even though aPTT is used most frequently, anti-Xa monitoring is the gold-standard due to its independence of coagulation protein levels, apart from Antithrombin III (ATIII). Therapeutic levels are usually 0.35-0.7 IU/ml. UFH related side effects include hemorrhage, heparin induced thrombocytopenia (HIT) and potentially osteoporosis.

A.2.1.2.2 LMWH

LMWH is often used after the initial phase of UFH administration during hospital stays or to bridge to other medications such as ASA or Coumadin. Its mode of action is comparable to UFH but with stronger inhibition of FXa and decreased dependence on AT III. It is administered subcutaneously with better bioavailability than UFH. LMWH clearance is purely renal, leading to contraindications in case of renal insufficiency. Its half-life is 3-6 hours. It is only partly reversible using protamine. Dosing is again age-dependent with a lot of controversy in the literature. *Chest* Guidelines recommend 1.5 mg/kg per dose for children younger than two months and 1.0 mg/kg per dose for older patients, Anti-Xa levels should be drawn 4-6 hours after dose administration (Monagle, Chan et al. 2012). There have been numerous studies however, reporting the need for higher doses (especially in preterm infants) in order to reach therapeutic Anti-Xa levels of 0.5-1.0 IU/ml (Fung and Klockau 2010, Schloemer, Abu-Sultaneh et al. 2014, McCormick, Parbuoni et al. 2015).

A.2.1.2.3 ASA

ASA is an irreversible cyclooxygenase (COX) inhibitor of COX-1 and COX-2 preventing the synthesis of thromboxane a2 and hence vasoconstriction and platelet aggregation. It is administered orally and its half-life is dose-dependent. It is most commonly used for thromboprophylaxis and treatment of arterial thrombosis in shunted patients, patients with stents and bioprosthetic valves. Doses recommended by *Chest* guidelines are 1-5 mg/kg per day (Monagle, Chan et al. 2012). Adequate ASA response can be monitored using multiple different assays, however, direct comparison between them showed poor

to no correlation (Schmugge, Speer et al. 2015). Thus, neither use has been recommended by guidelines for pediatric patients so far. Inadequate platelet inhibition measured with VerifyNow™, however, has been demonstrated to significantly correlate with thrombosis occurrence (Emani, Zurakowski et al. 2017). Israels et al. (2003) found clearance of ASA to be prolonged in newborns, potentially leading to an increased bleeding risk especially in combination with other anticoagulant agents. However, an additional effect on the neonatal platelet hyporeactivity could not be observed. In older children, ASA rarely causes major bleeding or other side effects in antiplatelet dosing. Rye syndrome for example seems to be dose-dependent and is associated with doses over 40 mg/kg (Starko, Ray et al. 1980, Baum 1983, Porter, Robinson et al. 1990).

A.2.1.2.4 Tirofiban

Tirofiban (Aggrastat®) is an antiplatelet drug preventing platelet aggregation by inhibiting Glycoprotein IIb/IIIa. Dosing guidelines have not been established for pediatric patients. The drug is administered intravenously, with dosing recommendations of 25 mcg/kg within 5 minutes and then 0.15 mcg/kg/min for up to 18 hours. Half-life is approximately 2 hours with mainly renal excretion. Side effects include hemorrhage, thrombocytopenia, bradycardia, dizziness and edema (MERCK & CO. 1998). Safety and efficacy for pediatric patients have not been established. However, some studies suggest safe use in children (Emani, Pereira et al. 2020) with monitoring done through platelet mapping (Emani, Kaza et al. 2015).

To monitor anticoagulation therapy and to be able to assess a patient's current state of coagulation in order to prevent both bleeding and thrombosis, numerous different assays are used in the CICU, as described in the following paragraphs.

A.2.2 Non-Surgical Bleeding: Anticoagulation Monitoring

While there are numerous different assays to monitor anticoagulation and antiplatelet therapy, the following descriptions are limited to aPTT, Anti-Xa and international normalizes ratio (INR), since they were primarily analyzed in this study in addition to platelet and fibrinogen levels. APTT and Anti-Xa are most commonly used to monitor heparin therapy in pediatric patients. However, no studies have been performed to establish the safety and efficacy of any laboratory test measuring anticoagulation therapy

specifically in children. Target values and heparin doses are extrapolated from adult studies and guidelines.

A.2.2.1 Activated Thromboplastin Time (aPTT)

APTT is a test based on the cascade model, reflecting the intrinsic and common pathway of in vitro coagulation. It is hence limited in its reliability and applicability to the in vivo human hemostatic processes. It can be used to monitor factors (I, II), V, VIII, IX, X, XI and XII and is most commonly used to monitor UFH therapy. Physiological aPTT values range between 20-40s but are defined by each laboratory individually. Target levels for therapeutic heparin are prolonged aPTT levels of 2-3 times baseline aPTT (Giglia, Massicotte et al. 2013). APTT is age-dependent and might be affected by various variables such as coagulation protein deficiencies, high C-reactive protein (CRP) levels (Devreese, Verfaillie et al. 2015) as well as circulating particles such as lupus anticoagulants (Ignjatovic, Furmedge et al. 2006). Some studies have found aPTT to overestimate the effect of heparin (Ignjatovic, Summerhayes et al. 2006, Kuhle, Eulmesekian et al. 2007, Chan, Black et al. 2008).

A.2.2.2 Anti-Factor Xa Activity (Anti-Xa)

Anti-Factor Xa Activity (Anti-Xa) is used to quantify the effects of UFH and LMWH (as well as some new oral anticoagulants) by measuring the heparin-antithrombin complex. It is thus highly dependent on AT III levels, but independent of other biological factors (Olson, Arkin et al. 1998, Garcia, Baglin et al. 2012, Price, Jin et al. 2013). Therefore, it is often used in pediatric patients in addition to aPTT. Recommended ranges for therapeutic UFH dosing are 0.35-0.7 U/ml and 0.5-1.0 U/ml for therapeutic LMWH (subcutaneous as well as continuous i.v. infusion). Even though correlation between aPTT and Anti-Xa levels is high in adults (Byun, Jang et al. 2016), they have been reported to poorly correlate with each other in younger heparinized pediatric patients (Ignjatovic, Summerhayes et al. 2007, Kuhle, Eulmesekian et al. 2007, Newall, Ignjatovic et al. 2010). Since AT III is low in infants (Andrew, Paes et al. 1987, Andropoulos and Fraser 2016, Manlhiot, Gruenwald et al. 2016), Anti-Xa levels are often low even with elevated aPTT (Andrew, Marzinotto et al. 1994, Kuhle, Eulmesekian et al. 2007, Newall, Ignjatovic et al. 2010). Some laboratories therefore add AT III before measuring Anti-Xa levels, which simultaneously obscures AT III deficiency (Nair, Oladunjoye et al. 2018). Also, AT III-independent anticoagulant effects of high heparin doses such as inhibition

of FX activation might not be measured by Anti-Xa (Garcia, Baglin et al. 2012), which might result in an underestimation of the heparin effect.

A.2.2.3 International Normalized Ratio (INR)

The INR is a standardized laboratory test for measuring the extrinsic pathway of coagulation and is derived from a patient's prothrombin time (PT). It avoids the problem of PT not being comparable due to different measurement methods. This is achieved by forming the quotient of a patient's PT and a reference PT which is then standardized for the international sensitivity index (ISI) generated by the World Health Organization (WHO). The INR is part of routine blood coagulation testing and is mainly used to monitor therapy with vitamin k antagonists such as Warfarin or Phenprocoumon. Target ranges vary according to underlying conditions but are usually set to 2.0-3.0 or 2.5-3.5 (Poller 2004). However, values for specific indications in pediatric patients are extrapolated from adult guidelines and haven not been tested in children specifically (Giglia, Massicotte et al. 2013).

A.2.2.4 Platelet Count

Platelets are essential for adequate clot formation in injured vessels and as a result target of multiple agents for the prevention of arterial thrombosis. Platelet counts are routinely measured in children as well as adults undergoing cardiac surgery. Depending on the literature, reference ranges are 150.000-400.000/µl or 150.000-450.000/µl, respectively (Giles 1981, Dacie and Lewis 1991). However, some studies suggest age-dependent target ranges, as there is evidence for declining platelet levels throughout life with the sharpest decrease during infancy (Carlo and Patrizia 2014). In addition to differences in number, infantile platelets seem to be physiologically hyporeactive to certain procoagulant agonists (Michelson 1998) and hence differ in function compared to adult platelets.

Children with CHD show even more platelet abnormalities: Various studies have shown decreased platelet levels in patients with CHD (Maurer, McCue et al. 1975, Colon-Otero, Gilchrist et al. 1987, Horigome, Hiramatsu et al. 2002). Especially children with cyanotic congenital heart disease seem to have lower counts as well as impaired platelet function. Platelet counts have also been shown to negatively correlate with hematocrit levels. Disseminated intravascular coagulation (DIC) has been hypothesized to be triggered by polycythemia and hyperviscosity and to be the reason for this thrombocytopenia (Maurer,

McCue et al. 1975, Horigome, Hiramatsu et al. 2002). In addition, platelet survival in children with cyanotic CHD seems to be decreased with a life-span of less than 80 hours, compared to 80-130 hours in physiological platelets (Waldman, Czapek et al. 1975, Peters, Rozkovec et al. 1982). On the other hand, hypoxemia and cyanosis are hypothesized to play a role in the activation of neutrophils, who subsequently activate platelets by releasing chemotactic and vasoactive agents that cause endothelial damage (McLeod, Martin et al. 1994). However, associations between platelet function or count and non-surgical related hemorrhage have not been studied in pediatric SV patients after congenital heart surgery.

A.2.2.5 Fibrinogen

Fibrinogen and fibrin play an important role in clot formation by contributing to platelet aggregation and building a fibrin network. Normal range is 200-400 g/l, however, ranges vary among laboratories (Oswald, Hunt et al. 1983).

Impaired fibrinogen function has been described in adults with CHD. Despite normally high fibrinogen levels, impaired fibrinogen function has been seen in adults with CHD using thromboelastography (TEG). TEG results were correlated negatively with hematocrit levels, indicating negative effects of hyperviscosity on fibrinogen function and clot formation. Hypocoagulability was found to be linked mainly to fibrinogen dysfunction. Thrombocytopenia on the other hand, if present, was not associated with major platelet dysfunction (Jensen, Johansson et al. 2013). In adults undergoing cardiac surgical procedures, fibrinogen levels of less than 220 mg/dl were found to significantly correlate with severe postsurgical bleeding (Ranucci, Pistuddi et al. 2016). Other studies suggest increased bleeding risks for pediatric patients with post-CPB fibrinogen levels of less than 150 g/l (Faraoni, Willems et al. 2014, Ranucci, Bianchi et al. 2019). Additionally, increased levels of d-dimers as shown in previous studies, indicate increased fibrinolysis in patients with cyanotic CHD (Goel, Shome et al. 2000, Levin, Wu et al. 2000).

No studies have been performed examining fibrinogen function specifically in SV children after cardiac surgery. There have also been no investigations to date of the relationship of low fibrinogen levels and the incidence of bleeding after pediatric congenital heart surgery for SVP.

A.2.2.6 Thromboelastography (TEG®)

In addition to standard laboratory tests such as aPTT, Anti-Xa, platelet count and fibrinogen levels, thromboelastography is a bedside test that can be used to depict the current state of coagulation and has gained importance especially in critically ill patients during surgery or in the CICU (Chitlur and Lusher 2010). This test helps to assess the interaction between coagulation proteins as well as fibrinogen and platelets and can be used to identify the underlying defect of the hemostatic system. It allows better understanding of a patient's individual hemostasis and need for specific blood products or medications and can hence ease difficult decisions in the treatment of bleeding (Dias, Sauaia et al. 2019).

A cup which has been adapted to the patient's body temperature is filled with 0.36 cc of whole or citrate blood. A pin is then introduced into the sample and an electronic device analyzes the properties of the blood sample while the cup is spinning and a clot is forming. The device generates a curve (compare Figure 3) with different parameters that depict the different stages of coagulation (Kouerinis, Kourtesis et al. 2008):

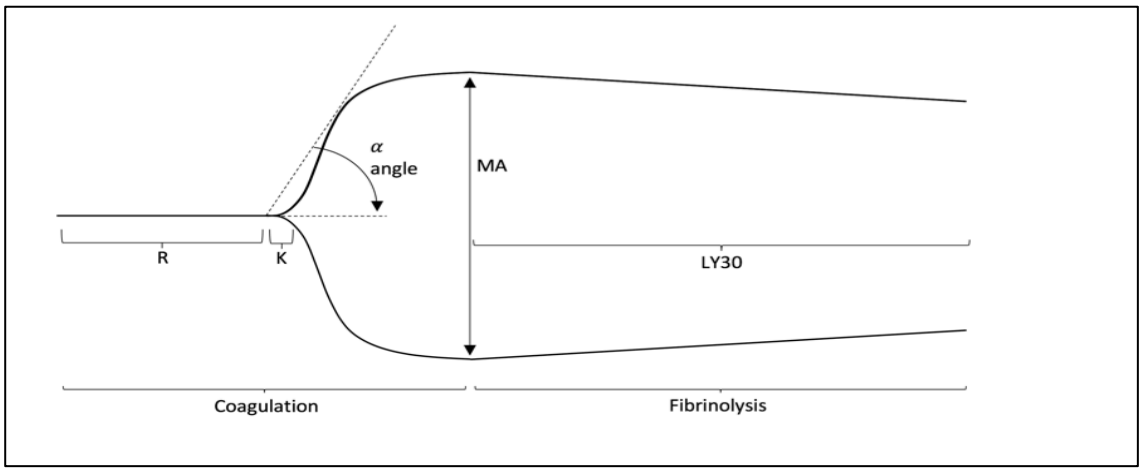

***FIGURE 3**: Normal Thromboelastography (TEG®).*

The main parameters that can be obtained and their normal ranges include (Vig, Chitolie et al. 2001):

1. Reaction time (R-time): The time from the start of coagulation to a 2 mm amplitude. This depicts the function of the intrinsic pathway: Anticoagulants such as heparin or coumadin lead to longer R-time, in case of hypercoagulability of any sort it is shortened. The normal range is between 4 to 8 min.

2. Kinetic clot time (K-time): The time from the begin of coagulation until the TEG amplitude reaches a clot strength of 20 mm. It is shortened in case of platelet hyperreactivity and prolonged in the presence of antiplatelet agents or in case of factor deficiency. Range: 1 to 4 min.
3. Alpha angle (α-angle): This angle shows the speed of fibrinogen cross-linking and represents the rate of clot formation indicating platelet and fibrinogen function. It is small in the presence of anticoagulants or platelet inhibiting agents and greater in case of high platelet and fibrinogen activity. Range: 47 to 74°.
4. Maximum amplitude (MA): MA is the maximum diameter of the clot and increases with higher platelet, fibrinogen and FXIII quality. It is decreased in case of platelet or fibrinogen dysfunction. Range: 55 to 73 mm.
5. G-Parameter: This parameter is derived from MA and is an indicator for clot strength. It is an absolute number and ranges from 6 to 13.
6. Clot lysis index (Ly30): An index that indicates the amount of clot showing fibrinolysis 30 minutes after MA. Its range is 0 to 8%.

As has been delineated, the relationship between both thrombocytopenia as well as hypofibrinogenemia and the occurrence of bleeding after pediatric congenital heart surgery for SV patients may be especially relevant for ameliorating post-operative care and have not received much attention in the literature, since most available data focuses on immediate postoperative hemorrhage occurring within 24 hours after surgery (Agarwal, Wolfram et al. 2014, Faraoni and Van der Linden 2014, Savan, Willems et al. 2014, Wolf, Maher et al. 2014). Therefore, the aim of the study at hand was to address these questions and conduct a statistical analysis of the described associations. A more in-depth explanation of the objectives of the research is given in the following.

B Objectives

Bleeding and thrombosis are severe complications which may occur during the postoperative period after congenital heart surgery for pediatric patients with SVP (Giglia, Massicotte et al. 2013). Associated factors like cyanosis, prematurity, complex surgical procedures and CPB associated coagulopathy as well as an immature and altered hemostatic system increase these patients' risk significantly (Guzzetta and Miller 2011, Manlhiot, Brandao et al. 2012).

While anticoagulation therapy in children can be quite difficult per se, it poses even greater challenges for the treating medical staff in this fragile patient population. There is only a small amount of research focusing on anticoagulation management in pediatrics, let alone in SV patients. Dosing and monitoring guidelines are mostly extrapolated from adult studies and limited in their applicability to this heterogenous pediatric group. Established laboratory parameters like aPTT and Anti-Xa seem to be unreliable, they tend to over- or underestimate actual heparin levels and often do not correlate with bleeding or thrombosis outcomes (Guervil, Rosenberg et al. 2011, Trucco, Lehmann et al. 2015, Oladunjoye, Sleeper et al. 2018).

The current study extended previous research on anticoagulation therapy and monitoring in SV patients after congenital heart surgery by analyzing associations with non-surgical-related bleeding events. The aim was to assess the most commonly used anticoagulation medications as well as well-established monitoring parameters and investigate the reliability of aPTT and Anti-Xa in heparinized pediatric patients with SVP. Furthermore, the aim was to assess the role of platelet and fibrinogen levels as well as their function in the development of spontaneous bleeding on the CICU. The hypothesis was that aPTT and Anti-Xa levels are not able to reliably depict a heparinized SV patient's coagulatory state and that thrombocytopenia and hypofibrinogenemia play important parts in the development of non-surgical related bleeding in pediatric patients with SVP.

C Materials and Methods

C.1 Study Design

This was a retrospective, single center, case-control study, conducted in the CICU at BCH. A database of all pediatric SV patients admitted to the CICU after open heart surgery for congenital heart disease at BCH was created with regards to anticoagulation therapy. This database was initiated for quality improvement purposes and all clinical care including laboratory measurements were part of routine practices.

C.2 Patient Cohort

Pediatric patients undergoing cardiac surgery for SVP at BCH who were postoperatively admitted to the CICU between November 2016 and March 2018 were included in this study.

Patients were excluded when older than 18 years or when they had a history of known coagulopathy before admission. Data of patients who were admitted to the CICU on ECMO or VAD were not collected. For patients on ECMO or VAD after CICU admission but during their CICU stay, all patient days (PD) on ECMO/VAD were excluded from analyses. All patients whose spontaneous bleeding event occurred on the day of surgery were excluded from analyses, due to the lack of laboratory values 24 hours prior to the event. All provoked bleeding events (see below for definition), as well as all PD following the first unprovoked bleeding event were excluded.

C.3 Data Collection

Data was collected from November 2016 to February 2018. Data for every patient was collected from the point of CICU admission after surgery until the day of discharge from the CICU. The need for informed consent was waived for this study. To review and report the results, the approval of BCH's Institutional Review Board (IRB) was obtained on 10/12/2016, IRB number: IRB-P00024192 for data collection and on 3/19/2018, IRB number: IRB-P00028336 for the research at hand. Data were collected according to the Geneva Convention. Study data were collected and managed using REDCap electronic data capture tools version 8.0.3 - © 2018 Vanderbilt University hosted at BCH (Harris, Taylor et al. 2009, Harris, Taylor et al. 2019). REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for

research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

A new anonymous record with a unique record-ID was created in REDCap for each patient who was admitted to the CICU. Into this record all relevant information regarding the patient's coagulation status was entered for admission and then for each day of the CICU stay.

C.3.1 Data Collection on Admission

On admission, demographic data, as well as information on diagnosis, surgery and coagulation were collected, as described in more detail in Tables 2 and 3.

TABLE 2. *Collected Demographic Data on Admission.*

Demographic Data	
MRN Date of birth Gender	Weight and Height on Admission Admission Date

MRN, medical record number.

TABLE 3. *Collected Diagnoses, Surgeries and clinical information on Admission.*

Data on Diagnoses and Surgeries	
Diagnosis • HLHS • DILV • DORV • CAVC • Unbalanced AVC • TOF • TGA • Valvular Disorders • TAPVR, Pulmonary Vein Stenosis • LVOTO • RVOTO • Shone's Complex • Ebstein's Anomaly • Coarctation • Tricuspid Atresia • Other	Type of Surgery • Stage I • Stage II • Fontan • BiV Conversion • Other Clinical Information • History of bleeding • History of thrombosis • Possible ongoing postsurgical bleeding

SV, Single Ventricle; HLHS, Hypoplastic Left Heart Syndrome; DILV, Double Inlet Left Ventricle; DORV, Double Outlet Right Ventricle; CAVC, Complete Atrioventricular Canal; AVC, Atrioventricular Canal; TOF, Tetralogy of Fallot; TGA, Transposition of Great Arteries TAPVR, ASD, Atrial Septal Defect; PFO, Persistent Foramen Ovale; PAPVR, Partial Anomalous Pulmonary Venous Return; VSD, Ventricular Septal Defect; PDA, Persistent Ductus Arteriosus; Total Anomalous Pulmonary Venous Return; LVOTO, Left Ventricular Outflow Tract Obstruction; RVOTO, Right Ventricular Outflow Tract Obstruction; IAA, Interrupted Aortic Arch; BiV, Biventricular; ASO, Arterial Switch Operation; AVR, Aortic Valve Replacement/ Repair; MVR, Mitral Valve Replacement/ Repair; PVR, Pulmonary Valve Replacement/ Repair; TVR, Tricuspid Valve Replacement/ Repair; VAD, Ventricular Assist Device.

For statistical reasons information on only one diagnosis and one surgical procedure was collected for every patient on admission. In case a patient had multiple different preoperative diagnoses, the leading diagnosis for which the patient received surgery was chosen.

C.3.2 Daily Data Collection

Information on medication and lines (see Table 4) as well as laboratory values (see Table 5) were collected for every SV patient admitted to the CICU.

C.3.2.1 Medications and Lines

TABLE 4: *Daily Collected Medications.*

Medication
UFH
No UFH
Low dose UFH (<15 u/hr/kg)
High dose UFH (≥ 15 u/hr/kg)
ASA (Aspirin ®)
Tirofiban (Aggrastat ®)
LMWH (Lovenox ®)
Bivalirudin (Angiomax ®)
Clopidogrel (Plavix ®)
Argatroban (Argatra ®)
Fondaparinux (Arixtra ®)
Warfarin (Coumadin ®)

UFH, unfractionated heparin; u/hr/kg, units/hour/kilogram; ASA, Acetylsalicylic acid; LMWH, Low Molecular Weight Heparin

The initial anticoagulation strategy was decided upon and initiated by the attending surgeon and CICU staff and then performed according to BCH's implemented anticoagulation protocol as shown in Figure 4 (Nair, Oladunjoye et al. 2018).

The strategical decision was made for each patient individually depending on the performed surgery, the patient's medical history, known coagulopathy, laboratory values and structural characteristics such as shunts or mechanical valves. Anticoagulation therapy was started six hours after admission if postsurgical bleeding was mild. Patients with a high risk for thrombosis (Stage I, Fontan, atrial-switch operation, prosthetic valves except pulmonary valve in a young child) received high dose UFH (≥ 15 u/hr/kg). If the surgeon and treating intensivists decided upon high dose UFH, a coagulation panel consisting of aPTT, INR, PT and ATIII was sent and evaluated before starting UFH with dosing based on the patient's age (<1 year: 28 u/hr/kg and >1 year: 20 u/hr/kg). Coagulation laboratory values were checked according to protocol.

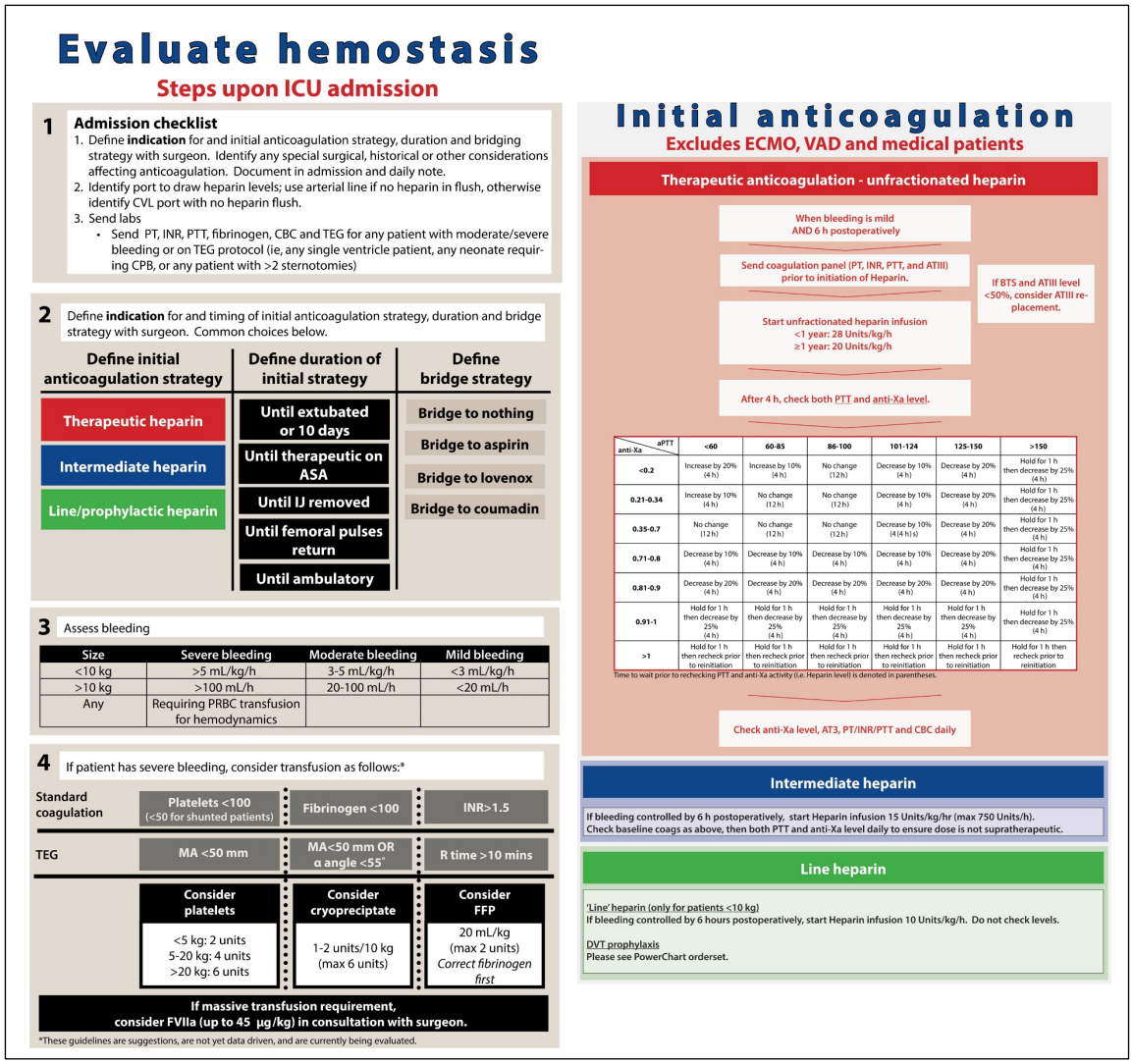

FIGURE 4. *Anticoagulation Strategies on the CICU at BCH.*

ICU, intensive care unit*; CVL,* central venous line*; PT,* prothrombin time*; INR,* international normalized *ratio; PTT,* partial thromboplastin time*; CBC,* complete blood count*; TEG,* thromboelastography*; CPB,* cardiopulmonary bypass*; ASA,* aspirin*; IJ,* internal jugular*; PRBC,* packed red blood *cells; MA,* maximum amplitude; *Max,* maximum*; FVIIa,* activated factor VIIa*; ECMO,* extracorporeal membrane oxygenation*; VAD,* ventricular assist device*; AT,* antithrombin*; BTS,* Blalock-Taussig shunt*; anti-Xa,* antifactor Xa activity*; CBC,* complete blood count*; DVT,* deep venous thrombosis.

Note: From "An anticoagulation protocol for use after congenital cardiac surgery" by A. Nair et al., 2018, *The Journal of Thoracic and Cardiovascular Surgery, 156, p. 345.* Copyright 2018 by The American Association for Thoracic Surgery. Reprinted with permission.

Low dose UFH (<15 u/hr/kg) was only administered in patients younger than ten years and levels were not routinely checked in this group. For data collection, the highest UFH dose given in 24 hours determined the overall UFH purpose (no UFH, low dose, high dose) for that day.

Bridging strategies were started either following extubation or by postoperative day 10, whichever came first. Starting doses of ASA were weight based: <10kg: 40.5 mg; 10-25 kg: 81 mg and >25 kg: 162 mg per oral or gastric route and checked with VerifyNow™ (Instrumentation Laboratory, Bedford, MA).

Bridging to LMWH was done according to protocol with a starting dose based on the patient's age: (1) Postmenstrual age (PMA) <37 weeks and <2 months: 2 mg/kg/dose subcutaneously administered every (SQ q) 12 hours. (2) PMA ≥37 weeks and <2 months: 1.7 mg/kg/dose SQ q 12 hours. (3) ≥ 2 months through adulthood: 1mg/kg/dose SQ q 12 hours. Monitoring was done based on Anti-Xa levels.

C.3.2.2 Laboratory Values

TABLE 5. *Collected Laboratory Values and Transfusions.*

Laboratory Values	
aPTT	Transfusions • pRBC • Platelets • Plasma • Cryoprecipitate • Albumin
Anti-Xa levels	
INR levels	
Fibrinogen levels	
Platelet counts	
TEG®	

aPTT, activated Partial Thromboplastin Time; Anti-Xa, Anti-factor Xa activity; INR, International Normalized Ratio; TEG, thromboelastography; pRBC, packed red blood cells.

Frequency, time and result of all laboratory values as well as given transfusions were collected. Therapeutic ranges for aPTT and Anti-Xa levels were defined as 60-80 s and 0.3-0.7 IU/ml, respectively. In certain high-risk patients defined by the treating surgeon, TEG® (Haemonetics, Braintree, MA) values were drawn 24 hours after admission to the CICU to assess a patient's coagulatory state. Other laboratory values like ATIII or VarifyNow™ were occasionally measured in patients but were not included in this study.

C.3.2.3 Bleeding Events

On every day of a patient's CICU stay, possible bleeding events were investigated. In order to study such events, a number of different factors in patients' medical records were examined on every day of their CICU stays:

1) A patient's hematocrit and hemoglobin levels within the past 24 hours as well as administration of blood products.
2) Diagnostic studies (Ultrasounds (US), Doppler US, Computed Tomography (CT) scans, Magnetic Resonance Imaging (MRI), Catheter reports etc. showing any signs of bleeding.
3) A change in anticoagulation therapy like decreases or increases in dosing.
4) Nursing notes, doctors' notes and consults with signs of possible bleeding events.

In case of a bleeding event, they were categorized and specified further, as described in the following.

Since the focus of this study lay on spontaneous bleeding events unrelated to surgery, all bleeding events were classified as either "provoked" (through a procedure) or "unprovoked" (possibly related to anticoagulation) during data collection. Bleeding events were classified as "provoked" if they were likely related to any procedural manipulation. This included all bleeding events within 36 hours after the initial surgery, as well as bleeding occurring after line removal or placement, catherization, chest closure, chest tube removal or placement, nasogastral/ nasojejunal placement, instrumentation, intubation, etc. However, bleeding events within 36 hours of surgery were seen as unprovoked if the initial bleeding after surgery had subsided and a new bleeding event occurred after UFH had been started. Events were generally classified as "unprovoked", if the bleeding occurred without any intervention. Bleeding events were then further specified as "minor", "clinically relevant non-major (crnm)" or "major". To categorize a bleeding event in this way, Table 6 was used (Nair, Oladunjoye et al. 2018).

TABLE 6. *Classification of Bleeding Events.*

Minor
Bleeding that is not actionable and does not cause unscheduled performance of studies or any treatment
Clinically relevant non-major
Any overt, actionable sign of hemorrhage that does not meet the criteria for "major" but does meet only of the following: - Non-surgical, medical intervention - Increased level of care (i.e. dressing change) - Prompting evaluation (laboratory or imaging) - Elective transfusion
Major
- Emergent transfusion of blood products for bleeding with hemodynamic instability - Bleeding requiring surgical intervention for control - New or acute on chronic intracranial hemorrhage (except germinal matrix hemorrhage) - Fatal bleeding

This table was used in order to achieve a consistent classification of the severity of bleeding events.

Note: Adapted from "An anticoagulation protocol for use after congenital cardiac surgery" by A. Nair et al., 2018, *The Journal of Thoracic and Cardiovascular Surgery, 156, p. 345*. Copyright 2018 by The American Association for Thoracic Surgery. Reprinted with permission.

C.4 Data Analysis and Statistical Methods

C.4.1 Analyzed Variables

Non-surgical related, unprovoked bleeding including all minor, crnm and major bleeding events served as the binary dependent variable (0=no bleeding, 1=bleeding). In total, 21 independent variables were used for descriptive statistics, univariate and multivariate analyses to identify possible risk factors for non-surgical related bleeding. Compare Table 7 for all variables included in analyses.

Table 7. *Analyzed Variables.*

Dependent Variable
Bleeding Patient (yes vs. no)
Independent Variables
Demographic Variables
Age
Male Gender (yes vs. no)
Weight
Height
Clinical Parameters
Diagnosis
HLHS
DORV
Valvular Disorders
Unbalanced AVC
DILV
RVOTO
Triscuspid Atresia
CAVC
Ebstein's Anomaly
TGA
Shone's Complex
LVOTO
Coarct
ToF
TAPVR
Other
Surgery Category
Stage I
Stage II
Fontan
BiV Conversion
Other
History of Bleeding

History of Thrombosis
Medication
UFH
LMWH
ASA
Tirofiban
Bivalirudin
Clopidogrel
Fondaparinux
Argatroban
Laboratory Values
aPTT
Anti-Xa
INR
Fibrinogen Level
Platelet Level

HLHS, Hypoplastic left heart syndrome; DORV, Double Outlet Right Ventricle; AVC, Atrioventricular Canal; DILV, Double Inlet Left Ventricle; RVOTO, Right Ventricular Outflow Tract Obstruction; CAVC, Complete Atrioventricular Canal; TGA, Transposition of Great Arteries; LVOTO, Left Ventricular Outflow Tract Obstruction; Coarct, Coarctation; ToF, Tetralogy of Fallot; TAPVR, Total anomalous venous return; BiV, biventricular; UFH, unfractionated heparin; LMWH, low molecular weight heparin; ASA, acetylsalicylic acid; aPTT, activated partial thromboplastin time; Anti-Xa, Anti-Factor Xa Activity; INR, international normalized ratio.

C.4.2 Definition of Laboratory Ranges and Group Administration

For the identification of risk factors for postoperative bleeding events in pediatric single ventricle patients, patients were retrospectively categorized into groups regarding their medications, UFH dosing, aPTT levels, Anti-Xa levels, INR levels, platelet counts and their fibrinogen levels.

Ranges were derived from the core laboratory with physiological levels serving as base for categorization (aPTT levels of 25-35s, Anti-Xa levels of 0.3-0.7 IU/ml, platelet counts of 200.000 - 400.000/µl and fibrinogen levels of 200 - 400 mg/dl). INR levels were categorized based on FFP transfusion triggers in case of bleeding (compare Figure 4) and literature review. See Table 8 for further information.

TABLE 8. *Patients' Group Administration for Statistical Analyses.*

UFH Dose	Anti-Xa
1. No UFH 2. < 15 u/kg/hr 3. ≥ 15 u/kg/hr	1. < 0.30 IU/ml 2. 0.30-0-70 IU/ml 3. > 0.70 IU/ml
aPTT	**INR**
1. < 25s 2. 25-35s 3. > 35s 4. > 70s 5. > 100s 6. > 150s	1. < 1.5 2. ≥ 1.5
	Platelet Level
	1. < 200.000/µl 2. 200.000-400.000/µl 3. > 400.000/µl
	Fibrinogen Level
	1. < 200 g/l 2. 200-400 g/l 3. > 400 g/l

UFH, Unfractionated heparin; *u/kg/hr*, units/kilogram/hour; *aPTT*, partial thromboplastin time; *s*, seconds; *Anti-Xa*, Anti-factor Xa; *IU/ml*, International Units per milliliter; *INR*, international normalized ratio; *µl*, microliter; *g/l*, gram/liter

In general, in bleeding patients all PD after the first unprovoked bleeding event were excluded from analyses. Regarding their UFH dose, patients were separated into three groups: no UFH, low dose UFH (<15 u/kg/hr) and high dose UFH (≥ 15 u/kg/hr). Patients without an adverse event were categorized based on the longest duration of a certain UFH dose given during the entire CICU stay. If a patient was in two different categories for the same amount of time, the higher dose determined group allocation. For bleeding patients, the highest dose given within 24 hours before the first event was used.

Regarding laboratory tests, non-bleeding patients were categorized according to their highest aPTT/ Anti-Xa/ INR levels and correspondingly to their lowest platelet/fibrinogen levels ever drawn during their CICU stays. For bleeding patients their highest aPTT/ Anti-Xa/ INR level and accordingly their lowest platelet/ fibrinogen level drawn within 24 hours ahead of the event were used.

C.4.3 Statistical Analysis

Data was analyzed using SPSS statistics versions 24 and 26 (IBM Corp., Armonk, NY). A *p*-value of less than 0.05 was used to demonstrate statistical significance in all analyses.

C.4.3.1 Missing Data

Due to the retrospective manner of this study, complete data could not be obtained for all variables. Therefore, missing values for all variables of interest were analyzed.

***TABLE 9**. Incomplete data stratified by bleeding status.*

Variable	Missing, n (%)		
	Total, n=266	**Bleeding Patients, n=49**	**Non-Bleeding Patients, n=217**
Dependent Variable			
Bleeding Outcome	0	0	0
Independent Variables			
Age	0	0	0
Gender	1 (0.4)	0	1 (0.5)
Weight	1 (0.4)	0	1 (0.5)
Height	1 (0.4)	0	1 (0.4)
Diagnosis	0	0	0
Surgery Category	0	0	0
History of Bleeding	1 (0.4)	0	1 (0.5)
History of Thrombosis	0	0	0
UFH	0	0	0
LMWH	0	0	0
ASA	0	0	0
Tirofiban	0	0	0
Bivalirudin	0	0	0
Clopidogrel	0	0	0
Fondaparinux	0	0	0
Argatroban	0	0	0
aPTT	22 (8.3)	14 (28.6)	8 (3.7)
Anti-Xa*	102/252* (40.5)	26/47* (55.3)	75/205* (36.6)
INR	38 (14.3)	13 (26.5)	25 (11.5)
Platelet Count	5 (1.9)	4 (8.2)	1 (0.5)
Fibrinogen Level	45 (16.9)	25 (51.0)	20 (8.1)
Additional Variables			
LOS	0	0	0
TEG^{+}	0	0	0

**only measured in heparinized patients (total n=252; bleeding patients n =47; non-bleeding patients n=205).*
$^{+}$only measured in patients on clinical TEG protocol (total n=110; bleeding patients n=27; non-bleeding patients n=83); aPTT, activated thromboplastin time; Anti-Xa, Anti-Factor Xa; INR, international normalized ratio; LOS, length of stay; TEG, thromboelastography.

C.4.3.2 Descriptive Statistics and Univariate Analysis

Baseline descriptive statistics were calculated for all variables of interest stratified by bleeding status. Distributions of possible risk factors were explored using histograms and boxplots for continuous variables and contingency tables and pie charts for categorical variables. Data are presented as median and interquartile range (IQR) in non-parametric variables, as mean and standard deviation (SD) in parametric variables or as counts and percentages (%) in categorical variables. Box plots are presented as median, IQR, minimum and maximum without outliers. Shapiro-Wilk tests and Q-Q plots were performed to assess normal distributions of continuous variables in bleeding and non-bleeding patients.

Univariate analyses were then performed to obtain initial estimates about the associations between potential risk factors and bleeding. *P*-values were determined using Mann-Whitney U or Kruskal-Wallis test for continuous and chi-square or fisher's exact test for categorical variables. Kruskal-Wallis test was used for the comparison of independent variables with more than two groups. Fisher's exact test was used for dichotomous variables, if expected values were below 5 in over 20% of cells (Bewick, Cheek et al. 2004). *P*-values for tables larger than 2x2 and up to 5x5 were calculated using chi-square test.

C.4.3.3 Multivariate Analysis

Multivariate binary logistic regressions were used to determine independent associations of variables with bleeding. Variables with a *p*-value of <0.05 in univariate analysis were considered for the model. Continuous variables with missing values exceeding 20% and categorical variables with a frequency of less than 5% were excluded from multivariate analysis to avoid the risk of model overdetermination. Assumptions for binary logistic regression were tested in all variables considered for the model, as appropriate: Multicollinearity was examined by using the variance inflation factor and bivariate correlation. The cutoffs were set at 10 and 0.70 (Tabachnick and Fidell 2001, Field 2013). Testing for linear associations between independent variables and the logit of the probability of the dependent variable was done using Box-Tidwell transformation. Therefore, the interactions of continuous predictors with their natural logarithm were included in the model as separate variables. The identification of outliers was performed by analyzing standardized residuals with a cut-off set at ±3 as well as Cook's distances

with a cut-off set at 1. Categorical variables with more than two categories were transformed into dummy variables and added to the model. The classification threshold for logistic regression was set at 0.4.

The results are presented as regression coefficients, Wald's χ^2, odds ratios (ORs) with 95% confidence intervals (CIs) and *p*-values. Adjusted predicted bleeding probabilities for significant continuous variables were extrapolated from the regression model. To demonstrate the model's accuracy, sensitivity, specificity, positive predictive value, negative predictive value and receiver operating characteristic (ROC) curve were calculated. Nagelkerke's R^2 is presented to demonstrate variance in bleeding explained by the model. Cut-off values for continuous variables with regard to bleeding were calculated using ROC curves with optimal values for sensitivity and 1-specificity. Values were stratified by categorical variables associated with the outcome.

D Results

D.1 Patient Cohort

A total of 288 patients with SVP were admitted to the CICU at BCH after undergoing cardiac surgery between November 2016 and February 2018. Twentytwo patients had to be excluded from analyses due to age, known coagulopathy, ECMO/VAD therapy or bleeding occurring on the day of surgery (compare C.2). This resulted in a total number of 266 SV patients included in analyses, as depicted in Figure 5.

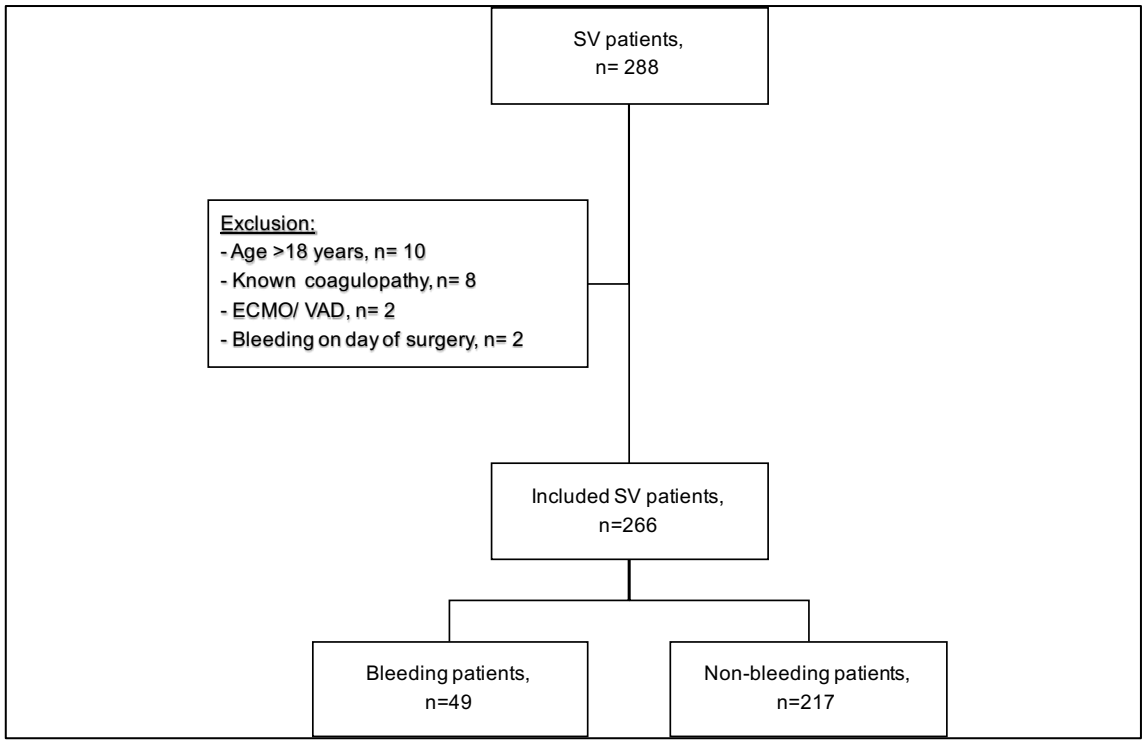

***FIGURE 5**. Criteria of Inclusion and Exclusion for Analyses.*

Flow diagram of patients included in this study. SV, Single Ventricle; ECMO, Extracorporeal Membrane Oxygenation; VAD, Ventricular Assist Device.

During these 266 SV patients' CICU stays following cardiac surgery, 105 bleeding events were observed, of which 63 were unprovoked. The majority of these unprovoked events was classified as clinically relevant non-major (32/63, 50.8%), followed by 30.2% (19/63) minor and 19.0% (12/63) major bleedings (compare Table 6 for definitions). In total, 18.4% (49/266) of patients developed one or more bleeding, occurring after a median of 4 days in the CICU. For further details compare Table 10.

TABLE 10. Adverse Events regarding Coagulation in SV Patients in the CICU.

Variable	Value n (%)
Bleeding Events	105
Unprovoked	63 (58.9)
Minor	19 (30.2)
Crnm	32 (50.8)
Major	12 (19.0)
Bleeding Patients	49 (18.4)
Minor	12 (24.5)
Crnm	27 (55.1)
Major	9 (18.4)
Day of Bleeding Event (IQR)	4 (2-6)

N, number; Crnm, clinically relevant non-major, IQR, interquartile range. Numbers presented as absolute numbers (%), continuous variables presented as median (IQR).

D.2 Descriptive Statistics: Patient Characteristics stratified by Bleeding Outcome

In the following, patient characteristics are presented stratified by bleeding outcome and assorted into demographic and clinical characteristics as well as medication and laboratory values.

D.2.1 Demographic Characteristics

The median age at point of surgery was 1 year (IQR 0.3-3.6), 59.8% (159/266) of patients were male. Median weight and height were 9.0 kg (IQR 5.2-14.6) and 72.8 cm (IQR 59.0-95.6), respectively. For further details on differences in demographic characteristics among bleeding and non-bleeding patients compare Table 11.

TABLE 11. Demographic characteristics of pediatric SV patients stratified by bleeding outcome.

	Total	Bleeding Patients	Non-Bleeding Patients
Patients, n	266	49	217
Age, y (IQR)	1.0 (0.3-3.6)	0.7 (0.04-3.7)	1.2 (0.4-3.6)
Age Group, n (%)			
Neonates	49 (18.4)	13 (26.5)	36 (16.6)
1mo-<1yr	84 (31.6)	14 (28.6)	70 (32.3)
1yr-<10yrs	115 (43.2)	8 (36.7)	97 (44.7)
≥10yrs	18 (6.8)	4 (8.2)	14 (6.4)
Male gender, n (%)	159 (59.8)	26 (53.1)	133 (61.3)
Weight, kg (IQR)	9.0 (5.2-14.6)	7.9 (3.4-14.5)	9.2 (5.4-14.6)
Height, cm (IQR)	72.8 (59.0-95.6)	66.5 (51.0-97.8)	74.3 (60.1-95.9)

N, number; y, years; IQR, interquartile range; mo, month; yr, year; yrs, years; kg, kilogram; cm, centimeter.

D.2.2 Clinical Characteristics

Most patients (30.8%, 82/266) were treated for HLHS, followed by 19.9% DORV (53/266) and 18.8% valvular disorders (50/266). Stages of the Fontan palliation were performed in 55.6% of patients (150/266), 25.2% patients underwent a biventricular repair/conversion (67/266). Compare Table 12 for further details.

TABLE 12. *Clinical characteristics of pediatric SV patients stratified by bleeding outcome.*

	Total	**Bleeding Patients**	**Non-Bleeding Patients**
Patients, n	266	49	217
		n (%)	
Diagnosis			
HLHS	82 (30.8)	17 (34.7)	65 (30.0)
DORV	53 (19.9)	5 (10.2)	48 (22.1)
Valvular Disorders	50 (18.8)	11 (22.4)	39 (18.0)
Unbalanced AVC	21 (7.9)	7 (14.3)	14 (6.5)
DILV	15 (5.6)	2 (4.1)	13 (6.0)
RVOTO	9 (3.4)	2 (4.1)	7 (3.2)
Triscuspid Atresia	9 (3.4)	2 (4.1)	7 (3.2)
CAVC	5 (1.9)	0	5 (2.3)
Ebstein's Anomaly	4 (1.5)	0	4 (1.8)
TGA	4 (1.5)	1 (2.0)	3 (1.4)
Shone's Complex	2 (0.8)	0	2 (0.9)
LVOTO	2 (0.8)	0	2 (0.9)
Coarct	1 (0.4)	0	1 0.5)
ToF	1 (0.4)	0	1 (0.5)
TAPVR	1 (0.4)	1 (2.0)	0
Other	7 (2.6)	1 (2.0)	6 (2.8)
Surgery Category			
Stage I	40 (15.0)	12 (24.5)	28 (12.9)
Stage II	67 (25.2)	5 (10.2)	62 (28.6)
Fontan	43 (16.2)	6 (12.2)	37 (17.1)
BiV Conversion	67 (25.2)	16 (32.7)	51 (23.5)
Other	49 (18.4)	10 (20.4)	39 (18.0)
History of Bleeding	13 (4.9)	3 (6.1)	10 (4.6)
History of Thrombosis	52 (19.5)	7 (14.3)	45 (20.7)

N, number; HLHS, Hypoplastic Left Heart Syndrome; DORV, Double Outlet Right Ventricle; AVC, Atrioventricular Canal; DILV, Double Inlet Left Ventricle; RVOTO, Right Ventricular Outflow Tract Obstruction; CAVC, Complete Atrioventricular Canal; TGA, Transposition of Great Arteries; LVOTO, Left Ventricular Outflow Tract Obstruction, Coarct, Coarctation; ToF, Tetralogy of Fallot; TAPVR, Total anomalous venous return; BiV, biventricular.

D.2.3 Medication

UFH, LMWH and ASA were most frequently given to pediatric patients during their postoperative CICU stay at BCH followed by Tirofiban, Bivalirudin, Clopidogrel, Fondaparinux and Argatroban. Four patients received Coumadin during their CICU stay, however, it was initiated after their first unprovoked bleeding event and therefore not

included in analysis. Compare Table 13 for further details on administered medications during pediatric SV patients' CICU stays.

TABLE 13. Administered medication in pediatric SV patients stratified by bleeding outcome.

	Total	Bleeding Patients	Non-Bleeding Patients
Patients, n	266	49	217
	n (%)		
UFH			
No UFH	40 (15.0)	2 (4.1)	38 (17.5)
Low Dose UFH	103 (38.7)	17 (34.7)	86 (39.6)
High Dose UFH	123 (46.3)	30 (61.2)	93 (42.9)
LMWH	35 (13.2)	5 (10.2)	30 (13.8)
ASA	46 (17.3)	23 (46.9)	197 (90.8)
Tirofiban	14 (5.3)	6 (12.2)	8 (3.7)
Bivalirudin	4 (1.5)	1 (2.0)	3 (1.4)
Clopidogrel	2 (0.8)	0	2 (0.9)
Fondaparinux	1 (0.4)	0	1 (0.5)
Argatroban	1 (0.4)	0	1 (0.5)

N, number; UFH, unfractionated heparin; LMWH, low molecular weight heparin; ASA, acetylsalicylic acid.

D.2.4 Laboratory Values

Regarding anticoagulation monitoring, INR, aPTT, Anti-Xa as well as platelets and fibrinogen levels were routinely measured in patients in the CICU. Therapeutic aPTT ranges (defined in the core laboratory) in patients receiving ≥15 u/kg/hr UFH were achieved in 83,6% of patients, therapeutic Anti-Xa ranges in 48.6%. For further details on differences regarding laboratory values among bleeding and non-bleeding patients compare Table 14.

TABLE 14. Laboratory tests and values in pediatric SV patients stratified by bleeding outcome.

	Total	Bleeding Patients	Non-Bleeding Patients
Patients, n	266	49	217
INR Level (IQR)	1.4 (1.3-1.7)	1.4 (1.1-1.6)	1.4 (IQR 1.3-1.7)
INR Category, n (%)	228 (85.7)	36 (73.5)	192 (88.5)
≥ 1.5	85 (37.3)	11 (30.6)	74 (38.5)
aPTT Level, s (IQR)	69.7 (44.0-130.8)	63.70 (45.6-112.6)	69.80 (43.1-135.4)
aPTT, Category, n (%)	244 (91.7)	35 (71.4)	209 (96.3)
25-35s	26 (10.7)	2 (5.7)	24 (11.5)
36-70s	98 (40.2)	17 (48.6)	81 (38.8)
71-90s	26 (10.7)	5 (14.3)	21 (10.0)
91-110s	17 (7.0)	2 (5.7)	15 (7.2)
>110s	77 (31.6)	9 (25.7)	68 (32.5)
Anti-Xa Level, IU/ml (IQR)	0.27 (0.01-0.43)	0.18 (0.01-0.36)	0.28 (0.01-0.45)
Anti-Xa, Category, n (%)	151 (56.8)	21 (42.9)	130 (59.9)

<0.30 IU/ml	81 (53.6)	14 (66.7)	67 (51.5)
0.30-0.70 IU/ml	57 (37.7)	6 (28.6)	51 (39.2)
>0.70 IU/ml	13 (8.6)	1 (4.8)	12 (9.2)
Fibrinogen Level, g/l (IQR)	378 (280-477)	281 ± 138 g/l*	387 (286-488)
Fibrinogen Category, n (%)	221 (83.1)	24 (49.0)	197 (90.8)
<200 g/l	19 (8.6)	9 (37.5)	10 (5.1)
200-400 g/l	108 (48.9)	12 (50.0)	96 (48.7)
>400 g/l	94 (42.5)	3 (12.5)	91 (46.2)
Platelet Count /µl (IQR)	212 (143-322)	112 (93-196)	225 (161-341)
Platelet Count Category, n (%)	261 (98.1)	45 (91.8)	216 (99.5)
<200 x10^3/µl	119 (45.6)	34 (75.6)	85 (39.4)
200-400 x10^3/µl	106 (40.6)	9 (20.0)	97 (44.9)
>400 x10^3/µl	36 (13.8)	2 (4.4)	34 (15.7)

*N, number; INR, international normalized ratio; IQR, interquartile range; aPTT, activated partial thromboplastin time; s, seconds; Anti-Xa, Anti factor Xa Activity; IU/ml, International Units per milliliter; g/l, gram per liter; µl, microliter. *mean with standard deviation.*

D.3 Univariate Analysis: Risk Factors for Non-Surgical Related Bleeding in Pediatric SV Patients in the CICU

Patients' demographic and clinical characteristics as well as administered medications and drawn laboratory values were analyzed comparing bleeding and non-bleeding patients in order to identify possible risk factors. In total, 21 possible risk factors were analyzed. Of these, six were significantly associated with bleeding. A summary of all variables and corresponding *p*-values can be found in Table 15.

***TABLE 15**. Possible risk factors for bleeding: Univariate Analysis.*

Variable	**Categorical Variables**		**Continuous Variables**
	Chi-Square test	Fisher's exact test	Mann-Whitney U test
Demographic Variables			
Age	-	-	0.435
Male Gender	0.334	-	-
Weight	-	-	0.428
Height	-	-	0.281
Clinical Parameters			
Diagnosis			
HLHS	0.608	-	-
DORV	0.074	-	-
Valvular Disorders	0.543	-	-
Unbalanced AVC	0.079	-	-
DILV	-	1	-
RVOTO	-	0.673	-
Triscuspid Atresia	-	0.673	-
CAVC	-	0.588	-

Ebstein's Anomaly	-	1	-
TGA	-	0.559	-
Shone's Complex	-	1	-
LVOTO	-	1	-
Coarct	-	1	-
ToF	-	1	-
TAPVR	-	1	-
Other	-	1	-
Surgery Category	**0.027**	-	-
Stage I	**0.040**	-	-
Stage II	**0.007**	-	-
Fontan	0.409	-	-
BiV Conversion	0.183	-	-
Other	0.691	-	-
History of Bleeding	-	0.717	-
History of Thrombosis	0.266	-	-
Medication			
UFH	**0.019**	-	-
No UFH	-	**0.015**	-
<15 u/kg/hr	0.627	-	-
≥15 u/kg/hr	**0.026**	-	-
LMWH	0.642	-	-
ASA	**<0.001**	-	-
Tirofiban	**0.015**	-	-
Bivalirudin	-	0.559	-
Clopidogrel	-	1	-
Fondaparinux	-	1	-
Argatroban	-	1	-
Laboratory Values			
aPTT	-	-	0.526
Anti-Xa	-	-	0.202
INR	-	-	0.072
Fibrinogen Level	-	-	**<0.001**
Platelet Level	-	-	**<0.001**

P-values of possible risk factors associated with bleeding.
HLHS, Hypoplastic left heart syndrome; DORV, Double Outlet Right Ventricle; AVC, Atrioventricular Canal; DILV, Double Inlet Left Ventricle; RVOTO, Right Ventricular Outflow Tract Obstruction; CAVC, Complete Atrioventricular Canal; TGA, Transposition of Great Arteries; LVOTO, Left Ventricular Outflow Tract Obstruction; Coarct, Coarctation; ToF, Tetralogy of Fallot; TAPVR, Total anomalous venous return; BiV, biventricular; UFH, unfractionated heparin; LMWH, low molecular weight heparin; ASA, acetylsalicylic acid; aPTT, activated partial thromboplastin time; Anti-Xa, Anti-Factor Xa Activity; INR, international normalized ratio.

D.4 Multivariate Analysis: Risk Factors for Non-Surgical Related Bleeding in Pediatric SV Patients in the CICU

D.4.1 Exclusion and Inclusion of Variables

Out of 21 analyzed possible risk factors, six met the inclusion criteria for statistical association, i.e. a significant group difference between bleeding and non-bleeding patients in that risk factor. These variables were surgery category (p=0.027), UFH dose (p=0.019), ASA (p<0.001), tirofiban (p=0.015), platelet level (p<0.001) and fibrinogen level (p<0.001).

None of these variables had missing values exceeding 20% or a frequency of less than 5% and had to be excluded.

D.4.2 Assumptions for Binary Logistic Regression

Dummy variables were created for the categorical variables surgery category and UFH dose.

D.4.2.1 Multicollinearity

Multicollinearity between all independent variables was tested using variance inflation factor (VIF) as well as bivariate correlation. Multicollinearity was not present between any of the variables (VIF < 10 and bivariate correlation <0.7 in all variables of interest).

D.4.2.2 Identification of Outliers

Outliers were identified through the analysis of standardized residuals and Cook's distances (see C.4.3.3). Two bleeding patients were excluded from further analyses due to standardized residuals above three, another bleeding patient and one non-bleeding patient due to Cook's distances above one.

D.4.2.3 Linear Associations

Linear associations between independent continuous variables and the logit of the probability of the dependent variable were tested using Box-Tidwell transformation. Crossproducts of platelet level and its natural logarithm as well as fibrinogen level and its natural logarithm were calculated and added to the model as a separate variable. *P*-values of 0.851 for platelet level and 0.982 for fibrinogen level were not significant and confirmed linear relations.

D.4.3 Binary Logistic Regression

Results from the multivariate logistic regression analysis can be found in Table 16. The variables platelet level, ASA and high dose UFH showed independent associations with the outcome variable. No surgical procedure was independently associated with bleeding, neither was Tirofiban. Fibrinogen levels showed a trend towards significance (OR 0.993 [CI 0.987;1,000] p=0.060). Significant differences could be observed regarding UFH dose categories: No difference in bleeding outcome was observed when comparing no UFH and low dose UFH (p=0.998). However, the odds for an unprovoked bleeding event were 8 times higher for patients on high dose heparin compared to low dose heparin (OR

8.074 [CI: 1.4; 46.9] p=0.020). Low dose UFH was chosen as reference group since the comparison of different UFH doses seemed more relevant in patients with an indication for anticoagulation than the comparison with no UFH at all.

TABLE 16. Multivariate Binary Logistic Regression.

	Bleeding Patients			
Predictor	**Regression Coefficient**	**Wald χ^2**	**Adjusted OR (95% CI)**	***P*-Value**
Constant	3.288	3.090	n.a.	0.079
Surgery Category				
Stage I	1.785	1.391	5.958 [0.307; 115.609]	0.238
Stage II	0.483	0.068	0.617 [0.016; 23.124]	0.794
Fontan	0.776	0.224	2.173 [0.087; 54.264]	0.636
BiV Conversion	0.774	0.254	2.168 [0.107; 43.992]	0.614
Other	Reference			
UFH				
No UFH	-17.210	<0.001	<0.001 [0.000; .]	0.998
Low Dose UFH	Reference			
High Dose UFH	2.089	5.418	8.074 [1.391; 46.868]	**0.020***
ASA	-3.896	16.515	0.020 [0.003; 0.133]	**<0.001***
Tirofiban	1.288	1.273	3.624 [0.387; 33.922]	0.259
Platelet Level/µl	-0.014	5.830	0.986 [0.975; 0.997)	**0.016***
Fibrinogen Level, g/l	-0.007	3.534	0.993 [0.987; 1.000)	0.060

*OR, odds ratio; n.a., not applicable; BiV, biventricular; UFH, unfractionated heparin; ASA, acetylsalicylic acid; µl, microliter; g/l, gram per liter. *statistically significant difference in variables at the 5% level. R^2=0.674.*

Patients on ASA showed one fifth of the bleeding odds of patients without ASA in this study (OR 0.020 [CI 0.003; 0.133] p<0.001). Inverting this OR, patients before the initiation of ASA had 5 times the odds of developing a bleeding.

Regarding platelet levels, the odds ratio was smaller than one (OR 0.986 [CI 0.975; 0.997] p<0.016), indicating a decrease in bleeding probability with increasing platelet levels. Bleeding odds decreased by 1.5% for every unit increase per microliter.

D.4.4 Model Validity

The presented model was significantly better at predicting bleeding than the intercept only, χ^2 (10, N=219) = 85.422, p<0.001. The classification threshold was set at 0.4 and

the model was able to correctly classify 72.7% of bleeding patients (sensitivity) and 98.0% of non-bleeding patients (specificity) with an overall success rate of 95.4%. The false positive and negative rates were 20.0% and 3.0%, respectively. According to the Hosmer-Lemeshow-test, the data fit the model well, χ^2 (8, N=219) = 4.521, p=0.807. Nagelkerke's R^2 was 0.674, which corresponds to a large effect according to Cohen (1992). ASA showed the highest contribution to the model with Wald's χ^2 of 16.515, followed by platelet level, high dose UFH and fibrinogen level. For the discriminatory ability of the model compare the area under the curve (AUC) depicted in Figure 6.

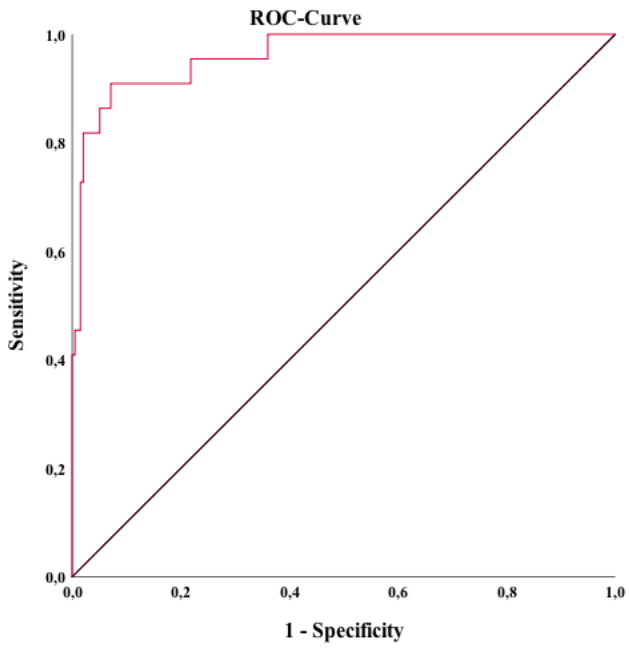

FIGURE 6. *Discriminatory Ability of the Model.*

*The discriminatory ability of the model corresponds to an AUC (area below red curve) of 0.962 (*95% CI 0.925-0.999; ***p*<0.001)** Black curve=reference. *AUC*, area under the curve; *ROC*, receiver operating characteristic.

D.5 Further Analyses

D.5.1 Evaluation of Platelet Cut-off Values Regarding Non-Surgical Related Bleeding

To evaluate critical platelet levels regarding bleeding, predicted bleeding probabilities adjusted for UFH dose categories as well as ASA categories were extrapolated from the regression model and can be found in Figures 7 and 8, respectively.

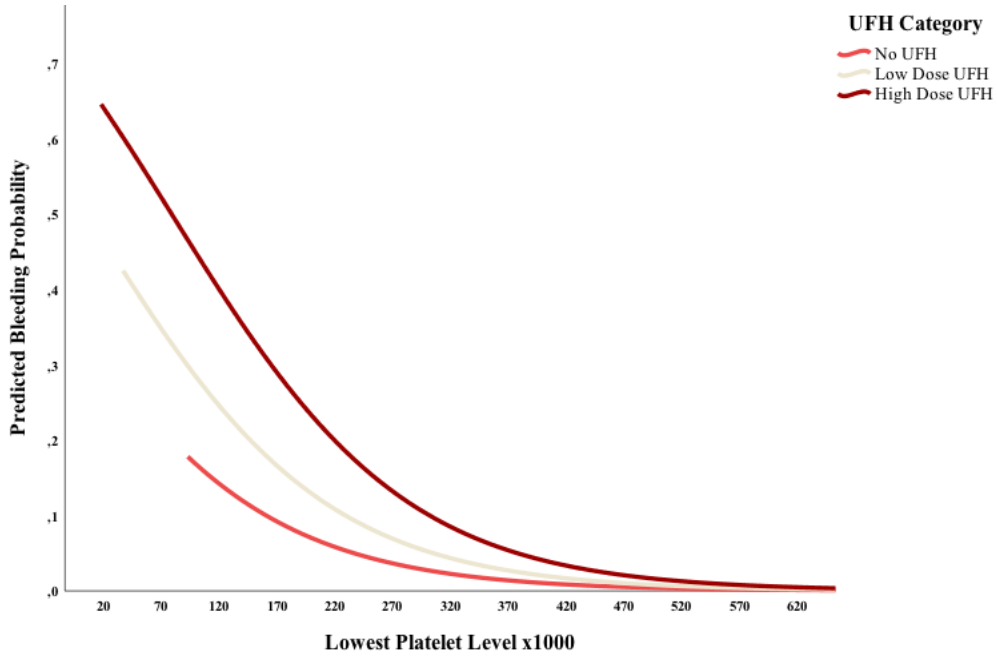

***FIGURE 7**. Predicted Bleeding Probability for Different Platelet Levels adjusting for UFH Dose Categories.*

UFH, unfractionated heparin.

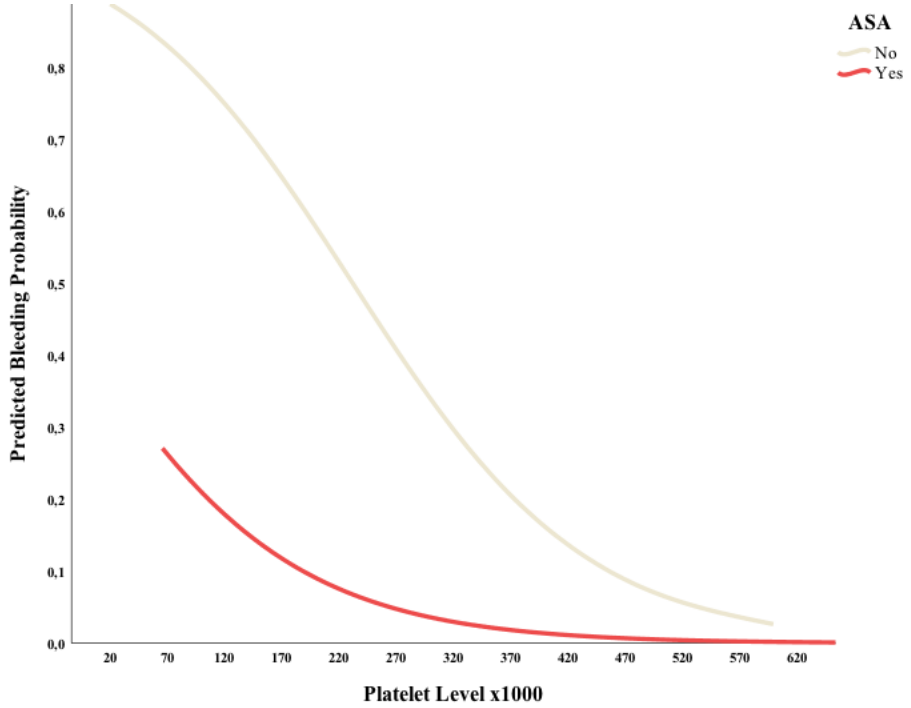

***FIGURE 8**. Predicted Bleeding Probability for Different Platelet Levels adjusting for ASA Categories.*

ASA, acetylsalicylic acid.

Cut-off values for platelet levels with regard to bleeding events were extrapolated from ROC curves for ASA and UFH categories as appropriate. Compare Table 17 for results.

TABLE 17. Platelet Level: Cut-off Values for Bleeding.

	Platelet Level Cut-off (n/µl)
Overall	160,500
ASA	160,500
No ASA	168,500
Low Dose UFH	118,000
High Dose UFH	171,000

Cut-off values for platelet levels with regard to bleeding outcome stratified by medication categories.
n, number; ASA, acetylsalicylic acid; UFH, unfractionated heparin.

D.5.2 Assessment of Possible Thrombocytopathy Using TEG

In order to assess possible coagulation dysfunction i.e. thrombocytopathy as an underlying cause of bleeding in addition to thrombocytopenia, TEG values drawn 24 hours after CICU admission were analyzed. Parameters in bleeding and non-bleeding patients were compared, however, no significant differences were found. All values including MA, of which 80% are derived from platelet function, were within target ranges (compare Tables 18 and 19).

TABLE 18. TEG values 24 hours after admission to the CICU.

Process	Available values, N	Normal range	Median (IQR)
Clotting time			
Reaction time = time to first fibrin strand formation (**R**), min	110	5-10	7.5 (6.1-8.8)
Kinetic clot time (**K**)	110	1-4	1.9 (1.6-2.5)
Clot kinetic			
α angle ($\boldsymbol{\alpha}$), °	110	53-72	63.9 (55.7-68.5)
Clot strength			
Maximum amplitude of clot strength (**MA**), mm	110	53-72	61.3 (56.6-65.1)
G- Parameter (**G**)	110	6-13	8.0 (6.5-9.3)
Clot stability			
Clot lysis within 30min (**LY30**), %	108	0-8	0.2 (0.0-1.6)

R, reaction time. α, alpha angle. MA, maximum amplitude. G, G-parameter. LY30, Clot lysis within 30 minutes. N, number. IQR, interquartile range. Min, minutes. Mm, millimeter. *P*-value calculated using Mann-Whitney-U test. *statistically significant difference in variables at the 5% level.

TABLE 19. *TEG values 24 hours after admission to the CICU stratified by bleeding status.*

	Available values, N	Normal Range	Bleeding Patients	Non-Bleeding Patients	*P*-Value
			Median (IQR)		
R, min	110	5-10	8.0 (5.9-9.8)	7.3 (6.1-8.7)	0.395
K, min	110	1-4	2,2 (1.6-3)	1,8 (1.6-2.4)	0.204
α angle (***α***), °	110	53-72	59.7 (53.5-69.1)	64.7 (57.6-68.5)	0.311
MA, mm	110	53-72	58.6 (54.8-62.9)	62.1 (58.3-65.8)	0.183
G	110	6-13	7.1 (6.1-8.5)	8.2 (7.0-9.6)	0.190
LY30, %	108	0-8	0.1 (0.0-1.4)	0.2 (0.0-1.7)	0.323

R, reaction time. *α*, alpha angle. MA, maximum amplitude. G, G-parameter. LY30, Clot lysis within 30 minutes. N, number. IQR, interquartile range. Min, minutes. Mm, millimeter. *P*-value calculated using Mann-Whitney-U test. *statistically significant difference in variables at the 5% level.

D.5.3 Differences in CICU LOS among Bleeding and Non-Bleeding Patients

Lastly, to demonstrate the importance of bleeding prevention in pediatric SV patients on the CICU, differences in LOS between bleeding and non-bleeding patients were calculated. The overall median LOS was 6 days (IQR 3-11). In bleeding patients, it was 11 days (IQR 6-21) compared to 5 days (IQR 2-9) in non-bleeding patients ($p<0.001$) with a maximum of 155 d and 72 d, respectively. An illustration of CICU LOS can be found in Figure 9.

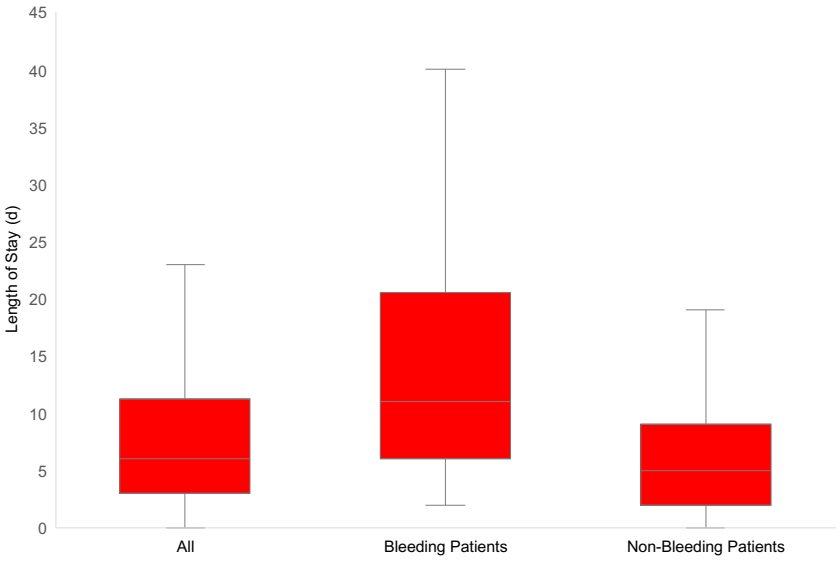

FIGURE 9*. Univariate Analysis: Length of Stay.*

Length of Stay (LOS) on the CICU in bleeding (N=49) and non-bleeding patients (N=217), ***p<0.001****. P-value calculated using Mann-Whitney U test. d, days.*

E Discussion

In this retrospective, single-center, case-control study previous research on anticoagulation management in pediatric patients following cardiac surgery was extended. It is to our knowledge the first study focusing specifically on bleeding's possible relation to anticoagulation in the immediate postoperative period occurring in patients with SVP. This study demonstrated the insufficient reliability of commonly used monitoring parameters in heparinized pediatric patients such as aPTT and Anti-Xa and highlighted the importance of adequately high platelet and fibrinogen levels, especially in presence of high dose heparin.

E.1 Bleeding Events

From 11/2016 to 02/2018 the inclusion criteria were met by 266 patients. A total of 63 unprovoked bleeding events occurred in 49 patients (18.4%). This finding is consistent with numbers reported for pediatric surgical patients with CHD by Nair et al. and Oladunjoye et al. (2018, 2018). However, other investigators report different incidence rates ranging from 1.5% to 24% (Andrew, Marzinotto et al. 1994, Schechter, Finkelstein et al. 2012, Trucco, Lehmann et al. 2015). This variation might be due to the inclusion of different kinds of CHD in other studies rather than focusing only on SVP as well as different definitions regarding bleeding events. In this study a standardized table was used to identify bleeding, its mechanism as well as its severity using objective criteria such as need for re-operation, blood product administration or discontinuation of anticoagulation medication (see Table 6).

E.2 Risk Factors for Non-Surgical Related Bleeding in Pediatric SV Patients in the CICU

E.2.1 Risk Factors: Demographic and Clinical Parameters

Age, Weight and Height

Neither age, nor weight or height were significantly associated with unprovoked bleeding events in analyses.

Regarding age, the literature shows heterogenous results concerning its associations with bleeding. Several studies identified younger age as well as low weight and height as risk

factors for postoperative bleeding (Williams, Bratton et al. 1999, Faraoni and Van der Linden 2014, Savan, Willems et al. 2014, Spiezia, Di Gregorio et al. 2017). However, the focus of these studies mostly lay on early postoperative bleeding occurring within 24 hours after surgery. Faraoni et al. (2014) found significant differences regarding bleeding only in children with cyanotic CHD up to 6 months of age. They hypothesized their immature coagulation system and especially impaired fibrinogen function in the presence of cyanotic disease to be one of the leading underlying pathophysiological mechanisms. Consistent with these findings, Ramírez-Flores et al. (2019) also found cyanotic heart disease to be significantly associated with bleeding, however age showed no significant association. Oladunjoye et al. (2018) investigated unprovoked bleeding events in pediatric patients after undergoing cardiac surgery at BCH not limited to SVP and also found no associations between age and bleeding.

These heterogenous results might be due to the inclusion of different kinds of CHD as well as differences in the definitions of bleeding events. Higher bleeding rates in younger patients might also be connected to the complexity of surgical procedures performed on smaller cardiac structures and not age alone. In this study, Stage I which was only performed in neonates, was associated with a higher bleeding incidence - however only in preliminary univariate analyses (compare paragraph on surgical procedures). In summary, our findings offer support for no significant association of age and bleeding.

Gender

Gender was not identified as a significant risk factor for spontaneous bleeding in this study. To date, no studies have been performed investigating associations between gender and postoperative spontaneous bleeding in pediatric SV patients specifically. However, Agarwal et al. (2014) investigated overall postoperative complications in pediatric cardiac surgery and found no influence of gender. Additionally, Savan et al. (2014) investigated postoperative blood loss within the first 6 postoperative hours and found no association between gender and bleeding. Based on these sparse data, gender does not seem to play a significant role in pediatric SV patients with regard to non-surgical related bleeding.

Diagnosis

None of the collected subgroups were significantly associated with postoperative bleeding events in this study. To date, no studies have investigated associations between

SV subgroups and spontaneous bleeding. However, multiple studies have identified cyanotic CHD in general as a risk factor for postoperative bleeding (Williams, Bratton et al. 1999, Guzzetta, Allen et al. 2015, Ramírez-Flores, Ibarra-Sarlat et al. 2019). Previously described abnormalities (compare paragraph A.2.1) in their coagulation system are one of the main hypothesized underlying causes.

Surgical Procedure

While univariate analyses yielded significant differences in bleeding frequencies with higher numbers after Stage I and fewer bleedings after Stage II, differences were not significant in subsequent adjusted multivariate regression.

To date, there is limited data comparing the incidence of non-surgical related bleeding after different surgical procedures for SVP. However, described mortality rates remain highest after Stage I and during the interstage I and II period (Jenkins, Gauvreau et al. 2002, Graham, Bradley et al. 2005, Welke, O'Brien et al. 2009). Hornik et al. (2011) described postoperative bleeding after Stage I Norwood limited to bleeding requiring reoperation and reported a prevalence of 8% with a consecutive increased mortality rate of 44.4%. Bartram et al. (1997) examined causes of death in 122 patients undergoing modified Norwood procedure. They found fatal bleeding leading to shock or pericardial tamponade in 8% of patients. However, 7 of these 9 patients died within the first 23 hours after surgery.

Such early postoperative bleeding events were excluded in this study, with the focus laying specifically on unprovoked hemorrhage possibly related to anticoagulation. As prevalence for both immediate postsurgical and spontaneous bleeding seem to be high after Stage I, the surgical complexity itself as well as postoperative anticoagulation regimes for shunt thrombosis prevention and the abnormal coagulation system in cyanotic neonates might put these patients at risk.

History of bleeding/thrombosis

Neither a history of bleeding nor of thrombosis were associated with bleeding in this study. However, numbers especially regarding a history of bleeding were low, limiting the predictive ability of these findings. No studies evaluating associations between history of bleeding or thrombosis and bleeding outcomes in pediatric SV patients have been performed to date. Therefore, the research at hand offers a first indication of the relevance of this association.

E.2.2 Risk Factors: Medication

UFH

To date, UFH is still the most used anticoagulant in pediatrics with 15% of children admitted to tertiary pediatric hospitals receiving UFH (Newall, Barnes et al. 2003). It is commonly used in the early postoperative period due to its short half-life and reversibility using protamine (Monagle, Chan et al. 2004). However, UFH effect is mostly dependent on the presence of AT III which is often decreased in neonates and patients with cyanotic CHD (Andrew, Paes et al. 1987, Andrew, Paes et al. 1988, Odegard, Zurakowski et al. 2009). In addition, age-related differences regarding heparin clearance or protein binding activity exist in children, which further complicates heparin therapy in pediatric patients (Deshaies, Poirier et al. 2018). Nevertheless, pediatric guidelines are mostly derived from adult data, resulting in worse clinical outcome for children compared to adults (24% major bleeding complications among pediatric patients receiving UFH vs. 1-11% in adults) (Hull, Raskob et al. 1990, Kuhle, Eulmesekian et al. 2007). Furthermore, responses to commonly used laboratory tests, such as aPTT and Anti-Xa, vary according to age and underlying health conditions (Deshaies, Poirier et al. 2018).

The analysis of different UFH doses in this study showed significant associations between high UFH doses and bleeding. Furthermore, it suggests special caution regarding high UFH doses in the presence of low platelet levels, since cut-off values for platelets are higher in patients receiving high dose UFH compared to low dose and no UFH (see Figure 7 and Table 19).

These findings are consistent with a study conducted by Kuhle et al. (2007). They investigated bleeding incidence in critically ill children receiving therapeutic UFH in the ICU, including patients with SVP. The median UFH dose administered was 27 u/kg/hr and major bleeding occurred in 24% patients (9/38). They found little agreement between UFH dose, aPTT and Anti-Xa levels. Moreover, two patients developed a new thrombotic complication while being on therapeutic UFH, raising questions about the actual efficacy of high UFH doses. These doubts are increased by a randomized controlled trial studying UFH therapy in children after cardiac catheterization (Hanslik, Kitzmüller et al. 2011): No differences in bleeding and thrombosis incidences were observed among patients receiving low and those receiving high dose UFH, indicating sufficient effectiveness of low dose UFH in the prevention of thrombosis. A recent study by Vorisek et al. (2019)

on bleeding and thrombosis in pediatric patients after congenital heart surgery even found higher bleeding and thrombosis odds in patients receiving high dose compared to low dose UFH.

These findings create doubts about the risk/benefit ratio of high dose UFH therapy for thrombosis prevention in pediatric cardiac patients and indicate careful evaluation of dosing, especially in presence of low platelet levels.

ASA

Significantly more bleeding events were observed in patients who did not receive ASA compared to those receiving the drug. Cut-off values for platelet levels regarding bleeding probabilities were also higher in patients not receiving ASA (see Table 19). While these findings might seem counterintuitive, the timing of patients' bleeding events might be a factor contributing to these results: Patients' first non-surgical related bleeding event occurred after a median of four days on the CICU (compare Table 10). However, ASA was not administered until extubation or by postoperative day 10, whichever came first. These findings suggest, that patients are most vulnerable and prone to spontaneous bleeding within the first days after surgery. Furthermore, patients at high risk for both bleeding and thrombosis received predominantly UFH in the immediate postoperative period due to its short half-life and reversability. Aspirin resistance, which has been described in adults and children before, might also be relevant in these patients. It has, however, rarely been studied in pediatric patients with CHD and is highly dependent on the assay used. Reported prevalence rates in SV patients range from 12% using VerifyNow^{TM} up to 80% using TEG (Heistein, Scott et al. 2008, Mir, Frank et al. 2015, Koh, Rodts et al. 2021). Dose increases have been described to overcome ASA resistance (Koh, Rodts et al. 2021).

In this study ASA response and resistance were not routinely checked. However, Emani et al. investigated ASA response in children undergoing cardiac surgery at BCH in 2017 using VerifyNow^{TM} and found suboptimal platelet response in 15% of patients. Subsequent dose adjustments were, however, successful in significantly reducing thrombosis rates.

Despite described ASA resistance, there is data supporting safe and efficient ASA use in children with SVP: Jacobs et al. (2002) examined the efficacy of long-term ASA in reducing thromboembolic events in patients after the Fontan procedure initiating it on the first postoperative day. They found no cases of ASA-related hemorrhage or thrombosis.

A recent study conducted by Attard et al. (2021) also found long term ASA administration to be superior to Warfarin therapy in pediatric patients with Fontan circulation.
Overall, ASA use seems to be safe in pediatric patients after congenital heart surgery. However, laboratory assays should be used in order to evaluate ASA response. Dose increases seem to overcome ASA resistance.

LMWH

No significant difference regarding the frequency of LMWH administration between bleeding and non-bleeding patients was observed. However, these findings are limited by low case numbers and can therefore only be interpreted to a limited extend.
Consistent with these findings a recent study by Ankola et al. (2021) has demonstrated safe and efficient use of LMWH in pediatric cardiac patients. However, higher dose requirements for neonates and infants compared to other age groups are suggested by multiple authors since dose escalations were more frequent and time until achievement of therapeutic ranges was often longer (Malowany, Knoppert et al. 2007, de Toledo, Gunawardena et al. 2010, Andrade-Campos, Montes-Limon et al. 2013, Chander, Nagel et al. 2013, Roeleveld, van der Hoeven et al. 2016). Murphy et al. (2018) on the other hand, investigated risk factors for thrombosis in pediatric patients undergoing cardiac surgery and found evidence, that LMWH might not be able to prevent central venous catheter-associated thrombosis. Described adverse events regarding LMWH therapy were mainly limited to neonates and involve primarily mild hemorrhage and osteopenia (Malowany, Knoppert et al. 2007).
Nevertheless, when accounting for higher dose requirements in neonates and infants, LMWH seems to be a relatively safe and efficient agent for thromboprophylaxis in pediatric cardiac surgery patients.

Tirofiban

Overall, Tirofiban was rarely administered in the CICU at BCH. In univariate analyses, significantly more bleeding patients had received tirofiban compared to non-bleeding patients. However, these results did not sustain in multivariate logistic regression and findings should be interpreted critically due to low case numbers.
Emani et al. (2020) recently demonstrated safe use of tirofiban in 52 pediatric patients undergoing aortopulmonary shunting with effective thrombosis prevention at BCH. Piekarski et al. (2019) studied tirofiban safety and efficacy in patients receiving modified

blalock-taussig shunts. Significantly less shunt thrombosis occurred in the 36 patients who received tirofiban in addition to standard of care anticoagulation (SOC) compared to 236 patients receiving only SOC. No differences in bleeding outcomes and mortality were observed.

Higher case numbers in these studies provide more reliable results compared to limited data available on tirofiban in this study. While these studies yield promising results, further prospective randomized, controlled trials are necessary to evaluate safety and efficacy in pediatric cardiac patients.

E.2.3 Risk Factors: Laboratory Values

aPTT and Anti-Xa

To monitor UFH effect, aPTT and Anti-Xa activity are most commonly used in heparinized pediatric patients. However, aPTT is age dependent and possibly influenced by other factors apart from UFH effect such as coagulation protein deficiencies, high CRP levels, lupus antigen, hypofibrinogenemia or vitamin K deficiency (Ignjatovic, Furmedge et al. 2006, Devreese, Verfaillie et al. 2015). Since measuring the heparin-AT complex, Anti-Xa is highly dependent on the presence of AT while being independent of other biological factors (Olson, Arkin et al. 1998, Garcia, Baglin et al. 2012, Price, Jin et al. 2013). Low Anti-Xa levels have been reported even in presence of elevated aPTT (Oladunjoye, Sleeper et al. 2018), which might be due to decreased AT levels in neonates and infants. Anti-Xa activity might hence underestimate AT-independent heparin effects especially in young pediatric patients. Thus, a combination of aPTT and Anti-Xa has become the monitoring strategy of choice in most pediatric institutions. Both methods have not been studied specifically in SV patients following cardiac surgery so far.

Target ranges for patients receiving therapeutic UFH doses were achieved in 83,6% of patients for aPTT and in 48,6% for Anti-Xa levels. However, the duration patients stayed in therapeutic ranges was not studied, since the focus of this study lay on the 24-hour period ahead of a bleeding event. Neither aPTT nor Anti-Xa levels were significantly associated with bleeding, showing similar values in bleeding and non-bleeding patients. Due to small numbers, aPTT and Anti-Xa levels were not compared regarding different severities of bleeding.

Various authors have reported aPTT and anti-Xa levels to poorly correlate with each other especially in neonates and infants: Guervil et al. (2011) demonstrated the superiority of

Anti-Xa levels over aPTT in a study with 100 pediatric patients reporting earlier achievement of therapeutic UFH levels and fewer UFH dosing adjustments. Trucco et al. (2015) investigated monitoring of UFH therapy using aPTT and Anti-Xa over a 1-year period each. They found no difference in the outcome of bleeding or thrombosis progression, while demonstrating slightly more stable monitoring using Anti-Xa. However, most studies examining the correlation between aPTT and Anti-Xa have been done on non-surgical patients, making it difficult to transfer these results to SV patients after cardiac surgery with the use of cardiopulmonary bypass. Oladunjoye et al. (2018) examined aPTT levels and Anti-Xa activity specifically in pediatric patients after surgery for CHD and found poor correlation between the two methods: 72.6% of matched aPTT and Anti-Xa levels were discordant. Elevated aPTT above 150s was significantly associated with bleeding, the odds of a crnm or major bleeding doubled with every 10s increase. However, Anti-Xa levels showed no relationship with bleeding and no threshold for an increased bleeding risk could be identified.

Overall, correlations between UFH doses, aPTT and Anti-Xa levels seem to be poor in pediatric cardiac patients and reliable predictions regarding the occurrence of bleeding cannot be made. Therefore, additional parameters are needed.

INR

INR values were determined as part of routine coagulation panels. No difference was observed comparing bleeding and non-bleeding patients. However, values ≥ 1.5 were present in 37.3% (85/266) of patients, indicating, in the absence of vitamin k antagonists, some sort of present coagulopathy.

Decreased levels of coagulation proteins such as proteins II, VII, IX and X have been described in patients with cyanotic CHD before (van Nieuwenhuizen, Peters et al. 1999, Odegard, McGowan et al. 2002, Odegard, McGowan et al. 2003). Liver congestion as a result of chronic venous hypertension or low cardiac output resulting in decreased perfusion as well as protein losing enteropathy have been hypothesized causes (Callegari, Christmann et al. 2019).

These findings suggest impaired integrity of the extrinsic pathway in some SV patients on the CICU. However, the INR is only a surrogate parameter and specific tests assessing coagulation function need to be performed to identify coagulopathy.

Platelets levels and function

Median platelet counts in this study were significantly lower in bleeding than in non-bleeding patients and differences were observed in both univariate and multivariate analyses. The calculated overall cut-off value regarding bleeding probability was 160,500/µl (compare Table 19 for further cut-off values accounting for medication). Among all bleeding patients, more than 2/3 had a platelet count of less than 200.000/µl within 24 hours prior to the event. No major bleeding event occurred in patients with platelet levels over 400,000/µl, while most could be observed in patients with levels lower than 200,000/µl. Of all major bleedings, 66.7% (6/9) occurred in patients with platelet levels lower than 100,000/µl. Predicted probabilities extrapolated from the regression model showed higher bleeding probabilities for low platelet counts, especially when patients received high dose heparin simultaneously. These findings are consistent with studies conducted by other investigators: Several studies found thrombocytopenia to be linked to increased rates of postsurgical bleeding in pediatric patients undergoing surgery for CHD (Kuhle, Eulmesekian et al. 2007, Trucco, Lehmann et al. 2015, Spiezia, Di Gregorio et al. 2017).

To identify possible platelet and fibrinogen dysfunction in addition to thrombocytopenia as underlaying causes of bleeding in this study, TEG results drawn 24 hours after admission to the CICU were compared between the bleeding and non-bleeding cohort. No significant differences were seen, and all values were within target ranges (compare Tables 18 and 19). This finding is consistent with a study conducted by Jensen et al. They found thrombocytopenia to be present in patients with CHD, however it was not linked to platelet dysfunction (Jensen, Johansson et al. 2013). Faraoni and Van der Linden (2014) found a significant association between cyanotic CHD and low platelet count. They also found significantly lower platelet levels after CBP compared to baseline. To examine platelet and fibrinogen function, they used ROTEM, but found no differences comparing cyanotic and non-cyanotic children.

A previous study conducted by Emani et al. at BCH however, found TEG in the operating room (OR) to be predictive of bleeding (Emani, Sleeper et al. 2018). They also showed associations with a reduction in bleeding outcomes in the ICU after prophylactic platelet transfusion in patients with intraoperative MA of less than 45 mm. Similarly, Romlin et al. found thromboelastometry (Rotem^{TM}) to adequately predict platelet dysfunction during, but not after CBP (Romlin, Soderlund et al. 2016).

In summary, these findings suggest adequate platelet function in SV patients in the CICU while thrombocytopenia is linked to a significantly increased bleeding risk. They highlight the importance of platelet levels as monitoring parameters and raise questions about adequate platelet transfusion triggers for SV patients, especially in the presence of anticoagulant agents like UFH.

Fibrinogen

Mean fibrinogen levels in bleeding patients were significantly lower than median levels in non-bleeding patients in univariate analyses. However, fibrinogen level did not quite reach statistical significance in multivariate logistic regression when including platelets, surgery category and medications.

Multiple studies have found hypofibrinogenemia to be linked to postoperative bleeding in children undergoing cardiac surgery. An early study conducted by Miller et al. (1997) found postprotamine platelet count and fibrinogen level to significantly correlate with 24-hour chest tube drainage in children weighing less than 8kg. Other more recent studies also found post-CPB fibrinogen levels of less than 150 g/l to be associated with increased blood loss in cardiac surgery children (Faraoni, Willems et al. 2014, Ranucci, Bianchi et al. 2019). Faraoni et al. (2014) also found reduced clot firmness in patients with low fibrinogen levels using ROTEM^{TM}. Accordingly, some authors have investigated blood product administration for hypofibrinogenemia and blood loss in pediatric cardiac surgery patients. A prospective study by Galas et al. (2014) has demonstrated safe and efficient use of fibrinogen concentrate as an alternative to plasma and cryoprecipitate without compromising outcomes and potentially reducing the risk of infection as well as immunologic reactions. There is also evidence that plasma on the other hand might increase the risk of thrombosis in pediatric patients undergoing cardiac surgery (Murphy, Benneyworth et al. 2018).

While these investigations focused on early postoperative bleeding occurring within 24 hours after surgery, this study was the first to demonstrate correlations of hypofibrinogenemia with spontaneous bleeding events in pediatric patients after cardiac surgery for SVP. SV patients seemed to be at risk for bleeding at higher fibrinogen levels than other pediatric patients undergoing cardiac surgery, while TEG could not detect significant coagulation abnormalities. Critical fibrinogen threshold levels for an increased bleeding risk are still a topic of debate and different transfusion triggers exist for pediatric patients depending on the clinical context (Crighton and Huisman 2021). Different

guidelines give different recommendations, however, levels of 100-150 mg/dl are usually an indication for the transfusion of Cryoprecipitate, fibrinogen concentrate or FFP in case of active bleeding in pediatric patients (Faraoni, Meier et al. 2019, Goobie, Gallagher et al. 2019). The current research raises doubts about whether these thresholds are applicable to pediatric SV patients. Further studies are needed to evaluate prophylactic and therapeutic transfusion triggers in this critically ill cohort, especially since there is data suggesting not only reduced postoperative bleeding but also a reduction of pRBC transfusions in cardiac and non-cardiac patients receiving supplementary fibrinogen concentrate (Fabes, Brunskill et al. 2018). Other authors also suggest that fibrinogen concentrate might improve clot firmness and reduce bleeding in presence of severe thrombocytopenia (Velik-Salchner, Haas et al. 2007).

E.3 Limitations

There are several limitations to this study. It was limited by its retrospective study design and small sample size; confirmation of these findings within a prospective randomized trial is thus warranted. Due to its study design, complete data could not be obtained for every variable and patient. Hence, numbers for some variables are small and the explanatory power of some results might be limited. However, model validity of the regression model was good (compare D.4.4 and Figure 6).

While this study design might be adequate for demonstrating associations between possible risk factors and bleeding, it did not allow to determine correlations between laboratory values and thrombosis outcomes. This was due to multiple reasons:

First, patients were not routinely screened for silent thrombosis. Second, the risk of thrombosis is likely related to a number of different factors like the type of cardiac lesion and cardiac surgery, infection, inflammation, cyanosis, cardiopulmonary bypass, cumulative effect of UFH dosing strategy that includes not only the monitoring methodology, but also duration of therapy and concomitant antiplatelet agents used. Third, unlike bleeding events that can easily be correlated with laboratory levels drawn within 24 hours prior to the event, thrombosis events are less likely to correlate with biomarkers within the previous 24-hour period. Furthermore, determination of the exact timing of a thrombosis event can be difficult. In patients with clinical, sonographic or echocardiographic signs of thrombosis, the exact timing of development of that thrombus may not be certain. Thus, associating a thrombosis event with laboratory values on a particular day is less relevant and hence this study was not designed to evaluate these

outcomes. To determine the effect of an anticoagulation monitoring strategy on the incidence of thrombosis, a prospective randomized trial would be necessary.

In terms of monitoring coagulation, the decision was made to focus on commonly used laboratory tests in order to obtain adequate statistical power. For future investigations, it might be of further interest, to additionally determine ATIII levels. Thromboelastography was analyzed in high risk patients 24 hours after CICU admission. While these results might be able to deliver a general idea of a present coagulopathy, they fail to depict a patient's coagulatory situation on the actual day of a bleeding event. Prospective trials investigating the value of routine thromboelastography to predict bleeding events in SV patients are necessary. Also, ASA resistance was not analyzed in this study due to small available numbers. However, Emani et al. (2017) analyzed ASA responsiveness and thrombosis rates in pediatric patients undergoing cardiac surgery at BCH and found inadequate platelet response in 15% of patients. Thrombosis rates could be decreased significantly through dose adjustments though, and the investigators recommended dosing based on platelet testing instead of weight.

E.4 Conclusion

Reported bleeding incidence rates as well as prolonged CICU-LOS demonstrate the importance of efficient yet safe anticoagulation in pediatric SV patients following surgery for CHD. Yet treatment and monitoring guidelines are often derived from adult studies and there are contradicting statements in the literature on anticoagulation recommendations.

This is the first study focusing primarily on bleeding events possibly related to anticoagulation after surgery for SVP. It demonstrated frequent usage of UFH for thrombosis prevention in the early postoperative period. However, high dose UFH was significantly associated with increased bleeding rates raising questions about its risk/benefit ratio compared to lower UFH doses and other available agents. Consistent with existing data, this study suggests safe ASA use in pediatric patients with SVP. Earlier postoperative administration might help to reduce UFH doses while effectively preventing thrombosis when accounting for possible ASA resistance. However, further studies examining the safety of early ASA administration in SV patients as well as ASA response are needed. Regarding anticoagulation monitoring, aPTT as well as Anti-Xa levels were not able to reliably predict bleeding events in SV patients on the CICU.

Thrombocytopenia and hypofibrinogenemia on the other hand, were frequent in bleeding patients and platelet levels of less than 160,500/µl were significantly associated with spontaneous bleeding. Additionally, platelet and fibrinogen function were assessed using TEG, however no altered function was demonstrated. Whether or not early administration of platelets, cryoprecipitate, FFP or fibrinogen concentrate can prevent major bleeding in this patient population remains controversial. However, some studies suggest decreased postoperative bleeding and improved TEG parameters without occurrence of thrombotic complications (Galas, de Almeida et al. 2014, Dennhardt, Sumpelmann et al. 2020). Considering the consequences of extensive hemorrhage, it seems therefore appropriate to investigate adapted transfusion triggers for pediatric patients with SV physiology in the future.

F Summary

Introduction: Pediatric patients with Single Ventricle Physiology (SVP) show numerous different risk factors leading to an increased risk for both bleeding and thrombosis during the immediate postoperative period following cardiac surgery (Giglia, Massicotte et al. 2013). Therefore, adequate anticoagulation therapy in these patients is essential. However, recommendations for frequently used medications and monitoring parameters are mostly derived from adult guidelines and little data on non-surgical related bleeding exists. The aim of this study was to identify possible risk factors for bleeding possibly related to anticoagulation in pediatric patients with SVP on the cardiac intensive care unit (CICU).

Methods: Patients with SVP admitted to the CICU after open heart surgery at Boston Children's Hospital were studied and reviewed regarding bleeding outcomes remote from surgery and independent of interventional procedures. Demographic and clinical characteristics as well as medications and laboratory values were compared between bleeding and non-bleeding patients. Significantly associated variables with bleeding in univariate analyses were included in a multivariate binary logistic regression model to identify independent associations.

Results: Among 266 patients undergoing cardiac surgery at BCH, 49 bleeding patients were identified. High dose unfractionated heparin (UFH) of ≥15 u/hr/kg, platelet counts of <160,500/µl and no administration of Aspirin were significantly associated with bleeding. Commonly used anticoagulation monitoring assays like activated partial thromboplastin time (aPTT), Anti-factor Xa activity and international normalized ratio (INR) on the other hand, showed no associations with bleeding in this study. Using thromboelastography, adequate fibrinogen and platelet function in SVP were demonstrated.

Conclusion: This study extended previous research on anticoagulation therapy in pediatric patients with SVP. It increases existing doubts about the safety of high dose UFH in pediatric patients with SVP on the CICU. Furthermore, future studies on the evaluation of platelet transfusion triggers are needed and might be able to help reduce extensive hemorrhage in SV patients.

G Zusammenfassung

Einleitung: Pädiatrische Patienten mit univentrikulären Herzen (UVH) weisen zahlreiche Risikofaktoren auf, die sowohl zu einem erhöhten Blutungs- als auch Thromboserisiko in der unmittelbar postoperativen Phase nach herzchirurgischen Eingriffen führen (Giglia, Massicotte et al. 2013). Die medikamentösen sowie laborchemischen Empfehlungen stammen jedoch zumeist aus Leitlinien für Erwachsene und es gibt wenig Daten über postoperative Blutungen in Zusammenhang mit Antikoagulanzien. Ziel dieser Studie war es daher, mögliche Risikofaktoren für Blutungen im Zusammenhang mit Antikoagulation bei pädiatrischen Patienten mit UVH auf der Intensivstation (ITS) zu ermitteln.

Methoden: Untersucht wurden alle Patienten mit UVH, die nach offenem herzchirurgischem Eingriff am Boston Children's Hospital auf die ITS aufgenommen wurden. Demographische und klinische Merkmale, sowie Informationen zu medikamentöser Therapie und Laborwerten wurden erhoben. Anschließend wurden Patienten, die eine Blutung erlitten, welche unabhängig von chirurgischen Eingriffen und interventionellen Verfahren auftrat, mit Patienten ohne Blutung verglichen. Variablen, die in univariaten Analysen signifikant mit Blutungen assoziiert waren, wurden in ein multivariates binäres logistisches Regressionsmodell aufgenommen, um unabhängige Zusammenhänge zu ermitteln.

Ergebnisse: Von 266 Patienten, die sich auf der ITS befanden, erlitten 49 Patienten eine spontane Blutung. Hohe Dosen unfraktioniertes Heparin (UFH), Thrombozytenzahlen von <160.500/µl und das Nicht-Verabreichen von Aspirin waren in multivariaten Analysen unabhängig mit dem Auftreten von Blutungen assoziiert. Partielle Thromboplastinzeit (aPTT), Anti-Faktor-Xa-Aktivität und die International Normalized Ratio (INR) zeigten hingegen keinen Zusammenhang mit dem Auftreten von Blutungen.

Schlussfolgerung: Diese Studie verstärkt bestehende Zweifel an der Sicherheit von hohen Dosen UFH bei pädiatrischen Patienten mit UVH auf der ITS. Außerdem gibt sie Anlass zur weiteren Evaluation von Thrombozytenkonzentrattriggern, um künftig das Auftreten von Blutungen zu verringern.

H Abbreviations

a	activated
α-angle	alpha angle
ADP	adenosine-diphosphate
Anti-Xa	anti-factor Xa activity
aPTT	activated thromboplastin time
ASA	acetylsalicylic acid
ASD	atrial septal defect
AT III	antithrombin III
AUC	area under the curve
AVC	atrioventricular canal
BCH	Boston Children's Hospital
BiV	biventricular
CAVC	Complete Atrioventricular Canal
CHD	congenital heart disease
CI	confidence interval
CICU	cardiac intensive care unit
Coarct	Coarctation
COX	cyclooxygenase
CPB	cardiopulmonary bypass
Crnm	clinically relevant non-major
CRP	c-reactive protein
CT	computed tomography
DIC	disseminated intravascular coagulation
DIRV	Double Inlet Right Ventricle
DORV	Double Outlet Right Ventricle
ECMO	extracorporeal membrane oxygenation
F	factor
FDPs	fibrin degradation products
HIT	heparin induced thrombocytopenia
HLHS	Hypoplastic Left Heart Syndrome
ICU	intensive care unit

INR	International Normalized Ratio
IRB	Institutional Review Board
ISI	international sensitivity index
IU/ml	international units/milliliter
IQR	Interquartile range
K-time	Kinetic clot time
LMWH	low-molecular weight heparin
LOS	length of stay
LVOTO	Left Ventricular Outflow Tract Obstruction
Ly30	Clot lysis index
MA	Maximum Amplitude/ Massachusetts
MBT	modified Blalock-Taussig shunt
MRI	Magnetic resonance imaging
mcg/kg	microgram/kilogram
mg/kg	milligram/kilogram
NO	nitric oxide
OR	Odd's Ratio Operating Room
PAB	pulmonary artery banding
PAI1	plasminogen activator inhibitor 1
PD	Patient Days
PDA	patent ductus arteriosus
PGI2	prostacycline
PMA	postmenstrual age
PT	Prothrombin time
R-time	Reaction time
ROC	receiver operating characteristic
RVOTO	Right ventricular outflow tract obstruction
RVPA	right ventricle to pulmonary artery shunt
SD	Standard deviation

SLVR	staged leftventricular recruitment
SOC	standard of care anticoagulation
SV	single ventricle
SVP	Single ventricle physiology
SQ q	Subcutaneously administered every
t-PA	tissue plasminogen activator
TAPVR	Total anomalous venous return
TEG	thromboelastography
TF	tissue factor
TFPI	tissue factor pathway inhibitor
TGA	Transposition of Great Arteries
ToF	Tetralogy of Fallot
u-PA	urokinase plasminogen activator
U/kg/h	units/kilogram/hour
UFH	unfractionated heparin
US	ultrasound
VIF	variance inflation factor
vWF	von Willebrand factor
WHO	World health organization

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L Appendix

L.1 Illustrations: Significantly associated Variables with Bleeding

UFH, ASA and platelet levels were significantly associated with bleeding in multivariate regression analysis. These associations are described in more detail in the following by depicting their descriptive properties.

L.1.1 UFH

UFH was frequently given to SV patients during their postoperative ICU stays. 95.1% of patients received UFH at least once during their entire stay. Regarding dose categories, significant differences were observed between patients who developed a bleeding and those who did not (overall p=0.019): 61.2% (30/49) of bleeding patients had received predominantly high dose UFH (≥15 u/kg/hr) leading up to the bleeding event compared to only 42.9% (93/217) of non-bleeding patients (p=0.026). No difference regarding bleeding was observed in patients receiving low dose UFH (<15 u/kg/hr): 34.7% of bleeding and 39.6% of non-bleeding patients (p=0.627). Significantly more non-bleeding patients were in the No UFH cohort compared to bleeding patients (17.5 % vs. 4.1 %, p=0.015). Compare Figure 10 for details.

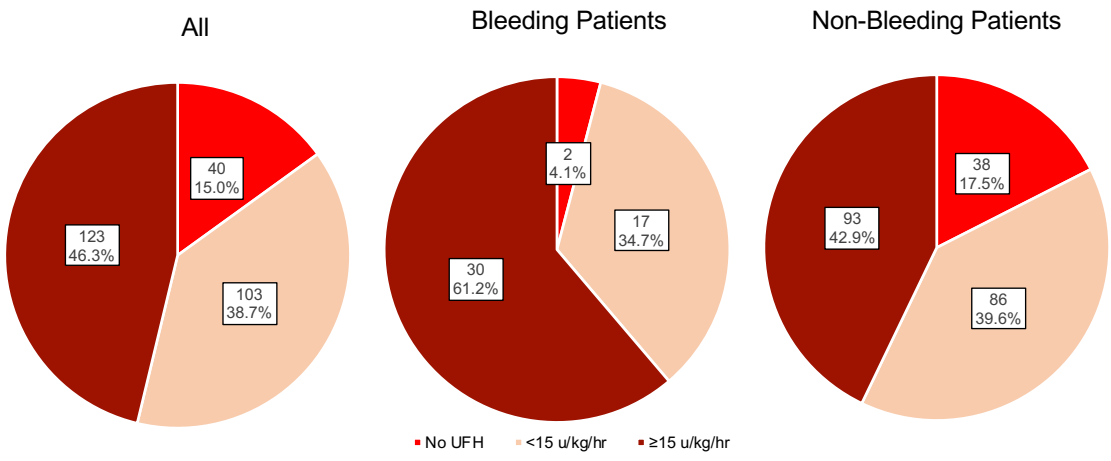

***FIGURE 10**. Univariate Analysis: UFH.*

*UFH dose categories among bleeding (N=49) and non-bleeding patients (N=217), overall **p=0.019**. Significances among dose categories: (1) No UFH vs. low and high dose UFH: **p=0.015** (2) Low Dose UFH vs. no and high dose UFH: p=0.627 (3) High Dose UFH vs no and low dose UFH: p=**0.019**. P-value calculated using chi-square test.*

In the high dose UFH cohort, 24.4% (30/123) of patients developed bleeding compared to only 16.5% (17/103) of patients in the low dose and 5% (2/40) of those in the no UFH group (compare Figure 11).

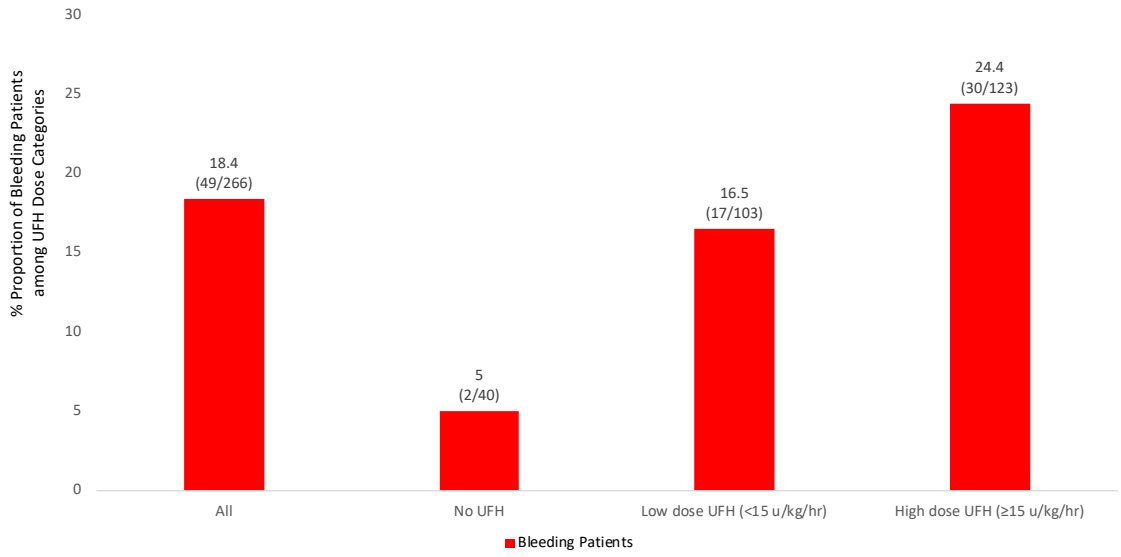

***FIGURE 11**. Proportion of Bleeding Patients among UFH Dose Categories.*

UFH, unfractionated heparin; u/kg/hr, units/kilogram/hour. N=49.

For distributions of bleeding severities among UFH dose categories compare Figure 12.

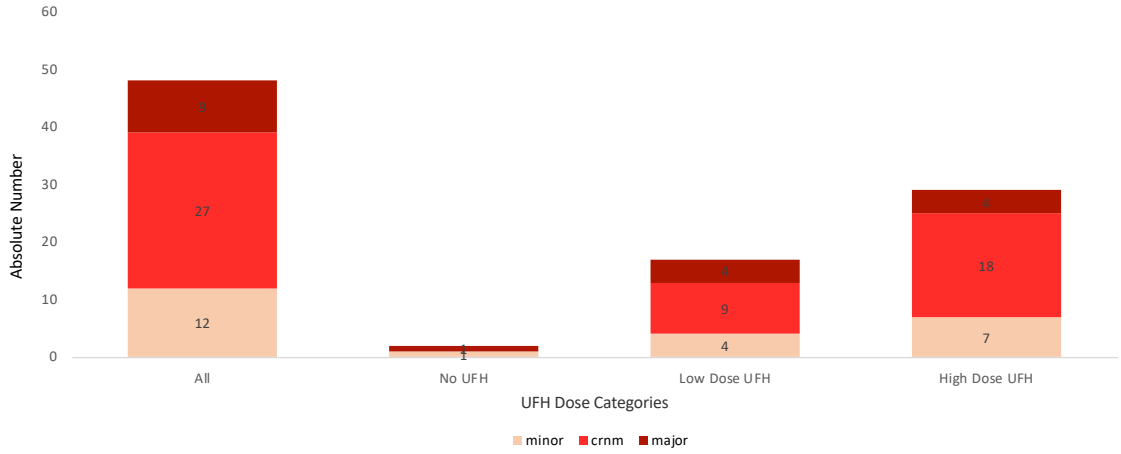

***FIGURE 12**. Distribution of Bleeding Severities among UFH Dose Categories.*

UFH, unfractionated heparin; crnm, clinically relevant non-major.

L.1.2 ASA

Overall, 17.3% (46/266) of patients received ASA. Significant differences in ASA administration between bleeding and non-bleeding patients were observed (p<0.001). Among bleeding patients 46.9% (23/49) received ASA within 24 hours prior to bleeding and 90.8% (197/217) of non-bleeding patients received ASA (compare Figure 13).

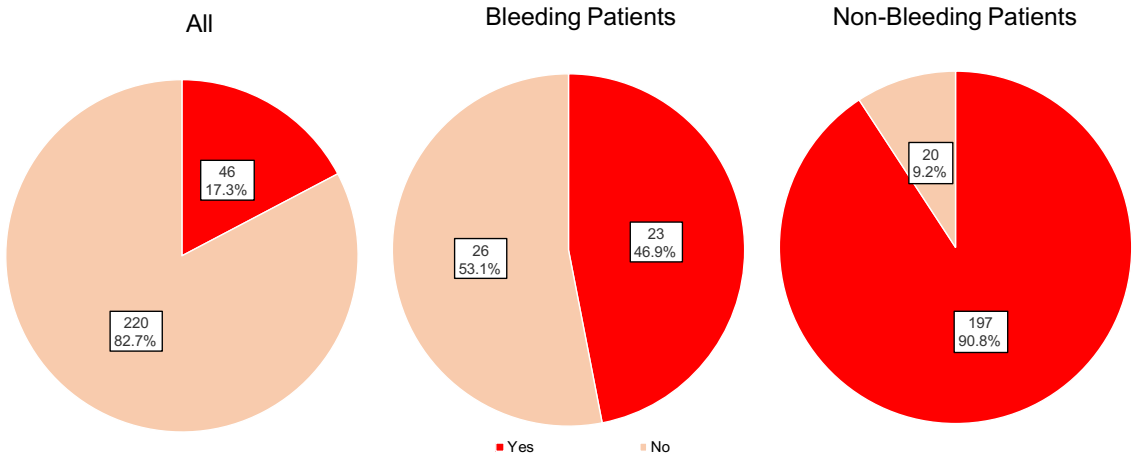

***FIGURE 13**. Univariate Analysis: ASA.*

*Distribution of ASA among bleeding (N=49) and non-bleeding patients (N=217), **p<0.001**. P-value calculated using chi-square test. ASA, acetylsalicylic acid.*

Absolute numbers were distributed relatively equally among patients receiving and those not receiving ASA (n=23 compared to n=26). However, proportions of bleeding patients differed with 10.5% bleeding among patients receiving ASA compared to 56.5% bleeding among those not receiving ASA. For distributions of bleeding patients among ASA categories compare Figure 14.

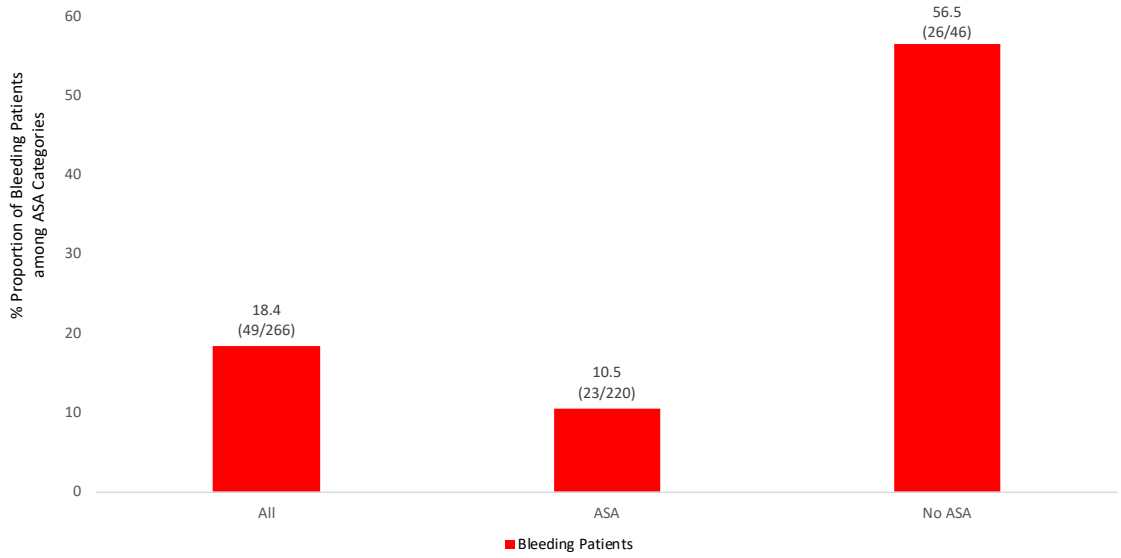

***FIGURE 14**. Proportion of Bleeding Patients among ASA Categories.*

ASA, acetylsalicylic acid. N= 49.

Bleeding severities were nearly equally distributed among patients receiving and those not receiving ASA (see Figure 15).

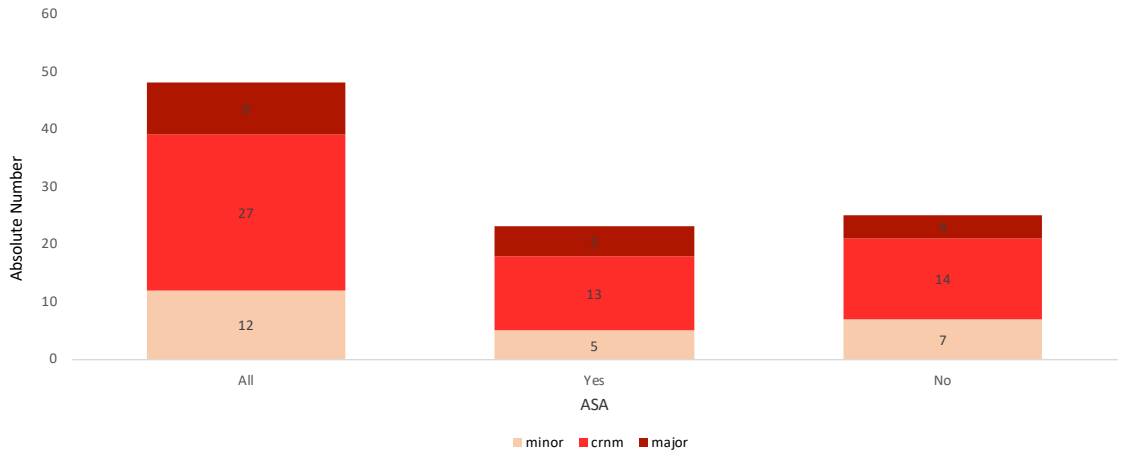

***FIGURE 15**. Distribution of Bleeding Severities among ASA Categories.*

ASA, acetylsalicylic acid; clinically relevant non-major.

L.1.3 Platelet Level

Platelet counts differed significantly among bleeding and non-bleeding patients (p<0.001). Median platelet counts in bleeding patients were 112,000/µl (IQR 93,000-196,000) and 225,000/µl (IQR 161,000-341,000) in non-bleeding patients. Compare Figure 16 for further details.

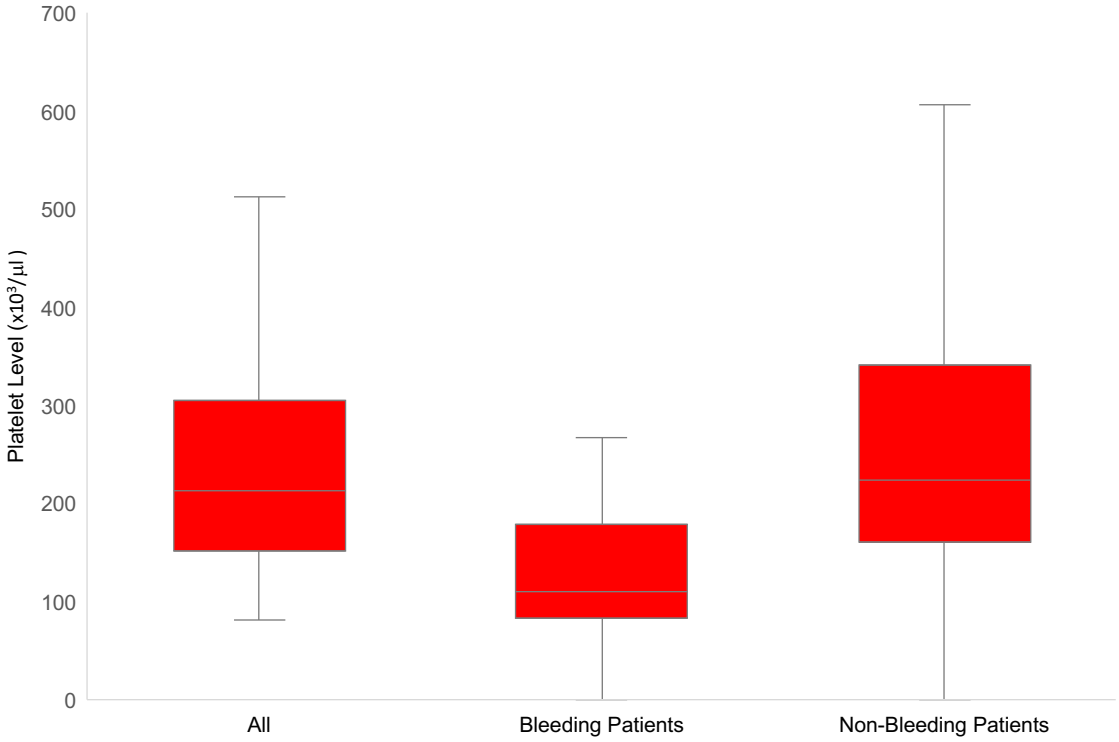

***FIGURE 16**. Univariate Analysis: Platelet Level.*

*Platelet Levels in bleeding (N=45) and non-bleeding patients (N=216), **p<0.001**. P-value calculated using Mann-Whitney U test. Shapiro-Wilk test: p<0.001 in bleeding and non-bleeding patients.*

Numbers of bleeding patients differed among platelet level categories (compare Figure 17).

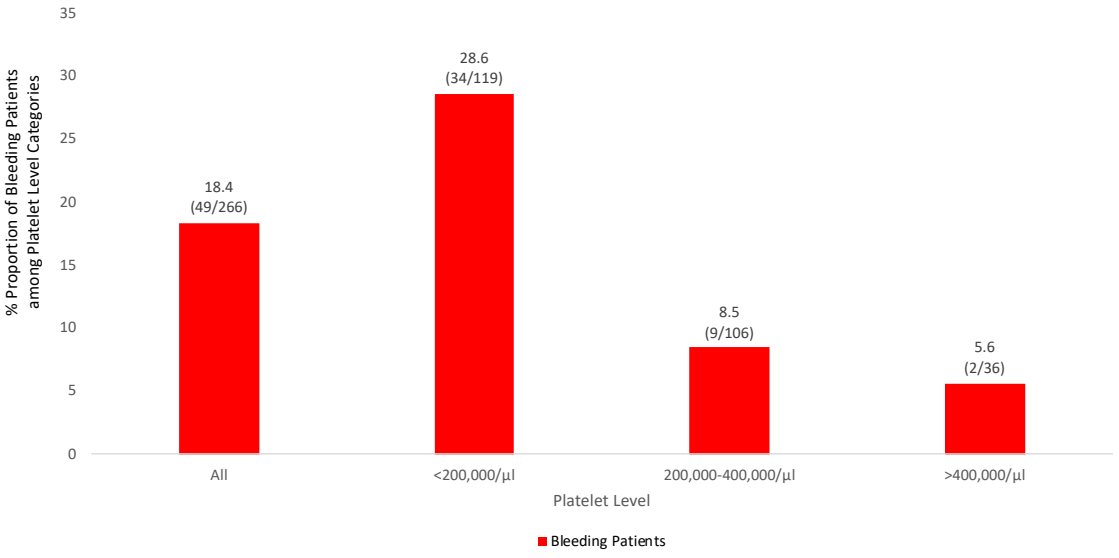

***FIGURE 17**. Proportion of Bleeding Patients among Platelet Level Categories.*

µl, microliter. N=49.

No major bleeding event occurred in patients with platelet levels over 400,000/µl, while seven could be observed in patients with levels lower than 200,000/µl (compare Figure 18. Of all major bleedings, 66.7% (6/9) occurred in patients with platelet levels lower than 100,000/µl.

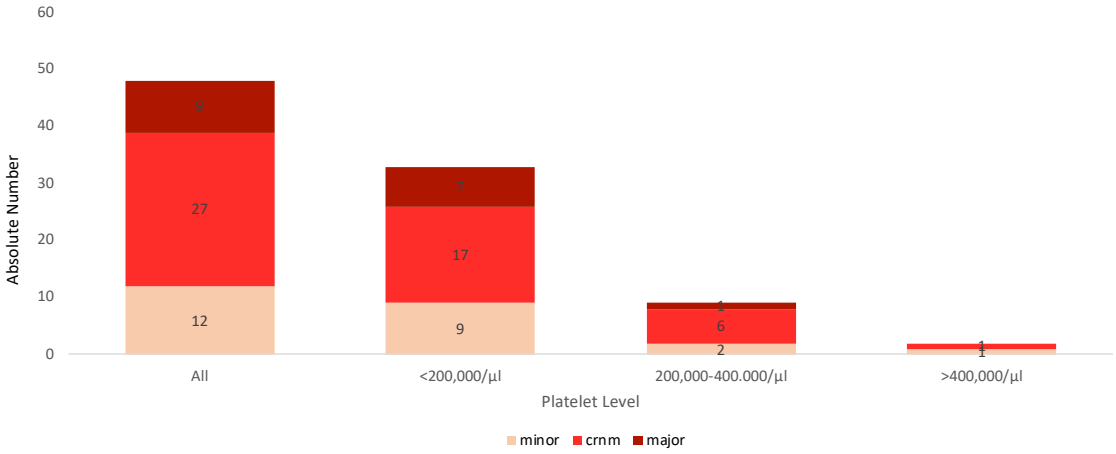

***FIGURE 18**. Distribution of Bleeding Severities among Platelet Level Categories.*

crnm, clinically relevant non-major. Five bleeding events are not included within platelet level categories due to missing platelet values 24 hours prior to the event, one bleeding was not categorized. N=48.

M Publikationsverzeichnis

Kongressbände

Seibold, C., Vorisek, C. N., Piekarski, B., Oladunjoye, O. O., & Emani, M. S. (2019). Evaluating Common Anticoagulation Laboratory Values in Single Ventricle Patients following Cardiac Surgery. *Thorac Cardiovasc Surg, 67*(S 02). doi:10.1055/s-0039-1679050

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Vorträge

02/2019 "Evaluating Common Anticoagulation Laboratory Values in Single Ventricle Patients following Cardiac Surgery", 51. Jahrestagung der Deutschen Gesellschaft für Pädiatrische Kardiologie, Wiesbaden

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