THESIS
Submitted to:
Charles Sturt University

For the:
Doctor of Philosophy
Faculty of Science

Submitted By:
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Title:
Individualised Heparin and Protamine Sulfate Management in Infants Undergoing Cardiac Surgery with Cardiopulmonary Bypass

August 31, 2010
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DECLARATION/CERTIFICATE OF AUTHORSHIP

“I hereby declare that this submission is my own work and that, to the best of my knowledge and belief, it contains no material previously published or written by another person nor material which to a substantial extent has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institution, except where due acknowledgement is made in the thesis. Any contribution made to the research by colleagues with whom I have worked at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

I agree that this thesis be accessible for the purpose of study and research in accordance with the normal conditions established by the Executive Director, Library Services or nominee, for the care, loan and reproduction of theses”.

Acknowledged this 31st day of August, 2010

Colleen Gruenwald
DEDICATION, ACKNOWLEDGEMENT AND CONTRIBUTIONS

This thesis is dedicated to children with heart disease. I am particularly indebted to those families who despite being in a very stressful situation took the time to consider participation in this research.

Acknowledgement

Enormous advances in the care of children with heart disease have developed over the past fifty years. The challenges in the care of these infants and children are complex and require the collaboration of a dedicated inter-professional team.

I would like to thank and acknowledge my many friends and colleagues at The Hospital for Sick Children. It is an inspiration to work with such intelligent, committed professionals.

Dedication

I am truly grateful to, and enriched by my family. To my husband, partner and best friend Falk Gruenwald, who demonstrates great patience, is a constant support all the while a joy to be with. To my children Matthew and Krista who understood from an early age the demands of this work and accepted it. Your unselfishness has moulded you both into beautiful young adults who will continue to contribute to society as you experience life in your own way. To my parents Murray and Marion Gauley who provided me with a solid foundation built from love and encouragement. I am forever thankful.
Contributions

I would like to thank my principle supervisor, Dr. Ross Richards for providing me with direction and support. I also extend my sincere gratitude to my associate supervisors, Helen Moriarty, Dr. Glen Van Arsdell and Dr. Anthony Chan.

I would like to acknowledge Helen Moriarty for her early contributions to this project and wish her a long and healthy retirement.

Dr. Glen Van Arsdell specifically encouraged me to do this. His support of the research focus was crucial. His skill as a surgeon and his leadership as chief of cardiovascular surgery have been central to this research.

Dr. Anthony Chan has been involved with our team for many years and has provided insight and expertise in haematology and coagulation research.

I am extremely grateful to these individuals for their scientific input, constructive feedback, guidance and thoughtful mentorship.

I would especially like to acknowledge the collaboration and camaraderie from my perfusion colleagues. I am fortunate to work with such outstanding professionals: they have made this possible. Leslie Jones is a fabulous administrative assistant and has provided invaluable help.
Individual Contributions

This work could not have been completed without significant contributions from the following individuals.

Dr. Helen Holtby has been a primary peer reviewer for this thesis. Her expertise as a paediatric cardiac anaesthesiologist was useful. However, her humour and candidness has shaped the thesis into its final (shorter) version (even more useful).

Lynn Crawford-Lean, Staff Cardiovascular Perfusionist has been a constant help in both the conduct of the research and the editing of this work. Her intellectual curiosity and dedication have been inspirational.

I am thankful for Cedric Manlhiot’s expertise in data analysis. As the clinical research program manager, Cedric has made valiant attempts to share his considerable knowledge of statistical analysis with me.

I am indebted to these individuals and appreciative of their good humour, tolerance and exceptional skill.

Funding

This work was supported by a generous grant from the Heart and Stroke Foundation of Ontario, Canada.
RESEARCH ETHICS APPROVAL

Ethics in Human Research Committee Office of Academic Governance, Charles Sturt University - **Protocol Number: 2007/136**

Research Ethics Board (REB), The Hospital for Sick Children - **REB File No. 1000007797**
ABSTRACT

Background

Bleeding and anticoagulation in infants undergoing heart surgery with cardiopulmonary bypass (CPB) is difficult to manage resulting in significant morbidity. The purpose of this study was to evaluate if the use of individualised heparin and protamine sulfate management and monitoring in infants resulted in improved anticoagulation and clinically relevant outcomes.

Objective

We sought to determine whether infants less than one year of age had similar clinical benefits with individualised anticoagulation management as older children and adults undergoing CPB.

Methods

Ninety infants less than one year of age undergoing CPB were enrolled in a randomised controlled trial comparing weight-based anticoagulation management utilising the activated clotting time (ACT) to individualised management using the Haemostasis Management System Plus® (HMS). Manufacturer’s guidelines were followed for the first 33 patients with a modified protocol used for the last 57 patients.

Results

The HMS device consistently underestimated plasma anti-Xa levels leading to high heparin doses. After a blinded interim analysis revealed poor
outcomes in the HMS group using the manufacturer guidelines, the safety committee suspended the study pending protocol modifications. The use of the HMS device following the modified protocol resulted in more stable anti-Xa levels during CPB with improved post-operative outcomes including reduced need for transfusions (71 vs. 80ml/kg, p=0.003), ventilation time (33 vs. 49 hours, p=0.04), intensive care (88 vs. 99 hours, p=0.003) and hospital length of stay (192 vs. 216 hours, p<0.001), compared with the weight-based protocol.

Conclusions

The HMS device is unsuitable for neonates and infants undergoing CPB when used according to the manufacturer’s instruction for use. This study supports the use of the HMS device, with a modified protocol for infants less than one year of age. Clinical guidelines for the use of the HMS device should be modified for infants less than one year of age.
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ABSTRACTS ACCEPTED/INVITED & PRESENTED

Primary Abstracts:

*Association between longer blood storage and worse post-operative outcomes in pediatric cardiac surgery*
- American Heart Association Scientific Sessions, November 14-18, 2009, Orlando, FL
- Published in Circulation – 2009;120:S_568 (Abstract 1906)

*Influence of heparin brand on immediate post-operative outcomes in children undergoing cardiac surgery*
- American Heart Association Scientific Sessions, November 14-18, 2009, Orlando, FL
- Published in Circulation – 2009; 120:S_557 (Abstract 1798)

*Individualized heparin and Protamine Sulphate management protocol in infants undergoing cardiopulmonary bypass for cardiac surgery improves immediate post-operative clinical outcomes*
- American Heart Association Scientific Sessions, November 8-12, 2008, New Orleans, LA
- Published in Circulation 2008,118:S_750 (Abstract 2598)

*Individualized heparin and Protamine Sulfate management protocol in infants undergoing cardiopulmonary bypass for cardiac surgery should consider the immaturity of the coagulation system*
- Canadian Cardiovascular Congress, October 25-29, 2008, Toronto, ON
- Published in Canadian Journal of Cardiology, October, 2008, Volume 24, Supplement SE (Abstract 0594)

*Response to heparin in infants undergoing cardiopulmonary bypass is associated with preoperative antithrombin level: implications of unbound heparin for clinical outcomes*
- Canadian Cardiovascular Congress, October 25-28, 2008, Toronto, ON
- Published in Canadian Journal of Cardiology, October, 2008, Volume 24, Supplement SE (Abstract 1019)
Should the activated coagulation time be used for infant patients undergoing cardiopulmonary bypass for cardiac surgery?

C. Gruenwald
- 4th International Conference on Pediatric Mechanical Circulatory Support Systems and Pediatric Cardiopulmonary Perfusion, May, 2008, Portland, OR

Co-author Abstracts:

Distribution and clinical signs of venous, arterial and intracardiac clots after pediatric cardiac surgery
L.R. Brandao, C. Manlhiot, I.B. Menjkz, A.K. Chan, C. Carew, C. E.
Gruenwald, S.M. Schwartz, V.B. Sivarajan and B.W. McCrindle
- American Society of Haematology, Annual Meeting December 5-8, 2009, New Orleans, Louisiana
- Published in Blood, 2009 114 (Abstract 3992)

Association between thromboembolic complications and increased mortality after pediatric cardiac surgery
- American Heart Association Scientific Sessions, November 14-18, 2009, Orlando, FL
- Published in Circulation, 2009; 120: S_557 (Abstract 1796)

Incidence and risk of thromboembolic complications associated with pediatric cardiac surgery
- Canadian Cardiovascular Congress, October 24-28, 2009, Edmonton, Alberta
- Published in the Canadian Journal of Cardiology, October 2009, Volume 25, Supplement SB (Abstract 529)
PUBLISHED MANUSCRIPTS

Review Article:

Management and Monitoring of Anticoagulation for Children Undergoing Cardiopulmonary Bypass in Cardiac Surgery
The Journal of ExtraCorporeal Technology (JECT) 2010; 42:9-19

Original Articles:

Randomized, Controlled Trial of Individualized Heparin and Protamine Management in Infants Undergoing Cardiac Surgery with Cardiopulmonary Bypass
Short title: Anticoagulation in Infant Heart Surgery
Colleen E. Gruenwald, Cedric Manlhiot, Anthony K. Chan, Lynn Crawford-Lean, Celeste Foreman, Helen M. Holtby, Glen S. Van Arsdell, Ross Richards, Helen Moriarty, Brian W. McCrindle
The Journal of American College of Cardiology, 2010; 56: 1794-1802

Reconstituted Fresh Whole Blood Improves Clinical Outcomes Compared with Stored Component Blood Therapy for Neonates Undergoing Cardiopulmonary Bypass for Cardiac Surgery: A Randomized Controlled Trial
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>α</td>
<td>alpha</td>
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<tr>
<td>α₂AP</td>
<td>alpha₂ antiplasmin</td>
</tr>
<tr>
<td>α₂-PI</td>
<td>a₂ –plasmin inhibitor</td>
</tr>
<tr>
<td>ACT</td>
<td>activated clotting time</td>
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<tr>
<td>ADP</td>
<td>adenosine diphosphate</td>
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<td>APC</td>
<td>activated protein C inhibitor</td>
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<td>AT</td>
<td>Antithrombin</td>
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<td>ATP</td>
<td>adenosine triphosphate</td>
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<td>CCCU</td>
<td>cardiac critical care unit</td>
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<td>CCHD</td>
<td>cyanotic congenital heart disease</td>
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<td>CHD</td>
<td>congenital heart disease</td>
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<td>CHF</td>
<td>congestive heart failure</td>
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<tr>
<td>CPB</td>
<td>cardiopulmonary bypass</td>
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<tr>
<td>DHCA</td>
<td>deep hypothermic circulatory arrest</td>
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<tr>
<td>EBV</td>
<td>estimated blood volume</td>
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<td>ECC</td>
<td>extracorporeal circulation</td>
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<td>ECMO</td>
<td>extracorporeal membrane oxygenation</td>
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<tr>
<td>EST</td>
<td>parameter estimate</td>
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<tr>
<td>EST±SE</td>
<td>parameter estimate plus or minus standard error</td>
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<tr>
<td>F</td>
<td>Factor</td>
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<tr>
<td>F 1.2</td>
<td>prothrombin fragment 1+2</td>
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<tr>
<td>FDP</td>
<td>fibrin degradation products</td>
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<td>FFP</td>
<td>fresh frozen plasma</td>
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<td>FPA</td>
<td>fibrinopeptide A</td>
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<td>FSP</td>
<td>fibrin split products</td>
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<td>fresh whole blood</td>
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<td>GP</td>
<td>glycoprotein</td>
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<td>HCII</td>
<td>heparin cofactor II</td>
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<td>Hct</td>
<td>haematocrit</td>
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<td>HEP</td>
<td>heparin concentration</td>
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<td>HMWK</td>
<td>high-molecular-weight-kininogen</td>
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<td>HMS</td>
<td>haemostasis management system</td>
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<td>HPT</td>
<td>heparin protamine sulfate titration</td>
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<td>ICU</td>
<td>intensive care unit</td>
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<td>IL</td>
<td>interleukin</td>
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<td>IU</td>
<td>international units</td>
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<td>Kg</td>
<td>kilogram</td>
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<td>KK</td>
<td>kallikrein</td>
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<td>KKI</td>
<td>kallikrein inhibitor</td>
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<td>MAP</td>
<td>mean arterial pressure</td>
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<td>mg</td>
<td>milligrams</td>
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<td>ml</td>
<td>millilitres</td>
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<td>mmHg</td>
<td>millimetres of mercury</td>
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<td>mmol/L</td>
<td>millimoles per litre</td>
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<tr>
<td>MUF</td>
<td>modified ultrafiltration</td>
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<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>OR</td>
<td>odds ratio</td>
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<tr>
<td>PAF</td>
<td>platelet-activating factor</td>
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<td>PAI</td>
<td>plasminogen activator inhibitor</td>
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<tr>
<td>PAI-1</td>
<td>plasminogen activator inhibitor 1</td>
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<td>PAI-2</td>
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<td>PAR-I</td>
<td>proteinase activated receptor – 1</td>
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<td>PAR-4</td>
<td>proteinase activated receptor – 4</td>
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<td>PDGF</td>
<td>platelet-derived growth factor</td>
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<td>PE</td>
<td>plasma equivalent</td>
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<td>PF3</td>
<td>phospholipid</td>
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<td>platelet factor 4</td>
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<td>PKK</td>
<td>prekallikrein</td>
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<td>PRBCs</td>
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<td>PTS</td>
<td>post-thrombotic syndrome</td>
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<td>RDS</td>
<td>respiratory distress syndrome</td>
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<td>rFVIIa</td>
<td>recombinant factor VIIa</td>
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<td>RFWWB</td>
<td>reconstituted fresh whole blood</td>
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<td>s</td>
<td>seconds</td>
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<td>SD</td>
<td>standard deviation</td>
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<td>standard error</td>
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<td>SEM</td>
<td>standard error of the mean</td>
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<td>SM-CPB</td>
<td>surface-modified CPB</td>
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<td>SIRS</td>
<td>systemic inflammatory response syndrome</td>
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<td>TAT</td>
<td>thrombin antithrombin</td>
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<td>TCs</td>
<td>thrombotic complications</td>
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<td>TF</td>
<td>tissue factor</td>
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<td>TFPI</td>
<td>tissue factor pathway inhibitor</td>
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<td>TNFα</td>
<td>tumour necrosis factor-alpha</td>
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<td>t-PA</td>
<td>tissue plasminogen activator</td>
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<td>TXA2</td>
<td>thromboxane A2</td>
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<td>U</td>
<td>unit</td>
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<td>unfractionated heparin</td>
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<td>U/ml</td>
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<td>urokinase plasminogen activator</td>
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<td>von Willebrand factor</td>
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<td>WB</td>
<td>whole blood</td>
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<td>°C</td>
<td>degrees Celsius</td>
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1.1 Background

Approximately 2000 children are born in Canada every year with congenital heart disease (CHD). The majority of them will require at least one cardiac surgery before their 5th birthday, and many will require multiple interventions throughout their lifetime (Hoffman and Kaplan 2002; Hoffman, Kaplan et al. 2004). Over the last two decades surgical practice has changed from staged palliations (to defer more complex interventions to an older age), towards early total correction of congenital heart malformations as the primary intervention. Therefore, the population undergoing surgery involving cardiopulmonary bypass (CPB) is now much younger and developmentally immature creating important challenges in terms of clinical management.

Cardiac surgical mortality in paediatric patients in the current era is low (Bazzani and Marcin 2007). The focus is now on preventing surgical complications and long-term morbidities (Boneva, Botto et al. 2001). There is limited data on bleeding and thrombotic complications (TCs) associated with cardiac surgery in paediatric patients (Chan, Deveber et al. 2003). Bleeding and TCs are intrinsic problems associated with cardiac surgery because of the competing influences of the CPB circuit promoting thrombin generation and clot formation on one side and anticoagulation aimed at preventing clot formation but promoting bleeding on the other side. Most of the knowledge on bleeding,
TCs and clinical protocols, including anticoagulation protocols used in children, are derived from adult studies and have not been re-validated for children (Revel-Vilk and Chan 2003). However, the results from adult trials should not be extrapolated to children because of important age-dependent physiologic differences in haemostasis (Andrew, Paes et al. 1987; Andrew, Paes et al. 1988; Andrew, Vegh et al. 1992; Monagle, Barnes et al. 2006). The effect of incomplete haemostatic development in neonates and infants on surgical management and outcomes are not clearly understood. Specific investigations into the differences in these distinct patient populations are required.

1.2 Introduction

Expected complications of CPB such as bleeding and thrombosis may be exacerbated by complex haemostatic defects and/or haemostatic system immaturity. Multiple mechanisms are involved including anticoagulation, haemodilution, platelet activation, coagulation and fibrinolysis (Woodman and Harker 1990; Chan, Leaker et al. 1997) all of which are affected to some degree by the developmental immaturity of very young patients undergoing cardiac surgery.

The haemostatic derangements caused by CPB can be a significant challenge in paediatric cardiac surgery and may result in increased risk of bleeding and clotting compared to adult patients. Specific influences include the immature coagulation system of the neonate, the effect of cyanosis on haemostasis, platelet hypercoagulability and decreased sensitivity to heparin, a greater
degree of coagulation factor haemodilution and the more frequent use of deep hypothermic circulatory arrest, which is known to affect platelet function (Mossinger and Dietrich 1998).

Maintaining haemostasis is a critical part of management during and after cardiac surgery with CPB especially in infants. There are a myriad of clinical consequences to the activation of the haemostatic and inflammatory systems. These include haemorrhage and need for blood transfusions, haemodynamic instability, and development of early and late thrombotic complications, coagulopathy, generalised oedema, renal impairment and neurological sequelae. To date, no single approach has proven successful in completely eliminating serious post-CPB complications however multiple management strategies have been shown to reduce the risk of those complications (Kern, Morana et al. 1992; Petaja, Lundstrom et al. 1996; Petaja, Peltola et al. 1996).

1.3 Normal Haemostasis

It is important to understand normal haemostasis to appreciate how cardiopulmonary bypass upsets the homeostasis. A thorough understanding of haemostatic derangements associated with CPB provides a framework to study methods to minimise the deleterious effects of this technology.

The primary function of the human coagulation system is to maintain vascular integrity without compromising blood flow. This is achieved by coagulation proteins that circulate in fluid in inactive forms (i.e. zymogens), endothelium
that is devoid of thrombogenic tissue factor and a brisk and laminar blood flow that removes any activated proteins and metabolises them in the liver (Mann, Nesheim et al. 1990).

Haemostasis at the site of blood vessel injury involves multiple defence mechanisms to limit blood loss. The haemostatic system consists of blood vessels, endothelial proteins, platelets, plasma coagulation proteins, fibrinolytic factors and their inhibitors. When a blood vessel is ruptured, three local mechanisms minimise the bleeding: transient vessel wall contraction, platelet haemostatic plug formation and initiation and maintenance of blood coagulation. All three mechanisms function in concert and are essential for normal haemostasis (Chen and Tsai 1948).

When the endothelium is compromised, tissue factor (TF) and thrombin initiate a chain reaction. Primary haemostasis is achieved by a combination of vasoconstriction and platelet adhesion and aggregation forming an immediate platelet plug at the site of injury. Secondary haemostasis occurs with coagulation proteins responding in a complex cascade to form fibrin strands which strengthen the platelet plug (Brewer 2006).

Haemostasis requires a phospholipid surface. Following vascular injury activated platelets expose a surface phospholipid known as platelet factor 3 (PF3). Since PF3 originates inside the blood vessel, the coagulation sequence
that occurs on this surface is known as the intrinsic pathway. The second source of phospholipid comes from injured cells outside the blood vessel wall. Tissue factor is a protein that initiates extrinsic pathway of coagulation (Nichols, Ungerleider et al. 2006). Phospholipids are required for the activation of both intrinsic and extrinsic pathways.

The fibrinolytic pathway mediated by plasmin regulates the clot size and gradual dissolution of the haemostatic plug. Fibrinolysis leads to restoration of normal blood flow however, premature or excessive lysis of the fibrin thrombus by accelerated fibrinolysis may cause re-bleeding (Gravlee, Davis et al. 1993).

1.4 Platelet Activation

Primary haemostasis refers to the interaction between the blood vessel wall and the platelet. Normal platelets circulate in the blood as free-floating cell fragments and do not usually adhere to normal vascular lining or to each other. When the endothelium is damaged, it releases proteins, most notably von Willebrand factor (VWF), that recruits factor (F) VIII, and platelets. Within seconds platelets accumulate at the site of vascular damage and adhere to subendothelial tissue forming the primary haemostatic plug. The platelet releases vasoactive factors that produce vasoconstriction of injured blood vessels. Finally, they provide a surface for specific coagulation factor reactions to occur. Platelets bind to adhesive proteins, such as collagen, with surface collagen-specific glycoprotein (GP) Ia/IIa receptors (Staatz, Walsh et al. 1990).
This association is strengthened by locally released proteins forming links between platelets and collagen fibrils. This process is described as platelet activation and adhesion. These activated platelets release the contents of their cytoplasmic granules into the blood plasma. The granule contents include adenosine diphosphate (ADP), adenosine triphosphate (ATP), calcium, serotonin, platelet-activating factor (PAF), platelet factor 4 (PF4), platelet-derived growth factor (PDGF), VWF, FV, fibrinogen, plasminogen activator inhibitor 1 (PAI-1), and thromboxane A2 (TXA2). These in turn activate more platelets. Activated platelets change shape from spherical to stellate. VWF links the platelet to the injured endothelium via glycoprotein Iβ. Fibrinogen binds to specific receptors on the platelet surface, glycoprotein IIb/IIIa (GP IIb/IIIa), and facilitates aggregation of adjacent platelets (Brewer 2006).

Activated platelets promote haemostasis in several ways: they aggregate at the site of blood vessel injury, provide a procoagulant surface to accelerate blood coagulation and inhibit the anticoagulant action of heparin by secreting PF4. In addition, platelets release PAI-1 preventing the premature lysis of a haemostatic plug (Shattil and Bennett 1981).

1.5 Blood Coagulation

The coagulation cascade leading to the generation of thrombin has classically been divided into three distinct pathways (intrinsic, extrinsic and common). More recently the coagulation cascade is described as a cell-based model with two distinct phases or pathways; the tissue factor pathway (formerly known as the extrinsic pathway), which leads to fibrin formation, and the contact
activation pathway (formerly known as the intrinsic pathway). It was called extrinsic because tissue factor is not found in blood. In contrast, the substances required for clotting via the intrinsic pathway are present within the blood and initiated via FXII. The common pathway is described as arising after the activation of FX.

The concept of two separate pathways is of practical value for understanding blood coagulation however it is now known that there are feedback mechanisms and interactions outside the classic schemes (Furie and Furie 2005). Furthermore, descriptions of the process are made more onerous to read due to the fact that while most of the coagulation proteins are numbered, unfortunately the biological reactions do not happen in numerical order.

Coagulation factors are enzymes that circulate in an inactive form. When the coagulation mechanism is triggered, a small portion of an inactive procoagulant molecule (factor, F) is cleaved off, producing an active serine protease. Serine proteases are enzymes that work by cleaving off a portion of their substrate. Cleavage of the inactive factor results in activation of that factor and is denoted by the suffix “a” after the Roman numeral (e.g. FXIa). The activated factor activates subsequent molecules in a series of reactions that eventually results in the generation of thrombin (Nichols, Ungerleider et al. 2006). Thrombin then acts on fibrinogen to convert it to fibrin, and a clot is formed which is then stabilised by FXIII.
1.5.1 Tissue Factor Pathway (Extrinsic Pathway)

Blood coagulation is initiated through the “tissue factor (TF) pathway”.

Following vessel injury, the damaged endothelium expresses TF. Tissue factor binds to FVII in plasma, and is rapidly converted to FVIIa-TF complex (Nemerson 1988). Factor VII is a trace plasma protein produced by the liver in the presence of vitamin K. Also, vascular endothelial cells and monocytes are able to produce and activate tissue factor when stimulated with interleukin-1 or endotoxin. This suggests that cytokines may modulate tissue factor expression and fibrin deposition at the site of inflammation (Moore, Andreoli et al. 1987).

The FVIIa-TF complex catalyses two reactions; first the conversion of FX to FXa and secondly FIX to FIXa. FXa activates prothrombin to thrombin, the primary mediator of coagulation (Drake, Morrissey et al. 1989). The prime function of thrombin in haemostasis is the conversion of soluble fibrinogen into insoluble fibrin. Fibrin monomers polymerize near the platelet plug and are strengthened further through cross-linking by FXIII (Sharathkumar and Pipe 2008). The activation of FX occurs by two mechanisms: direct activation by the FVIIa-TF complex and indirect activation by FIXa. The latter requires FVIII, phospholipid, and calcium ions (Rao, Robinson et al. 1992).

The activity of the FVIIa-TF complex is neutralised by tissue factor pathway inhibitor (TFPI) which is a plasma protein (Rapaport 1991). The plasma concentration of TFPI is low. TFPI first binds to FXa, and these complexes then react with the FVIIa-TF complex (Wesselschmidt, Likert et al. 1993).
TFPI directly blocks FXa as well as the generation of FXa and FIXa by the FVIIa-TF complex, but it does not inhibit production of FXa by FIXa. This is feedback inhibition of the tissue factor pathway but not of the direct activation by FIXa. Factors IX and VIII significantly reduce the inhibition of FX activation by TFPI via FVIIa-TF. It seems that direct activation of FXa by the tissue factor pathway is short-lived because of the action of TFPI. It is insufficient to sustain haemostasis. Thus, FIX activation in the (contact pathway) is important for continued FXa generation and effective haemostasis (Furie and Furie 2005). Factor X occupies a key position in the sequence of blood clotting as it is at that point that the contact and tissue factor pathways converge (common pathway). Factor X is a glycoprotein that, like prothrombin, requires vitamin K during its biosynthesis in the liver for its biologic activity (see Figure 1-1).
Figure 1-1 Haemostatic System

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1.5.2 Contact Activation Pathway (Intrinsic Pathway)

The contact activation pathway is initiated by contact of four clotting factors (factor XII, XI, prekallikrein, and high-molecular-weight kininogen (HMWK)) with a surface other than normal vascular endothelium or circulating blood cells. This interaction of the four proteins with such a surface results in the conversion of FXI to FXIa. Factor XIa in turn activates FIX to FIXa, which then catalyses the conversion of FX to FXa in the presence of FVIII, phospholipids and calcium ions (Rao, Robinson et al. 1992).

Factor XII is surface bound and is converted to FXIIa by proteolytic cleavage. It then activates surface-bound prekallikrein to form kallikrein by cleavage of a single peptide bond. Kallikrein, in turn, converts surface-bound factor XII to factor XIIa. This positive feedback loop amplifies the contact activation reaction (Gallin and Snyderman 1999).

A study by (Ratnoff and Saito 1979) demonstrated that the contact activation reaction appears to participate in other systems for host defence, such as kinin generation and fibrinolysis (Ratnoff and Saito 1979). Bradykinin, a potent vasoactive substance is released from HMW kininogen by the activation of FXII and the generation of kallikrein. Plasma kallikrein also activates urokinase and enhances fibrinolytic activity (Gallin and Snyderman 1999). Factor XIIa is also a weak activator of plasminogen.
Factor XI circulates in plasma as a protein complex with HMW kininogen. Factor XIa activates FIX. Factor IX can be converted to its serine protease form (FIXa) both by the contact pathway, FXIa and by the tissue factor pathway FVIIa-TF complex. Evidence suggests that FIX is principally activated by the tissue factor pathway (Bauer, Kass et al. 1990). Once activated, FIXa converts FX to its active form in the presence of FVIIIa, phospholipids, and calcium ions. The clotting factors form a multimolecular complex (the “tenase” complex) on the surface of phospholipid (PF3) found on stimulated platelets. Factors IXa and Xa mediate the reaction (Leytus, Foster et al. 1986). Factor VIII is a cofactor and requires activation by thrombin to FVIIIa before it can function in the complex (Mann, Jenny et al. 1988). Thrombin converts FVIII to FVIIIa by a specific proteolytic reaction that separates FVIII from VWF (Hill-Eubanks, Parker et al. 1989). Factor Xa also activates FVIII. Activated protein C degrades FVIIIa. Thus FVIII must be activated for haemostasis, and it must be inactivated to maintain the fluidity of blood (Furie and Furie 2005) (see Figure 1-1).

1.5.3 Plasma Inhibitors

Plasma contains serine protease inhibitors which limit and localize thrombosis and fibrinolysis. These include; antithrombin (heparin co-factor), heparin co-factor II, α2-plasmin inhibitor, plasminogen activator inhibitor I, activated protein C inhibitor, and tissue factor pathway inhibitor.
Antithrombin (AT) inhibits thrombin by forming a stable complex between an arginine residue of AT and the serine active site of thrombin. AT also inhibits factors XIIa, XIa, Xa, IXa, VIIa-TF, plasma kallikrein, and plasmin. Its action is greatly accelerated by heparin. Heparin binds to AT and induces a conformational change in AT that renders arginine at the reactive site more readily available for interaction with thrombin (Rosenberg and Rosenberg 1984).

Heparin cofactor II (HCII) selectively inhibits thrombin by forming a complex (Tollefsen and Blank 1981). The activity of this inhibitor, like that of AT, is stimulated in the presence of heparin. HCII differs from AT in two respects. First, HCII does not inhibit the activity of other activated clotting factors and second, HCII is stimulated by dermatan sulphate (Maimone and Tollefsen 1990).

α₂-plasmin inhibitor (α₂-PI) is a fast reacting plasmin inhibitor that forms a 1:1 complex interfering with the binding of plasminogen to fibrin (Aoki and Harpel 1984).

Plasminogen activator inhibitor I (PAI-1) is produced by a variety of cells such as endothelial cells, fibroblasts, hepatocytes and platelets. It is present at very low concentrations in plasma. PAI-1 inhibits both tissue plasminogen activator (t-PA) and urokinase by forming a complex with the enzyme. It plays a critical role in regulation of the activity of the fibrinolytic system. Another inhibitor of t-PA and urokinase is plasminogen activator inhibitor 2 (PAI-2) (Loskutoff, Sawdey et al. 1993).
Activated protein C (APC) inhibitor, inhibits the activity of activated protein C by forming a complex with this enzyme. This protein, also known as plasminogen activator inhibitor 3, inhibits urokinase as well (Heeb, Espana et al. 1987).

Tissue factor pathway inhibitor (TFPI) inhibits coagulation initiated by the FVIIa-TF complex (Rapaport 1991). The plasma concentration of TFPI is low. Most of it is bound to the vascular endothelium. Tissue factor pathway inhibitor interacts with FXa to form Xa-TFPI complexes that then forms a Xa-TFPI-VIIa-TF complex with resultant loss of the activity of FVIIa-TF. Tissue factor pathway inhibitor is synthesised by vascular endothelial cells and by hepatocytes (Broze and Miletich 1987; Rapaport 1991). Plasma levels of TFPI increase two to four fold following the infusion of heparin (see Figure 1-1).

1.6 Formation of Fibrin

Fibrinogen (factor I) is a large plasma protein that is synthesised in the liver. Fibrinogen is an acute phase reactant so the plasma concentration increases markedly in inflammation (Huber, Laurent et al. 1990). The conversion of fibrinogen to fibrin occurs in three stages. The first step is the cleavage of four small peptides – two fibrinopeptides A (FPA) and two fibrinopeptides B (FPB) – from the α and β chains respectively of the fibrinogen molecule by the action of thrombin. Fibrinogen from which FPA and FPB have been severed forms fibrin monomer. The second step in the transformation is the polymerisation of fibrin monomers. These aggregate to form fibrin polymers (fibrin strands) (Budzynski, Olexa et al. 1983). The third step results in the production of a
tough, insoluble form of the protein. It requires the action of FXIII (fibrinogen stabilising factor) and calcium ions. Factor XIII is activated by thrombin and cross-links fibrin polymers by forming covalent bonds (Lorand, Jeong et al. 1993) (see Figure 1-1).

1.7 Activation of Prothrombin and Generation of Thrombin

The final process in the coagulation cascade is the generation of thrombin. Prothrombin (factor II) is the precursor of thrombin. Prothrombin is a single-chain glycoprotein that is produced by the liver in the presence of vitamin K. Prothrombin is converted to thrombin by FXa. Factor Xa splits two internal peptide bonds in the prothrombin molecule and generates thrombin. This reaction is accelerated by FVα, phospholipids and calcium ions (Mann, Jenny et al. 1988). Formation of this multimolecular complex (the prothrombinase complex) brings molecules of prothrombin and FXa together and increases their chance of interacting (Furie and Furie 1992).

Thrombin, a serine protease enzyme, plays several important roles in haemostasis. It cleaves fibrinogen, and factors XIII, V and VIII, prothrombin, and protein C. Thrombin aggregates platelets, facilitating the formation of the haemostatic plug. Thrombin can stimulate vascular endothelial cells to increase their production and secretion of VWF, prostacyclin (PGI2) and tissue plasminogen activator inhibitor (de Groot, Gonsalves et al. 1984; Gelehrter and Sznycer-Laszuk 1986). Finally, thrombin can promote inflammation by a variety of mechanisms (Lorant, Patel et al. 1991) and can directly and indirectly promote cell proliferation (Coughlin 1994) (see Figure 1-1).
1.8 Fibrinolysis

The coagulation and fibrinolytic systems are two separate, but interlinked enzyme cascades that regulate the production and breakdown of fibrin. Once a stable fibrin clot has formed and the damaged tissues have been repaired, the fibrinolytic system metabolises fibrin, and restores normal blood flow.

Fibrinolysis is primarily achieved by plasmin, a serine protease enzyme produced in the liver and present in normal plasma. The inert precursor of plasmin is plasminogen. Plasminogen is converted to plasmin by the action of specific enzymes collectively known as plasminogen activators. Plasmin hydrolyses susceptible arginine and lysine bonds in many proteins, including fibrin, fibrinogen, FV and FVIII. The transformation of plasminogen to plasmin occurs preferentially on fibrin surfaces. When fibrin is formed, plasminogen is bound to it via its lysine-binding sites. Tissue plasminogen activator is also adsorbed on the fibrin surface and efficiently converts fibrin-bound plasminogen to plasmin, thereby providing localised activation of fibrinolysis (van Zonneveld, Veerman et al. 1986).

When fibrin is digested by plasmin, a series of fragments known as fibrin degradation products (FDP) or fibrin split products (FSP) are produced (Marder, Shulman et al. 1969). Fibrin degradation products inhibit both platelet aggregation and the action of thrombin on fibrinogen which may contribute to bleeding during fibrinolysis (Kowalski 1968).
Intrinsic activators include FXII and other contact factors such as prekallikrein and HMW kininogen. Extrinsic activators are widely distributed in almost all body tissues, including vasculature endothelium. They include tissue plasminogen activator (t-PA), urokinase PA (uPA), and streptokinase (Sprengers and Kluft 1987). Tissue plasminogen activator has a high affinity for fibrin, and this property seems to be crucial for the dissolution of fibrin. Tissue plasminogen activator may be released from the endothelial cells as a result of local or systemic stimuli, such as local thrombotic venous occlusion, physical exercise or thrombin (Thorsen, Glas-Greenwalt et al. 1972).

The fibrinolytic system is tightly controlled by the presence of plasma inhibitors that limit the generation and action of plasmin. There are at least two physiologically important inhibitors: plasminogen activator inhibitor I (PAI-I) and α₂-plasmin inhibitor (α₂-PI). PAI-I rapidly inactivates both tPA and urokinase, thereby regulating the activation of plasminogen. α₂-PI is a potent inhibitor of plasmin; it reacts instantaneously by forming a stable, inactivated complex with this enzyme (Narita, Bu et al. 1995).

The fibrinolytic system is activated and regulated to achieve dissolution of unwanted fibrin deposits without causing premature breakdown of the haemostatic plug. It is generally assumed that fibrinolysis is controlled by production and release of t-PA and PAI-1 from vascular walls, clearance of t-PA by the liver, activation of plasminogen, and inhibition of the activation and action of plasmin (Narita, Bu et al. 1995) (see Figure 1-1).
1.9 Summary

In summary, both the formation and dissolution of clots, and the maintenance of vascular integrity are governed by complex enzymatic reactions which have both positive and negative feedback loops. At the end of the thrombotic process a stable fibrin clot is enmeshed throughout a platelet plug. It is ultimately dissolved by fibrinolysis after tissue repair.

Abnormalities of haemostasis, coagulation and fibrinolysis have all been reported in patients with congenital heart disease. This observation is exaggerated in the newborn and small infant patients undergoing heart surgery utilising CPB. The use of cardiopulmonary bypass causes haemodilution of the patients’ circulating blood volume and is associated with derangements of all aspects of the haemostatic system. One of the major haemostatic abnormalities associated with CPB is platelet dysfunction. Although heparin is used to limit the activation of blood by an artificial system (CPB) it does not prevent a thrombogenic stimulus that contributes to post-bypass bleeding and clotting complications that result in significant morbidity in these patients.
Chapter 2
Cardiopulmonary Bypass Effects on Haemostasis

2.1 Introduction

Over fifty years ago two major innovations made surgical treatment of cardiovascular disease possible: the development of the heart-lung machine and the discovery and commercial production of the anticoagulant heparin.

There are numerous differences between infants and adults that affect the physiologic response to cardiopulmonary bypass (CPB). Technological limitations have meant that infants and children are exposed to relatively larger CPB circuits. This means that there is more haemodilution during open heart surgery. Surgical needs to visualise small structures have meant that techniques involving extreme hypothermia have been employed.

The coagulation systems are altered with the use of CPB through dilution of coagulation factors and platelets along with activation of the contact and tissue factor pathways. These abnormalities are exaggerated in infants compared to adults and can manifest at the end of CPB as a multifactorial coagulation defect.

2.2 History of Cardiopulmonary Bypass (CPB)

During the early 1900’s many physicians and researchers were interested in the challenges associated with the circulation of blood outside the body. In 1931, John Gibbon and his wife Mary began many years of research into the
development of a heart-lung bypass machine. Gibbon was the first to establish
the concept of combining extracorporeal circulation (ECC) and oxygenation of
the blood by artificial means (Gibbon 1954). In 1935 his heart-lung machine
supported the heart and lungs of a cat for almost four hours. The apparatus
used for this experiment was further improved and continued to be tested on
laboratory animals. Following these additional trials, Gibbon successfully
utilised ECC in a young woman to facilitate open cardiac repair of an atrial
septal defect (Gibbon 1954). Unfortunately, Gibbon’s next five patients did
not survive the procedure and he subsequently abandoned this technique.
Concurrently, experiences from other groups between 1951 and 1953 were
uniformly dismal, (see Table 2-1) (Nichols, Ungerleider et al. 2006) with 17
patient deaths after the initial success by Gibbon, discouraging continued
attempts at CPB (Nichols, Ungerleider et al. 2006).

The next milestone in the development of circulatory support was in 1954 when
Lillehei began using controlled cross-circulation (see Figure 2-1 and 2-2)
(Gravlee, Davis et al. 1993) using living relatives as the pump-oxygenator to
repair congenital heart defects. His initial experiment included 45 patients, with
success in 28 (Lillehei 1955). Astonishingly there was no donor mortality or
long-term morbidity (see Table 2-2) (Gravlee, Davis et al. 1993). Most
impressive is the 30 year follow-up of the original successful patient cohort; 22
out of the 28 patients were still alive and well (Lillehei, Varco et al. 1986).
Newer and safer mechanical extracorporeal systems led to the discontinuation of
cross-circulation. Kirklin’s group at the Mayo clinic developed the stationary
film oxygenator (Kirklin, Dushane et al. 1955) that spawned the continued development of artificial oxygenators (e.g. bubble oxygenator) into the late 1950s and 1960s (Nichols, Ungerleider et al. 2006). Membrane oxygenators came into practice in the 1970s due to increased safety and fewer complications. This enabled more complex repairs, with longer CPB exposure time.

Table 2-1

Open-Heart Surgery with Total Cardiopulmonary Bypass: Results of All Reported Cases 1951-1954 (Before cross-circulation, March 26, 1954)

<table>
<thead>
<tr>
<th>Surgeon</th>
<th>N</th>
<th>Oxygenator Type</th>
<th>Year</th>
<th>Survived</th>
<th>Died</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dennis</td>
<td>2</td>
<td>Film</td>
<td>1951</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Helmsworth</td>
<td>1</td>
<td>Bubble</td>
<td>1952</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Gibbon</td>
<td>6</td>
<td>Film</td>
<td>1953</td>
<td>1*</td>
<td>5</td>
</tr>
<tr>
<td>Dodrill</td>
<td>1</td>
<td>Autogenous</td>
<td>1953</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mustard</td>
<td>5</td>
<td>Monkey Lungs</td>
<td>1951-1953</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Clowes</td>
<td>3</td>
<td>Bubble</td>
<td>1953</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>18</td>
<td></td>
<td></td>
<td>1 (5%)</td>
<td>17 (95%)</td>
</tr>
</tbody>
</table>

*Atrial septal defect repair

Nichols DG, Ungerleider RM, Spevak PJ, Greeley WJ. Critical heart disease in infants and children. 2nd edition ed: Mosby; 2006. (Table 20-1 with permission) (Nichols, Ungerleider et al. 2006)
Table 2-2

Results of Direct-Vision Intra-cardiac Operations with CPB by Cross-Circulation in 45 Patients, from March 26, 1954, to July 9, 1955

<table>
<thead>
<tr>
<th>Abnormality</th>
<th>Corrective Operations</th>
<th>Patients</th>
<th>Hospital</th>
<th>Late (30 years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSD</td>
<td>Suture closure</td>
<td>27</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>PDA (with severe pulmonary hypertension)</td>
<td>Exploratory ventriculotomy; division of ductus</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>Closure of VSD; correction of infundibular/valvular pulmonary stenosis</td>
<td>10</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Atrioventricular is communis</td>
<td>Closure of ostium primum, VSD; repair of valvular deformities</td>
<td>5</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Isolated infundibular pulmonary stenosis</td>
<td>Resection of infundibulum</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
| Pulmonary stenosis, ASD, anomalous pulmonary venous return | Pulmonary valvotomy; ventricular and atrial cardioto
tomies; transposition of anomalous pulmonary veins; closure of septal defects | 1        | 1        | 0               |
| Totals:                                  |                                                             | 45       | 17       | 6               |

Cross-circulation was used exclusively from its inception through February 1955. Beginning March 1, 1955, other bypass methods (bubble oxygenator, dog-lung oxygenator, and arterial reservoir) were employed for lower-risk patients. Cross-circulation was reserved for high-risk patients. By July 1955, the bubble oxygenator had become the sole method. VSD, ventricular septal defect; PDA, patent ductus arteriosus; ASD, atrial (secundum) septal defect.

Gravlee GP, Davis RF, Utley JR, Cardiopulmonary Bypass Principles and Practice. Williams and Wilkins; 1993. (Table 1.6 with permission) (Gravlee, Davis et al. 1993)
Figure 2-1 Schematic of Cross-Circulation
A depiction of the method of direct-vision of intra-cardiac surgery utilising ECC by means of controlled cross-circulation. Gravlee GP, Davis RF, Utley JR, Cardiopulmonary Bypass Principles and Practice, Williams and Wilkins; 1993. (Figure 1.2 with permission) (Gravlee, Davis et al. 1993)

Figure 2-2 Operating Room Scene
The scene on March 26, 1954, during the first controlled-cross-circulation operation performed by Dr. C.W. Lillehei at the University of Minnesota Medical Center. A ventricular septal defect was successfully visualized by ventricular cardiotomy and closed on an infant with his father serving as the extracorporeal oxygenator. Gravlee GP, Davis RF, Utley JR, Cardiopulmonary Bypass Principles and Practice, Williams and Wilkins; 1993. (Figure 1.3 with permission) (Gravlee, Davis et al. 1993)
2.3 Cardiopulmonary Bypass Circuit Description

In the typical arrangement of the CPB circuit, blood drains by gravity into a venous reservoir through cannulae usually placed in the vena cavae. There is a filter between the venous reservoir and the oxygenator that removes particles from the circulation. The blood is then pumped through an oxygenator, and returned to the aorta (Lobato, Gravenstein et al. 2007). An additional filter is placed in the arterial line (returning blood to the patient) to trap any air or particulate emboli. High potassium solution is administered to stop the heart for the surgical procedure (see Figure 2-3). There are numerous artificial materials that comprise the bypass circuit: polyvinyl-chloride plastic tubing, polycarbonate plastics, polypropylene, polyester and nylon. Cardiopulmonary bypass permits the patient’s blood to bypass the heart and the lungs, achieving the desired operative field for surgical repair of the heart.

Figure 2-3 Schematic of Cardiopulmonary Bypass System
The disposable circuitry (tubing, oxygenator, filters etc.) must be filled with a priming solution, to de-air the system prior to use. The diameter and length of the tubing, size of the oxygenator, filters and cardioplegia system together, dictate the total volume of prime solution required. This mixes with the patient’s blood upon initiation of CPB. The decrease in the red cell mass, plasma protein concentration and clotting factors in the patient, after going onto bypass is referred to as haemodilution. Haemodilution is more profound in infants compared to the adult due to technological and practical limitations. The priming volume may exceed the blood volume of a neonate by as much as 100% to 200% compared to an adult patient for whom the prime volume may reflect only 20% to 30% of the patient’s blood volume.

2.4 Infants and Cardiopulmonary Bypass

Early observations of the consequences of blood flowing outside the body through the ECC demonstrated the need to prevent blood clotting, both on a gross scale and also microscopically. However, despite significant improvements in care over the last 50 years, deleterious effects remain associated with the interaction between the human body and the non-physiologic surface of the bypass circuit, especially significant in neonatal patients.

The use of CPB stimulates the haemostatic and inflammatory systems mainly by the contact of blood with the artificial surfaces of the bypass circuit (see Figure 2-4) (Gruenwald, Manlhiot et al. 2010). In addition, from the time of surgical incision there is activation of the tissue pathway. This is exacerbated by
reinfusion of tissue factor from the surgical field throughout the duration of CPB. Direct activation of the contact pathway causes thrombin generation, platelet activation and also stimulates the inflammatory response (Despotis, Avidan et al. 2001). Platelets and plasma coagulation factors are activated and consumed throughout bypass, often leading to consumptive coagulopathy (Despotis, Avidan et al. 2001; Mojcik and Levy 2001). This is despite the use of large doses of heparin as an anticoagulant during CPB. Clinical consequences are haemorrhage and the necessity for blood transfusion, development of a systemic inflammatory response, and organ failure (Leal-Noval, Rincon-Ferrari et al. 2001; Edmunds 2004). This response produces a wide range of cytotoxins, cell-signalling proteins, and vasoactive substances that circulate and disrupt interstitial fluid balance and homeostasis (Edmunds 2004).

2.5 Activation of Coagulation and Fibrinolytic Systems during CPB
The extensive contact between the CPB circuit and the patient’s blood leads to activation of the contact activation pathway (Tanaka, Takao et al. 1989). Activation of FXI initiates the coagulation cascade; cleavage of high-molecular-weight kininogen by kallikrein produces bradykinin, and cleavage of plasminogen by kallikrein and tissue plasminogen activator produces the fibrinolytic enzyme, plasmin. Heparin prevents coagulation during CPB by blocking the coagulation cascade at several levels. However, it does not inhibit the contact activation of FXII, with subsequent activation of the coagulation, fibrinolytic, and plasma kallikrein-kinin systems (Tanaka, Takao et al. 1989). The clinical consequences of activation of these systems may be responsible for some of the coagulation
disorders and inflammatory sequelae seen following CPB (Stibbe, Kluft et al. 1984; Holloway, Summaria et al. 1988). Platelet dysfunction associated with CPB is thought to be a major cause of haemostatic abnormalities in the post-bypass period (Harker, Malpass et al. 1980; Campbell 1991). Platelet abnormalities are both qualitative and quantitative. The activation of platelets produces functionally impaired platelets in the post-operative period. In addition, haemodilution causes a quantitative disorder of the platelets that is pronounced in neonates and infants (Kern, Morana et al. 1992).

Figure 2-4 Association between cardiopulmonary bypass, anticoagulation, bleeding, and thrombosis.

(AT, antithrombin; CHD, congenital heart disease; ACT, activated clotting time; INR, international normalised ratio; aPTT, activated partial thromboplastin time; IL, interleukin; TNFα, tumour necrosis factor alpha)

In depth analyses of haemostasis have characterised the specific effects of CPB in the paediatric population (Saatvedt, Lindberg et al. 1995; Chan, Leaker et al. 1997; Mossinger and Dietrich 1998). Activation of both the coagulation and fibrinolytic systems occur during CPB in neonates and children. The coagulation markers thrombin-antithrombin (TAT), prothrombin fragment F1+2 and D-dimers increase significantly, peaking at the end of CPB. The progressive increase in the plasma concentration of these markers confirm the generation of both thrombin and plasmin (Saatvedt, Lindberg et al. 1995; Chan, Leaker et al. 1997; Mossinger and Dietrich 1998). Saatvedt et al have shown a significant increase in plasma kallikrein (KK) values with decreased levels of prekallikrein (PKK) and kallikrein inhibitor (KKI) after heparin injection; KK level remained elevated throughout CPB (Saatvedt, Lindberg et al. 1995).

Chan et al (Chan, Leaker et al. 1997) showed a 50% decrease, in the concentration of all haemostatic proteins following the initiation of CPB. However, the majority of procoagulants, inhibitors of coagulation and some components of the fibrinolytic system (plasminogen, α2-antiplasmin) remained stable during CPB. They hypothesised that low plasma concentrations of heparin (<2.0 U/ml) and global decreases of components of the coagulation and fibrinolytic systems are primarily due to haemodilution and secondly to consumption (Chan, Leaker et al. 1997). Furthermore, the younger the patient, the more pronounced the reduction in plasma concentrations of coagulation factors (Kern, Morana et al. 1992). Inappropriate activation of the coagulation and fibrinolytic systems may contribute to the greater incidence of bleeding in
paediatric cardiac surgery (Saatvedt, Lindberg et al. 1995; Chan, Leaker et al. 1997; Mossinger and Dietrich 1998).

Activation of the fibrinolytic system during CPB has been documented in both adults (Tanaka, Takao et al. 1989; Spiess 1991) and children (Dietrich, Mossinger et al. 1993a; Saatvedt, Lindberg et al. 1995; Chan, Leaker et al. 1997). In one study of children, levels of both plasminogen and $\alpha_2$-antiplasmin ($\alpha_2$ AP) remained stable during bypass, while the ratio of tissue plasminogen activator (tPA) to plasminogen activator inhibitor (PAI) and levels of D-dimer increased; suggesting that the fibrinolytic system was activated during CPB. Post-operatively, levels of plasminogen and $\alpha_2$ AP increased but did not reach pre-operative levels while the ratio of tPA to PAI decreased and D-dimer levels stabilised; suggesting that the fibrinolytic system was shutting down (Chan, Leaker et al. 1997). In contrast, an earlier study by Petaja et al (Petaja, Peltola et al. 1996) demonstrated that fibrinolysis was not activated during CPB in neonates but showed late activation by measuring elevated levels of D-dimer on post-operative day three. They concluded that in neonates fibrinolysis is rarely seen during CPB because tissue plasminogen activator and its inhibitor both increase, effectively creating a physiologic fibrinolytic shutdown (Petaja, Peltola et al. 1996).

Williams et al (1998) showed that fibrinolysis was associated with poor tissue perfusion in critically ill infants (Williams, Bratton et al. 1998a). The patients that developed fibrinolysis during bypass were characterised by younger age,
greater degree of haemodilution and hypothermia utilised for more complex surgery and the need for longer duration of CPB (Williams, Bratton et al. 1998a).

It seems that despite anticoagulation, the artificial surface of the bypass circuit activates the fibrinolytic, coagulation and plasma kallikrein-kinin systems during open heart surgery in children (Saatvedt, Lindberg et al. 1995; Chan, Leaker et al. 1997; Mossinger and Dietrich 1998).

2.6 Heparin and Thrombin during CPB

Optimal administration of heparin therapy during CPB is important since excess heparin increases bleeding complications (Jumean and Sudah 1983; Levine 1986b; Levine 1986a) and suboptimal heparinisation may result in thrombin generation and fibrin deposition in the microvasculature (Bull, Huse et al. 1975; Bull, Korpman et al. 1975; Young, Kisker et al. 1978).

Unfractionated heparin is useful because it causes rapid thrombin inhibition and is reversed by protamine sulfate. A major disadvantage of heparin is that it fails to completely prevent thrombin formation during CPB (Boisclair, Lane et al. 1993; Brister, Ofosu et al. 1993).

CPB affects thrombin generation and inhibition by several mechanisms: heparin anticoagulation, activation via the CPB circuit, haemodilution, and
inadequate protamine sulfate neutralization of heparin (Levin, Wu et al. 2000). Thrombin induced platelet activation plays an important role in coagulation and fibrinolytic responses to CPB. In normal haemostasis, platelets exert anti-fibrinolytic activity through the release of PAI-1. After activation, platelets provide a specialised surface for the activation of both the coagulation and fibrinolytic systems (Levin, Wu et al. 2000). Therefore, thrombin generation during CPB may contribute to post-operative bleeding by activating platelets thus providing increased anticoagulant properties of the platelets at the same time (Lu, Soria et al. 1991; Lu, Lijnen et al. 1991; Winters, Santoro et al. 1991).

Thrombin normally acts locally at the site of blood vessel injury, however during CPB thrombin is circulated and may transform a local process into a systemic reaction. Specifically, the principle actions of thrombin during CPB are the cleavage of fibrinogen into fibrin, activation of FXIII to crosslink fibrin, activation of platelets by specific thrombin receptors, proteinase activated receptor-1 (PAR-1) and PAR-4, and stimulation of endothelial cells to release tissue plasminogen activator protein (t-PA) and von Willebrand factor (Koutts, Walsh et al. 1978; Colman, Marder et al. 2005).

Once thrombin formation is initiated by the FVIIa/tissue factor complex, the production of thrombin by the contact pathway is mediated by platelets (Walsh 2004). During bypass platelets are diluted by the priming volume and activated by low concentrations of thrombin in the pericardial wound and in the extracorporeal circuit (Gikakis, Khan et al. 1996). Although, the heterogeneity
of the circulating platelet mixture varies between patients and extracorporeal circuits, all patients do exhibit increased bleeding times and markers of granulocyte release and diminished platelet function following CPB (Edmunds, Ellison et al. 1982).

Rinder et al (1994) determined that children with CHD undergoing CPB showed a significant decrease in platelet glycoprotein IB (GP1B) adhesive receptor, and a high degree of platelet activation. Furthermore, cyanotic patients demonstrate a baseline deficit in the platelet adhesion receptor GP1B. This may explain the relatively high prevalence of a prolonged bleeding time in patients with cyanotic heart disease (Rinder, Gaal et al. 1994). Cardiopulmonary bypass produces a consumptive coagulopathy and reduces platelet numbers and function. The clinical consequences of the thrombotic response are thrombosis, fibrin-platelet microemboli and bleeding (Edmunds and Colman 2006).

A study in children with CHD undergoing open heart surgery examined the in-vitro capacity of plasma to both generate and inhibit thrombin before and after CPB (Turner-Gomes, Mitchell et al. 1994). The investigators identified that thrombin regulation was normal in these children before surgery. After CPB, the capacity to generate thrombin decreased by 50%, primarily as a result of haemodilution. Similar decreases were noted in the levels of AT, heparin cofactor II, and α2-macroglobulin following CPB. Levels of TAT and D-dimer increased after surgery, indicating that thrombin had been generated. The authors identified that there is a relative imbalance in the capacity of plasma to
regulate thrombin production with relative preservation of its thrombin-inhibitory capacity as compared with its thrombin-generating capacity. They concluded that these patients were at greater risk of bleeding rather than thrombotic complications following CPB (Turner-Gomes, Mitchell et al. 1994).

2.7 The Systemic Inflammatory Response during CPB

The systemic inflammatory response to CPB is multifaceted. Along with direct activation of the coagulation cascade, CPB triggers a systemic inflammatory response with positive feedback which leads to further activation of the coagulation system (Koster, Fischer et al. 2002; Heying, van Oeveren et al. 2006; Kozik and Tweddell 2006). The systemic response stems from several humoral and cellular cascades: the acute phase proteins, granulocytes and plasma proteinase systems of coagulation and fibrinolysis (Steinberg, Kapelanski et al. 1993; Borowiec, Hagman et al. 1995; Ernofsson, Thelin et al. 1997). Other factors contributing to this process include hypotension with non-pulsatile perfusion, relative anaemia, heparin and protamine sulfate administration as well as blood product administration (Nichols, Ungerleider et al. 2006).

Complement activation (Sonntag, Dahnert et al. 1998) results in the formation of C3a and C5a (Kouchoukos, Blackstone et al. 2003) which are important anaphylotoxins and chemotactic agents for neutrophils, and inflammatory mediators. C5a formation results in the production of other cytokines and activation of cellular elements such as platelets and macrophages (Kirklin, Westaby et al. 1983; Butler, Rocker et al. 1993). Complement activation also
results in the elaboration of many different cytokines from monocytes and endothelial cells that mediate the inflammatory reactions (Finn, Naik et al. 1993). Interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor-alpha (TNFα) are detectable in the immediate post-operative period. Tissue ischaemia and reperfusion of the heart enhances release of inflammatory cytokines IL-1, IL-6, IL-8 and TNFα by polymorph nuclear leukocytes (Brix-Christensen 2001; Carvalho, Maluf et al. 2001; Seghaye 2003). Elevated cytokines have been associated with prolonged CPB duration (Steinberg, Kapelanski et al. 1993).

The pro-inflammatory cytokine response is accompanied by an almost simultaneous anti-inflammatory release. This balance is thought to be critical in determining the extent of tissue injury and clinical outcomes in children (Chew, Brandslund et al. 2001). Chew et al (2001) showed that most of the pro-inflammatory cytokines present pre-operatively do not increase significantly during CPB, and that the anti-inflammatory cytokines increase during CPB with a peak response following CPB. This study demonstrated that both neonates and infants were capable of mounting a cytokine release and inflammatory response to CPB and cardiac surgery, in addition to activation of the coagulation system (Chew, Brandslund et al. 2001).

Although heparin inhibits the formation of clot during CPB it does not prevent the expression of tissue factor on endothelial cells. Tissue factor activation results in the production of thrombin, which by itself has significant thrombotic
and inflammation properties (Boyle, Verrier et al. 1996). The combined inflammatory and thrombotic response produces thousands of microscopic particles. These microemboli, consisting of platelet aggregates, fibrin fragments, fat, and other particulate matter obstruct arterioles, and in conjunction with cytotoxins, temporarily disturb organ function (Edmunds 2004). In uncomplicated cases, the inflammatory response is a temporary event representing a physiologic reaction to tissue injury. In more complex cases it manifests as generalised oedema and respiratory disturbances, and in severe cases multi-system organ failure. This process is exaggerated in infants and neonates compared to adults (Finn, Naik et al. 1993; Seghaye, Duchateau et al. 1993; Saatvedt, Lindberg et al. 1995; Butler, Pathi et al. 1996; Seghaye, Grabitz et al. 1996; Ashraf, Tian et al. 1997b).

There may be an ongoing inflammatory stimulus for tissue factor activation that extends into the post-operative period in infants undergoing open heart surgery (Jaggers, Neal et al. 1999). Greeley et al (Greeley, Bushman et al. 1988) demonstrated a significant increase in thromboxane levels during bypass in infants. Endothelial injury promotes release of vasoconstrictors such as thromboxane and endothelin and impairs release of important vasodilators such as prostacyclin and nitric oxide (Bui, Hammerman et al. 1991; Cave, Manche et al. 1993; Finn and Dreyer 1993). Furthermore, abnormalities in endothelial function promote increased capillary permeability and increased interstitial oedema (Seghaye, Grabitz et al. 1996).
In infants and neonates, the inflammatory response is characterised by respiratory distress syndrome (RDS), total body oedema, pulmonary hypertension, myocardial dysfunction, coagulation abnormalities and haemodynamic instability. Clinically this means prolonged ventilation, renal dysfunction, prolonged inotropic support, bleeding and later thrombosis, and if severe, the inability to close the chest in the operating room and possible need for long-term mechanical support (Dietrich, Mossinger et al. 1993b; Nichols, Ungerleider et al. 2006).

2.8 Circuit Coating to Address Inflammation During CPB

There have been many attempts to minimise the surface contact activation by CPB using technology. Industry has researched and developed coating techniques for extracorporeal circulation to improve haemocompatibility, reduce complement activation and suppress the inflammatory response to CPB. There is supportive data in the adult literature pertaining to the benefit of heparin-coated circuits (Videm, Svennevig et al. 1992; Gu, van Oeveren et al. 1993; Aldea, O'Gara et al. 1998; Tamim, Demircin et al. 1999; Wendel and Ziemer 1999).

Similar research has been undertaken in children, including the use of heparin coated systems for paediatric cardiac surgery. The results are equivocal. Several investigators have shown the advantages of heparin coated circuits in paediatric cardiac operations: less platelet activation (Schreurs, Wijers et al. 1998; De Somer, Francois et al. 2000), reduced levels of circulating C3a, an
activated complement factor with intrinsic endothelial-damaging capability (Olsson, Siegbahn et al. 2000), reduced inflammatory cytokine response (determined by levels of TNFα, IL-6 and IL-8) (Ashraf, Tian et al. 1997a; Kagisaki, Masai et al. 1997; Ozawa, Yoshihara et al. 2000) and less activation of fibrinolysis (Jensen, Andreasson et al. 2004). Some of the same investigators found no significant advantages related to key variables, such as leukocyte and platelet counts, improved post-operative blood loss, intubation time, or ICU length of stay (Ozawa, Yoshihara et al. 2000).

Two surface-modified CPB (SM-CPB) circuits are available for paediatric patients: the PMEA circuit, which utilises an amphiphilic polymer coating (poly-2-methoxyethylacrylate) on the tubing and oxygenator (Terumo Corporation, Tokyo, Japan), and the SMART circuit, which uses a combination of phosphorylcholine coating with polycaprolactone-polydimethylsiloxilane additives to the base resin (COBE Cardiovascular, Inc, Arvada, Colo). In a prospective study Kirschbom et al (2006) found no significant improvements in platelet counts, platelet function, post-operative bleeding, or blood product use when SM-CPB circuits were utilised when compared to uncoated circuits in elective paediatric cardiac surgery (Kirshbom, Miller et al. 2006).

2.9 Summary

The past 50 years has seen CPB evolve from an experimental heroic effort with high mortality to a safe means of ECC support for many children undergoing complex cardiac procedures throughout the world. Advances in
technology and health care have played a role in improving surgical outcomes for the most complex of problems. However, CPB is still responsible for significant morbidity related to bleeding, coagulation abnormalities and inflammation. This is particularly troublesome in neonates and young children.
Chapter 3

Bleeding, Transfusion and Thrombosis

3.1 Introduction

Advancements in the care of children with congenital heart disease (CHD) have allowed more complex anomalies to be surgically corrected at an earlier age, with reduced peri-operative mortality (Jaggers, Neal et al. 1999; McElhinney and Wernovsky 2001). Bleeding has been recognised as one of the most serious complications of cardiopulmonary bypass (CPB) for open heart surgery since the early use of these techniques. This has been attributed to alterations in haemostatic mechanisms caused by CPB. It has been recognised that the complexity of the process precludes a simple solution (Kern, Morana et al. 1992).

There are also risks of thrombosis with these operations (Petaja, Lundstrom et al. 1995; Chambers, Cohen et al. 1996; Jaggers, Neal et al. 1999) that contribute to morbidity and mortality (Guay and Rivard 1996). The imbalance between pro and anti-thrombotic activity during CPB is not well defined. The risk and incidence of thrombosis during and after cardiac surgery in children is currently unknown (Heying, van Oeveren et al. 2006). Therefore, both bleeding and thrombosis are of concern in children, especially when long-term sequelae are considered.
3.2 Quantifying the Problem

For both children (Greeley, Bushman et al. 1988; Miller, Mochizuki et al. 1997) and adults (Belisle and Hardy 1996), blood loss and transfusion practices after cardiac surgery vary widely between institutions. There are extreme variances in the literature regarding amount of bleeding observed in paediatric patients during the post-operative period, and no consensus on what is acceptable. The most recent incidence for excessive bleeding following congenital heart surgery is approximately 5% to 10% (Nichols, Ungerleider et al. 2006).

When comparing bleeding complications between adults and children: blood loss over 24 hours in adults undergoing cardiac surgery, including re-operations, ranged from 10-40 ml/Kg (Jobes, Nicolson et al. 1993). Studies in infants less than 2 years of age undergoing complex surgical repairs have reported blood loss as high as 63-110 ml/Kg in the first 24 hours (Manno, Hedberg et al. 1991; Reynolds, Nicolson et al. 1993). One publication defined excessive haemorrhage as chest tube drainage of more than 10% of the patient’s estimated blood volume (EBV) per hour during the first 2-3 hours after leaving the operating room (Kern, Morana et al. 1992). Another group (Stark, de Leval et al. 2006) defined excessive blood loss as measured intra-operative loss greater than 50% of the patient’s EBV or post-operative chest tube loss greater than 20% of EBV during the initial 2 hours in the ICU, greater than 20% of EBV 2-6 hours in the ICU, or greater than 30% during 6-12 hours in the ICU (Stark, de Leval et al. 2006).
In more recent publications severe post-operative bleeding was considered when chest tube volume loss was 10ml/Kg/hr or greater for at least a one hour time period (Agarwal, Bennett et al. 2007). Malviya et al (Malviya 1997) suggested that bleeding in excess of 10 ml/Kg/hr warrants the need for surgical re-exploration in children once other factors are eliminated i.e. neutralization of heparin, coagulation factor replacement (Malviya 1997). Others consider re-exploration for blood loss greater than 10% of the child’s circulating blood volume in any hour or blood loss greater than 5% of blood volume per hour for more than three consecutive hours (Nichols, Ungerleider et al. 2006).

Without well defined criteria for excessive bleeding the paediatric patient is at risk of unnecessary therapies such as increased allogeneic transfusion or delay of appropriate therapy, such as re-exploration.

3.3 **Factors Associated with Blood Loss and Transfusion during CPB**

CPB is associated with a reduction in fibrinogen levels, depression of platelets and increased red cell destruction. Patients who had previous heart surgery or more complex procedures had significantly more bleeding compared to first time or simple procedures (Phillips, Malm et al. 1963; Douglas, McNicol et al. 1966; Gans, Castaneda et al. 1967; Gomes and McGoon 1970).

Bleeding following CPB is multifactorial. The infant undergoing CPB is subjected to additional risk factors (Kern, Morana et al. 1992; Jobes, Nicolson et al. 1993) for excessive post-operative bleeding. The pre-operative risk factors
for excessive bleeding in the infant include age less than 2 years (Manno, Hedberg et al. 1991) and weight less than 8Kg (Miller, Mochizuki et al. 1997) which have been shown to be the most important factors associated with bleeding and transfusions following open heart surgery (Williams, Bratton et al. 1998b). Additional risk factors for bleeding include immaturity (Massicotte, Mitchell et al. 1986; Andrew, Paes et al. 1987; Williams, Bratton et al. 1998b; Monagle, Barnes et al. 2006), polycythemia (increased haematocrit, a surrogate measure of cyanotic heart disease), congestive heart failure (CHF) and the use of prostaglandin E which is a platelet inhibitor (Ekert and Gilchrist 1968; Gross, Keefer et al. 1968; Mauer, McCue et al. 1972; Moncada and Vane 1979).

A prospective study on consecutive children less than 18 years of age who underwent open heart surgery found post-operative blood loss was inversely related to age (Williams, Bratton et al. 1998b) as was blood product transfusion (Williams, Bratton et al. 1998b).

In another prospective study of 548 children undergoing open heart surgery, the factors associated with bleeding and transfusions varied with specific age categories. Lower weight and body core temperature during bypass was highly associated with blood loss and transfusions in infants less than 1 year of age; whereas, pre-operative CHF, prolonged duration of bypass and re-sternotomy were significant factors associated with bleeding and transfusions in children greater than 1 year of age. Intra-operative bleeding predicted excessive post-operative chest tube drainage (Williams, Bratton et al. 1999a).
Most procedures performed in infants are more complex than in adults (Manno, Hedberg et al. 1991; Guay and Rivard 1996; Williams, Bratton et al. 1998b; Williams, Bratton et al. 1999a), require longer duration of CPB (Seear, Wadsworth et al. 1989; Williams, Bratton et al. 1998b; Williams, Bratton et al. 1999a) and have multiple extra-cardiac suture lines. Perfusion techniques such as haemodilution, deep hypothermic temperature and circulatory arrest, have also been shown to influence the amount of bleeding (Kern, Morana et al. 1992; Turner-Gomes, Andrew et al. 1992; Jobes, Nicolson et al. 1993; Petaja, Lundstrom et al. 1995; Andropoulos, Stayer et al. 2010).

Platelet counts during CPB are independently associated with peri-operative blood loss and blood product transfusion (Williams, Bratton et al. 1999b). Platelet counts at the time of protamine sulfate administration correlates with chest tube drainage in children (Miller, Mochizuki et al. 1997) and adults (Despotis, Filos et al. 1996b). These qualitative and quantitative abnormalities are greatly exaggerated in infants compared to adults (Nichols, Ungerleider et al. 2006).

Other studies have confirmed an inverse correlation between body temperature during CPB and increased fibrin degradation products at the end of CPB (Manno, Hedberg et al. 1991; Guay and Rivard 1996); putting infants at a higher risk of bleeding (Jobes, Nicolson et al. 1993; Mossinger and Dietrich 1998) and thrombosis (Manno, Hedberg et al. 1991; Guay and Rivard 1996; Williams, Bratton et al. 1999a) following heart surgery.
3.4 Pharmacologic Therapy

Antifibrinolytic drugs have been utilised to minimise bleeding (Kneyber, Hersi et al. 2007). Aprotinin and tranexamic acid have both demonstrated a blood sparing effect in children (Reid, Zimmerman et al. 1997; Mossinger, Dietrich et al. 2003). Aprotinin is a serine protease inhibitor, inhibits kallikrein and the conversion of plasminogen to plasmin. It has been used during cardiac surgery to diminish blood loss and suppress inflammatory responses to CPB (Mossinger, Dietrich et al. 2003). A recent report of a negative mortality trend associated with aprotinin in high-risk patients undergoing cardiac surgery has resulted in the withdrawal of the product by the manufacturer, due to safety concerns demonstrated in adults (Fergusson, Hebert et al. 2008). Paediatric patients were not considered as a distinct population that may not be affected by the same factors. Synthetic lysine analogues, such as tranexamic acid, are now widely used to reduce blood loss (Brown, Birkmeyer et al. 2007).

Aside from antifibrinolytic drugs there are limited pharmacologic therapies available to treat excessive bleeding in infants following cardiac surgery (Agarwal, Bennett et al. 2007). Recombinant factor VIl a (rFVIl a) has been used to treat bleeding in haemophilia patients since the 1980s (Hedner and Kisiel 1983). High-dose rFVIl a enhances tissue factor initiated coagulation at the site of blood vessel injury through two mechanisms: first, FVIl a is capable of directly activating factor X on the surface of activated platelets resulting in increased thrombin generation at the site of injury, secondly, rFVIl a increases the concentration of FVIl a at the site of injury resulting in a higher number of
FVIIa-tissue factor complexes that are available to activate factors IX and X (Monroe, Hoffman et al. 2000; Lisman and De Groot 2003).

Although rFVIIa has been used off-label for treatment of severe bleeding for adult cardiac surgery (Al Douri, Shafi et al. 2000; Hyllner, Houltz et al. 2005; Steiner, Key et al. 2005) there are few studies evaluating the efficacy in children. Case reports and small case series in children (Egan, Lammi et al. 2004; Pychynska-Pokorska, Moll et al. 2004; Razon, Erez et al. 2005; Agarwal, Bennett et al. 2007) suggest that rFVIIa reduces severe post-operative bleeding, but there are serious concerns related to major thrombotic complications (Agarwal, Bennett et al. 2007; Guzzetta, Huch et al. 2009). Future randomised, controlled trials are needed to evaluate the potential indications, benefits and adverse effects of rFVIIa administration to infants following CPB.

3.5 Morbidity Associated with Bleeding following CPB

Bleeding abnormalities following adult cardiac surgery are well documented (Levin, Wu et al. 2000). These problems also affect children. Post-operative coagulopathy following CPB in infants is frequent and remains prevalent in older children (Williams, Bratton et al. 1998a; Williams, Bratton et al. 1999a).

Early investigations into complications associated with excessive bleeding reported the need for re-operation due to cardiac tamponade (Phillips, Malm et al. 1963; Douglas, McNicol et al. 1966; Gans, Castaneda et al. 1967; Gomes and McGoon 1970). Haemorrhage and tamponade are poorly tolerated in
infants therefore surgical haemostasis needs to be meticulous (Dietrich, Mossinger et al. 1993a; Jobes, Nicolson et al. 1993).

In addition to the sequelae of allogeneic blood donor exposures (Belisle and Hardy 1996; Stainsby, Jones et al. 2008), infants are more prone to complications related to infusion rate and volume (Motoyama and Davis 2005) of blood product transfusion ((Miller, Mochizuki et al. 1997; Agarwal, Bennett et al. 2007; Guzzetta, Huch et al. 2009).

Bleeding in children under 2 years of age is often accompanied by haemodynamic compromise (Jobes, Nicolson et al. 1993). Bleeding and transfusion of blood products are important causes of morbidity and mortality in the young (Petaja, Lundstrom et al. 1995; Chambers, Cohen et al. 1996; Williams, Bratton et al. 1998b).

Excessive post-operative bleeding prolongs hospital length of stay (Woodman and Harker 1990; Miller, Mochizuki et al. 1997). Less than half the patients who undergo re-operation actually have obvious surgical sources for bleeding (Moulton, Creswell et al. 1996; Dacey, Munoz et al. 1998; van de Watering, Hermans et al. 1998; Agarwal, Bennett et al. 2007). Re-exploration is associated with negative clinical outcomes such as renal failure, sepsis, increased hospital stay and mortality (Moulton, Creswell et al. 1996; Dacey, Munoz et al. 1998; van de Watering, Hermans et al. 1998; Arnold, Fergusson et al. 2006).
3.6 Transfusion and Risks

The availability of a safe and sufficient blood supply has enabled enormous advances to be made in cardiac surgery and intensive care including the use of extra-corporeal membrane oxygenation (ECMO).

Transfusion of allogeneic blood can be associated with adverse events including the transmission of infective agents (Goodnough, Brecher et al. 1999), acute and delayed haemolytic transfusion reactions, transfusion-related acute lung injury, transfusion-associated graft-versus-host disease (Nichols, Ungerleider et al. 2006; Kneyber, Hersi et al. 2007) and increased mortality (Kneyber, Hersi et al. 2007).

A recent study (Stainsby, Jones et al. 2008) reported the morbidity and mortality related to blood transfusion in children in the United Kingdom. Out of the total 3,239 reports, 321 (10%) were related to transfusion of children under 18 years and 147 (4.5%) to infants less than 12 months of age. There were 264 cases of “incorrect blood component transfused” resulting from errors at all stages in the transfusion chain; 26 suffered actual morbidity. Thirty acute transfusion reactions, three delayed transfusion reactions, 20 cases (3 fatal) of transfusion-related acute lung injury, two cases (both fatal) of transfusion-associated graft-versus-host disease and two transfusion transmitted infections were also reported (Stainsby, Jones et al. 2008).
Kneyber et al (Kneyber, Hersi et al. 2007) demonstrated that red blood cell transfusion in critically ill children is independently associated with increased mortality and prolonged mechanical ventilation, prolonged infusion of vaso-active agents and prolonged ICU stay. In addition, higher mortality rates were found among patients with multiple transfusions (Kneyber, Hersi et al. 2007).

The infant undergoing CPB for cardiac surgery requires blood product transfusion (Chambers, Cohen et al. 1996; Williams, Bratton et al. 1998b; Williams, Bratton et al. 1999a; Slonim, Joseph et al. 2008) as part of the priming volume, and to treat bleeding. Children undergoing heart surgery are more likely than adults to be transfused (Chambers, Cohen et al. 1996; Williams, Bratton et al. 1998b; Williams, Bratton et al. 1999a; Slonim, Joseph et al. 2008).

### 3.7 Type of Transfusion

Neonates undergoing cardiac surgery were transfused with fresh whole blood (FWB) (Lavee, Martinowitz et al. 1989; Manno, Hedberg et al. 1991; Kern, Morana et al. 1992; Jobes, Nicolson et al. 1993; Guay and Rivard 1996). Fresh whole blood is useful in replenishing deficient coagulation factors associated with CPB (Kern, Morana et al. 1992; Chambers, Cohen et al. 1996). In neonates, FWB has several advantages: the transfused blood product is from a single donor, is less than 48 hours old and is a balanced product of red cells, platelets, and coagulation factors. It avoids red cell dilution, which may occur during platelet or fresh frozen plasma transfusion in infants (Manno, Hedberg et al. 1991; Jobes, Nicolson et al. 1993) and has not been depleted of
2, 3-diphosphoglycerate (the major regulator of the haemoglobin-oxygen dissociation curve) and thus has optimal oxygen carrying capacity (Collins 1980).

When fresh whole blood is not available, Kern et al (Kern, Morana et al. 1992) suggests that treatment should be initiated empirically with platelets and followed by fresh frozen plasma if bleeding persists. In neonates, cryoprecipitate may be useful to avoid volume overload and further dilution of platelets (Kern, Morana et al. 1992). Studies have shown improved platelet function and reduced transfusion requirement utilising FWB compared with component therapy (Mohr, Martinowitz et al. 1988; Manno, Hedberg et al. 1991).

Fresh whole blood is generally not available due to logistical issues. An alternative approach using “reconstituted” fresh whole blood (RFWB) demonstrated independent factors that were associated with improvements in outcome (Gruenwald, McCrindle et al. 2008). These included blood less than 48 hours old, fewer donor exposures (single donor), and higher platelet count during bypass (Gruenwald, McCrindle et al. 2008). The study benefits may be replicated by using a fresh blood component therapy protocol reconstituting packed red blood cells (PRBCs), fresh frozen plasma and platelets when single donor RFWB is unavailable. The addition of platelets in the prime achieves a higher platelet count on bypass which was an independent predictor for less bleeding (Gruenwald, McCrindle et al. 2008). Platelet count during CPB was validated as a significant variable associated with blood loss in children by others (Williams, Bratton et al. 1999b).
3.8 Blood Storage

Blood storage is associated with decreased function and increased platelet activation which increases the risk of thrombosis (Cauwenberghs, van Pampus et al. 2007). Transfusion of PRBCs that had been stored for more than 14 days was associated with a significantly increased risk of post-operative complications and reduction of both short-term and long-term survival after adult cardiac surgery (Koch, Li et al. 2008; Lelubre, Piagnerelli et al. 2009). The risk of adverse outcomes increases incrementally with each unit of red cells transfused (Koch, Khandwala et al. 2006; Koch, Li et al. 2006a; Koch, Li et al. 2006b).

The storage time of RBCs used for priming the CPB circuit for newborns and infants is an independent risk factor for post-operative morbidity (Ranucci, Carlucci et al. 2009) including pulmonary complications, acute renal failure and infections (Ranucci, Carlucci et al. 2009). Our group studied a cohort of 1,037 consecutive paediatric cardiac surgery cases and found that older age of blood is associated with increased chest tube loss, PRBC transfusion, prolonged intubation, ICU and hospital stay (Menjak, Manlhiot et al. 2009). Each additional day of PRBC storage increased the odds of re-operation for bleeding and in-hospital mortality (Menjak, Manlhiot et al. 2009). The freshest possible blood is suggested for use peri-operatively (Menjak, Manlhiot et al. 2009; Ranucci, Carlucci et al. 2009).
3.9 Thrombosis

Venous clots can cause pulmonary embolisms and post-thrombotic syndrome. Arterial clots can cause myocardial infarctions, limb/organ ischaemia or leg length discrepancy, and arterial ischemic stroke. Clots from the venous system can embolise to the arterial system through septal defects and shunts in children with CHD. (See Figure 3-1)

**Figure 3-1** Arterial and Venous Clot Placement and Symptoms

There is very limited data on thrombotic complications (TCs) associated with paediatric cardiac surgery. The actual incidence of TCs is not currently known (Andrew, Monagle et al. 2000; Chan, Deveber et al. 2003). Most paediatric studies have used retrospective and/or administrative identification of TCs, both of which are susceptible to ascertainment bias (clinically evident events only) and reporting bias. In a recent study, the incidence of venous TCs in hospital discharge summaries increased from 34 to 58 events per 10,000 paediatric admissions between 2002 and 2007 (Raffini, Huang et al. 2009). The Canadian Paediatric Thrombosis Registry documented that approximately
25% of all children (50% of those less than 6 months of age) diagnosed with venous thrombosis had a congenital heart condition (Monagle 2003). In a cross-sectional study of 1,542 patients who underwent cardiac surgery between 2004 and 2007, it was found that there was an incidence of TCs of 13.2 events per 100 surgeries, of which venous TCs were 7.9 events per 100 surgeries (Manlhiot, Brandao et al. 2009; Manlhiot, Menjak et al. 2009). Clinical and surgical factors associated with increased risk of TCs included elements of blood hypercoagulability, resistance to anticoagulation, haemostatic disruption and extreme haemodilution of coagulation factors, disruption of blood flow and compromise of vascular wall integrity and vascular injury (see Table 3-1).

Table 3-1  Factors Associated with Thrombotic Complications

<table>
<thead>
<tr>
<th>Factor</th>
<th>OR (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior thromboembolism</td>
<td>3.1 (2.1-4.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Congenital prothrombotic disorder</td>
<td>3.0 (1.1-8.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>Number of prior surgeries</td>
<td>1.6 (1.2-1.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number of previous catheterization</td>
<td>1.5 (1.2-1.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age at surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-31 days</td>
<td>1.8 (1.2-2.7)</td>
<td>0.003</td>
</tr>
<tr>
<td>&gt;31 days - 1 year</td>
<td>1.3 (0.8-2.3)</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;1 - 5 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5 - 10 years</td>
<td>0.2 (0.1-0.7)</td>
<td>0.02</td>
</tr>
<tr>
<td>&gt;10 - 18 years</td>
<td>1.3 (0.6-2.8)</td>
<td>NS</td>
</tr>
<tr>
<td>Single ventricle physiology</td>
<td>1.6 (1.1-2.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>Transposition of the great arteries</td>
<td>1.6 (1.1-2.5)</td>
<td>0.03</td>
</tr>
<tr>
<td>Aortic arch abnormalities</td>
<td>3.1 (1.1-9.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Truncus arteriosus</td>
<td>3.0 (1.3-7.0)</td>
<td>0.01</td>
</tr>
<tr>
<td>Total anomalous pulmonary venous connections</td>
<td>3.5 (1.5-8.1)</td>
<td>0.02</td>
</tr>
<tr>
<td>Lower O2 saturation (/5%)</td>
<td>1.2 (1.1-1.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Stage I or II palliation</td>
<td>2.0 (1.1-3.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>Longer cross-clamp time</td>
<td>1.2 (1.1-1.3)</td>
<td>0.003</td>
</tr>
<tr>
<td>Use of deep hypothermia</td>
<td>1.6 (1.1-2.5)</td>
<td>0.04</td>
</tr>
<tr>
<td>ECMO before surgery</td>
<td>2.6 (1.3-5.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>ECMO after surgery</td>
<td>4.0 (2.3-6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Greater number of central venous lines</td>
<td>1.8 (1.1-2.9)</td>
<td>0.02</td>
</tr>
<tr>
<td>Greater number of days with central venous lines (/5 days)</td>
<td>1.2 (1.1-1.3)</td>
<td>0.003</td>
</tr>
</tbody>
</table>
There is a lack of consensus on clinical and laboratory signs or symptoms of active thrombosis and on which patients should be routinely screened for TCs. Many episodes are asymptomatic or have non-specific symptoms. In a prospective 2 year registry of 99 children with TCs in the Netherlands, 35% of patients were symptom free (van Ommen, Heijboer et al. 2001). Thrombosis may be clinically evident by a number of acute symptoms including swelling and discoloration of the affected limb, pain, diminished or absent peripheral pulses, decreased skin temperature, and prolonged capillary refill time (Chan, Deveber et al. 2003; Price and Massicotte 2003). Venous thrombosis may present as pulmonary embolism, chylothorax, chylopericardium, and superior vena cava syndrome (Dhande, Kattwinkel et al. 1983; Graham and Gumbiner 1984; Andrew, David et al. 1994; Schmidt and Andrew 1995; Kurekci, Kaye et al. 1998; Massicotte, Dix et al. 1998; Chait, Dinyari et al. 2001). The majority of children with arterial thrombosis are clinically asymptomatic (Andrew, Monagle et al. 2000). A large proportion of arterial thromboses are diagnosed on autopsy; this is also the most common method of diagnosis of pulmonary embolism in children (Andrew, Monagle et al. 2000). The average time from surgery to diagnosis of venous thrombosis ranges from 13-17 days, although many episodes present between post-operative days 2-4 (Berman, Fripp et al. 1991; Petaja, Lundstrom et al. 1996). Even without validated screening protocols, the number of identified thrombotic episodes has been increasing possibly due to better awareness (Sandoval, Sheehan et al. 2008; Raffini, Huang et al. 2009). High risk patients are not routinely screened for thrombosis as there is no validated protocol. This can be detrimental for
asymptomatic patients (Price and Massicotte 2003; Revel-Vilk, Sharathkumar et al. 2004). There is no validated protocol for the use of diagnostic imaging to detect clots, and even high risk patients are not routinely screened for thrombosis. This can be particularly detrimental for patients with silent thrombosis (Price and Massicotte 2003; Sandoval, Sheehan et al. 2008) and those with non-specific clinical signs. Because of the lack of specific symptoms in many cases, the index of suspicion is often low and many cases can go unnoticed until they manifest in a more severe or acute manner. Still today, a large proportion of arterial thromboses are diagnosed on autopsy, which is also the most common method of diagnosis of pulmonary embolism in children (Andrew, Monagle et al. 2000). This lack of clinical suspicion is evident in that the average time from surgery to diagnosis of venous thrombosis ranges from 13-17 days, when many episodes present between post-operative days 2-4 (Berman, Fripp et al. 1991; Petaja, Lundstrom et al. 1996). Nonetheless, many TCs are likely still to be ignored or undetected, and TCs after paediatric cardiac surgery might be much more frequent than previously estimated.

Thrombosis can be treated using pharmaceutical and mechanical interventions (Revel-Vilk and Chan 2003; Cynamon, Stein et al. 2006; Manco-Johnson 2006; Motsch, Walther et al. 2006; Schobess, During et al. 2006). Most studies of thrombosis treatment are inadequately powered randomised controlled trials and prospective cohort or retrospective reviews. Many current treatment guidelines are extrapolated directly from adult studies and are inappropriate for
children (Massicotte, Julian et al. 1999; Chan, Deveber et al. 2003; Kuhle, Eulmesekian et al. 2007; Chan, Black et al. 2008; Ignjatovic, Summerhayes et al. 2008). The use of thrombolytic drugs in children is rare and often only partially effective (Asante-Korang, Sreeram et al. 1992; Gupta, Leaker et al. 2001). The margin of safety is thought to be narrow; the reported frequency of major bleeding varies from 5% to 40% (Leaker, Massicotte et al. 1996; Petaja and Peltola 1997).

Outcomes of TCs are suboptimal. Reported rates of resolution of thrombosis in children vary between 36-67%. The outcome is influenced primarily by the site and degree of occlusion, and less by age and treatment (Andrew, David et al. 1994; Leaker, Massicotte et al. 1996; Petaja, Lundstrom et al. 1996; Hauser, Hubner et al. 2001; Kearon 2003; Kuhle, Koloshuk et al. 2003; Revel-Vilk, Sharathkumar et al. 2004; Brandao, Manlhiot et al. 2009). Patients with thrombosis are at risk of severe complications including pulmonary embolism, strokes, haemorrhage, cardiorespiratory arrest/failure and death (Prandoni and Bernardi 1999; Male, Kuhle et al. 2003; Kaulitz, Ziemer et al. 2005). Venous thrombotic events in children with CHD result in a mortality of 7-9% (Monagle 2003; Kenet, Kirkham et al. 2007). Pulmonary embolism has a mortality up to 20% (Biss, Brandao et al. 2008). Arterial strokes and sinovenous thrombosis have a high mortality and up to two thirds of patients have permanent neurologic damage (deVeber, MacGregor et al. 2000; deVeber, Andrew et al. 2001). Henke et al. (2007) found the length of hospital stay for cardiac surgical patients was longer by 68-126% in patients with thrombosis,
significantly increasing the cost of care. Thrombosis after surgery is linked to a 3.4 fold increase in mortality (Brown, Ridout et al. 2003; Gillespie, Kuijpers et al. 2006; Henke, Froehlich et al. 2007).

In a cross-sectional review of 1,542 paediatric cardiac surgeries (Manlhiot, Brandao et al. 2009; Manlhiot, Menjak et al. 2009), 233 patients had TCs. Of these 87 (42%) either had an intervention or died prior to hospital discharge. Treatment included intravascular tissue plasminogen activator (tPA), re-operation or re-catheterization. This composite outcome was more likely in patients with clots in multiple systems, odds ratio (OR) (OR: 1.9, p=0.02), clots in the arterial system (OR: 2.6, p=0.02) and for clots in the carotid artery, brachiocephalic artery or cerebral arteries (OR: 4.4, p=0.03). Asymptomatic clots were slightly less likely to be associated with complications (OR: 0.5, p=0.09) (Brandao, Manlhiot et al. 2009). Autopsy report listed TCs as primary or secondary cause of death in 12/23 (52%) patients with TCs who died and in 12/57 (21%) of all deaths in this cohort. Patients with either isolated intra-cardiac TCs or with TCs in multiple vascular systems had a significantly higher associated risk of cardiac arrest (31% vs. 14%, p=0.04) and mortality (31% vs. 10%, p=0.009) than those with isolated intra-venous or intra-arterial TCs. In multivariate regression models, intravascular TCs were independently associated with longer ICU stay (p<0.001), longer hospital stay (p<0.001), cardiac arrest (OR: 6.9 (3.2 - 14.6), p<0.001), all cause re-operation (OR: 4.4 (2.4 - 8.2), p<0.001) and higher odds of hospital death (OR: 2.8 (1.2 - 6.5), p<0.001).
Long-term complications of TCs manifest as post-thrombotic syndrome (PTS). Signs of PTS can be present in 30-70% of children after venous thrombosis, are clinically significant in 10-20% of all children post venous thrombosis and in 50% of children when thrombosis occurred after surgery (Monagle, Adams et al. 2000; Anton and Massicotte 2001; Marzinotto, Choi et al. 2001; van Ommen, Ottenkamp et al. 2002; Kuhle, Koloshuk et al. 2003). The incidence of PTS has been increasing in recent years (Vu, Nobuhara et al. 2008). The true rate of PTS in children is probably underestimated because of the high frequency of unidentified TCs (van Ommen, Heijboer et al. 2001; Chan, Deveber et al. 2003). Clinical manifestations of PTS include varicose veins, oedema, skin hyperpigmentation, and skin ulcers. Pain or heaviness in the affected leg, chronic limb oedema and/or venous stasis ulcers also occurs (Manco-Johnson, Nuss et al. 2000; Ziegler, Schillinger et al. 2001; Barnes, Newall et al. 2002; van Ommen, Ottenkamp et al. 2002; Kuhle, Koloshuk et al. 2003; Cynamon, Stein et al. 2006; Pesavento, Bernardi et al. 2006). PTS is caused by residual obstruction at the site of proximal deep venous thrombosis and venous hypertension. It is associated with the development of venous valvular insufficiency (Monagle, Adams et al. 2000). Venous hypertension results in widening of the endothelial cellular junctions and extravasations of red cells, fibrinogen, and inflammatory mediators, resulting in painful discoloured skin and brawny indurations of subcutaneous tissue (Labropoulos, Leon et al. 1994; Johnson, Manzo et al. 1995; Rutherford 1996). Symptoms of PTS may develop over months to years following the initial thrombotic event (Piovella and Barone 1999). The management of established post-thrombotic
syndrome is challenging. Surgical therapy is often unsuccessful (Ackroyd and Browse 1986). Patients with PTS report decreased quality of life, and the severity of the disease increases over time (Ziegler, Schillinger et al. 2001; Kahn and Ginsberg 2002).

3.10 Summary

Cardiopulmonary bypass causes a significant disruption to haemostasis in children which increases the risk of both bleeding and thrombosis. Blood loss is associated with specific patient and peri-operative factors. Younger age was most significantly associated with bleeding and blood product transfusions following heart surgery. Deep hypothermic circulatory arrest and prolonged bypass periods tend to result in low platelet counts. All of these factors have been identified as being associated with increased bleeding and transfusion in children following heart surgery.

Understanding these factors may assist pre-operative formulation of haemostasis strategies and guide peri-operative coagulation management (Williams, Bratton et al. 1999b).

Bleeding post-CPB is treated with transfusion of allogeneic blood products and pharmacologic therapy including antifibrinolytic drugs. Due to the small blood volume in the infant compared to the CPB prime requirement, the use of allogeneic blood products is mandated in most cases. The type of transfusion
(component versus whole blood), age of blood and the number of donor products used all influence clinical outcome.

Ultimately, both surgery and bleeding contribute to thrombosis, which is a serious complication. Outcomes of thromboembolic complications are guarded. Treatment is difficult and associated with high risk of adverse events.
4.1 Introduction

Infants who undergo CPB for cardiac surgery have particular risks due to their age and size, such as the immaturity of the haemostatic system, greater levels of haemodilution, varied pharmacokinetic responses to anticoagulants and the use of deep hypothermic circulatory arrest to support complex surgical repairs (Oliver 2003).

Insufficient anticoagulation promotes platelet dysfunction, thrombin formation, bleeding and thrombosis. Too much anticoagulation results in bleeding and transfusion complications in the post-operative period (Guzzetta, Bajaj et al. 2008). Cardiac surgery presents both pro-thrombotic and pro-haemorrhagic influences. The level of anticoagulation on bypass can impact clinical outcomes therefore careful management and monitoring is important (Heying, van Oeveren et al. 2006).

Early monitoring of anticoagulation during CPB involved direct observation of fibrin strands in the bypass circuit. Initial monitors of anticoagulation during CPB consisted of simple tests that measured the time it takes for whole blood to form a clot. More sophisticated and complex monitoring systems are emerging.
4.2 Immaturity of the Coagulation System in Infants

The haemostatic system in infants and children differs significantly from that of adults although it should be considered physiologically functioning (Andrew, Vegh et al. 1992; Monagle, Chan et al. 2004). The rate of blood protein production increases with gestational age and throughout the first year of life when the plasma activity for most coagulation factors becomes comparable with that of adults (Sharathkumar and Pipe 2008).

The significance of the developmental differences on the mechanisms of anticoagulation in the infant during cardiac surgery is not well understood. Andrew et al (1987,1992) identified age-related differences in the haemostatic system in normal infants and children compared to adults (Andrew, Vegh et al. 1992). This was confirmed by Monagle et al (2006).

The concentration of many coagulation proteins and inhibitors are lower at birth than in adults (Andrew, Paes et al. 1987; Monagle, Barnes et al. 2006). Vitamin K-dependent factors (II, VII, IX, X) and contact factors (XII, XI, prekallikrein (PK) and high molecular weight kininogen (HMWK) are approximately 50% of adult values and remain 10-20% reduced throughout childhood. In contrast, values for fibrinogen, FVIII and FV at birth are similar to adult values (see Table 4-1) (Andrew, Paes et al. 1987; Monagle, Barnes et al. 2006).

The inhibitors of coagulation, such as antithrombin (AT), protein C and S as well as heparin cofactor II (HCII) are significantly less than adult values at
birth. Some haemostatic components remain reduced throughout childhood i.e. protein C (Andrew, Paes et al. 1987; Monagle, Barnes et al. 2006). In contrast, AT and protein S levels reach adult values by approximately three months of age. Levels of α2-macroglobulin and α1-antitrypsin (inhibitors of coagulation) are at adult values at birth with α2-macroglobulin values continuing to rise throughout childhood. Cl-esterase inhibitor is low at birth and rises above adult values by six months of age (Andrew, Paes et al. 1990; Mitchell, Piovella et al. 1991; Petaja, Lundstrom et al. 1996).

Although the age-related changes in coagulation proteins are now well defined throughout childhood, the functional and structural differences are yet to be determined (Monagle, Barnes et al. 2006). Monagle et al (2006) observed that absolute values of measured haemostatic components for most assays were proportionally increased in all age-groups compared to Andrew’s work. The authors highlight the importance of determining age-related reference ranges, and the issues of measurement differences for various reagents and analysers utilised (Monagle, Barnes et al. 2006).

Alterations in concentration of specific coagulation proteins may increase the risk of haemorrhage or thrombotic complications (TC’s) (Andrew, Paes et al. 1990; Andrew, Vegh et al. 1992). Neonatal plasminogen does not bind as well to cellular receptors because of differences in carbohydrate composition from the adult form. This results in decreased fibrinolytic activity (Edelberg, Enghild et al. 1990). Plasma prothrombin concentrations are 10-20% lower in
young children (Andrew, Paes et al. 1990; Andrew, Vegh et al. 1992) and the
capacity to generate thrombin is decreased by 26% (Andrew, Mitchell et al.
1994). This balance between pro and anti-thrombotic states as seen in the
neonatal coagulation system might explain why infants are generally less
susceptible to clots than adults (Andrew 1995), but also why infants are at
higher risk of both bleeding and thrombotic complications following CPB
(Heying, van Oeveren et al. 2006).

4.3 Cyanotic Congenital Heart Disease (CHD)
Cyanotic congenital heart disease (CHD) is associated with increased risk of
stroke and thromboembolism (Kajimoto, Nakazawa et al. 2007; Manlhiot,
Brandao et al. 2009; Manlhiot, Menjak et al. 2009) compared to other young
children with CHD. The understanding of the significance of haemostatic
abnormalities and platelet activation has improved (Levin, Wu et al. 2000).

Unusual circulation patterns and high haematocrit may affect haemostasis.
Odegard et al (2002) found that lower levels of FVII, protein C and S were
positively correlated with the length of time between the Glenn and Fontan
procedure in patients with single ventricle physiology. Factors II, V, VII, X;
plasminogen; and AT were significantly lower in these patients when
compared to age-matched controls prior to completion of the cavopulmonary
connection (Odegard, McGowan et al. 2002).
A recent study reported that heparin co-factor II, and α-2 macroglobulin levels are significantly lower in infants with CHD compared to healthy age-matched controls (McEwan 2007). The normal ratio of these inhibitors is approximately twice AT levels in healthy infants. This may account for higher levels of thrombin generation during CPB (McEwan 2007). There is a correlation between patients with cyanotic CHD and higher levels of P-selectin on platelets and elevated thrombin-antithrombin (TAT) compared to normal subjects. These findings were associated with an increase in risk of thromboembolic events (Kajimoto, Nakazawa et al. 2007). The increased coagulability and platelet activation demonstrated in patients with cyanotic CHD appears to promote a prothrombotic milieu (Kajimoto, Nakazawa et al. 2007).

Low AT levels in cyanotic CHD patients correlated with higher thrombin generation as indicated by elevated prothrombin F1+2 levels (Heying, van Oeveren et al. 2006). These studies identify factors that may contribute to the coagulation abnormalities seen in patients with cyanotic CHD.

4.4 Haemodilution

The use of crystalloid solution to prime the bypass circuit and avoid transfusions for open heart surgery, was introduced in 1959 by Panico and Neptune (Panico and Neptune 1960). At the time, hypothermia was proposed to decrease oxygen consumption. The inevitable haemodilution from this approach reduces plasma proteins and clotting factors, decreases colloid osmotic pressure, activates inflammatory mediators, produces electrolyte imbalances and results in release of stress hormones (Nichols, Ungerleider et al. 2006).
The safe lower limit of haematocrit is not known, especially during neonatal and infant heart surgery. The priming volume of the bypass circuit and the blood volume of the patient dictate the level of haemodilution during CPB. In infants, the prime volume of the circuit is relatively large. This mandates the use of donor blood products in the prime to avoid excessive haemodilution.

The optimal level of haemodilution during CPB is not known. The goal is to provide adequate oxygen delivery while minimizing blood product exposure. Shin’oka et al (Shin'oka, Shum-Tim et al. 1996) have shown that higher haematocrit (30%) during CPB is associated with improved cerebral recovery after deep hypothermic circulatory arrest (Shin'oka, Shum-Tim et al. 1996). More recently, higher haematocrit levels (35-40%) during hypothermic CPB have been advocated on the basis of potentially improved neurologic outcome (Gruber, Jonas et al. 1999).

Dilution of haemostatic components such as AT and platelets cause significant problems during and following CPB in infants (Kern, Morana et al. 1992; Jobes, Nicolson et al. 1993; Chambers, Cohen et al. 1996; Guay and Rivard 1996; Chan, Leaker et al. 1997; Kirshbom, Miller et al. 2006). As previously noted, neonates normally have lower levels of haemostatic components. CPB reduces coagulation factor levels and AT by approximately 50%, and platelet counts fall up to 70%, creating serious deficiencies in most elements of haemostasis (Kern, Morana et al. 1992; Chan, Leaker et al. 1997).
## Table 4-1 Reference values for coagulation tests in healthy full-term children

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 3</th>
<th>1 month – 1 year</th>
<th>1 – 5 years</th>
<th>6 – 10 years</th>
<th>11 – 16 years</th>
<th>Adults</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>APTT results (sec)</strong></td>
<td></td>
<td></td>
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<tr>
<td>PTT-A</td>
<td>38.7 (34.3-44.8)</td>
<td>36.3 (29.5-42.2)</td>
<td>39.3 (35.1-46.3)</td>
<td>37.7 (33.6-43.8)</td>
<td>37.3 (31.8-43.7)</td>
<td>39.5 (33.9-46.1)</td>
<td>33.2 (28.6-38.2)</td>
</tr>
<tr>
<td>CK Prest</td>
<td>NA</td>
<td>NA</td>
<td>34.4 (31.1-36.6)</td>
<td>32.3 (29.8-35.0)</td>
<td>32.9 (30.8-34.8)</td>
<td>34.1 (29.4-40.4)</td>
<td>29.1 (25.7-31.5)</td>
</tr>
<tr>
<td>Actin FSL</td>
<td>NA</td>
<td>NA</td>
<td>37.4 (33.4-41.4)</td>
<td>36.7 (31.8-42.8)</td>
<td>35.4 (30.1-40.4)</td>
<td>38.1 (32.2-42.2)</td>
<td>30.8 (27.1-34.3)</td>
</tr>
<tr>
<td>Platelin L</td>
<td>NA</td>
<td>NA</td>
<td>36.5 (33.6-40.4)</td>
<td>37.3 (32.5-43.8)</td>
<td>35 (31.0-39.3)</td>
<td>39.4 (32.6-49.2)</td>
<td>31.3 (27.2-35.4)</td>
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<tr>
<td><strong>Coagulation tests</strong></td>
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<td></td>
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<td></td>
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<tr>
<td>TCT (sec)</td>
<td>NA</td>
<td>NA</td>
<td>17.1 (16.3-17.6)</td>
<td>17.5 (16.5-18.2)</td>
<td>17.7 (16.7-17.9)</td>
<td>18.0 (16.9-18.0)</td>
<td>17.1 (16.1-18.5)</td>
</tr>
<tr>
<td>PT (sec)</td>
<td>15.6 (14.4-16.4)</td>
<td>14.9 (13.5-16.4)</td>
<td>13.1 (11.5-15.3)</td>
<td>13.3 (12.1-14.5)</td>
<td>13.4 (11.7-15.1)</td>
<td>13.6 (12.7-16.1)</td>
<td>14.0 (11.5-14.5)</td>
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<tr>
<td>INR</td>
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<td>1.00 (0.86-1.22)</td>
<td>1.03 (0.92-1.14)</td>
<td>1.04 (0.87-1.20)</td>
<td>1.08 (0.97-1.30)</td>
<td>1.00 (0.80-1.20)</td>
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<td>Fibrinogen (g/L)</td>
<td>2.80 (1.92-3.74)</td>
<td>3.30 (2.83-4.01)</td>
<td>2.42 (2.02-3.83)</td>
<td>2.82 (2.62-4.01)</td>
<td>3.04 (2.09-4.09)</td>
<td>3.15 (2.42-4.33)</td>
<td>3.1 (2.14-4.3)</td>
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<tr>
<td><strong>Coagulation factors (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>54 (41-69)</td>
<td>62 (50-73)</td>
<td>90 (62-103)</td>
<td>89 (70-109)</td>
<td>89 (67-110)</td>
<td>90 (61-107)</td>
<td>110 (78-138)</td>
</tr>
<tr>
<td>V</td>
<td>81 (64-103)</td>
<td>122 (92-154)</td>
<td>113 (94-141)</td>
<td>97 (67-127)</td>
<td>99 (56-141)</td>
<td>89 (67-141)</td>
<td>118 (78-152)</td>
</tr>
<tr>
<td>VII</td>
<td>70 (52-88)</td>
<td>86 (67-107)</td>
<td>128 (83-160)</td>
<td>110 (70-156)</td>
<td>113 (70-156)</td>
<td>118 (69-200)</td>
<td>129 (61-199)</td>
</tr>
<tr>
<td>VIII</td>
<td>182 (105-329)</td>
<td>159 (83-274)</td>
<td>94 (54-145)</td>
<td>94 (44-127)</td>
<td>96 (48-145)</td>
<td>111 (64-216)</td>
<td>130 (59-254)</td>
</tr>
<tr>
<td>IX</td>
<td>48 (35-56)</td>
<td>72 (44-97)</td>
<td>71 (43-121)</td>
<td>85 (44-127)</td>
<td>96 (48-145)</td>
<td>111 (64-216)</td>
<td>130 (59-254)</td>
</tr>
<tr>
<td>X</td>
<td>55 (46-67)</td>
<td>60 (46-75)</td>
<td>95 (77-122)</td>
<td>98 (72-125)</td>
<td>97 (68-125)</td>
<td>91 (53-122)</td>
<td>124 (96-171)</td>
</tr>
<tr>
<td>XI</td>
<td>30 (7-41)</td>
<td>57 (24-79)</td>
<td>89 (62-125)</td>
<td>113 (65-162)</td>
<td>113 (65-162)</td>
<td>111 (65-162)</td>
<td>112 (67-196)</td>
</tr>
<tr>
<td>XII</td>
<td>58 (43-80)</td>
<td>53 (14-80)</td>
<td>79 (20-135)</td>
<td>85 (36-135)</td>
<td>81 (26-137)</td>
<td>75 (14-117)</td>
<td>115 (35-207)</td>
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<td><strong>Coagulation inhibitors (%)</strong></td>
<td></td>
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</tr>
<tr>
<td>AT</td>
<td>76 (58-90)</td>
<td>74 (60-89)</td>
<td>109 (72-134)</td>
<td>116 (101-131)</td>
<td>114 (95-134)</td>
<td>111 (96-126)</td>
<td>96 (66-124)</td>
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<tr>
<td>Protein C Chromogenic</td>
<td>36 (24-44)</td>
<td>44 (28-54)</td>
<td>71 (31-112)</td>
<td>96 (65-127)</td>
<td>100 (71-129)</td>
<td>94 (65-118)</td>
<td>104 (74-164)</td>
</tr>
<tr>
<td>Protein C clotting</td>
<td>32 (24-40)</td>
<td>33 (24-51)</td>
<td>77 (28-124)</td>
<td>94 (50-134)</td>
<td>94 (64-125)</td>
<td>88 (59-112)</td>
<td>103 (54-166)</td>
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<tr>
<td>Protein S clotting</td>
<td>36 (28-47)</td>
<td>49 (33-67)</td>
<td>102 (29-162)</td>
<td>101 (67-136)</td>
<td>109 (64-154)</td>
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<tr>
<td>D-Dimers (ug/ml)</td>
<td>1.47 (0.41-2.47)</td>
<td>1.34 (0.58-2.74)</td>
<td>0.22 (0.11-0.42)</td>
<td>0.25 (0.09-0.53)</td>
<td>0.26 (0.10-0.56)</td>
<td>0.27 (0.16-0.39)</td>
<td>0.18 (0.05-0.42)</td>
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<td><strong>TFPI ref values</strong></td>
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<tr>
<td>TFPI free (ng/ml)</td>
<td>NA</td>
<td>NA</td>
<td>7.13 (5.63-8.44)</td>
<td>10.16 (5.06-9.05)</td>
<td>6.69 (4.29-9.31)</td>
<td>7.66 (5.15-8.74)</td>
<td>10.70 (6.12-12.34)</td>
</tr>
<tr>
<td>TFPI total (ng/ml)</td>
<td>NA</td>
<td>NA</td>
<td>77.49 (69.42-85.58)</td>
<td>76.33 (61.27-89.80)</td>
<td>73.99 (59.13-88.02)</td>
<td>74.09 (61.63-87.36)</td>
<td>87.49 (63.64-104.38)</td>
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<tr>
<td><strong>ETP ref values</strong></td>
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<tr>
<td>ETP (pM.min)</td>
<td>4865 (2653-7162)</td>
<td>4429 (2537-6084)</td>
<td>5363 (2719-8938)</td>
<td>7593 (3373-7516)</td>
<td>8475 (7043-10205)</td>
<td>8506 (7043-10205)</td>
<td>8526 (7043-10205)</td>
</tr>
</tbody>
</table>

Adapted with permission from Monagle P et al. Developmental haemostasis. Impact for clinical haemostasis laboratories. Thromb Haemost. 2006;95:362–372 (8) aPTT, activated partial thromboplastin time; INR, international normalised ratio; PT, prothrombin time; AT, antithrombin; TFPI, tissue factor pathway inhibitor; ETP, endogenous thrombin potential; pM.min is a measurement unit. PTT-A (Dianostica Stago), CK Prest (Diagnostica Stago), FSL (Dade Behring), TCT (thrombin clotting time, Diagnostica Stago), Platelin L (Organon Teknika)
The addition of colloid to the bypass prime may prevent some of the capillary leak of fluid and result in improved organ function after CPB (Nichols, Ungerleider et al. 2006). Maintaining normal colloid osmotic pressure may improve survival in infants undergoing CPB (Haneda, Sato et al. 1985).

The use of whole blood can aid in maintaining colloid osmotic pressure while increasing the level of circulating red cells, clotting factors and platelets. The use of packed red blood cells and fresh frozen plasma may be suitable alternatives when whole blood is unavailable. New technology and techniques for paediatric perfusion have focused on reducing prime volumes.

4.5 **Heparin and Protamine Sulfate – Brief Overview**

Heparin was first discovered in 1916, and commercially accessible in 1935, when a purified preparation of the physiological reversible agent became available. The safe and effective anticoagulation of human patients (Best 1959) was a prerequisite for CPB. Heparin remains the most universally used anticoagulant (Hudson 1959; Mc 1959; Rothnie and Kinmonth 1960) for CPB.

Heparin is required not only to prevent gross clotting of the bypass circuit but also to prevent more subtle activation and consumption of coagulation system components. Although the thrombotic response is attenuated by heparin, it does not completely prevent clotting or the activation of the inflammatory response (Edmunds 2004).
The principal pharmacological agent used during CPB is unfractionated heparin (UFH). UFH is a polysaccharide mixture of low and high-molecular-weight fractions (i.e., molecules ranging from 1,000 to 50,000 Daltons) that differ functionally relative to molecular weight. Fractions with minimum chain length of 18 oligosaccharide units and a molecular weight of approximately 4,500 Daltons or higher preferentially inhibit thrombin (Bray, Lane et al. 1989). UFH is an indirect anticoagulant; it requires simultaneous binding of thrombin and AT by heparin. Only one in three unfractionated heparin molecules have the critical pentasaccharide sequence required for binding to AT (Choay, Petitou et al. 1983). The heparin-induced conformational change in AT results in a 1000-fold increase in AT inhibition of coagulation protein activity compared with antithrombin alone (Pratt and Church 1991). The heparin-AT complex also inhibits factor Xa and several steps in the contact and tissue factor pathways (Olds, Lane et al. 1993). By inhibiting factor Xa, the amplified burst of thrombin generation that occurs when one molecule of factor Xa generates more than 1,000 thrombin molecules can be prevented (Mann, Brummel et al. 2003).

Heparin binds to a wide range of additional proteins including platelet factor 4, VWF, macrophages and endothelial cells. This complicates pharmacokinetics and limits bioavailability (Hirsh, Raschke et al. 1995).

In the final step of the coagulation cascade, thrombin converts fibrinogen into insoluble fibrin, leading to the formation of a stable clot. Clot-bound thrombin is
relatively protected from inhibition by heparin because the heparin-binding site on thrombin is inaccessible when the enzyme is bound to fibrin. The large size of the heparin-AT complex prevents it from penetrating the clot (Weitz, Hudoba et al. 1990).

There is substantial variation in heparin anticoagulant responsiveness when a wide range of heparin dose-response curves were measured in patients undergoing heart surgery (Despotis, Hogue et al. 1995; Despotis, Joist et al. 1997). Heparin resistance is often attributed to AT deficiency, but significant variability may also result from differences in heparin binding to endothelial cells, platelets, white cells or proteins (Teoh, Young et al. 1993).

A major advantage of heparin for CPB is the fact that it is reversible when haemostasis is required. Protamine sulfate was first identified as having anti-heparin properties as early as 1937. Its use became widespread with the advent of CPB (Blumberg, Winterscheid et al. 1960).

Protamine sulfate has significant side effects including, hypotension, complement activation, and cardiac function depression. Serious allergic reactions are relatively frequent. These adverse reactions raise concerns about the use of protamine sulfate (Blumberg, Winterscheid et al. 1960; Shigeta, Kojima et al. 1999). Although previously thought to be rare, cardiopulmonary compromise following protamine sulfate administration occurs in children (Boigner, Lechner et al. 2001). In a retrospective cohort analysis of the
incidence of adverse events in children following protamine sulphate, Seifert and colleagues reported a similar incidence to adults (Seifert, Jobes et al. 2003). Female sex, smaller heparin and larger protamine sulfate doses were independent risk factors for adverse events (Seifert, Jobes et al. 2003).

4.6 Heparin Dosing and Anticoagulation Monitoring

Heparin has been used to manipulate haemostasis during cardiopulmonary bypass since the first procedures performed in the 1950’s.

Early experience reported heparin dosing based on weight. A loading dose of 2-5 milligrams (mg) heparin per kilogram (Kg) of body weight was given immediately prior to perfusion (Rothnie and Kinmonth 1960; Bahnson, Spencer et al. 1962; Roberts 1962; Castaneda 1966). Various maintenance doses were given throughout bypass (Bahnson, Spencer et al. 1962; Berger, Ramaswamy et al. 1968). The therapeutic goal was to prevent the formation of visible clots in the circuit. At the time, there were no tests to monitor anticoagulation. The perfusion circuit was inspected visually to ensure ongoing fluidity and lack of clot formation (Doty, Knott et al. 1979). Lower doses (1.5 mg/Kg to the patient) resulted in the formation of gelatinous clots on the filters of the perfusion circuit (Rothnie and Kinmonth 1960).

Bleeding caused significant morbidity and mortality. This was the impetus for investigating heparin management and monitoring of anticoagulation (Babka, Colby et al. 1977). The first test described for the purpose of measuring whole
blood coagulation time was the Lee-White clotting-time (Rothnie and Kinmonth 1960). Following this, Hattersley (Hattersley 1966) investigated and refined the technique for measuring the activated coagulation time (ACT). Hattersley established a range of normal clotting times and causes of variability between patient samples (Hattersley 1966). The use of the ACT device as a bedside monitor of anticoagulation improved management of anticoagulation during surgery. Too much heparin during CPB had already been identified as a major factor in excessive post-operative bleeding (Babka, Colby et al. 1977). Conversely, in a multivariate analysis of 487 consecutive adult surgeries it was demonstrated that lower initial heparin dosage was associated with increased blood loss and transfusion requirements (Despotis, Filos et al. 1996a).

Although monitoring of heparin and protamine sulfate therapy eventually became routine practice during CPB for open heart surgery, there were no standard protocols (Bull, Korpman et al. 1975). Bull et al collected more than 30 protocols for heparin therapy (Bull, Korpman et al. 1975) during an observational study.

The use of empiric heparin dosing protocols results in a decline in free heparin concentration with increasing time on CPB (Horkay, Martin et al. 1992; Guzzetta, Miller et al. 2005). The individual response to heparin was proposed to extrapolate dosing throughout CPB (Bull, Huse et al. 1975). Bull’s group observed empirically that there were no clots in the bypass circuit when the ACT exceeded 300 seconds and consequently established an “adequate ACT
range of 300 to 600 seconds” as their safe zone. They subsequently used a
target ACT of 480 seconds for all patients (Bull, Korpman et al. 1975). This
recommended ACT target was corroborated in a study on rhesus monkeys that
found when the ACT fell below 400 seconds fibrin monomer was detected
(Young, Kisker et al. 1978).

The use of the ACT as a monitor of anticoagulation caused a reduction in post-
operative chest tube drainage. In one clinical study, investigators showed that
significantly less heparin and protamine sulfate was required in the ACT group
compared to empiric dosing. This resulted in a 43% reduction in blood loss in
the first 48 hours after surgery (Babka, Colby et al. 1977). The use of the ACT
for monitoring anticoagulation reduced transfusion in the post-operative period
(Akl, Vargas et al. 1980). These improved clinical outcomes were observed in
both adults and children (Doty, Knott et al. 1979; Litwin, Mitra et al. 1981;
Jumean and Sudah 1983).

Many cardiac centres have adopted the practice of administering 300-400U/Kg
heparin to all patients in efforts to achieve an ACT > 450 seconds. The ACT is
then measured at regular intervals during CPB, with supplementary heparin
administered to maintain the ACT > 450 seconds.
4.6.1 Limitations of the ACT for Monitoring Anticoagulation

The ACT is a relatively crude and non-standardised bedside test of anticoagulation, yet it remains the most widely used device for monitoring the level of anticoagulation during CPB. The prolongation of the ACT as a measure of anticoagulation does not account for factors unrelated to heparin activity, including haemodilution of contact factors and platelets, reduced platelet function and hypothermia (Jobes, Schwartz et al. 1981; Despotis, Santoro et al. 1994; Owings, Pollock et al. 2000; Guzzetta, Monitz et al. 2010).

A study in children measured contact factors during CPB and demonstrated decreases by an average of 50% secondary to haemodilution (Chan, Leaker et al. 1997). Haemodilution affects the ACT as it is dependent on uniform activation of the contact system (Chan, Leaker et al. 1997). Many of the physiologic factors affecting the ACT are exacerbated in infants (Andrew, MacIntyre et al. 1993; Chan, Leaker et al. 1997; Guzzetta, Monitz et al. 2010).

Reproducibility is affected by the choice of activator, operator technique, and physiologic condition (Hattersley 1966; Jobes, Schwartz et al. 1981; Moorehead, Westengard et al. 1984; Gravlee, Arora et al. 1994; Moliterno, Califf et al. 1995). There is also considerable variation between ACT devices so results are not interchangeable (Schriever, Epstein et al. 1973; Ogilby, Kopelman et al. 1989; Ferguson 1992; Andrew, MacIntyre et al. 1993; Svenmarker, Appelblad et al. 2004). ACT values do not correlate with plasma heparin levels. It has been shown that ACT values increase on bypass, while plasma heparin levels decrease (Culliford, Gitel et al. 1981; Despotis, Santoro et al. 1994). In paediatric studies ACT values did not correlate with plasma
heparin concentrations during CPB (Andrew, MacIntyre et al. 1993; Chan, Leaker et al. 1997; Guzzetta, Bajaj et al. 2008; Guzzetta, Monitz et al. 2010). Prolongation of the ACT should represent an acceptable anticoagulation state, yet the heparin level may be inadequate.

### 4.6.2 Heparin Dosing and Monitoring in Infants and Children

Adults and children respond differently to heparin during CPB (Turner-Gomes, Nitschmann et al. 1997). Paediatric patients metabolise heparin faster and seem to require a higher heparin dose to achieve the same anticoagulation level (Jaberi, Bell et al. 1974; Moliterno, Califf et al. 1995; Owings, Pollock et al. 2000). This is related to blood volume, differences in protein binding and lower levels of coagulation factors (McDonald, Jacobson et al. 1981; Guzzetta, Bajaj et al. 2008). Newall et al (2010) reported age–related responses of increasing anti-Xa effect of UFH with age; infants achieving much lower activity than older children for a given dose of UFH (Newall, Ignjatovic et al. 2010).

The physiologic impact of CPB is greater in children than adults. Anticoagulation practices for children have been extrapolated from adult protocols (Martindale, Shayevitz et al. 1996; Turner-Gomes, Nitschmann et al. 1997; Williams, Bratton et al. 1999a). This may be less than ideal.
Due to the limitations of ACT monitoring and the differences above, methods of anticoagulation and monitoring based on the individual patient’s dose-response to heparin have been re-evaluated.

4.7 Individualised Heparin and Protamine Sulfate Management

There is a wide variability in heparin response between individuals (Congdon, Kardinal et al. 1973; Altshuler, Altshuler et al. 1974; Bull, Korpman et al. 1975; Friesen and Clement 1976) which increases with decreasing patient weight (Olshove, Langwell et al. 1994; D'Errico, Shayevitz et al. 1996). A point-of-care whole blood (WB) haemostasis management system (HMS) (Medtronic, Inc. Minneapolis, MN) has been clinically tested and validated as a device to manage and monitor anticoagulation during CPB in adults (Horkay, Martin et al. 1992; Despotis, Joist et al. 1995; Raymond, Ray et al. 2003).

A prospective randomised trial of 254 adult patients was conducted (Despotis, Joist et al. 1995) comparing HMS to an ACT-based protocol. An empiric dosing regimen for heparin and protamine sulfate was used in the control arm utilising the ACT for monitoring, whereas the intervention protocol was based on heparin dose-response, ACT and WB heparin concentration values. Patients in the intervention arm received 25% more heparin and smaller protamine sulphate-to-heparin ratios when compared to control patients. Control patients had longer chest closure times, more mediastinal chest tube drainage and twice as many required transfusion in the intensive care unit (Despotis, Joist et al. 1995).
In additional studies, higher heparin concentrations lead to a significant reduction in thrombin generation, fibrinolysis, and neutrophil activation (Weitz, Hudoba et al. 1990; Despotis, Filos et al. 1996a; Okita, Takamoto et al. 1997; Koster, Fischer et al. 2002). Maintenance of higher stable heparin concentrations during CPB preserve platelet function and decrease platelet activation (i.e., lower platelet factor 4 and β-thromboglobulin levels) (Despotis, Filos et al. 1996a; Okita, Takamoto et al. 1997).

This strategy may be more important in children given the greater variability in weight, blood volume, and metabolic rate (McDonald, Jacobson et al. 1981). Codispoti et al (2001) compared the use of an empiric weight-based heparin protocol (300 IU/Kg) to individualised dosing in children (3-6 years of age) following the HMS (Codispoti, Ludlam et al. 2001). Individualised protocol patients received more heparin than empirically dosed patients. These patients demonstrated less activation of coagulation proteins, fibrinolysis and bleeding post-operatively and received fewer blood transfusions (Codispoti, Ludlam et al. 2001).

Guzzetta et al (2008) compared empirical heparin dosing to an individualised heparin regimen in infants (3 to 6 months of age) and found that the requirement for heparin was again significantly higher in the individualised group. These patients demonstrated less thrombin generation as measured by prothrombin fragment 1.2 and reduced factor VIII consumption (Guzzetta, Bajaj et al. 2008). However, these same patients had an increase in 24 hour
chest tube drainage as well as one additional blood donor exposure. The current practice trends continue to be empiric based heparin dosing in most paediatric cardiac centres (Codispoti and Mankad 2000; Taneja, Fernandes et al. 2008).

As with heparin, various methods of dosing protamine sulfate have been described. Historically a range of tests, whole blood clotting time, thrombin clotting time, and prothrombin clotting times, were employed to determine the presence of excess heparin after protamine sulfate administration (Perkins, Osborn et al. 1956; Rothnie and Kinmonth 1960; Jaberi, Bell et al. 1974). Most protamine sulfate dosing was based on the initial heparin dose and calculated for patient body weight (Rothnie and Kinmonth 1960). Recommended doses of protamine sulfate to heparin were in a ratio of 2:1 (Rothnie and Kinmonth 1960). Additional doses in a 1:1 ratio were recommended if tests indicated the presence of un-neutralised heparin. There is a great variation in the protamine sulfate dose required to fully neutralize heparin. Ratios of total protamine sulfate to heparin ranged from 1:1 and up to 5:1 (Hudson 1959; Rothnie and Kinmonth 1960). Heparin elimination may vary from patient to patient (Rothnie and Kinmonth 1960; Berger, Ramaswamy et al. 1968; Horkay, Martin et al. 1992). There is also a variation in the potency between protamine sulfate preparations (Hudson 1959; Rothnie and Kinmonth 1960). As a result, delivery of a standard dose of protamine sulfate could lead to significant under or overdosing. Teoh et al (Teoh, Young et al. 1993) observed less bleeding following surgery when patients were given low
dose protamine sulfate infusions for six hours following initial heparin neutralisation. Protamine sulfate has anticoagulant properties and overdosing may be responsible for bleeding complications post-operatively (Ni Ainle, Preston et al. 2009). Ni Ainle et al (2009) showed an increase in anti-Xa levels with increasing doses of protamine sulfate (Ni Ainle, Preston et al. 2009). Studies have shown that smaller doses of protamine sulfate (0.5-0.66:1 protamine sulphate: heparin ratio) were associated with significantly less bleeding and less platelet dysfunction following bypass (Berger, Ramaswamy et al. 1968; Shigeta, Kojima et al. 1999; Ni Ainle, Preston et al. 2009).

Codispoti et al (2000) reported great variability in heparin (dose used in prime and throughout CPB) and protamine sulfate dosing strategies in children among paediatric centres (Codispoti and Mankad 2000).

4.8 Summary

While much has been done to elucidate the unique characteristics of the developing haemostatic system and the differences between healthy children and those with congenital heart disease, many questions remain unanswered regarding the influence of coagulation factor abnormalities on bleeding and thromboembolism in infants and children undergoing CPB.

The need for anticoagulation for CPB is certain. At present, the primary pharmacological agent is heparin. Age-related variability in response combined with differences in protein and endothelial cell binding and
unpredictable metabolism make heparin a less than ideal anticoagulant. 
Bleeding remains an important post-operative complication in the cardiac patient; excessive or inadequate heparin and protamine sulfate dosing may exacerbate this. There is a need for new anticoagulants for use during CPB. An ideal anticoagulant would be specific, have predictable pharmacodynamics and pharmacokinetics (Bauer 2006). Although a significant amount of research is being devoted to direct thrombin inhibitors as potential anticoagulants, there is currently no replacement for UFH during CPB for open heart surgery (Hirsh, Bauer et al. 2008).

Clinically, monitoring of anticoagulation for CPB presently involves measuring heparin effect or, less commonly, heparin concentration. Using ACT to measure effect is an indirect measure of thrombin suppression that is affected by many other factors not related to heparin anticoagulation. Bedside tests measuring whole blood heparin concentration have shown to improve some clinical outcomes in children but further refinement of specific regimens is needed for neonates and infants.
5.1 Objectives and Research Questions

The objective of this study was to determine whether using the Heparin Management System (HMS) device to guide and monitor heparin management in infants having heart surgery would maintain stable heparin levels throughout cardiopulmonary bypass (CPB). We hypothesised this would result in decreased laboratory markers of activation and improved clinical haemostasis following surgery, resulting in reduced bleeding and the need for blood product transfusions.

Specific research hypothesis and research questions were:

1. Inadequate heparin dosing and monitoring in infants results in inadequate anticoagulation during CPB as shown by low blood heparin concentration, haemostatic activation, increased bleeding, an exaggerated inflammatory response and poor clinical outcomes.

2. The use of the HMS Plus® Hemostasis Management System (HMS) device during infant heart surgery will increase the total amount of heparin administered during CPB resulting in higher and stable heparin concentration confirmed by anti-Xa levels during CPB.

3. Improved anticoagulation will lead to reduced consumption of coagulation proteins and platelets. There will be less fibrinolysis and overall improved haemostasis following CPB.
4. Clinical benefits will include a reduction in post-operative bleeding, donor blood product transfusions and associated clinical complications.

5.2 Study Design

This study was a single centre prospective double-blind randomised clinical trial comparing two methods of anticoagulation dosing and monitoring during CPB for infant heart surgery. Patients randomised to the control group received heparin and protamine sulfate doses based on current clinical protocols using empiric weight based calculations and were monitored following the ACT alone. Patients randomised to the treatment group received heparin and protamine sulfate doses based on the heparin dose-response calculation and ongoing monitoring by the HMS.

The first period of enrolment (hereafter HMS 1) utilised manufacturer guidelines for the use of the HMS device, while the second period of enrolment (hereafter HMS 2) utilised a modified protocol for the use of the HMS device.

5.2.1 Patient Population

- Inclusion criteria:
  - Cardiac surgery utilising CPB
  - <1 year of age at the time of elective surgery

- Exclusion criteria:
  - Non-elective surgery
  - Weight <2.0 kilograms
- Gestational age at birth <36 weeks
- Preoperative use of anticoagulants (i.e. heparin or coumadin at a therapeutic dose)
- Pre-operative antithrombin replacement therapy
- Known history of pro-haemorrhagic disorders
- Known history of pro-thrombotic disorders
- Clinical renal failure (creatine, normal for age)
- Clinical liver failure (bilirubin, normal for age)
- Pre-operative use of ECMO or ventricular assist device (VAD)
- Refusal of parental consent

5.2.2 Dates of Enrolment

The study began in August 2006 (HMS 1), and was suspended in June 2007 by the study’s independent data safety committee following a blinded interim analysis. The study resumed with a revised protocol in October 2007 (HMS 2) with enrolment completed in February 2009.

5.3 Clinical Protocol - HMS 1

5.3.1 Anticoagulation Protocol – Control Group

➢ Prime and anaesthesia heparin dosing: Patients <5 Kg received 400 U/Kg of heparin and patients >5 Kg received 300 U/Kg of heparin with a target ACT of 480 seconds prior to initiating CPB. An additional 2 U/ml of heparin was added to the final prime and 2 U/ml of heparin was added to the fresh frozen plasma (FFP) prior to transfusion during CPB.
Monitoring of anticoagulation during CPB: The ACT was monitored following the initial heparin bolus, 10 minutes following the initiation of CPB and every 30 minutes for the duration of CPB. A target ACT of 480 seconds was required prior to initiating CPB.

CPB dosing: Additional heparin was given to maintain the ACT > 480 seconds in a standardised way (ACT 400-480s – add 100 U/Kg; ACT < 400s – add 150 U/Kg).

Protamine sulfate dosing: Protamine sulfate was given in a 1:1 ratio based on the initial heparin dose (patients <5 Kg – 4 mg/Kg; patients >5 Kg – 3mg/Kg). To confirm reversal of heparin, the ACT was performed with additional protamine sulfate given in a standardised way if necessary (1mg/Kg protamine sulfate if ACT ≥ 10% of baseline or > 140 seconds).

5.3.2 Anticoagulation Protocol – Treatment Group

Prime and anaesthesia heparin dosing: Following induction of anaesthesia and arterial line insertion, a blood sample was taken to measure the heparin dose response assay (HDR) on the HMS. The HDR predicted the minimum amount of heparin required to achieve an ACT of greater than 480 seconds and the required heparin concentration to be maintained during CPB. The initial patient and pump prime heparin dose was given as determined by the HDR.

Monitoring of anticoagulation during CPB: The heparin concentration during CPB was monitored through the use of the heparin protamine sulfate
titration assay (HPT). The HPT also calculates additional heparin required to maintain protocol heparin concentration.

- CPB dosing: Heparin was administered throughout bypass as recommended by the HMS to maintain the heparin concentration predicted by the HDR.

- Protamine sulfate dosing: Protamine sulfate was given based on the results from the last HPT test just prior to the termination of CPB. Total (patient + pump) protamine sulfate dose was administered. An HPT assay was used to confirm heparin reversal with a zero heparin concentration measured. Additional protamine sulfate was administered as suggested by the HMS.

5.4 Clinical Protocol – HMS 2

5.4.1 Anticoagulation Protocol – Control Group

- Prime and anaesthesia heparin dosing: Patients <30 days received a bolus of 400 U/Kg of heparin with 4 U/ml added to the final prime and patients >30 days received a bolus of 300 U/Kg of heparin with 3 U/ml added to the final prime. 4U/ml and 3U/ml of heparin was added to the FFP prior to transfusion during CPB for patients <30 days and >30 days respectively.

- Monitoring of anticoagulation during CPB: The ACT was monitored following the initial heparin bolus, 10 minutes following the initiation of CPB and every 30 minutes for the duration of CPB. A target ACT of 480 seconds was required prior to initiating CPB.
CPB dosing: Additional heparin was given to maintain the ACT > 480 seconds in a standardised way (ACT 400-480s – add 100 U/Kg; ACT < 400s – add 150 U/Kg).

Protamine sulfate dosing: Protamine sulfate was given in a 1:1 ratio based on the initial heparin dose (patients <30 days – 4 mg/Kg; patients >30 days – 3mg/Kg). To confirm reversal of heparin, the ACT was performed with additional protamine sulfate given in a standardised way if necessary (1mg/Kg protamine sulfate if ACT ≥ 10% of baseline or > 140 seconds).

5.4.2 Anticoagulation Protocol – Treatment Group

Prime and anaesthesia heparin dosing: Following induction of anaesthesia and arterial line insertion, a blood sample was taken to measure the heparin dose response assay (HDR) on the HMS. The target heparin concentration to be achieved and maintained throughout CPB was 4.0U/ml for patients <30 days of age and 3.5U/ml for patients >30 days of age. For each patient an initial heparin bolus was administered based on these target heparin concentrations. 4U/ml and 3U/ml of heparin was added to the final prime for patients <30 days and > 30 days of age respectively.

Monitoring of anticoagulation during CPB: The heparin concentration during CPB was monitored through the use of the HPT.

CPB dosing: Heparin was administered throughout CPB to maintain a heparin concentration of 3.0U/ml for all patients. A maximum heparin dose of 1500U/Kg for patients < 30 days and 900U/Kg for patients greater than 30 days was not to be exceeded.
Protamine sulfate dosing: For heparin reversal, protamine sulfate was given based on the results from the last HPT test just prior to the termination of CPB. One and one half the total (patient + pump) protamine sulfate dose was administered. To confirm heparin reversal, the heparin concentration was tested using an HPT test and the recommended protamine sulfate dose was given if the concentration was not zero.

5.5 Clinical Protocol – Common

5.5.1 Transfusion Guidelines – Perfusion

- Packed red blood cells (PRBC) were added to the prime and throughout CPB to achieve and maintain the HCT at 28-30%. Prior to termination of CPB the HCT was increased to 32-35% for cyanotic patients.
- One ½ unit of FFP was added to the prime and the second ½ unit was added prior to the removal of the aortic x-clamp.
- Modified ultrafiltration (MUF) was performed for 15-20 minutes for all patients following CPB.
- Residual blood from the MUF circuit was collected post CPB for transfusion at the discretion of the anaesthetist.
5.5.2 Transfusion Guidelines – Anaesthesia

Table 5-1 Transfusion Guidelines Anaesthesia

<table>
<thead>
<tr>
<th>Trigger</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hct &lt; 40%, if cyanotic</td>
<td>Transfuse packed red blood cells</td>
</tr>
<tr>
<td>Hct &lt; 30% if acyanotic</td>
<td></td>
</tr>
<tr>
<td>Platelet count &lt; 100,000 mm$^3$</td>
<td>Transfuse 1 U/ 5 Kg platelets</td>
</tr>
<tr>
<td>with evidence of clinical bleeding</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen &lt; 1.0 gm/l</td>
<td>Transfuse 1 U/ 5 Kg cryoprecipitate</td>
</tr>
</tbody>
</table>

* If as determined by the clinicians, there was still significant bleeding after following the above protocol, heparin reversal was assessed first. Additional blood products were given according to the clinicians’ discretion.

5.5.3 Transfusions Guidelines – CCCU

- Post-operative transfusion of blood products was at the discretion of the CCCU team, which was blinded to the patients’ study group.

5.5.4 Steroid Administration

- Steroids were given pre-operatively for patients less than 30 days old at the time of surgery and for procedures requiring deep hypothermic circulatory arrest.
5.6 Research Methodology

5.6.1 Sample Size Calculation

- HMS 1: Sample size estimation was based on a previous reported trial of 13 subjects per group. Chest drain loss in the first 24 hours after surgery was shown to be 26.4 (SEM 4.7) ml/Kg in the control group verses 15.2 (SEM 3.7) ml/Kg in the treatment group (Codispoti, Ludlam et al. 2001). Based on alpha of 0.5 and beta of 0.20, sample size was estimated at 29 patients per group. Variation of the beta to 0.10 and reduction in the variance estimate yielded similar sample sizes.

- HMS 2: Same sample size calculation as HMS 1

5.6.2 Randomisation Methodology

- The person creating the randomisation assignment numbered envelopes was independent of recruitment and performance of the study manoeuvres. Subjects were randomised in random blocks of 2, 4 and 6 using a random number generator, with two sets of envelopes to reflect the stratified randomisation according to two age groups (birth to one month and one month to 12 months). Envelopes were tamperproof, and were not opened until informed consent had been obtained and on the planned day of surgery.

5.6.3 Blinding in Operating Room

- All clinicians were blinded to the patients’ group assignment with the exception of the perfusionist. The anaesthetist drew the baseline sample of
blood at the beginning of the case and the perfusionist performed the appropriate tests based on the group to which the patient was assigned. Heparin was drawn up and diluted by the perfusionist to a consistent final volume for all patients. A second perfusionist confirmed the heparin dose. An ACT was checked and reported to the anaesthetist in both groups of patients to confirm that it had passed the safe target of 480 seconds prior to initiating CPB.

➢ The perfusionist drew up the protamine sulfate reversal dose following termination of CPB based on the group to which the patient was assigned and once again diluted to a consistent final volume for all patients. A second perfusionist confirmed the protamine sulfate dose. The perfusionist determined heparin reversal as stated above and prepared extra protamine sulfate to be given by the anaesthetist as required by the study protocol. The HPT assay and ACT was repeated at 45 minutes following protamine sulfate administration to assess for any heparin rebound.

5.6.4 Blinding in CCCU

➢ CCCU staff was not aware of the treatment assignment at any times.

5.6.5 Blinding in Data Collection

➢ Data collection was done by study staff completely blinded to the treatment assignment and who had no clinical responsibilities for the enrolled patient.
5.7 Laboratory Measurements

5.7.1 Frequency of Laboratory Measurements in Operating Room

- baseline following induction of anaesthesia
- 10 minutes following the initiation of CPB
- Every 30 minutes throughout the CPB period
- just prior to termination of CPB
- post-protamine sulfate administration

Frequency of Laboratory Measurements in CCCU

- 4 hours after arrival in the CCCU
- 24 hours after arrival in the CCCU

5.7.2 HMS Device and Assay Descriptions

Principle

- The HMS is a microprocessor based, multichannel, clot timing instrument, with automated syringe handling for pipe ting blood into single use cartridges. This integrated system contains a component for computing the results of the clot detection tracking mechanism. The cartridge instructs the system, through an optical code, as to the type of test being performed. It also determines calculations and format required for results and volume of samples needed for each channel.
- The detection process uses the plunger assembly within the cartridge. This assembly is lifted and dropped through the sample/reagent mixture, by a lifting mechanism in the HMS actuator as the sample
clots, a fibrin web forms around the daisy, located on the bottom of the plunger assembly and impedes the assembly descent rate. This change in fall rate is detected by photocells within the actuator of the instrument. The end point of the test is the time at which fibrin formation is detected.

The HMS provides the following information:

- Indication of heparin response via the Heparin Dose Response (HDR) cartridge.
- Heparin calculation based on dosing protocol, patient blood volume and the CPB circuit volume.
- Simultaneous, quantitative, and functional evaluation of heparin via the Heparin Protamine Titration Assay (HPT) and the Activated Clotting Time (HR-ACT) cartridges.
- Calculation of additional heparin required to maintain the patient at an adequate heparin concentration.
- Calculation of the protamine sulfate dose required to reverse the circulating heparin.
- Calculation of protamine sulfate reversal.

**Heparin Dose Response Assay (HDR)**

- The HDR cartridge is a modification of an activated clotting time (ACT). The test is designed to identify patients who may be unusually sensitive or resistant to heparin anticoagulation. Each cartridge reagent chamber
contains 88 microliters of kaolin reagent (with calcium in a HEPES buffer). Channels 1 and 2 also contain porcine mucosal heparin to reach a blood heparin concentration of 2.5 U/ml while channels 3 and 4 contain sufficient heparin to reach a concentration of 1.5 U/ml. Channels 5 and 6 do not contain heparin.

➢ The HMS individualises heparin dosing to each patient taking into account the patient’s size and haemodilution by the pump prime volume. The HMS determines a heparin dose-response for the patient through the HDR assay. Based on the dose-response, the HMS calculates the necessary amount of heparin required to reach the specified ACT target of 480 seconds and the target heparin concentration for CPB.

**Heparin Protamine Titration Assay (HPT)**

➢ The HPT test uses the principle of heparin/protamine titration to quantitatively determine the concentration of heparin in the sample. It is a four or six channel test, with each channel of the cartridge containing a different amount of protamine sulfate and a constant amount of thromboplastin, for activation of the test. The first channel to clot is the one in which the amount of protamine sulfate most closely neutralises the heparin in the blood, without an excess of either heparin or protamine sulfate.
During CPB, a heparin protamine titration (HPT) assay determines the patients’ blood heparin concentration. This result is used to calculate the amount of heparin required to maintain the target heparin concentration. The data is also used to calculate protamine sulfate required for neutralisation.

**Activated Clotting Time, high range (HR-ACT)**

- Anticoagulation effect is monitored by an automated whole blood activated clotting time (ACT). Heparin administration requires monitoring due to varying volume of distribution and clearance of heparin as well as varying levels of heparin resistance (Despotis, Gravlee et al. 1999). The HMS HR-ACT uses a two channel cartridge containing kaolin reagent that measures the time to clot formation in whole blood.

- **Low Cartridge HPT Assay**

  Following heparin reversal with protamine sulfate the low cartridge HPT test is used to determine the heparin concentration.

**5.7.3 Measures of Blood Haemostasis Assay Descriptions**

**Anti-Xa, antithrombin, fibrinogen:**

- Anti-Xa and antithrombin (AT) were measured to assess anticoagulation.
  - Anti-Xa was measured using a Coamatic ® Heparin assay by Chromogenix.
  - AT levels were measured using the Chromogenic Biophen ® Antithrombin assay. These may be complicated in infants due to increased heparin
clearance and lower AT (Heying, van Oeveren et al. 2006; Kajimoto, Nakazawa et al. 2007). These tests were used during the surgical period in order to better understand the level of anticoagulation required to maintain optimal haemostasis.

- Fibrinogen was measured as a guide for appropriate treatment of coagulopathy post CPB. Siemens Multifibren™ U assay was used to measure fibrinogen.

**Complete Blood Count (CBC)**

- CBC was used as a laboratory measure of blood formed elements. The CBC was used to assess the haemoglobin, haematocrit, and platelet count. The Abbott CELL-DYN Sapphire™ Analyser was utilized to measure the CBC.

**D-dimers, Thrombin-Antithrombin (TAT), Prothrombin Fragment 1.2 (F1.2)**

- TAT and F1.2 were used as markers of thrombin generation. These markers were measured to assess baseline levels and peak values. Measurements were done with Siemens Healthcare Diagnostics Ltd. kits: Enzygnost® TAT ELISA and Ezygnost® F1.2 (Monoclonal) ELISA assays.

- D-dimer is a sensitive test for the presence of thrombosis (Scarvelis, Palareti et al. 2008). This parameter was measured to assess baseline levels and peak values. D-dimer levels were measured with Instrumentation Laboratory HemosIL® d-dimer assay.
5.8 Pre-Operative and Intra-operative Measurements

5.8.1 Patient Demographics and Previous Clinical History

- A detailed medical history, including cardiac anatomy, co-morbidities, previous history of TCs and current medications was obtained from the patients’ medical record.

5.8.2 Surgical Procedure and Complexity (Aristotle score) and Operative Parameter

- At the induction of anaesthesia but prior to heparinisation the HMS was used to obtain the heparin dose response. Baseline heparin concentration, ACT, anti-Xa, AT, CBC, fibrinogen, F1.2, TAT and d-dimer levels were measured following the loading heparin dose. Once CPB was initiated blood was drawn to evaluate the level of anticoagulation and haemodilution. Throughout surgery heparin concentration and ACT were measured at regular intervals according to the protocol. Other procedural variables were recorded.

- Procedures were classified according to the International Nomenclature for Congenital Heart Surgery (Lacour-Gayet, Maruszewski et al. 2000) and procedure complexity was classified according to the Aristotle (Lacour-Gayet, Clarke et al. 2004) and RACHS scores (Jenkins, Gauvreau et al. 2002).

- All blood transfusions intra-operatively were recorded.
5.9 **Outcome Measures – Primary Outcome**

- Packed red blood cells (PRBC) transfusion in the CCCU: volume of PRBC indexed to body weight given (ml/Kg) in the first 24 hrs post-operatively.

5.10 **Outcome Measures, Secondary Outcomes**

5.10.1 **Heparin Dose - indexed to body weight (U/Kg)**

- CPB prime
- prior to CPB initiation
- during CPB
- total dose with and without CPB prime dose

5.10.2 **Protamine Sulfate Dose**

- Initial protamine sulfate dose, additional protamine sulfate dose and total protamine sulfate dose indexed to body weight (mg/Kg) given after CPB termination.

5.10.3 **Protamine Sulfate to Heparin Ratio**

- ratio of total protamine sulfate dose to total heparin dose excluding CPB prime
- ratio of total protamine sulfate dose to total heparin dose including CPB prime
- ratio of initial protamine sulfate dose indexed to body weight to total dose of heparin excluding CPB prime
- ratio of initial protamine sulfate dose to total dose of heparin including CPB prime
5.11 Outcome Measures, Secondary Outcomes (Laboratory)
- Serial antithrombin and anti-Xa
- Serial platelet count, haemoglobin and haematocrit
- Serial fibrinogen, d-dimer, TAT and F1.2
- ACT

5.12 Outcome Measures – Secondary Outcomes (Clinical)

5.12.1 Bleeding and Blood Product Transfusions
- PRBC transfusions (ml/Kg) in the operating room
- Platelet transfusions (ml/Kg) in the operating room and CCCU
- FFP and cryoprecipitate (ml/Kg) in the operating room and CCCU
- Total blood product transfusion volume
- Total units of blood products transfused
- Chest tube volume loss (ml/Kg) at 4 and 24 hours in CCCU

5.12.2 Inotropic Support Score in CCCU
- 4, 12 and 24 hours after CCCU arrival (dosages expressed in mcg/Kg/min, dopamine + (milrinone x 10) + (epinephrine/norepinephrine x 100))

5.12.3 Thromboembolism
- Use of recombinant Factor VIIa peri-operatively
- Use of Vitamin K in the post-operative period
- Thromboembolic complication
5.12.4 Chest Closure and Hospitalisation

- Time from protamine sulfate to sternal closure (in minutes if chest closed in the operating room)
- Sternum closed in the operating room
- Sternum reopened in CCCU
- Duration of ventilation in hours
- Duration of CCCU stay in days
- Duration of hospital stay in days

5.12.5 In-hospital Morbidity and Mortality at Last Follow-up

5.13 Regulatory Issues

5.13.1 Ethics Approval

- Study protocol was reviewed and approved by the Research Ethics Board (REB) at The Hospital for Sick Children, REB study approval #1000007797, the REB approval was renewed yearly. In addition, research ethics approval was granted by Charles Sturt University - Protocol Number: 2007/136.

5.13.2 Patient Consent

- The primary investigator Colleen Gruenwald, Director, Perfusion Services/ECLS Program was the lead professional approaching parents/guardians to obtain consent.
5.13.3 Data Safety Monitoring Board (DSMB) and Blinded Interim Analyses

- DSMB was planned after enrolment of 50% of patients in HMS 1. The study was prematurely stopped by the DSMB and upon re-approval mandated safety review of the first 10 patients, with immediate termination of HMS 2 if any study-related serious adverse events occurred in those patients. A second DSMB was performed after 50% enrolment in HMS 2.

5.14 Statistical Analysis

5.14.1 General

- Data are described as frequencies, medians with ranges and means with standard deviations as appropriate. Cross-sectional between-group comparisons of characteristics and outcomes were compared with Fisher’s exact chi-square, Wilcoxon non-parametric tests and student’s t-tests assuming unequal variance between samples as appropriate. Data from both protocols (HMS 1 and 2) were analysed separately. All analyses were performed using SAS statistical software version 9.2 (SAS Institute, Inc, Cary, NC).

5.14.2 Between-groups Comparison

- Comparison of outcomes was performed in multivariable logistic or linear regression models (maximum likelihood algorithm for parameter estimation) adjusted for age at surgery, surgeon and Aristotle score. Distribution of skewed variables was normalised through logarithmic or exponential transformation as necessary.
5.14.3 Association between Anti-Xa Levels and Chest Tube Volume Loss

- Association modelled in 8 linear regression models (4 time points and 2 age groups), 5 variations were created for each model (no transformation, log, square root, cube or inverse) and the model with the best fit was selected. The regression equation was solved to determine the anti-Xa level associated with the age-appropriate maximum bleeding level allowable.

5.14.4 Association between Haematocrit Level and Anti-Xa and HMS Measurement Discrepancy

- Association modelled in 2 multivariable linear regression models (2 age groups), 5 variations were created for each model (no transformation, log, square root, cube or inverse) and the model with the best fit was selected. Anti-Xa and haematocrit were used as independent variables and the HMS heparin concentration as the dependent variable. Models were solved for different anti-Xa levels and 4 different haematocrit concentrations.

5.14.5 Association between Heparin Dose and Chest Tube Volume Loss

- Associations modelled in 18 linear regression models (9 time points/doses and 2 age groups), 5 variations were created for each model (no transformation, log, square root, cube or inverse) and the model with the best fit was selected. The regression equation was solved to determine the heparin dose associated with the age-appropriate maximum bleeding level allowable for each time point.
5.14.6 Association between Antithrombin and Heparin Dose Required to Maintain Target Anti-Xa

- Association modelled in 2 multivariate linear regression models (2 age groups). Heparin dose and AT levels were used as independent variables (as an interaction term) with adjustments for post-heparin anti-Xa; anti-Xa at termination was used as the dependent variable. Models were solved for different heparin doses with varying AT concentrations and heparin doses required to maintain age-appropriate target anti-Xa were calculated.

5.14.7 Effect of Unbound Heparin on Clinical Outcomes

- Multiple linear regression models were created for different outcomes in which both anti-Xa and the cumulative heparin dose at different intervals were used as independent variables and the post-operative outcome was used as the dependent variable. All models were adjusted for age at surgery, surgeon and surgery Aristotle score. Models where the heparin dose remained significant despite adjusting for anti-Xa at termination of CPB were considered evidence of unbound heparin effect.

5.14.8 Trends over Time in Laboratory Measurements for HMS 2

- Statistical significance of the difference between HMS and control groups was assessed in individual linear regression models adjusted for age at surgery, Aristotle score and surgeon. Platelet count, TAT and F1.2 were log transformed in those regression models.
6.1 Enrolment and Demographics

A total of 90 patients were enrolled, 33 in the original protocol (HMS I) and 57 in the modified protocol (HMS II). In HMS I 16/33 (48%) patients were randomised to the HMS group compared to 28/57 (49%) patients randomised to the treatment group with the HMS II protocol. Baseline and surgical patient characteristics are detailed (Table 6-1) and were comparable between groups for both protocols. In HMS 1, 15/33 (45%) were neonates <1 month of age compared to 21/57 (37%) in HMS 2.

6.2 Clinical Results from HMS 1

Patients in the HMS group had similar demographics and surgery compared to patients in the ACT group. The HMS patients received significantly more heparin (7252 vs. 3760 units, p=0.001) and more protamine sulfate (34.0 vs. 17.6 mg, p=0.001) than the ACT group. Patients in the HMS group had significantly higher anti-Xa levels throughout the procedure, but similar levels of AT, d-dimer, fibrinogen, haemoglobin and haematocrit. No clinically relevant trends were observed for any other factors.

HMS patients had higher inotropic scores leaving the operating room (9.5 vs. 8.5, p=0.62), at 4hrs (9.5 vs. 8.7, p=0.76), 12hrs (9.1 vs. 8.2, p= 0.64) and 24hrs (7.7 vs. 6.6, p=0.54), higher volume of chest tube loss in the first 4
hours (18.9ml/Kg vs. 9.5ml/Kg, p=0.06) and in the first 24 hours (23.0ml/Kg vs. 16.9ml/Kg) following surgery. They also had higher transfusion requirements in the operating room (102ml/Kg vs. 42ml/Kg, p=0.39) and in the CCCU (42ml/Kg vs. 24ml/Kg, p=0.02). Infants in the HMS group spent more time on the ventilator (100hrs vs. 48hrs, p=0.62) and in the CCCU (160hrs. vs. 95hrs, p=0.34), hospital length of stay was similar between groups. Recombinant Factor VIIa, (indicative of severe bleeding) was given to 3 patients in the HMS group. Thrombus developed in 3 patients, all in the HMS group. Of 8 patients who underwent 2 bypass runs, 6 were in the ACT group.

Linear regression models (maximum likelihood models, logarithmic transformation for skewed variables) were created in order to assess the influence of heparin dose-response (measure of anticoagulation resistance), surgeon and age at surgery on the difference between experimental groups. Infants in the HMS group had similar inotropic score to those in the ACT group. They experienced significantly larger blood loss (16ml/Kg vs. 12ml/Kg, p=0.02) at 4 hours in the CCCU. They had longer ventilation (95hrs vs. 56hrs, p<0.001), CCCU stay (154hrs vs. 100hrs, p<0.001), hospital stay (154hrs vs. 100hrs, p<0.001), more transfusions in the operating room (107ml/Kg vs. 51ml/Kg, p<0.001) and in the CCCU (35ml/Kg vs. 32 ml/Kg, p=0.003). Based on these results the trial was prematurely suspended.
6.3 Insights on Anticoagulation in Infants based on HMS 1 Results

There is little information on anticoagulation for children undergoing open heart surgery especially regarding target heparin dose, target anti-Xa levels and the effect of AT on heparin binding. The HMS I trial provided valuable information on anticoagulation in this population as the 33 infants enrolled received widely varying heparin doses with drastically different clinical outcomes. Multiple models were created using target estimates of bleeding at 24 hours given specific anticoagulation measurements at different times during surgery. All models presented in this document use the same statistical basis and all relationships were tested for the following 4 transformations: logarithmic, squared, square root or inverse. Based on these observations guidelines for anticoagulation in infants were derived assuming that the clinically acceptable target for bleeding was 20ml/Kg for neonates and 15ml/Kg for infants at 24 hours post-operatively.
### Table 6-1 Demographics and Surgical Characteristics

<table>
<thead>
<tr>
<th>Demographic and clinical</th>
<th>HMS I (N=33)</th>
<th>HMS II (N=57)</th>
<th>p</th>
<th>CTRL (N=17)</th>
<th>HMS (N=16)</th>
<th>p</th>
<th>CTRL (N=29)</th>
<th>HMS (N=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>11 (65%)</td>
<td>8 (50%)</td>
<td>0.50</td>
<td>14 (48%)</td>
<td>18 (64%)</td>
<td>0.29</td>
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</tr>
<tr>
<td>Age at surgery (days)</td>
<td>163 (6-262)</td>
<td>65 (2-367)</td>
<td>&lt;0.001</td>
<td>96 (5-238)</td>
<td>120 (5-280)</td>
<td>0.02</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>&lt;1 month old at surgery</td>
<td>7 (41%)</td>
<td>8 (50%)</td>
<td>0.74</td>
<td>10 (34%)</td>
<td>11 (39%)</td>
<td>0.61</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight at surgery (Kg)</td>
<td>5.2±1.9</td>
<td>5.1±2.6</td>
<td>0.89</td>
<td>4.7±1.3</td>
<td>5.2±1.8</td>
<td>0.27</td>
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<tr>
<td>Diagnosis/surgical category</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Septal defects</td>
<td>1 (6%)</td>
<td>6 (38%)</td>
<td>0.04</td>
<td>8 (28%)</td>
<td>4 (14%)</td>
<td>0.33</td>
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<tr>
<td>Pulmonary venous anomalies</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00</td>
<td>3 (10%)</td>
<td>0 (0%)</td>
<td>0.24</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TOF/DORV/PAVSD</td>
<td>9 (53%)</td>
<td>3 (19%)</td>
<td>0.04</td>
<td>6 (21%)</td>
<td>9 (32%)</td>
<td>0.38</td>
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</tr>
<tr>
<td>Single ventricle (stage I)</td>
<td>0 (0%)</td>
<td>2 (13%)</td>
<td>0.23</td>
<td>3 (10%)</td>
<td>1 (4%)</td>
<td>0.62</td>
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</tr>
<tr>
<td>Single ventricle (stage II)</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>1.00</td>
<td>0 (0%)</td>
<td>1 (4%)</td>
<td>0.50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transposition of great arteries</td>
<td>6 (35%)</td>
<td>4 (25%)</td>
<td>0.71</td>
<td>7 (24%)</td>
<td>8 (29%)</td>
<td>0.77</td>
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<tr>
<td>Thoracic vein and arteries abnormalities</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00</td>
<td>2 (7%)</td>
<td>5 (18%)</td>
<td>0.25</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgical</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Aristotle score</td>
<td>9.5 (7.5-11.0)</td>
<td>9.0 (7.0-14.5)</td>
<td>0.59</td>
<td>9.0 (6.0-14.5)</td>
<td>9.5 (6.0-14.5)</td>
<td>0.94</td>
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</tr>
<tr>
<td>Aristotle category 4 (score &gt;9.9)</td>
<td>8 (47%)</td>
<td>7 (44%)</td>
<td>1.00</td>
<td>12 (41%)</td>
<td>14 (50%)</td>
<td>0.60</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgeon A</td>
<td>12 (71%)</td>
<td>4 (25%)</td>
<td>0.02</td>
<td>10 (34%)</td>
<td>14 (50%)</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgeon B</td>
<td>5 (29%)</td>
<td>11 (69%)</td>
<td>0.04</td>
<td>6 (21%)</td>
<td>5 (18%)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgeon C</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
<td>0.49</td>
<td>4 (14%)</td>
<td>1 (4%)</td>
<td>0.36</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Surgeon D</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00</td>
<td>9 (31%)</td>
<td>8 (29%)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peri-operative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Cardiopulmonary bypass time (minutes)</td>
<td>112±40</td>
<td>116±39</td>
<td>0.75</td>
<td>105±40</td>
<td>106±38</td>
<td>0.55</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cross clamp time (minutes)</td>
<td>69±19</td>
<td>67±28</td>
<td>0.85</td>
<td>76±33</td>
<td>73±31</td>
<td>0.24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deep hypothermic circulatory arrest</td>
<td>0 (0%)</td>
<td>2 (6%)</td>
<td>0.17</td>
<td>4 (14%)</td>
<td>5 (18%)</td>
<td>0.73</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Selective cerebral perfusion</td>
<td></td>
<td></td>
<td></td>
<td>5 (17%)</td>
<td>5 (18%)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aprotinin</td>
<td>6 (35%)</td>
<td>7 (44%)</td>
<td>0.73</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tranexamic acid</td>
<td>11 (65%)</td>
<td>9 (56%)</td>
<td>0.73</td>
<td>28 (97%)</td>
<td>28 (100%)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Steroids given in operating room</td>
<td>5 (29%)</td>
<td>7 (44%)</td>
<td>0.49</td>
<td>12 (41%)</td>
<td>10 (36%)</td>
<td>0.79</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vasopressin</td>
<td>0 (0%)</td>
<td>3 (19%)</td>
<td>0.11</td>
<td>5 (17%)</td>
<td>4 (14%)</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Tetralogy of Fallot, TOF; Double outlet right ventricle, DORV; Pulmonary atresia with ventricular septal defect, PAVSD
6.4 Summary of Results

1. Target anti-Xa should be 3.8 U/ml in neonates and 3.4 U/ml in infants.

2. There is a difference between plasma anti-Xa level and heparin concentration as measured by the HMS. It is affected by the haematocrit in neonates but not in infants. For neonates, the HMS device should be set respectively at 3.2, 3.0, 2.9 and 2.8 for haematocrit levels of 0.2, 0.3, 0.4 and 0.5 to maintain a target anti-Xa of 3.8 U/ml. For infants, the HMS device should be set at 2.9 U/ml regardless of the haematocrit level to maintain a target anti-Xa of 3.4 U/ml.

3. In neonates the total heparin dose should not exceed 960U/Kg prior to CPB, and not more than 1370U/Kg for the entire procedure. In infants the combined heparin dose should not exceed 500U/Kg prior to CPB, and no more than 760U/Kg for the entire procedure.

4. The protamine sulfate dose was calculated using heparin concentration as measured by the HMS device. Limited conclusions could be reached regarding protamine sulfate doses.

5. Lower AT levels in patients adversely affect anti-Xa response to heparin. Higher heparin doses are needed to maintain target anti-Xa levels. This effect is pronounced in neonates.

6. Unbound heparin (heparin not contributing to anti-Xa) is most strongly associated with blood loss at 24 hours, duration of ventilation and duration of hospital stay. Appropriate heparin dosing is crucial.

7. Patients managed with the ACT method are under anticoagulated, receive insufficient heparin doses and have lower than required anti-Xa levels.
8. Young children, especially neonatal patients are at risk of being over-anticoagulated if managed following the HMS method by manufacturer guidelines.

6.5 Target Plasma Anti-Xa Level

Although it is known that low levels of anti-Xa are detrimental, the HMS 1 trial demonstrated that high levels of anti-Xa are also detrimental. Regression models established a target anti-Xa of approximately 3.8 U/ml for neonates (Figure 6-1) and 3.4 U/ml for infants (Figure 6-2) at the initiation of CBP as measured by the HMS. Infants monitored via the ACT have lower than optimal anti-Xa (Table 6-2) and infants monitored via the HMS device have higher than optimal anti-Xa levels (Table 6-3). Importantly, while monitoring with the HMS device results in higher median anti-Xa than the established targets, the spread of values is quite important and the mean is strongly influenced by outliers. Current clinical practice of ACT monitoring is not optimal, so there is a need for more appropriate devices such as the HMS. However, strict maximum anti-Xa levels should be set while using the HMS device in order to prevent over dose of heparin and excessively high anti-Xa levels.
Figure 6-1: Association between anti-Xa levels (U/ml) and chest tube volume loss per Kg at 24 hours post-operatively in neonates

Table 6-2: Association between anti-Xa levels (U/ml) and chest tube volume loss per Kg at 24 hours post-operatively (EST±SE) with predicted target anti-Xa level to maintain chest tube volume loss below 20ml/Kg/24 hours (target) and comparison with actual anti-Xa levels recorded in the HMS 1 trial

<table>
<thead>
<tr>
<th></th>
<th>EST ± SE*</th>
<th>p</th>
<th>Target</th>
<th>ACT</th>
<th>HMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-heparin (s)</td>
<td>1.152 ± 0.343</td>
<td>0.001</td>
<td>5.7</td>
<td>4.8 (4.3 – 6.6)</td>
<td>6.3 (2.7 – 7.5)</td>
</tr>
<tr>
<td>CPB initiation (s)</td>
<td>1.447 ± 0.601</td>
<td>0.02</td>
<td>3.8</td>
<td>2.8 (2.6 – 4.0)</td>
<td>4.3 (2.9 – 6.3)</td>
</tr>
<tr>
<td>30mins CPB (s)</td>
<td>0.955 ± 0.491</td>
<td>0.05</td>
<td>3.0</td>
<td>2.5 (0.9 – 3.0)</td>
<td>4.3 (2.7 – 6.8)</td>
</tr>
<tr>
<td>CPB termination (l)</td>
<td>2.354 ± 1.518</td>
<td>0.12</td>
<td>2.8</td>
<td>2.5 (2.2 – 2.8)</td>
<td>4.8 (3.8 – 6.7)</td>
</tr>
</tbody>
</table>

(s) = squared, (l) = log/10
* EST±SE = Parameter Estimate Plus or Minus Standard Error
Figure 6-2: Association between anti-Xa levels (U/ml) and chest tube volume loss per Kg at 24 hours post-operatively in infants

Table 6-3: Association between anti-Xa levels (U/ml) and chest tube volume loss per Kg at 24 hours post-operatively (EST±SE) with predicted target anti-Xa level to maintain chest tube volume loss below 15ml/Kg/24 hours (target) and comparison with actual anti-Xa levels recorded in the HMS 1 trial

<table>
<thead>
<tr>
<th>Event</th>
<th>EST ± SE*</th>
<th>p</th>
<th>Target</th>
<th>ACT</th>
<th>HMS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-heparin (s)</td>
<td>0.521 ± 0.162</td>
<td>0.002</td>
<td>4.4</td>
<td>4.4 (3.6 – 4.9)</td>
<td>4.2 (3.0 – 7.3)</td>
</tr>
<tr>
<td>CPB initiation (s)</td>
<td>0.723 ± 0.353</td>
<td>0.04</td>
<td>3.4</td>
<td>2.9 (2.7 – 4.1)</td>
<td>3.8 (3.0 – 5.6)</td>
</tr>
<tr>
<td>30mins CPB (s)</td>
<td>0.419 ± 0.202</td>
<td>0.04</td>
<td>3.4</td>
<td>2.8 (2.4 – 3.8)</td>
<td>4.0 (2.9 – 6.9)</td>
</tr>
<tr>
<td>CPB termination (l)</td>
<td>0.575 ± 0.191</td>
<td>0.003</td>
<td>3.8</td>
<td>2.7 (2.3 – 4.4)</td>
<td>4.1 (3.0 – 6.5)</td>
</tr>
</tbody>
</table>

(s) = squared
*EST±SE = Parameter Estimate Plus or Minus Standard Error
6.6 Relationship between Anti-Xa and HMS Heparin Concentration

The HMS device underestimated plasma anti-Xa (Table 6-4). This relationship is affected by the haematocrit level in neonates (Figure 6-3) but not in infants (Figure 6-4). In neonates, for a target anti-Xa of 3.8, the HMS device should be set respectively to 3.2, 3.0, 2.9 and 2.8 for haematocrit levels of 0.2, 0.3, 0.4 and 0.5. The difference between HMS-measured heparin concentration and anti-Xa increased as heparin concentration increased suggesting lower reliability at the higher end of measurement. For infants, the HMS device should be set at 2.9 for a target anti-Xa of 3.4 regardless of the haematocrit level.

Figure 6-3: Relationship between HMS device measurement and plasma anti-Xa confounded by haematocrit in neonates. Vertical line represents target anti-Xa of 3.8U/ml for this group
Table 6-4: Confounding effect of haematocrit on anti-Xa level and HMS measurement discrepancies in neonates and infants

<table>
<thead>
<tr>
<th></th>
<th>EST ± SE*</th>
<th>p</th>
<th></th>
<th>EST ± SE*</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neonates</strong></td>
<td></td>
<td></td>
<td><strong>Infants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intercept</td>
<td>-2.159 ± 0.968</td>
<td>0.03</td>
<td>Intercept</td>
<td>-1.751 ± 1.441</td>
<td>0.23</td>
</tr>
<tr>
<td>Anti-Xa (r)</td>
<td>2.371 ± 0.274</td>
<td>&lt;0.001</td>
<td>Anti-Xa (r)</td>
<td>2.510 ± 0.400</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HCT (l)</td>
<td>-0.998 ± 0.479</td>
<td>0.04</td>
<td>HCT (l)</td>
<td>-0.031 ± 0.774</td>
<td>0.97</td>
</tr>
</tbody>
</table>

*(r) = square-root; (l) = log
*EST±SE = Parameter Estimate Plus or Minus Standard Error

Figure 6-4: Relationship between HMS device measurement and plasma anti-Xa confounded by haematocrit in infants. The vertical line represents target anti-Xa of 3.4U/ml for this group
6.7  Target Heparin Dose

In the HMS trial, heparin dose was dependent on the experimental group, neonates in the control group received 400 U/Kg and infants 300 U/Kg of heparin prior to CPB, with 2 U/ml of heparin in the final prime and 2 U/ml of heparin for all subsequent FFP transfusion with the ACT monitoring thereafter determining subsequent doses. In the HMS group, the HMS device was programmed to predict a heparin concentration (anti-Xa) of 4.0 U/ml in neonates and 3.0 U/ml in infants, and fixed levels of heparin were added to the prime. These two methodologies resulted in greatly varying heparin doses between the two groups. Target levels of heparin to be given at different stages of the surgery are detailed in Figures 6-5 and 6-6, along with target cumulative heparin doses at different time points during surgery. Tables 6-5 and 6-6 compare the estimated target heparin level with the level in each experimental group. From this analysis it is clear that infants monitored via the ACT method are not anticoagulated enough, while some infants monitored via the HMS device received much more heparin than required.
Table 6-5: Association between heparin dose (U/Kg) and chest tube volume loss per Kg at 24 hours post-operatively (EST±SE) in neonates with predicted target heparin dose to maintain chest tube volume loss below 20ml/Kg/24 hours (target for age group) and comparison with actual heparin dose recorded in the HMS 1 trial

<table>
<thead>
<tr>
<th></th>
<th>EST ± SE</th>
<th>p</th>
<th>Target</th>
<th>ACT</th>
<th>HMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumulative dose (U/Kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaesthesia/prime (s)</td>
<td>0.117±0.054</td>
<td>0.03</td>
<td>920</td>
<td>670 (630 – 820)</td>
<td>1309 (964 – 1975)</td>
</tr>
<tr>
<td>Prior CPB initiation</td>
<td>1.827±0.899</td>
<td>0.05</td>
<td>960</td>
<td>811 (630 – 826)</td>
<td>1711 (1000 – 2755)</td>
</tr>
<tr>
<td>30 mins CPB</td>
<td>1.489±0.835</td>
<td>0.08</td>
<td>1020</td>
<td>811 (630 – 826)</td>
<td>1904 (1082 – 2905)</td>
</tr>
<tr>
<td>60 mins CPB (s)</td>
<td>0.042±0.021</td>
<td>0.04</td>
<td>1260</td>
<td>811 (630 – 826)</td>
<td>2131 (1368 – 3055)</td>
</tr>
<tr>
<td>Total given (s)</td>
<td>0.043±0.018</td>
<td>0.02</td>
<td>1370</td>
<td>811 (631 – 931)</td>
<td>2131 (1475 – 3205)</td>
</tr>
<tr>
<td><strong>Individual dose (U/Kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaesthesia (s)</td>
<td>2.115±0.783</td>
<td>0.007</td>
<td>410</td>
<td>400 (399 – 416)</td>
<td>436 (321 – 600)</td>
</tr>
<tr>
<td>Prime (s)</td>
<td>0.190±0.093</td>
<td>0.06</td>
<td>520</td>
<td>266 (217 – 420)</td>
<td>872 (643 – 1450)</td>
</tr>
<tr>
<td>Post-heparin (s)</td>
<td>2.986±1.077</td>
<td>0.006</td>
<td>130</td>
<td>100 (0 – 156)</td>
<td>147 (0 – 390)</td>
</tr>
<tr>
<td>Termination (s)</td>
<td>6.520±1.676</td>
<td>&lt;0.001</td>
<td>90</td>
<td>0 (0 – 160)</td>
<td>54 (0 – 313)</td>
</tr>
</tbody>
</table>

(s) = square
*EST±SE = Parameter Estimate Plus or Minus Standard Error
Figure 6-5: Association between heparin dose (U/Kg) and chest tube volume loss per Kg at 24 hours post-operatively in neonates

Figure 6-6: Association between heparin dose (U/Kg) and chest tube volume loss per Kg at 24 hours post-operatively in infants
Table 6-6: Association between heparin dose (U/Kg) and chest tube volume loss per Kg at 24 hours post-operatively (EST±SE) in infants with predicted target heparin dose to maintain chest tube volume loss below 15ml/Kg/24 hours (target for age group) and comparison with actual heparin dose recorded in the HMS 1 trial

<table>
<thead>
<tr>
<th></th>
<th>EST ± SE</th>
<th>p</th>
<th>Target</th>
<th>ACT</th>
<th>HMS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cumulative dose (U/Kg)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anaesthesia/prime (s)</td>
<td>2.345±1.512</td>
<td>0.12</td>
<td>500</td>
<td>463 (425 – 821)</td>
<td>600 (408 – 879)</td>
</tr>
<tr>
<td>Prior CPB initiation (s)</td>
<td>0.151±0.061</td>
<td>0.02</td>
<td>600</td>
<td>563 (452 – 821)</td>
<td>706 (464 – 1209)</td>
</tr>
<tr>
<td>30 mins CPB (s)</td>
<td>0.106±0.049</td>
<td>0.04</td>
<td>650</td>
<td>572 (452 – 974)</td>
<td>768 (524 – 1319)</td>
</tr>
<tr>
<td>60 mins CPB (s)</td>
<td>0.090±0.038</td>
<td>0.02</td>
<td>700</td>
<td>620 (452 – 974)</td>
<td>832 (524 – 1538)</td>
</tr>
<tr>
<td>Total given (s)</td>
<td>0.054±0.017</td>
<td>0.002</td>
<td>760</td>
<td>716 (452 – 974)</td>
<td>878 (611 – 2088)</td>
</tr>
</tbody>
</table>

| **Individual dose (U/Kg)** |          |       |        |              |              |
| Anaesthesia (i)          | -8.021 ± 3.627 | 0.03  | 250    | 300 (297 – 410) | 291 (231 – 440) |
| Prime (s)                | 0.446 ± 0.412 | 0.28  | 180    | 160 (125 – 410) | 288 (177 – 440) |
| Post-heparin             | 1.252 ± 4.069 | 0.76  | <100   | 97 (0 – 150)     | 97 (0 – 112)    |
| Termination (l)          | -0.144 ± 0.508 | 0.78  | <100   | 0 (0 – 166)      | 14 (0 – 167)    |

(s) = square; (l) =log; (i) =inverse
*EST±SE = Parameter Estimate Plus or Minus Standard Error

6.8 Target Protamine Sulfate Dose

The protocol for protamine sulfate administration was different in both groups making it difficult to analyse the effect on bleeding. Infants in the control group all received protamine sulfate in a 1:1 ratio of the heparin dose. Very little variation is observed in this group (protamine sulfate to heparin ratio of 0.9 to 1.3). Infants monitored via the HMS device received a dose of protamine sulfate based on heparin concentration at termination of CPB. Therefore the ratio of protamine sulfate to heparin varies from 1.0 to 3.6. Higher ratios are associated with higher heparin doses, creating a negative
association between higher protamine sulfate to heparin ratio and clinical outcomes. This relationship does not remain significant if either total heparin dose or anti-Xa at termination of CPB is taken into account. It is not possible to determine the effect of protamine sulfate to heparin ratios on bleeding or to establish target ratios. The discrepancies between the HMS ratio and actual anti-Xa levels make it difficult to recommend protamine sulfate doses purely on the HMS reading without a correction.

6.9 Effect of Antithrombin level on Heparin-dose Response

The effect of AT on the patient response to heparin was investigated. Regardless of patient age, lower AT was associated with a lower dose-response to heparin (i.e. for a given dose of heparin, anti-Xa will have a greater response at higher levels of AT). Neonates had lower levels of AT than infants (Table 6-7), which means that they require quite high doses of heparin to reach a target anti-Xa level. Figures 6-7 and 6-8 depict the relationship between the heparin dose to maintain target anti-Xa and patient AT levels. Table 6-8 estimates heparin dose by patient age and AT levels after the initial heparin dose. This analysis demonstrates that lower AT levels are associated with lower heparin dose response (i.e. higher amount of unbound heparin circulating in the blood). Target heparin levels calculated are close to prediction for AT levels of 1.0 in the infant but closer to AT of 0.8 in neonates. The AT level may be an important factor for heparin anticoagulation in neonatal patients.
Figure 6-7: Relationship between antithrombin and heparin dose required to maintain a target anti-Xa of 3.8 U/ml in neonates

Table 6-7: Antithrombin levels throughout surgery by age category in HMS 1

<table>
<thead>
<tr>
<th></th>
<th>Neonates</th>
<th>Infants</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-heparin</td>
<td>0.60 (0.43 – 0.90)</td>
<td>0.83 (0.66 – 0.97)</td>
<td>0.001</td>
</tr>
<tr>
<td>CPB initiation</td>
<td>0.38 (0.24 – 0.53)</td>
<td>0.45 (0.37 – 0.58)</td>
<td>0.004</td>
</tr>
<tr>
<td>30 minutes CPB</td>
<td>0.33 (0.20 – 0.52)</td>
<td>0.45 (0.30 – 0.65)</td>
<td>0.001</td>
</tr>
<tr>
<td>Termination CPB</td>
<td>0.42 (0.31 – 0.57)</td>
<td>0.53 (0.40 – 0.65)</td>
<td>0.003</td>
</tr>
</tbody>
</table>
Figure 6-8: Relationship between antithrombin and heparin dose required to maintain a target anti-Xa of 3.4 U/ml in infants

Table 6- 8: Association between antithrombin level and total heparin dose required to maintain target anti-Xa, accounting for post-heparin anti-Xa (below) and predicted required heparin dose by antithrombin levels next page.

<table>
<thead>
<tr>
<th></th>
<th>EST ± SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept</td>
<td>1.383 ± 0.053</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Anti-Xa post-heparin</td>
<td>-0.243 ± 0.021</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total heparin dose * Antithrombin (r)</td>
<td>0.027 ± 0.006</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

(r)=square root
*EST±SE = Parameter Estimate Plus or Minus Standard Error
### Total heparin dose (U*100/Kg)

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<th>Infants</th>
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#### 6.10 Effects of Unbound Heparin

The significance of unbound heparin is not known. If it is inert then AT levels are of no importance because the heparin dose only has to be increased in order to maintain the desired target anti-Xa level. On the other hand, if unbound heparin detrimentally affects clinical outcomes, then low AT is extremely important: more heparin will remain unbound and a greater amount of heparin will be required to obtain the desired target anti-Xa level.

The following models look at the effect of unbound heparin (i.e. heparin dose not accounted for by changes in the anti-Xa model) after adjusting for patient age, surgeon and heparin dose-response. The columns on the left side are the effect of anti-Xa levels at different points in time on clinical outcomes adjusted for patient age, heparin dose-response and surgeon. The columns on the right side are the effect of heparin dose (per Kg) adjusted for anti-Xa, patient age,
heparin dose-response and surgeon on clinical outcomes (i.e. variability in clinical outcomes attributed to heparin dose not accounted for by any of the 3 confounders or anti-Xa level) (Table 6-9).

Table 6-9: Effect of unbound heparin on clinical outcomes. Unbound heparin is defined as the variance in clinical outcomes explained by heparin dose beyond contribution to anti-Xa

<table>
<thead>
<tr>
<th>Outcome</th>
<th>EST</th>
<th>SE</th>
<th>p</th>
<th>EST</th>
<th>SE</th>
<th>p</th>
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<td>0.6341</td>
<td>0.8100</td>
<td>0.0029</td>
<td>0.0024</td>
<td>0.2190</td>
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<td>0.6139</td>
<td>0.0027</td>
<td>0.0020</td>
<td>0.1917</td>
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<td>0.0035</td>
<td>0.0022</td>
<td>0.1101</td>
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<tr>
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<td>0.4993</td>
<td>0.0002</td>
<td>0.0028</td>
<td>0.9285</td>
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<td>0.0138</td>
<td>0.0064</td>
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<td>0.0031</td>
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<td>1.0100</td>
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Table 6-9 (continued)

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<th>p</th>
<th>EST</th>
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</tr>
<tr>
<td>Post-heparin</td>
<td>16.3333</td>
<td>7.8601</td>
<td><strong>0.0377</strong></td>
<td>0.0130</td>
<td>0.0297</td>
<td>0.6626</td>
</tr>
<tr>
<td>Induction</td>
<td>8.2377</td>
<td>13.4471</td>
<td>0.5401</td>
<td>0.0158</td>
<td>0.0269</td>
<td>0.5575</td>
</tr>
<tr>
<td>30 mins CPB</td>
<td>0.8224</td>
<td>12.3613</td>
<td>0.9470</td>
<td>0.0175</td>
<td>0.0301</td>
<td>0.5608</td>
</tr>
<tr>
<td>60 mins CPB</td>
<td>-37.2918</td>
<td>12.3078</td>
<td><strong>0.0024</strong></td>
<td>0.0768</td>
<td>0.0291</td>
<td><strong>0.0082</strong></td>
</tr>
<tr>
<td>90 mins CPB</td>
<td>-3.6709</td>
<td>12.2550</td>
<td>0.7645</td>
<td>0.0126</td>
<td>0.0190</td>
<td>0.5063</td>
</tr>
<tr>
<td>Termination</td>
<td>-22.5098</td>
<td>15.0181</td>
<td>0.1339</td>
<td>0.0662</td>
<td>0.0322</td>
<td><strong>0.0401</strong></td>
</tr>
</tbody>
</table>

After accounting for anti-Xa, patient age, patient heparin dose-response and surgeon, higher heparin doses (i.e. unbound heparin) was found to be associated with higher chest tube volume loss, longer length of ventilation, CCCU stay, hospital stay, greater transfusion requirements in the CCCU and overall. Heparin dose does not affect inotropic scores or transfusion
requirements in the operating room. The adverse effect of the heparin dose (above that of anti-Xa) on clinical outcomes seems greatest after surgery. The data suggests that the lowest possible amount of heparin to achieve a given target anti-Xa is beneficial: unbound heparin has a negative effect on clinical outcomes. A trial of AT supplementation is justified on the assumption that it will reduce the amount of heparin required to reach target anti-Xa levels and diminish the adverse effects of unbound heparin.

6.11 Clinical Results

6.11.1 Clinical Outcomes of HMS I

The HMS device underestimated laboratory plasma anti-Xa levels in infants. This overestimated the required dose of heparin (Table 6-10). The result was adverse clinical outcomes including greater chest tube volume loss (Table 6-11), transfusion requirement (Table 6-12), and intravascular thrombosis along with greater ventilation time; longer CCCU and hospital stay (Table 6-11).

6.11.2 Clinical Outcomes of HMS II

In HMS 2, patients in the treatment group received significantly more heparin and protamine sulfate (all p<0.001) than control patients during the perioperative period but much less than in HMS I (Table 6-10). Patients in the treatment group were more likely to receive periodic doses of heparin throughout CPB, which was a rare occurrence in the control group (Figure 6-9). As a result, the plasma anti-Xa concentrations were more stable throughout CPB (Figure 6-10).
A statistically significant increase in the ratio of protamine sulfate to pre-CPB heparin dose was observed in the treatment group vs. control (Table 6-10).

![Heparin dose (U/Kg) during CPB for HMS 2](image)

**Figure 6-9: Heparin dose (U/Kg) during CPB for HMS 2**

### 6.11.3 Laboratory Measurements

Treatment patients had significantly higher platelet counts at all time points throughout CPB, significantly lower levels of TAT complexes upon arrival and at 24 hours in the CCCU and significantly lower levels of F1.2 after protamine sulfate, upon arrival and at 24 hours in the CCCU (Figure 6-10). There was no significant difference between groups in serial measurements of AT, fibrinogen, d-dimers, haemoglobin or haematocrit.
6.11.4 Bleeding and Transfusions

There was no significant difference between groups in chest tube loss at both time points (4 and 24 hours after CCCU arrival) (Table 6-11). Patients in the treatment group received significantly less transfusions in the operating room and over the first 24 hours following surgery. We observed a reduction in the number of patients who received >5 units of blood in the operating room, any transfusions in the CCCU and >7 units of blood overall (Table 6-12) in the treatment group. This did not reach statistical significance.

6.11.5 Morbidity and Hospital Stay

Patients in the treatment group had significantly shorter ventilation times, CCCU and hospital lengths of stay when compared to the control group. Five (17%) control patients, (including one patient who received rFVIIa), had thrombosis during their hospital stay compared to 1 (4%) in the treatment group. There were no significant differences in inotropic requirements or in-hospital mortality between groups (Table 6-11).

6.12 Summary

Heparin management in the HMS 1 group followed the device calculations at induction of anaesthesia. Patients in the treatment group received significantly more heparin and protamine sulfate. This resulted in worse clinical outcomes including increased chest tube volume losses, increased transfusion, and longer ventilation time, CCCU and total hospital length of stay. The HMS overestimated the amount of heparin required to achieve adequate anticoagulation during CPB in neonates and infants.
As a result of HMS 1, specific protocols were established for heparin loading and prime doses as well as heparin concentration targets while on CPB. The goal was to maintain the equivalent plasma heparin concentration (anti-Xa) of 4.0 U/ml. The rationale for HMS 2 resulted in a protocol for all treatment patients where the HMS target was 3.0 U/ml for the duration of CPB. Patients in the treatment group received significantly more heparin than patients in the control group both at induction and throughout CPB to maintain steady levels. This led to significantly higher and more stable plasma anti-Xa levels and improved clinical outcomes.
<table>
<thead>
<tr>
<th></th>
<th>CTRL (N=17)</th>
<th>HMS I (N=33)</th>
<th>HMS (N=16)</th>
<th>p</th>
<th>CTRL (N=29)</th>
<th>HMS II (N=57)</th>
<th>HMS (N=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope of heparin dose response</td>
<td>86±25</td>
<td>90±28</td>
<td>0.42</td>
<td></td>
<td>95±30</td>
<td>93±29</td>
<td>0.75</td>
<td></td>
</tr>
<tr>
<td>Baseline heparin concentration (U/Kg)*</td>
<td>4.5±1.6</td>
<td>4.4±1.7</td>
<td>0.83</td>
<td></td>
<td>4.1±1.8</td>
<td>4.1±1.4</td>
<td>0.91</td>
<td></td>
</tr>
<tr>
<td>Initial heparin dose - anaesthesia (U/Kg) (1)</td>
<td>395 (297-416)</td>
<td>324 (231-600)</td>
<td>0.005</td>
<td></td>
<td>316 (294-427)</td>
<td>304 (225-519)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Initial heparin dose - prime (U/Kg)</td>
<td>222 (125-420)</td>
<td>451 (177-1450)</td>
<td>&lt;0.001</td>
<td></td>
<td>320 (199-645)</td>
<td>291 (160-846)</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Additional heparin given before CPB (U/Kg)</td>
<td>99 (0-156)</td>
<td>102 (0-390)</td>
<td>&lt;0.001</td>
<td></td>
<td>0 (0-213)</td>
<td>54 (0-206)</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Heparin given on CPB (U/Kg)</td>
<td>105 (0-482)</td>
<td>574 (0-1188)</td>
<td>&lt;0.001</td>
<td></td>
<td>75 (0-303)</td>
<td>272 (48-772)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Total heparin dose (U/Kg) (2)</td>
<td>739 (452-1050)</td>
<td>1448 (618-3205)</td>
<td>&lt;0.001</td>
<td></td>
<td>810 (521-1355)</td>
<td>927 (667-1593)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Total protamine sulfate dose (mg/Kg)</td>
<td>391 (297-505)</td>
<td>701 (285-1333)</td>
<td>&lt;0.001</td>
<td></td>
<td>404 (286-762)</td>
<td>666 (185-1385)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Ratio protamine sulfate dose to (1)</td>
<td>1.00 (0.95-1.27)</td>
<td>1.99 (0.95-5.13)</td>
<td>0.02</td>
<td></td>
<td>1.11 (0.94-1.79)</td>
<td>2.19 (0.79-3.21)</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>Ratio protamine sulfate dose to (2)</td>
<td>0.50 (0.29-0.71)</td>
<td>0.51 (0.16-1.47)</td>
<td>0.88</td>
<td></td>
<td>0.50 (0.30-0.98)</td>
<td>0.72 (0.28-1.08)</td>
<td>0.39</td>
<td></td>
</tr>
</tbody>
</table>

Cardiopulmonary bypass, CPB
Table 6-11 Clinical Outcomes (p-values from models adjusted for age at surgery, surgery Aristotle score and surgeon)

<table>
<thead>
<tr>
<th></th>
<th>HMS I (N=33)</th>
<th>HMS II (N=57)</th>
<th>p</th>
<th>HMS I (N=16)</th>
<th>HMS II (N=28)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CTRL (N=17)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chest closure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time protamine sulfate to sternal closure (min)*</td>
<td>58 (23-125)</td>
<td>49 (30-134)</td>
<td>0.001</td>
<td>50 (13-144)</td>
<td>64 (20-110)</td>
<td>0.04</td>
</tr>
<tr>
<td>Sternum closed in OR</td>
<td>17 (100%)</td>
<td>11 (69%)</td>
<td>0.02</td>
<td>25 (86%)</td>
<td>24 (86%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Sternum reopened in CCCU</td>
<td>1 (6%)</td>
<td>1 (6%)</td>
<td>1.00</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00</td>
</tr>
<tr>
<td><strong>Thrombosis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor VIIa given in OR</td>
<td>0 (0%)</td>
<td>3 (19%)</td>
<td>0.11</td>
<td>1 (3%)</td>
<td>0 (0%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>2 (12%)</td>
<td>2 (12%)</td>
<td>1.00</td>
<td>4 (14%)</td>
<td>2 (7%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Intravascular clot</td>
<td>0 (0%)</td>
<td>3 (19%)</td>
<td>0.11</td>
<td>5 (17%)</td>
<td>1 (4%)</td>
<td>0.20</td>
</tr>
<tr>
<td><strong>Inotrope support</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inotropic score in OR</td>
<td>8.7±2.7</td>
<td>9.3±4.5</td>
<td>0.99</td>
<td>9.9±4.1</td>
<td>8.6±3.4</td>
<td>0.16</td>
</tr>
<tr>
<td>Inotropic score 4 hours in CCCU</td>
<td>8.6±3.4</td>
<td>9.7±4.3</td>
<td>0.56</td>
<td>8.1±3.6</td>
<td>7.6±2.9</td>
<td>0.56</td>
</tr>
<tr>
<td>Inotropic score 12 hours in CCCU</td>
<td>8.1±3.1</td>
<td>9.5±3.9</td>
<td>0.99</td>
<td>8.2±2.7</td>
<td>7.6±3.4</td>
<td>0.52</td>
</tr>
<tr>
<td>Inotropic score 24 hours in CCCU</td>
<td>6.9±3.2</td>
<td>8.2±4.2</td>
<td>0.38</td>
<td>5.7±3.3</td>
<td>5.6±3.5</td>
<td>0.89</td>
</tr>
<tr>
<td><strong>Bleeding</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest tube loss 4 hours CCCU (ml/Kg)</td>
<td>5.1 (2.7-57.2)</td>
<td>13.6 (2.5-93.7)</td>
<td>0.05</td>
<td>9.1 (3.0-23.3)</td>
<td>7.6 (1.1-43.2)</td>
<td>0.66</td>
</tr>
<tr>
<td>Chest tube loss 24 hours CCCU (ml/Kg)</td>
<td>18.1 (9.6-129.0)</td>
<td>28.1 (8.0-162.7)</td>
<td>&lt;0.001</td>
<td>21.9 (11.0-61.3)</td>
<td>21.5 (7.5-70.0)</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Hospitalisation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventilation length (hours)</td>
<td>47 (5-144)</td>
<td>70 (5-528)</td>
<td>&lt;0.001</td>
<td>49 (5-237)</td>
<td>33 (4-234)</td>
<td>0.04</td>
</tr>
<tr>
<td>CCCU length of stay (hours)</td>
<td>70 (27-384)</td>
<td>105 (20-645)</td>
<td>&lt;0.001</td>
<td>99 (20-356)</td>
<td>88 (27-312)</td>
<td>0.003</td>
</tr>
<tr>
<td>Total length of stay (hours)</td>
<td>196 (114-916)</td>
<td>307 (117-3480)</td>
<td>&lt;0.001</td>
<td>216 (97-692)</td>
<td>192 (97-2376)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Mortality</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In-hospital mortality</td>
<td>0 (0%)</td>
<td>1 (6%)</td>
<td>0.49</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Deceased at last follow-up</td>
<td>2 (12%)</td>
<td>3 (19%)</td>
<td>0.66</td>
<td>2 (7%)</td>
<td>0 (0%)</td>
<td>0.50</td>
</tr>
</tbody>
</table>

* Chest closure in OR only
Operating room, OR; Cardiac critical care unit, CCC
Table 6-12. Blood Transfusions (p-values from models adjusted for age at surgery, surgery Aristotle score and surgeon)

<table>
<thead>
<tr>
<th></th>
<th>CTRL (N=17)</th>
<th>HMS I (N=33)</th>
<th>p</th>
<th>HMS II (N=57)</th>
<th>CTRL (N=29)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>PRBC total in OR (ml/Kg)</td>
<td>86 (35-147)</td>
<td>85 (37-434)</td>
<td>&lt;0.001</td>
<td>65 (40-224)</td>
<td>64 (32-167)</td>
<td>0.01 †</td>
</tr>
<tr>
<td>PRBC CCCU (ml/Kg)</td>
<td>6 (0-112)</td>
<td>20 (0-107)</td>
<td>0.06</td>
<td>10 (0-37)</td>
<td>0 (0-29)</td>
<td>0.05 †</td>
</tr>
<tr>
<td>PRBC total (ml/Kg)</td>
<td>97 (37-205)</td>
<td>112 (46-465)</td>
<td>&lt;0.001</td>
<td>80 (49-224)</td>
<td>71 (32-186)</td>
<td>0.003 †</td>
</tr>
<tr>
<td>Platelets total in OR (ml/Kg)</td>
<td>16 (0-46)</td>
<td>14 (1-102)</td>
<td>&lt;0.001</td>
<td>20 (0-83)</td>
<td>17 (0-54)</td>
<td>0.09 †</td>
</tr>
<tr>
<td>Platelets CCCU (ml/Kg)</td>
<td>0 (0-54)</td>
<td>0 (0-20)</td>
<td>&lt;0.001</td>
<td>0 (0-10)</td>
<td>0 (0-20)</td>
<td>0.80</td>
</tr>
<tr>
<td>Platelets total (ml/Kg)</td>
<td>18 (0-56)</td>
<td>19 (1-102)</td>
<td>&lt;0.001</td>
<td>24 (0-93)</td>
<td>25 (0-54)</td>
<td>0.13 †</td>
</tr>
<tr>
<td>FFP and cryoprecipitate total (ml/Kg)</td>
<td>60 (30-124)</td>
<td>91 (24-297)</td>
<td>&lt;0.001</td>
<td>61 (28-133)</td>
<td>49 (26-113)</td>
<td>0.64</td>
</tr>
<tr>
<td>All blood products total (ml/Kg)</td>
<td>169 (79-374)</td>
<td>238 (94-865)</td>
<td>&lt;0.001</td>
<td>157 (93-438)</td>
<td>153 (75-354)</td>
<td>0.006 †</td>
</tr>
<tr>
<td>Total units transfused in OR</td>
<td>8 (3-12)</td>
<td>9 (4-21)</td>
<td>0.20</td>
<td>5 (3-9)</td>
<td>5 (1-8)</td>
<td>0.41</td>
</tr>
<tr>
<td>&gt;5 units transfused in OR</td>
<td>13 (76%)</td>
<td>12 (75%)</td>
<td>0.34</td>
<td>12 (41%)</td>
<td>7 (25%)</td>
<td>0.17</td>
</tr>
<tr>
<td>Total units transfused CCCU</td>
<td>0 (0-12)</td>
<td>2 (0-6)</td>
<td>0.94</td>
<td>1 (0-3)</td>
<td>0 (0-8)</td>
<td>0.20</td>
</tr>
<tr>
<td>&gt;0 units transfused in CCCU</td>
<td>8 (47%)</td>
<td>14 (88%)</td>
<td>0.07</td>
<td>17 (59%)</td>
<td>12 (43%)</td>
<td>0.26</td>
</tr>
<tr>
<td>Total units transfused OR and CCCU</td>
<td>9 (3-17)</td>
<td>11 (5-23)</td>
<td>0.25</td>
<td>6 (3-9)</td>
<td>6 (1-12)</td>
<td>0.84</td>
</tr>
<tr>
<td>&gt;7 units transfused OR and CCCU</td>
<td>13 (76%)</td>
<td>12 (75%)</td>
<td>0.33</td>
<td>9 (31%)</td>
<td>5 (18%)</td>
<td>0.20</td>
</tr>
</tbody>
</table>

† Favouring HMS
Packed red blood cells, PRBC; Fresh frozen plasma, FFP; Cardiopulmonary bypass, CPB; Operating room, OR; Critical care unit, CCU
Figure 6-10 Trends in laboratory measurements of anticoagulation, platelet count and thrombin generation. Red lines represent control patients, black lines represent patients monitored with the HMS device. Time point with (*) represent statistically significant differences. Data presented for HMS II only.

Legend:
PST HEP: post heparinisation
CPB IND: induction of cardiopulmonary bypass
30M CPB: cardiopulmonary bypass time 30 minutes
60M CPB: cardiopulmonary bypass time 60 minutes
90M CPB: cardiopulmonary bypass time 90 minutes
END CPB: end of cardiopulmonary bypass
PST PROT: 5 minutes after protamine sulfate administration
AAR CCU: critical care unit arrival
24H CCU: 24 hours after critical care unit arrival

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Chapter 7

Discussion and Conclusions

7.1 Introduction

Infants less than one year of age who undergo complex cardiac surgery are at higher risk of morbidity and mortality associated with cardiopulmonary bypass (CPB). The combination of multiple risk factors results in increased bleeding, transfusion and thrombotic complications in these patients compared to older children and adults (Williams, Bratton et al. 1998b).

This study has investigated the management and monitoring of anticoagulation in infants undergoing heart surgery with CPB. It highlights a common problem in paediatric medicine: The application of medical knowledge from adult studies to paediatric patients without further evaluation is often inappropriate. The use of a modified protocol utilising the HMS device to guide heparin dosing and monitoring is associated with improved clinical outcomes.

7.2 Heparin Dosing and Monitoring for CPB

It has been shown that infants require more heparin than older children and adults to achieve adequate anticoagulation (Doty, Knott et al. 1979). The predominant practice of heparin dosing for both children and adults undergoing CPB is mostly empirical. Patients receive an initial heparin bolus and thereafter anticoagulation is monitored with the ACT throughout CPB. The optimal heparin dosing for CPB is uncertain because the target heparin level
for paediatric patients has not been established. The use of the ACT for monitoring anticoagulation during CPB has been criticised because it does not correlate with the circulating heparin concentration. Heparin concentrations ranging from 1.3 to 5.0 U/ml have been advocated (Weitz, Hudoba et al. 1990; Hashimoto, Yamagishi et al. 1994). Consequently, heparinisation regimens for paediatric cardiac surgery vary widely between institutions (Oliver 2003). Due to this variation, investigators have suggested that monitoring the heparin concentration during CPB may be more accurate. It was confirmed that when patients are managed through empirical heparin dosing with anticoagulation monitored with the ACT device alone, they receive insufficient heparin doses and have insufficient anti-Xa levels to limit post-operative complications.

Previous studies in paediatric patients have demonstrated that individualised heparin concentration-based protocols for anticoagulation and protamine sulfate reversal appear superior to weight-based protocols (Codispoti, Ludlam et al. 2001; Guzzetta, Bajaj et al. 2008). A heparin concentration-based heparin management protocol resulted in higher, more constant heparin concentration than a weight-based heparin protocol monitoring with the ACT. Each concluded that higher heparin concentrations were associated with greater suppression of haemostatic activation, less fibrinolysis and factor consumption (Codispoti, Ludlam et al. 2001; Guzzetta, Bajaj et al. 2008). In older children (mean age 4.8 years) there was less blood loss and transfusion (Codispoti, Ludlam et al. 2001). In infants three to six months of age there was an increase in twenty-four hour chest tube drainage and a higher blood product exposure in
the treatment arm. Both authors suggested that further research is needed to better define the clinical impact from this approach to anticoagulation specifically in this population (Codispoti, Ludlam et al. 2001; Guzzetta, Bajaj et al. 2008).

The research confirmed some of the previous observations. Patients in the treatment group of HMS 1 and 2 received significantly more heparin than in the control arms. Following the manufacturer’s recommendations as in HMS 1, patients received doses of heparin and protamine sulfate that resulted in greater bleeding and poorer clinical outcomes than in the control arm. In HMS 2, patients in the treatment group received more heparin than controls but less than in HMS 1 and were also more likely to receive periodic doses of heparin throughout CPB, which rarely occurred in the control group. As a result the plasma anti-Xa concentrations were more stable throughout CPB and the patients had improved clinical outcomes.

As a result of HMS 1, target heparin concentrations and total heparin dose limits were established. This resulted in a modified protocol for HMS 2. Target heparin concentrations were achieved and maintained resulting in stable anti-Xa levels and improved clinical outcomes. This study confirms that empirical dosing of heparin and ACT monitoring results in lower total doses of heparin and inadequate anticoagulation. It also demonstrates that the proprietary algorithm by the manufacturer is inappropriate for neonates and
infants and results in heparin overdose. The modified HMS protocol results in higher total doses of heparin and improved clinical outcomes.

7.3 Limitations of the HMS Device

7.3.1 HMS Heparin Concentrations Compared with Plasma Anti-Xa Assay

The HMS device results were lower than laboratory plasma anti-Xa levels. Agreement between whole blood heparin concentration measured by the HMS device and laboratory plasma heparin concentration using an anti-Xa assay has been found to be excellent in adult patients (Despotis, Santoro et al. 1994; Hashimoto, Sasaki et al. 1999; Raymond, Ray et al. 2003). In infants over three months old analysis showed >95% of samples were within the limits of agreement following the heparin bolus, and 93% of values were within range prior to termination of CPB (Guzzetta, Monitz et al. 2010). As previously reported in adult (Raymond, Ray et al. 2003) and infant studies (Guzzetta, Monitz et al. 2010) it was found that the difference between the HMS calculated heparin concentration and anti-Xa increased as the heparin concentration increased suggesting lower reliability at the higher end of measurement. Some of this difference may be due to the various commercially available anti-Xa assays. In a recent publication it has been shown that the Coamatic Chromogenic anti-Xa assay results are higher than the Coatest. The authors suggest that this difference may be due to the addition of dextran sulfate in Coamatic assay (Mouton, Calderon et al. 2003; Ignjatovic, Than et al. 2011). Furthermore, other anti-Xa assays require the addition of exogenous AT to account for variability in endogenous patient AT levels (Guzzetta,
Monitz et al. 2010). Patients with low levels of AT could be expected to have variation between anti-Xa and HMS measurements due to the artificial adjustments to the anti-Xa assays. Ignjatovic et al recommend that the use of anti-Xa assay kits without the addition of dextran sulfate or AT may be the best representation of in vivo anti-Xa activity (Ignjatovic, Than et al. 2011).

7.3.2 HMS Overestimates the Amount of Heparin Required

The HMS underestimated heparin concentration therefore overestimated the amount of heparin required to achieve adequate anticoagulation during CPB. The reason for this is not clear but several mechanisms are possible. The standard algorithm of the HMS device may have an inappropriate blood volume calculation for infants. The immaturity of the coagulation system, and acquired anomalies that occur as a result of CHF and cyanosis may affect the performance of the device.

7.3.3 HMS Results are Dependent on Haematocrit Level

The discrepancy between the HMS and the plasma anti-Xa level is also affected by the haematocrit level in the neonate but not in infants. Based on our results of this study we suggest that the HMS measurements be adjusted for the haematocrit level. Codispoti et al, (Codispoti, Ludlam et al. 2001) recommended the following formula to adjust for the haematocrit level: whole blood (WB) heparin concentration [Hep] measurements converted into their plasma equivalent (PE) by:

\[ PE_{\text{Hep}} = \frac{\text{WB } [\text{Hep}] \times 100}{100 - \text{HCT}} \]
7.3.4 Heparin Protamine Titration (HPT) - Low Range Cartridge

The HMS cartridge used for detection of heparin reversal is limited to a range of 0 U/ml to 0.4 U/ml of heparin. Almost half the patients in both HMS groups had laboratory plasma anti-Xa levels of 0.3 U/ml or greater following protamine sulfate reversal despite a heparin protamine sulfate titration measurement of 0 U/ml from the HMS. However, over half of patients in the control groups also demonstrated therapeutic anti-Xa levels following protamine sulfate reversal. This level of heparin concentration following reversal with protamine sulfate may contribute to bleeding in the immediate post-bypass and post-operative period. It is difficult to differentiate if the bleeding is caused by continued heparin effect or from the sequelae of CPB such as poor platelet function or low fibrinogen levels.

7.4 Paediatric Revalidation of Adult Protocols

This study reconfirmed the potential dangers of utilising technology and protocols developed for adult patients in infants and children. The extrapolation of findings from adult studies to paediatric patients without further validation is a common occurrence that should be discouraged (Smyth and Weindling 1999; Conroy, Choonara et al. 2000; Crawley, Kurz et al. 2003). This principle has multiple examples in pharmacology, often to the detriment of children (tetracycline, thalidomide, and chloramphenicol). There have been legislative efforts to address the depth of appropriate research within the pharmaceutical industry, but no parallel initiatives for manufacturers of medical devices. HMS technology, which was shown to have benefits in older
children and adults, should not be directly applied to infant patients undergoing heart surgery with CPB.

The physiology of infants less than one year of age is fundamentally different. The effects of CPB on the infant are significantly greater than that of older children and adults. These factors must be considered when developing trials and adapting protocols from older children or adult data. This research exemplified the importance of validating devices and protocols originally derived from older patients. The HMS device cannot be safely used in infants with the manufacturer suggested protocol.

7.5 Antithrombin Role in Anticoagulation

This trial has provided new insight into dosing and monitoring anticoagulation during CPB in infants. It provided a better understanding of developmental biology in infants, and the possible role of low levels of AT (which appears physiologic), but is distinctly disadvantageous in the un-physiologic scenario of CPB.

Heparin alone has no effect on coagulation. The anticoagulant effect is predominantly mediated by binding to AT through a specific pentasaccharide sequence (Choay, Petitou et al. 1983). AT subsequently inactivates several activated coagulation factors, most importantly factors ll a and Xa (Hirsh, Raschke et al. 1995). Heparin catalyses the inactivation of thrombin by acting as a template by which the enzyme and inhibitor bind to form a complex and prevent clot formation.
Decreases in AT levels during CPB have been reported in adults (Despotis, Joist et al. 1997) with even greater decreases observed in children (Turner-Gomes, Andrew et al. 1992) and infants (Kern, Morana et al. 1992). There are multiple reasons for this: developmental immaturity (Andrew, Paes et al. 1987; Andrew, Paes et al. 1988; Andrew, Vegh et al. 1992; Monagle, Barnes et al. 2006) smaller blood volume, greater degree of haemodilution on CPB and decreased plasma volume in cyanotic CHD.

Low AT concentrations imply that less of the heparin is bound to AT contributing to a potentially lower anti-Xa level. Unbound heparin binds to other proteins in the blood. Thus a fraction of heparin, inversely proportional to the amount of AT in the blood, will remains unbound and has no anticoagulant effect (Gruenwald 2008c). Patients with low AT will therefore require higher heparin doses to achieve target anti-Xa levels necessary for adequate anticoagulation.

Our results showed that the use of large doses of heparin to reach nominal target anti-Xa levels during CPB for infant cardiac surgery is associated with poor post-operative outcomes including increased bleeding (Gruenwald 2008b; Gruenwald 2008a). Conversely, not achieving target anti-Xa levels was associated with increased clotting. From this study, an anti-Xa dose-response curve to heparin was derived at various AT levels. To achieve a target anti-Xa level, the dose of heparin required is directly proportional with the AT concentration.
These findings provide a rationale for supplementation of AT in patients with low levels. It should increase the heparin response and reduce the amount of heparin required to maintain target anti-Xa levels throughout surgery. Unbound heparin (heparin not contributing to anti-Xa) was found to be the most responsible factor in blood loss and poor clinical outcomes in these research results. Enhanced anticoagulation should prevent both excessive bleeding and reduce the likelihood of thrombotic complications.

In a small case series of adult and paediatric patients, AT supplementation prior to bypass maintained preoperative levels of AT and resulted in suppressing coagulation (Hashimoto, Yamagishi et al. 1994). Levels of fibrinopeptide A were suppressed by maintaining normal AT levels during CPB. This was especially significant in the paediatric patients. Similar heparin levels caused different anticoagulant responses depending on the AT levels. The authors concluded that maintaining AT levels >80% not only effectively suppressed coagulation activity, but may reduce the amount of heparin required (Hashimoto, Yamagishi et al. 1994).

The optimal AT level during and following CPB has not been determined. AT levels <58% of normal have been shown to be associated with adverse events, including mediastinal re-exploration, prolonged hospitalisation and thromboembolic complications in adults (Ranucci, Carlucci et al. 2009). There is no data available for neonates or children.
AT supplementation may overcome some of the challenges associated with anticoagulation in the infant but there are no studies. A placebo-controlled trial was conducted on premature infants with respiratory distress syndrome (RDS) who were randomised to test whether AT concentrate would reduce thrombin formation, improve gas exchange and decrease the duration of mechanical ventilation and oxygen therapy (Schmidt, Gillie et al. 1998). The results demonstrated that treatment of AT deficiency associated with neonatal RDS in premature infants did not improve clinical outcomes (Schmidt, Gillie et al. 1998). In treated infants, AT activity was raised to means of 1.69 and 2.25 U/ml at 24 and 48 hours respectively, compared to means in control infants that were 0.37 and 0.44 U/ml. The authors suggested that further controlled trials are required to determine the safety and efficacy of AT concentrate in other groups of critically ill patients (Schmidt, Gillie et al. 1998).

### 7.6 Summary and Conclusions

Post-operative bleeding and the restoration of haemostasis in infants are the result of interactions between many mechanisms. Therefore there will not be a single solution, but careful attention to each aspect of management in these challenging patients will be needed to improve outcomes. There are few clinical studies that have investigated strategies for effective anticoagulation and monitoring in infants or the subsequent effects on haemostasis and clinical outcomes. This is especially true for neonates. The use of a modified protocol to guide heparin and protamine sulfate management and monitoring with the
HMS device, taking the physiological differences of infant patients into account, is associated with improved clinical outcomes.

This prospective randomised controlled trial highlights the importance of evaluating medical devices for different patient populations. It also confirms the benefit of achieving and maintaining adequate anticoagulation in patients less than one year of age during CPB. The use of the HMS device with a modified protocol is a useful component of an anticoagulation strategy in infant patients. Furthermore, this study demonstrated that both low and very high levels of heparin concentration lead to worse clinical outcomes.

7.7 Future

A well-designed prospective randomised trial should test AT supplementation in infant patients having heart surgery utilising CPB. We propose a prospective double blind randomised controlled trial to evaluate the use of AT concentrate in infants. All infants undergoing CPB for non-emergent cardiac surgery will be screened for this study. Following informed consent patients with an AT concentration less than 0.85U/ml will be randomised to one of two groups. The intervention group will receive AT concentrate following induction of anaesthesia and the control group will receive a placebo. We hypothesise that the administration of AT concentrate prior to heparinisation will decrease the amount of heparin required to achieve optimal anticoagulation (as defined by anti-Xa levels) during CPB. Patients receiving supplemental AT will experience a decrease in activation and consumption of
coagulation proteins, platelets and subsequent fibrinolysis resulting in improved haemostasis following CPB in those patients. Finally, the clinical benefits would include a reduction in post-operative bleeding and associated clinical complications. This should in turn reduce the number of allogeneic blood product transfusion and improve overall clinical outcomes.
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