University of Alberta

Profiles of Systemic Inflammatory Response Indicated by C-reactive protein in Children Undergoing Ventricular Assist Device Support and Heart Transplantation

by

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A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of

Master of Science

Medical Sciences - Pediatrics

Spring 2013
Edmonton, Alberta

DEDICATION

To my parents

ABSTRACT

C-reactive protein (CRP) is a biomarker of systemic inflammatory response (SIR) which is associated with the extent and activity of disease. The profiles of SIR and the changes of CRP remain unknown in children undergoing ventricular assist device (VAD) support and heart transplantation (HTx). We examined the perioperative measurements of CRP and the clinical implications in children undergoing VAD support and HTx. Preoperative CRP levels were elevated in both groups due to the mechanical circulatory support prior to surgery. VAD implantation was associated with a prolonged SIR, which may also influenced by an insufficient hemodynamics. The SIR was more pronounced in children receiving bi-ventricular than left-ventricular support. HTx induced an intensified SIR, which may be associated with longer cardiopulmonary bypass duration and insufficient hemodynamics. CRP did not predict the length of stay in the intensive care unit or hospital or death in children undergoing VAD support and HTx.

ACKNOWLEDGEMENTS

I would like to thank Dr. Jia Li for her mentoring and for the opportunity to participate in her research. She helped me to improve my presentations skills, scientific writings and critical thinking.

I would like to thank Dr. Lori West for providing her expertise in pediatric heart transplantation. She led me to this fascinating and challenging field that is worth spending a lifetime exploring.

I want to show my gratitude to Dr. Po-Yin Cheung, for his invaluable advice and supervision of both my research and graduate study. I would like to thank him most for his encouragement and help during my defense preparation.

Thank you, Dr. Shaohua Wang for providing me the opportunity to come to Canada. I would not be able to achieve what I have accomplished without your support for all these years.

I would like to express my thankfulness to Dr. Sujata Persad, the Graduate Education Director in the Department of Pediatrics, for her patience and support to help me out of the difficult situation I faced in the last few months of study.

I sincerely acknowledge Dr. Andriy Chepesh for his assistance in research, and Ms. Trish kryzanowski for her constant help to graduate students.

Lastly, I wish to express my gratitude to my mom and dad for their spiritual encouragement and endless love, especially during my time away from home.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

BiVAD	bi-ventricular assist device
CPB	cardiopulmonary bypass
CRP	C-reactive protein
ECMO	extracorporeal membrane oxygenation
HTx	heart transplantation
ICU	intensive care unit
LVAD	left ventricular assist device
POD	postoperative day
RVAD	right ventricular assist device
SIR	systemic inflammatory response
VAD	ventricular assist device

Chapter 1 INTRODUCTION

1.1 Systemic inflammatory response

Systemic inflammatory response (SIR) is the reaction of body to a pathogenic insult, such as virus, bacteria, injury, surgery and critical illness. The SIR is initiated by the tissue damage caused by pathogenic insults, during which the macrophages are activated. The activated macrophages then release cytokines, such as IL-1, IL-6 and TNF-α, which activate leukocytes to move to the injury site and eliminate the insults. Meanwhile, the cytokines affect on major organs, such as brain, bone marrow and liver, resulting in hormone secretion and protein synthesis. During the acute-phase of SIR, the liver increasingly synthesize acute-phase proteins, such as fibrinogen, haptoglobin, serum amyloid A protein and C-reactive protein (CRP) [Figure 1.1] [1].

Measurement of acute-phase proteins is a useful marker of SIR, given that SIR is associated with the severity of illness and the outcomes of critical ill patients, including severe burn injury [2], cardiovascular disease [3, 4] and surgical trauma [5-7].Among the acute-phase proteins, the speed and quantity of the synthesis of CRP is outstanding. It rises rapidly up to 1000-fold in the acute phase response within 48 hours after a severe stimulus such as myocardial infarction, bacterial infection, major trauma, or surgery [8]. These properties make CRP one of the most sensitive biomarker of SIR.

1.2 C-reactive protein

1.2.1 The structure of CRP

CRP was discovered by Oswald Avery in 1929 and named because of its interaction with the somatic C-polysaccharide of pneumococci [9]. The CRP molecule consists of five identical non-glycosylated polypeptide subunits, each containing 206 amino acid residues [Figure 1.2] [10].

1.2.2 The function of CRP

CRP is an acute phase protein synthesized by the liver after a severe stimulus such as bacterial infection, major trauma, or surgery [11]. CRP gene expression is regulated by IL-6 in the human hepatoma cell line Hep 3B cells [12]. Serum CRP concentration in healthy individuals is less than 8 mg/L [8]. It rises rapidly up to 1000-fold in the acute phase response within 48 hours following a stimulus [8]. The speed of change and incremental range of CRP concentration are exceptional among all the acute phase proteins except for serum amyloid A protein which behaves in a similar fashion. The half-life of CRP in the circulation is 19 hours [13]. The level of CRP in circulation is only determined by the rate of synthesis, which is dependent on the duration and intensity of the illness. Therefore, the serum CRP level usually closely reflects the extent and activity of disease. These properties underlie the value in clinical practice of precise measurement of the serum CRP concentration [14].

1.2.3 CRP and cardiovascular diseases

CRP has been recognized as an independent predictor of clinical outcomes in patients with cardiovascular diseases, such as atrial fibrillation, cardiomyopathy, myocardial infarction and heart failure. Tagnoski et al. demonstrated that the CRP in patients with permanent atrial fibrillation was significantly higher than patients with sinus rhythm (5.8 mg/L vs. 2.7 mg/L, p=0.001) [15]. In adult patients with dilated cardiomyopathy, the CRP level was similar to patients with atrial fibrillation but significantly higher than adults with epicardial coronary arteries (5.5 vs. 2.4 mg/L, $p<0.001$) [16]. Gabriel and colleagues examined CRP in a series of patients undergoing acute myocardial infarction [3]. CRP was already high (around 25 mg/L) at admission. The peak CRP concentration appeared on the second day after acute myocardial infarction. CRP returned to normal at 6 weeks and remained in a normal range until 12 weeks after infarction. Interestingly but not surprisingly, CRP in patients with heart failure is much higher than in patients with other cardiac diseases. According to the report from Alonso-Martinez et al., mean CRP in patients with heart failure at admission was 40 mg/L. On discharge, CRP levels increased in relation to NYHA class: I: 7.4; II: 37.8; III: 74; IV: 122 mg/L. During an 18 month follow-up, mean CRP in patients requiring readmission was 52 mg/L, whereas in those who were not readmitted it was 40 mg/L. They also demonstrated that CRP level was an independent predictor of readmission. For patients who were readmitted, those presenting CRP levels >9 mg/L were readmitted to hospital earlier than those with levels below 9 mg/L [4].

1.2.4 CRP in patients undergoing non-cardiac surgery

CRP has been studied in both adults and children undergoing non-cardiac surgery, such as abdominal, pancreatic, and elective general surgeries including resection of necrotic bowel and the repair of gastroschisis [5, 17-21]. In one study in adults undergoing major abdominal surgery [21], CRP peaked to 220 mg/L at postoperative day 2, and decreased to around 50 mg/L at postoperative day 12. Cole et al. reported that in 164 adults undergoing elective general surgery, 91% had a preoperative CRP less than 20 mg/L, 56% of whom had < 6 mg/L [20]. Median CRP peaked at 130 mg/L at postoperative day 2. They also demonstrated that, preoperatively, most patients with no co-morbidity had a normal or only slightly elevated CRP. Where co-morbidities, such as cancer, diabetes and morbidity in cardiovascular, respiratory and renal system, were present, CRP was more likely to be abnormal [20]. It is also well established that CRP is a diagnostic tool to detect infection and predict clinical outcomes [5, 17, 22]. Warschkow and colleagues' study of adults undergoing pancreatic surgery reported that CRP was significantly higher in patients who developed postoperative inflammatory complications before and after 10 days of surgery [17]. Van Genderen et al. showed that in patients undergoing esophagectomy with gastric tube reconstruction, increased CRP levels were associated with the occurrence of postoperative complications and higher 1-year mortality [5].

CRP has also been studied in children undergoing non-cardiac surgery, such as orthopedic operation, repair of inguinal hernia, resection of necrotic bowel and the repair of gastroschisis. Chwals et al. showed that in children undergoing general surgery, including resection of necrotic bowel and the repair of gastroschisis [18], CRP values

were within a normal range before surgery. Immediate postoperative CRP peaked at 80 mg/L in survivors, whereas it peaked at 140 mg/L in non-survivors. In children undergoing minor surgery, such as repair of inguinal hernia or undescended testis, CRP was also normal before surgery (median 2.4 mg/L). It increased right after surgery (median 23.9 mg/L) and returned to normal by postoperative day 5 (median 4.8 mg/L) [23]. Limpisvasti et al. studied 22 children undergoing orthopedic operation and found that preoperative CRP was also within normal range. It increased after surgery and returned to normal value three weeks postoperatively [24]. Similar to its role with adult patients, CRP has also been shown to predict outcomes in children undergoing non-cardiac surgery. In one study carried out by Chwals et al., peak CRP concentration significantly and positively correlated with the length of stay in the intensive care unit (ICU) of surgical infants [6]. Chwals and colleagues also demonstrated that peak CRP levels were significantly higher in non-survivors after general surgery, which indicated a correlation between CRP and mortality in these groups of children [18].

1.2.5 CRP in patients undergoing cardiac surgery

CRP has been extensively studied in adults and children undergoing cardiopulmonary bypass (CPB) surgery. Fransen et al. reported a study of 593 adults undergoing CPB surgery, including coronary artery bypass grafting operations, valve operations, combined coronary artery bypass grafting and valve operations [25]. They showed mean preoperative CRP was 9.4 mg/L, which was close to normal. Postoperative CRP peaked to 200 mg/L on day 3 and decreased to 78 mg/L on day 6 postoperatively. This study has also illustrated that preoperative CRP >8 mg/L is a risk factor for postoperative infection. Another study has also showed normal values of preoperative

CRP in adult patients undergoing CPB surgery [26]. The postoperative CRP was 199 mg/L in day 1-3, and remain elevated (41 mg/L) in day 7-14. It is also demonstrated that peak CRP >180 mg/L is an independent predictor of major adverse cardiac events in adults undergoing CPB surgery [27].

In children undergoing cardiac surgery for the repair of congenital heart defects, CRP was also normal before surgery [7, 28]. Postoperative CRP was around 80 mg/L at 48 hours after surgery, 50 mg/L on postoperative day 5, and remained higher than preoperative level 10 days after surgery. CRP has also been demonstrated to be predictive of outcomes and mortality in adults and children undergoing cardiac surgery [6, 7, 27, 29]. Pons Leite et al. reported postoperative CRP may predict the length of stay in pediatric ICU [7]. In addition, Fellahi et al. demonstrated that the 10-year-survival rate in children undergoing CPB surgery who have preoperative CRP > 10 mg/L is 10% less than those with preoperative CRP < 10 mg/L [29].

1.2.6 Drugs that influence serum CRP level

Statins have anti-inflammatory and lipid-lowering properties, and thus have effects on reducing CRP and cholesterol levels in patients with cardiovascular diseases. In the Pravastatin or Atorvastatin Evaluation and Infection Therapy–Thrombolysis in Myocardial Infarction 22 (PROVE IT–TIMI 22) study [30], intensive statin therapy (80 mg of atorvastatin orally per day) and moderate statin therapy (40 mg of pravastatin orally per day) was randomly assigned to patients with acute coronary syndrome. The results showed that pravastatin reduced CRP from 11.9 to 2.3 mg/L in one month; whereas in the same period, atorvastatin reduced CRP from 12.2 to 1.6 mg/L. The study also showed that patients who have CRP levels less than 2 mg/L in addition to LDL

cholesterol of less than 1.8 mmol/L have better clinical outcomes than those with higher CRP levels.

The same investigators later carried out the JUPITER trial, assessing the effects of rosuvastatin 20 mg versus placebo on clinical outcomes in healthy men and women, instead of patients with cardiovascular disease [31]. This approach allowed the study to be free of potential confounding effects that might have accrued in the PROVE IT–TIMI 22 study. The JUPITER trial suggested that in healthy men and women starting rosuvastatin therapy achievement of target concentrations of LDL cholesterol less than 1.8 mmol/L and CRP less than 2 mg/L was associated with improved event-free survival compared with achievement of neither target or with achievement of reduced LDL cholesterol alone.

In addition, epinephrine increases serum CRP dose-dependently, the mechanism of which is unclear. Although it probably via a receptor mechanism [32].

1.2.7 Summary

CRP is a useful biomarker of SIR in critically ill children. In children undergoing non-cardiac or CPB surgeries, the preoperative CRP concentrations are within normal range. Postoperatively, CRP peaks to around 80 mg/L in survivors after non-cardiac surgery and CPB surgery. In non-survivors after non-cardiac surgery, the peak CRP is 140 mg/L. However, CRP returns to normal faster in non-cardiac patients. In adult patients, there are similar patterns of CRP changes regarding the normal preoperative concentrations and the early normalization in non-cardiac surgery group, except for the

higher peak concentration in CPB surgery. CRP is an independent predictor of clinical outcomes in both adult and pediatric patient populations.

Although the changes of CRP and the profile of SIR have been extensively studied in various patient cohorts, they remain unknown in children undergoing ventricular assist devices support (VAD) and heart transplantation (HTx). The course of illness in these two groups of patients is distinctive as compared to children undergoing other cardiac surgeries. On the other hand, there are also similarities between the two groups of patients.

1.3 Characteristics of children undergoing VAD and HTx

1.3.1 Diagnosis and preoperative management of children undergoing VAD support

VAD, as a bridge to HTx, shares the same patient population as HTx. According to North American experience [33], the diagnoses of children receiving VAD support include dilated cardiomyopathy (58%), myocarditis (10%), congenital heart disease (26%) and other cardiomyopathy (6%). All patients received inotropes support before VAD implantation and 73% of patients were ventilator dependent. One third of patients received ECMO support before implantation. Left ventricular assist devices (LVAD) were implanted in 57% of children, whereas 43% were implanted with bi-ventricular assist devices (BiVAD).

1.3.2 Device modes and implantation procedures of VAD

Berlin Heart EXCOR® is the most popular pediatric VAD which enables to bridge children of all size to HTx. Figure 1.3 shows Berlin Heart EXCOR® pumps (A) and cannulae (B) with different stroke volumes, and other types of pumps including Jostra (C) and Levitronix (D).

Figure 1.4 shows the procedures of LVAD and BiVAD implantations. Under general anesthesia with the patient intubated, the chest wall is prepped and draped and a standard median sternotomy incision is performed. After opening the pericardium, the ascending aorta and the right atrium are cannulated allowing the patient to be placed on CPB with systemic cooling. A left ventricular vent is inserted through the right superior pulmonary vein and the heart is electrically fibrillated. The inflow cannula is connected

to the left atrium or the left ventricular apex. The outflow cannula is directed to the ascending aorta. Both cannulae are brought out through the abdominal wall and connected to the pump. After successfully weaning off CPB and obtaining a satisfactory hemodynamics, the standard sternal closure is performed.

When both left and right ventricles require mechanical support, a BiVAD is applied. The procedure includes the implantation of a LVAD and a right ventricular assist device (RVAD). For the RVAD implantation, the inflow cannula is connected to the right atrium, whereas the outflow cannula is directed to the main pulmonary artery.

1.3.3 Outcomes of children undergoing VAD support

As reported by 17 institutions in North America, out of 73 children implanted with VAD, 67% were bridged to HTx, 7% recovered with the VAD explanted, 4% were discharged with the VAD implanted, and 22% deceased during VAD support [33]. The major adverse events after VAD implantation include non-device infection, sepsis, right ventricular dysfunction, bleeding, atrial thrombosis and ventricular arrhythmia [34]. All of the major events may have impact on the SIR, leading to an elevated CRP level pre- and post-operatively in this group of patients.

1.3.4 Diagnosis and preoperative management of children undergoing HTx

The International Society of Heart and Lung Transplantation [35] reported that the most common indications for HTx are congenital heart disease and cardiomyopathy. The proportions are 62% and 35% in infants younger than 1-year-old and 37% and 56% in children between 1-year and 17-years old, respectively. The preoperative extracorporeal membrane oxygenation (ECMO) supports were provided to 7.6% of

patients. The use of VAD as a bridge to HTx was found in 15% of patients, including 8.6% LVAD and 4.6% BiVAD.

1.3.5 Donor brain death and donor heart ischemia of children undergoing HTx

Brain death evokes a cascade of hematologic, autonomic, endocrinologic, and metabolic effects. Animal experiments suggested that mRNA of macrophage-associated products become highly expressed after brain death, such as IL-6, the regulator of CRP [36]. Brain death also causes sympathetic storm resulting in myocardial injury of donor heart [37]. Due to transportation, the donor heart usually experiences prolonged ischemia. Subsequently, the duration of CPB is prolonged, which may induce a much greater SIR than which in patients undergoing other CPB surgeries [38, 39].

1.3.6 Surgical procedures of pediatric HTx

The surgical techniques include bi-atrial, bi-caval and total orthotopic HTx. The bi-caval technique provides anatomic and functional advantages, with improvements in survival rates and hemodynamics. This technique involves the left atrial, inferior vana caval and superior vana caval anastomoses [Figure 1.5].

1.3.7 Postoperative immunosuppression in children undergoing HTx

Immunosuppressants were used for induction and maintenance to prevent allograft rejections after transplantation. The most commonly used immunosuppressants include Anti-thymocyte globulin, prednisone, mycophenolate and tacrolimus. These medications suppress the functions of the immune system in children undergoing

transplantation, which may subsequently influence the SIR, leading to an increment of CRP level.

1.3.8 Outcomes of children undergoing HTx

Reported by The International Society of Heart and Lung Transplantation [35], a total number of 8830 children received HTx from 1982 to 2009. The overall mortality after HTx was 15% at 1-year, 30% at 5-years, 40% at 10-years and 50% at 15-years. Children receiving a transplanted heart in early infancy have lower long-term mortality than those who had heart transplantation in older age [Figure 1.6]. The major causes of death are graft failure, rejection, infection, multiple organ failure and primary failure. Risk factors for mortality include infection, ischemia, congenital diagnosis and the use of ECMO prior to HTx.

1.3.9 Summary

There are similarities between children undergoing VAD support and HTx, such as the overlapping portion of patient population, preoperative use of inotropes and ECMO, as well as the intraoperative CPB. However, children undergoing HTx had a unique course of illness, in terms of using VAD as a bridge to transplantation, the impact from donor heart, and the postoperative immunosuppression. As such, the profile of SIR in these two groups of patients may be distinctive from each other.

1.4 Knowledge gaps, hypotheses and objectives

1.4.1 Knowledge gaps

The profile of SIR in children undergoing VAD support and HTx is unknown. The SIR in the two groups of patients may have similarities, since both groups have experienced prolonged end-stage heart failure, and some of them received ECMO support preoperatively. On the other hand, both groups have their own unique characteristics of illness. Children with VAD support may induce persistent SIR, because of the body's contact with the implantable device. The postoperative course of children undergoing HTx may be affected by the duration of donor heart injury caused by sympathetic storm at brain death [37] and prolonged ischemia during transportation. In addition, children undergoing HTx routinely receive immunosuppressants, which may have impacts on SIR as well.

Moreover, Serum CRP has been demonstrated to predict clinical outcomes, such as mortality and the length of stay in the intensive care unit in children undergoing non-cardiac and cardiac surgery. However, the predicting value of serum CRP to the clinical outcomes in children undergoing VAD and HTx remain unknown.

1.4.2 Hypotheses

The hypotheses are as followes: a) Perioperative serum CRP level in children undergoing VAD implantation is elevated, and the elevation is prolong; b) serum CRP level is elevated during perioperative period in children undergoing HTx, suggesting SIR; and c) the elevated CRP levels in this study setting may be associated with poor outcomes, such as longer stay in ICU, death.

1.4.3 Objectives

Therefore, the objectives of the projects are to examine the perioperative measurements of serum CRP in children undergoing VAD support and HTx, and to examine the correlation between CRP and other variables, including the length of stay in ICU and hospital and death, in children undergoing VAD support and HTx.

Figure 1.1 Acute phase of systemic inflammatory response

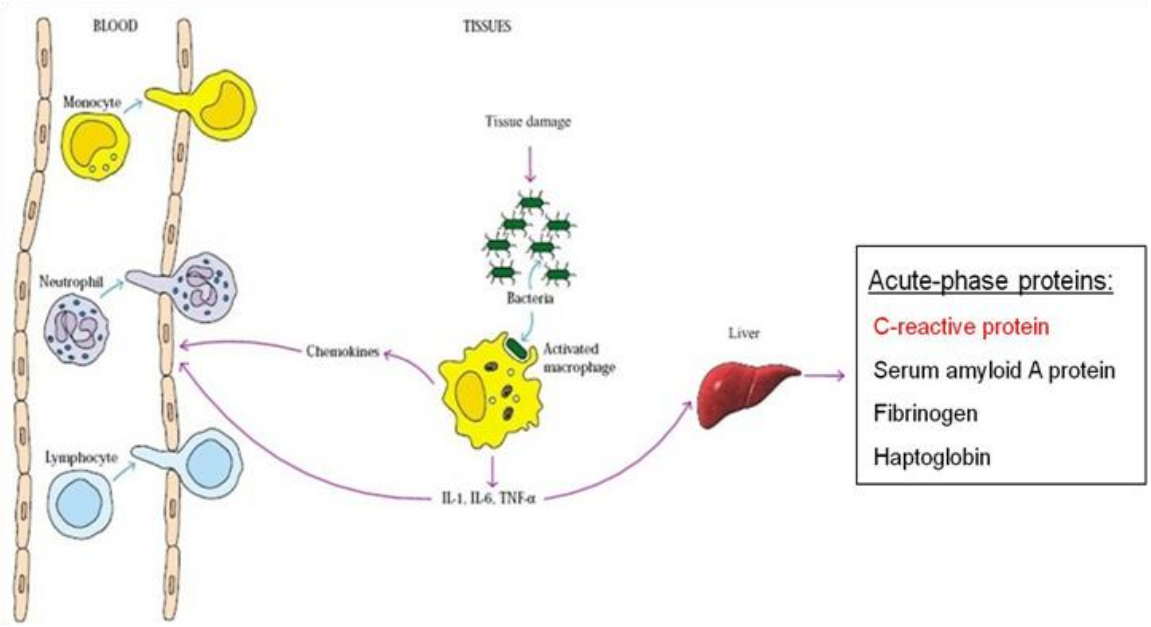


Figure 1.2 Three-dimensional structure of human C-reactive protein

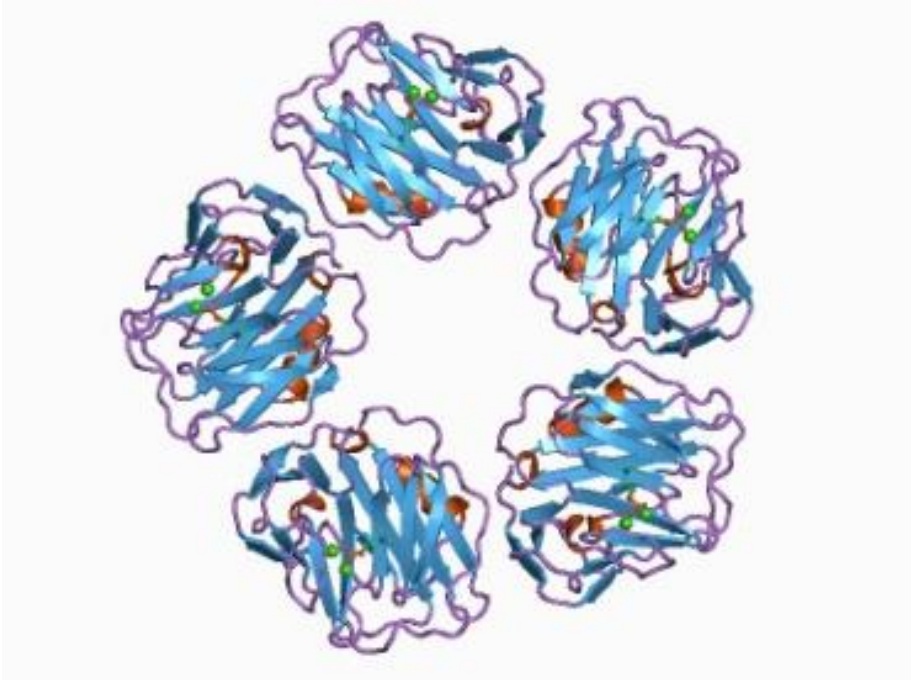
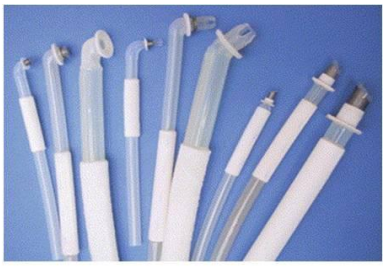
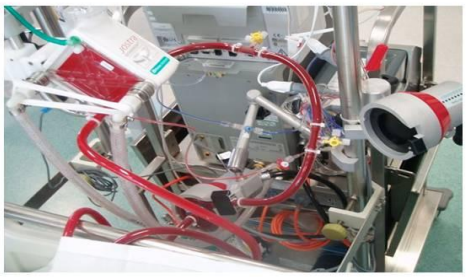


Figure 1.3 Pediatric Berlin Heart EXCOR®(A), cannulas (B), Jostra pump (C) and Levitronix (D)

A



B



C

D

Figure 1.4 Modes of implantation of the Berlin Heart Excor biventricular assist device

(A) In the earlier period, atrial cannulation was the rule. (B) More recently, apical cannulation was introduced and is now preferred owing to better left ventricular unloading and reduced afterload to the right ventricle. Consequently, in many instances, a left ventricular assist device only is sufficient. *Hetzer R et al. Improvement in survival after mechanical circulatory support with pneumatic pulsatile ventricular assist devices in pediatric patients. Ann Thorac Surg. 2006 Sep;82(3):917-24.*

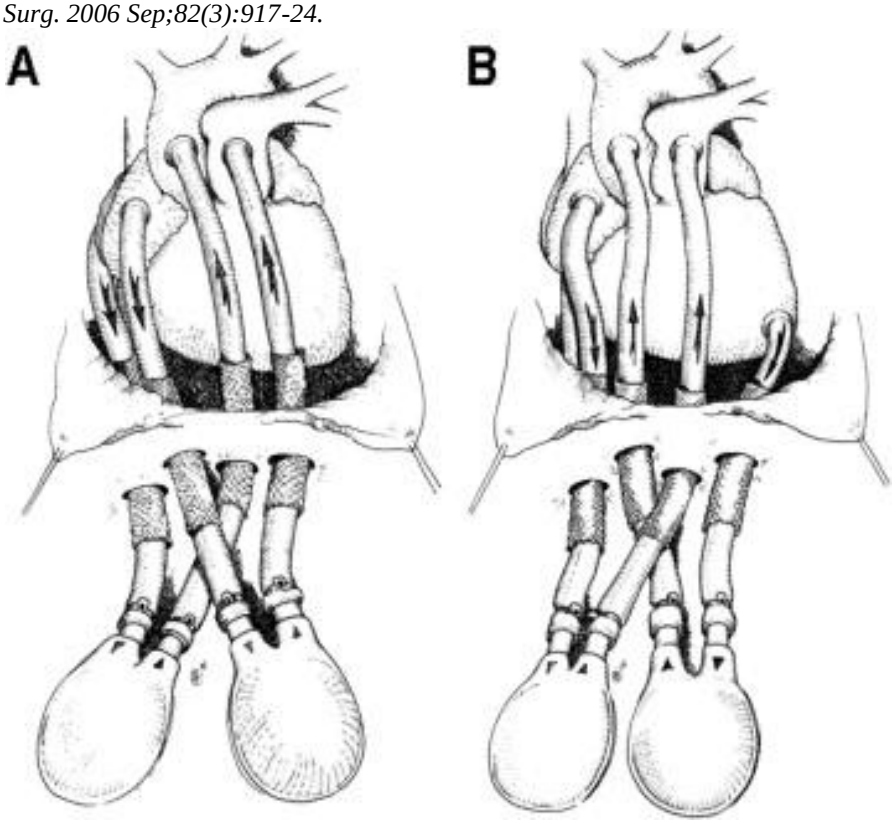


Figure 1.5 Bi-caval orthotopic heart transplantation

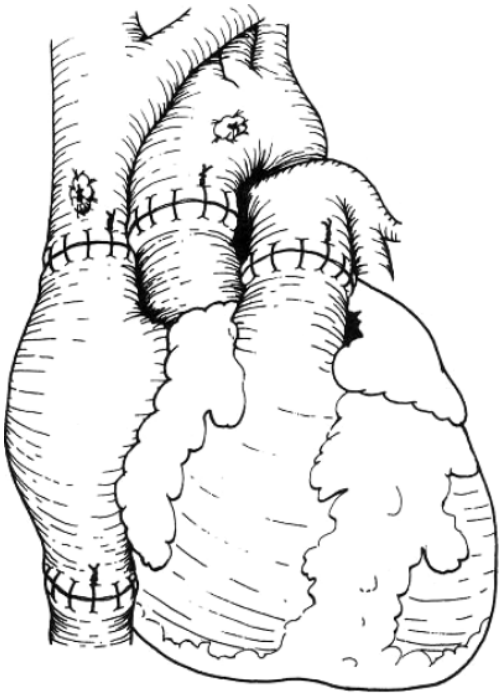
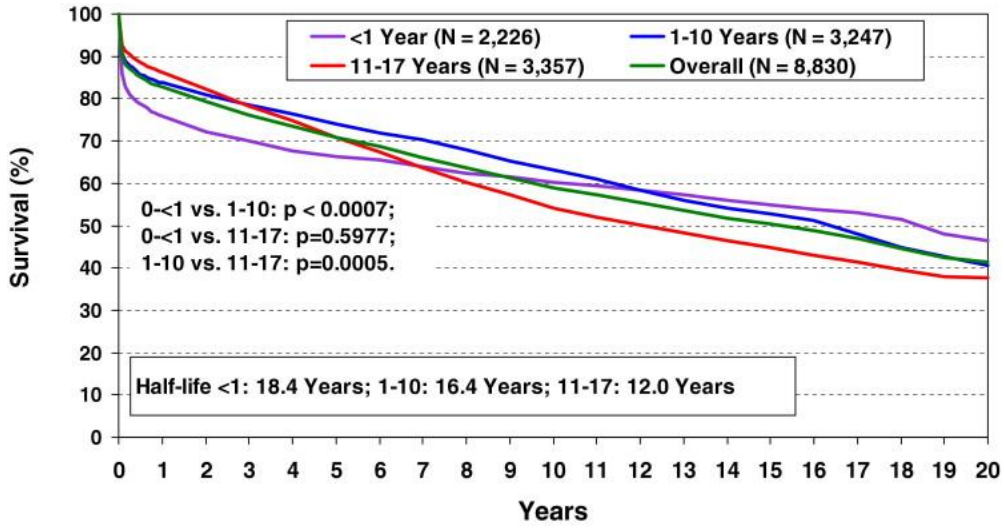


Figure 1.6 Kaplan-Meier survival for pediatric heart transplantations from January 1982 through June 2009

Kirk R et al. J Heart Lung Transplant. 2011 Oct;30(10):1095-103.



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Chapter 2 THE PROFILE OF SYSTEMIC INFLAMMATORY RESPONSE IN CHILDREN UNDERGOING VENTRICULAR ASSIST DEVICE SUPPORT[1]

1 A version of this chapter has been published in *Interactive CardioVascular and Thoracic Surgery 2012 May 22 [Epub ahead of print]* by Yu X, Larsen B, Rutledge J, West LJ, Ross DB, Rebeyka IM, Buchholz H, Li J.

2.1 ABSTRACT

Objectives: Serum C-reactive protein (CRP) has been used as a systemic inflammatory response (SIR) marker in critical ill including children after cardiopulmonary bypass surgery. Ventricular assist devices (VAD) have been increasingly used as a bridge support to heart transplantation in children. We aimed to examine the profiles of CRP in children receiving VAD support.

Methods: Charts of 13 children receiving Berlin Heart EXCOR® in 2005-2009 were reviewed. Data obtained prior to and during VAD support included: CRP, white blood cells, inotropes and steroid use, VAD mode and duration of VAD support. Ten patients received left VAD (LVAD) and 3 biventricular VAD (BiVAD).

Results: The median duration of VAD support was 59 days (ranged 3-678 days). Pre-VAD CRP was 35±51mg/L and increased to 109±59 mg/L on day 1-3 after VAD implantation (p=0.01), then gradually decreased to 28±28mg/L by 4 months, and normalized by 5 months (p<0.0001). CRP was higher in BiVAD than in LVAD patients throughout the study period (p=0.003). CRP positively correlated with the doses of epinephrine, norepinephrine and monocyte count, and negatively correlated with lymphocyte count. Lymphocyte count was $2.5 \pm 0.4 * 10^9$/L prior to implantation, decreased to $2.1 \pm 1.3 * 10^9$/L on day 1-3 (p=0.5), then to $0.6 \pm 0.1 * 10^9$/L by 6 months (p=0.08). It trended lower in BiVAD patients (p=0.06).

Conclusions: SIR exists in children prior to VAD support. VAD implantation is associated with a significant and prolonged increase in CRP and a decrease in lymphocyte count indicating suppressed immune function, being more pronounced in BiVAD patients.

2.2 INTRODUCTION

Ventricular assist devices (VAD) have been increasingly used as a bridge to heart transplantation or to cardiac recovery in patients with end-stage heart failure [1]. Berlin Heart EXCOR® pediatric VAD has enabled the use in children with satisfactory outcomes. In 73 children from 17 North American institutions in 2000 to 2007 [1], 57% received a left VAD (LVAD), and the remainder received biventricular VAD (BiVAD). Overall 70% were bridged to transplantation and 7% to recovery. Mortality remains substantial, being 35% in BiVAD patients and 14% in LVAD patients. While the factors that contribute to mortality are certainly complex, the systemic inflammatory response (SIR) may play an important role.

SIR can be evoked by critical illness [2]. It involves an acute-phase reaction with increased synthesis of acute-phase proteins, such as C-reactive protein (CRP). CRP is a SIR marker and an independent predictor of outcomes in varied group of patients, including those after cardiopulmonary bypass (CPB) [3, 4]. However, the changes of CRP in children undergoing VAD support remain unknown. This group of children have a distinctive course of critical illness. Prior to VAD implantation, they have experienced end-stage heart failure, and some have had cardiogenic shock and received extracorporeal membrane oxygenation (ECMO) support. VAD implantation may require prolonged CPB. VAD itself, as an implantable device with external artificial components, may further induce a persistent SIR. This study examined the profile of SIR by evaluating the perioperative changes of CRP in children undergoing VAD support.

2.3 METHODS

2.3.1 Patients

After approval by the Institutional Health Research Ethics Board, the charts of 13 children (aged 72±87 months) receiving VAD at the Stollery Children's Hospital between 2005 and 2009 were reviewed. Table 2.1 provides the details of demographic data.

2.3.2 Devices

Thirteen patients received Berlin Heart EXCOR® including 2 patients having LVAD Jostra for 12 days (Patient 12) or LVAD Levitronix for 10 days (Patient 10) prior to Berlin Heart EXCOR®. There were 10 LVAD including the two patients with Jostra or Levitronix (aged 46±80 months) for 89±77 days and 3 BiVAD (aged 159±44 months p=0.02) for 108±96 days (p=0.80). Our clinical protocol is to measure CRP twice weekly (Monday and Thursday) during VAD support, but the practice overall varied widely among clinicians.

2.3.3 Data Collection

CRP was measured prior to VAD implantation (1 to 4 days, median 1 day), and twice weekly during support. Additional laboratory data obtained concurrently with CRP included: total white blood cell, neutrophil, lymphocyte and monocyte counts. Doses of inotropes and vasodilators (epinephrine, norepinephrine, milrinone and nitroprusside) and steroid (hydrocortisone) were recorded within 8 hours prior to CRP measurements. Cultures of blood and body fluids (sputum, urine nasopharyngeal, sternum, throat and rectal swabs respectively) were also collected. Other demographic and clinical data

included age, weight, gender, diagnosis, use of ECMO prior to VAD, and duration of CPB. CRP was measured using UniCel DxI 800 (Beckman Coulter, Brea, CA, USA). Reference range of CRP is < 8mg/L.

2.3.4 Data Analysis

Data are expressed as mean ± SD. Mixed linear regression for repeated measures was used to compare the measurements prior to and the first time after VAD implantation, and also to determine the nature of any time trend of the variables during the support period. This method was also used to analyze correlations of CRP with other laboratory and clinical variables. The strength and trend of correlations were indicated by parameter estimate and p value. All data analysis was performed with SAS statistical software version 9.2 (SAS Institute, Inc, Cary, NC). P values of less than 0.05 were considered significant.

2.4 RESULTS

2.4.1 Clinical data

Table 2.1 showed the clinical data of the 13 patients. Seven patients received ECMO prior to VAD implantation. Among them, one had weaned from ECMO for 10 days prior to VAD, and the remaining 6 were converted directly to VAD. Inotropes used concurrently with CRP measurements during VAD support included epinephrine (0.005-0.25 mcg/kg/min) in 7 patients, norepinephrine (0.01-0.2 mcg/kg/min) in 4 patients, milrinone (0.25-0.75 mcg/kg/min) in 9 patients, and nitroprusside (0.25-5 mcg/kg/min) in 8 patients. Hydrocortisone (1 mg/kg Q6-8 hours) was used in 5 patients. During VAD support, positive blood culture was found in 4 patients and positive cultures of other body fluids in 7 patients including sputum, urine and sternum swabs. Patient 1 developed embolic strokes and died on day 25 of VAD support. Patient 9 had bilateral above-knee amputation on day 26 on VAD due to progressive tissue deterioration resulting from the cardiac arrest that occurred 5 days prior to VAD requiring ECMO support. Ten patients (7 LVAD and 3 BiVAD) were successfully bridged to HTx.

2.4.2 Changes in CRP and other laboratory data before and after VAD implantation

CRP varied widely both between and within patients throughout the study period. Overall, prior to VAD implantation, CRP measured 35±51 mg/L (n=12). CRP was significantly higher in the group of patients converted from ECMO to VAD support (73.6±61.7 mg/L, n=6) compared to those that either did not require ECMO (n=6) or weaned from ECMO for 10 days prior to VAD (n=1) (CRP 7.2±5.9 mg/L, p=0.02). All 3

patients subsequently receiving BiVAD had been on ECMO. Pre-VAD CRP was not significantly different between patients receiving BiVAD (67±51 mg/L) versus LVAD (24±49 mg/L, p=0.3). Lymphocyte count was 2.5±0.4*10^9/L in all patients. It was significantly lower in patients requiring pre-VAD ECMO (1.4±0.4 vs. 3.0±1.5*10^9/L, p=0.03) and not significantly different between BiVAD vs. LVAD patients (1.4±0.5 vs. 2.8±1.5*10^9/L, p=0.3). Monocyte and neutrophil counts were 1.2±0.7 and 7.9±3.1*10^9/L in all patients respectively with no difference between those with pre-VAD ECMO or those with BiVAD vs. LVAD. There were no significant correlations between pre-VAD levels of CRP, lymphocyte and monocyte and during-VAD levels.

After VAD implantation, CRP increased significantly to 109±59 mg/L on day 1-3 (p=0.01, n=13), then decreased to 52±53 mg/L by 1 month (n=8), 28±28 mg/L by 4 months (n=5), and normalized by 5 months and thereafter (p<0.0001, n=2) [Figure 2.1]. Lymphocyte count decreased insignificantly to 2.1±1.3*10^9/L on day 1-3 (p=0.5), then gradually decreased to 0.6±0.1*10^9/L by 6 months (p=0.08) [Figure 2.2]. Monocyte count was 1.0±0.5*10^9/L on day 1-3 of VAD support (p=0.7), and then gradually and significantly decreased to 0.7±0.1*10^9 /L by 6 months (p<0.001) [Figure 2.3]. Neutrophil count increased to 9.2±4.0*10^9 /L on day 1-3 after implantation, and significantly decreased to 7.8±2.4*10^9 /L by 6 months (p<0.0001).

2.4.3 Comparisons between LVAD and BiVAD

Comparisons were made between the two groups of patients receiving BiVAD and LVAD support. CRP was significantly higher in those who received BiVAD compared to LVAD throughout the entire period of support (p=0.003) [Figure 2.4]. Lymphocyte counts in children with BiVAD trended lower than in those on LVAD

(p=0.06) [Figure 2.5]. There were no significant differences between the two groups in monocyte and neutrophil counts, dosage of inotropes and duration of CPB. Comparisons were also made between the groups with pre-VAD ECMO versus those without previous ECMO support showing no significant differences in the measured variables.

2.4.4 Correlations of CRP with other laboratory and clinical variables during VAD

Table 2.2 shows the statistical results of correlations of CRP with other clinical variables during VAD support. CRP correlated negatively with lymphocyte count (p=0.02), and correlated positively with monocyte count (p=0.02) and the doses of epinephrine (p<0.0001) and norepinephrine (p=0.002). No significant correlations were found between CRP and cultures of blood or body fluids. In addition, there were no significant correlations between cultures in blood or other body fluids and WBC, neutrophil, lymphocyte or monocyte counts. CRP did not significantly correlate with the length of stay in ICU and hospital. CRP in the deceased patient (Patient 1) was lower than average before and in the first 18 days on VAD, but acutely increased to 69 mg/l on day 20 (2 days after the stroke), and then continuously increased to 138 mg/l until death.

In Patient 9 who had a cardiac arrest prior to VAD and consequent progressive tissue deterioration of the legs during VAD, pre-VAD CRP was 102 mg/l. Post-VAD CRP was persistently higher than average, being 200 mg/l until a bilateral above-knee amputation on day 26 of support. It decreased to 50 mg/l by 1 week after the amputation, maintained at this level until 4 months of support and normalized from the fifth month of support.

2.5 DISCUSSION

This study examined the profile of SIR by evaluating the changes in serum CRP concentration in children undergoing VAD support. Prior to VAD implantation, CRP was significantly higher in patients on ECMO. VAD implantation was associated with an intensified and prolonged SIR and immunosuppression, as indicated by the significantly elevated CRP and decreased lymphocyte count in the first five months. The SIR and immunosuppression trended to be greater in children with BiVAD than those with LVAD, as indicated by the significantly higher CRP and lower lymphocyte count throughout the entire period of VAD support.

CRP has been extensively used to indicate SIR in a wide range of patients including those undergoing ECMO and CPB [5-7]. The three cardiovascular support devices (CPB, ECMO and VAD) share a common interaction of blood with the artificial surfaces of the system and thus a common mechanism for inducing SIR [8]. ECMO and VAD may induce a SIR with similar magnitude but longer duration than CPB. A paucity of data has been reported about CRP in children undergoing CPB for the repair of congenital heart defects [3, 4] and even less data in ECMO patients [7]. In children undergoing CPB, preoperative CRP was normal, and peaked to a mean of 50-80 mg/L after CPB and returned to normal within 3-10 days [3, 4] In one report of ECMO patients, CRP was persistently elevated in the first 7 days of support, but no data was available afterward [7]. This is supported by our data. Pre-VAD CRP was significantly higher in patients on ECMO, whereas it was normal in those who were either weaned from ECMO or did not require ECMO at all. A recent study has reported that a high preoperative CRP level is associated with increased hospital mortality in children with VAD support [9].

Immediately after VAD implantation, CRP increased to a similar magnitude as previously reported in ECMO and post-CPB patients. Subsequently, there was a prolonged elevation of CRP up to five months after VAD implantation, indicating a persistent SIR. Of note, SIR in the three BiVAD patients was significantly greater than in LVAD patients as indicated by the significantly higher CRP throughout the VAD support period. This may be due to more contact with artificial surfaces as well as severer myocardial dysfunction in BiVAD patients. The latter factor is supported by the positive correlation between CRP and the dose of epinephrine and norepinephrine infusions, indicators of poor cardiovascular status, indicating the role of cardiovascular functional status in SIR and vice versa [10].

SIR is associated with a depression in immune function [11]. This is supported by the reduced level of lymphocyte count during VAD support, along with the negative correlation between CRP and lymphocyte count. In concordance with the CRP profiles, lymphocyte count trended lower in BiVAD patients than in LVAD patients. There was no significant difference in monocyte count however, despite a positive correlation between CRP and monocyte count.

This study examined the correlations of CRP with clinical outcomes. CRP has been reported to independently predict adverse clinical outcomes [3, 12]. The conventional indicates of clinical outcomes such as the length of ICU and hospital stay may not be appropriate in VAD patients, since their stay largely depends on the opportunity of HTx. Nonetheless, higher levels in CRP were associated with adverse events in our patients. In the deceased patient, CRP rapidly increased after stroke until death. In the patient undergoing bilateral above-knee amputation, CRP also substantially

increased with tissue deterioration until amputation. It subsequently decreased but remained relatively high by 4 month of support. Our findings have several important clinical implications. The profile of changes in CRP provides quantitative information about the intensity and duration of SIR in children undergoing VAD support. On the other hand, CRP has been shown to have direct effect in promoting inflammation [13]. Treatment strategies to reduce SIR and CRP may improve clinical outcomes in patients undergoing VAD support and potentially subsequent HTx [14, 15].

2.6 SUMMARY

Prior to VAD implantation, serum CRP was significantly higher, while lymphocyte count was lower, in patients undergoing ECMO support than in those not undergoing ECMO. After VAD implantation, CRP significantly increased in day 1-3 of support, and returned to normal by 5 months of support. Monocyte significantly decreased by 6 months of support. CRP was significantly higher in BiVAD patients than in LVAD patients. Lymphocyte count trended to be lower in BiVAD patients than in LVAD patients. CRP positively correlated with monocyte count, doses of epinephrine and norepinephrine, and device mode; negatively correlated with lymphocyte count. CRP did not significantly correlate with the length of stay in ICU or hospital, or death.

2.7 CONCLUSIONS

Prior to VAD implantation, CRP was elevated in patients requiring ECMO support. VAD implantation was associated with a significant and prolonged increase in CRP. The elevated CRP level was associated with increased monocyte count and

decreased lymphocyte count, which may suggest a prolonged SIR. The higher CRP levels in BiVAD patients may suggest that the SIR is more pronounced in children receiving BiVAD than LVAD. The correlation of CRP and the dose of epinephrine may suggest that elevated CRP levels are associated with insufficient hemodynamics. CRP did not predict the length of stay in ICU or hospital, or death in children undergoing VAD implantation.

Table 2.1 Clinical data of the 13 patients undergoing VAD support

Patient No.	Age	Gender	Diagnosis	ECMO	CPB (min)	VAD mode	VAD duration (day)	ICU stay (day)	Outcome
1	4m	M	Cardiomyopathy	No	119	LVAD	25	25	Deceased
2	15y	F	Cardiomyopathy	No	122	LVAD	145	191	Recovery
3	1m	M	Myocarditis	Weaned	112	LVAD	35	44	HTx
4	9y	M	Cardiomyopathy	Yes	166	BiVAD	3	20	HTx
5	3m	M	Cardiomyopathy	No	164	LVAD	56	72	HTx
6	17y	M	Cardiomyopathy	Yes	102	LVAD	678	84	Discharge with LVAD
7	16y	M	Friedreichs ataxia, cardiaogenic shock	Yes	222	BiVAD	128	158	HTx
8	3y	M	HLHS Failed Fontan	Yes	150	LVAD	173	195	HTx
9	15y	M	Rheumatic mitral & aortic valve disease, bilateral above-knee amputation	Yes	465	BiVAD	192	515	HTx
10	1m	M	Myocarditis	Yes	101	LVAD (Levitronix for 12 days)	59	32	HTx
11	5m	M	Cardiomyopathy	No	136	LVAD	28	47	HTx
12	2m	F	Cardiomyopathy	No	150	LVAD (Jostra for 10 days)	106	28	HTx
13	11m	F	Cardiomyopathy	No	102	LVAD	23	75	HTx

BiVAD: biventricular assist device; **CPB**: cardiopulmonary bypass; **ECMO**: extracorporeal membrane oxygenation; **HLHS**: hypoplastic left heart syndrome; **HTx:** heart transplantation; **ICU:** intensive care unit; **LVAD**: left ventricular assist device; **VAD**: ventricular assist device.

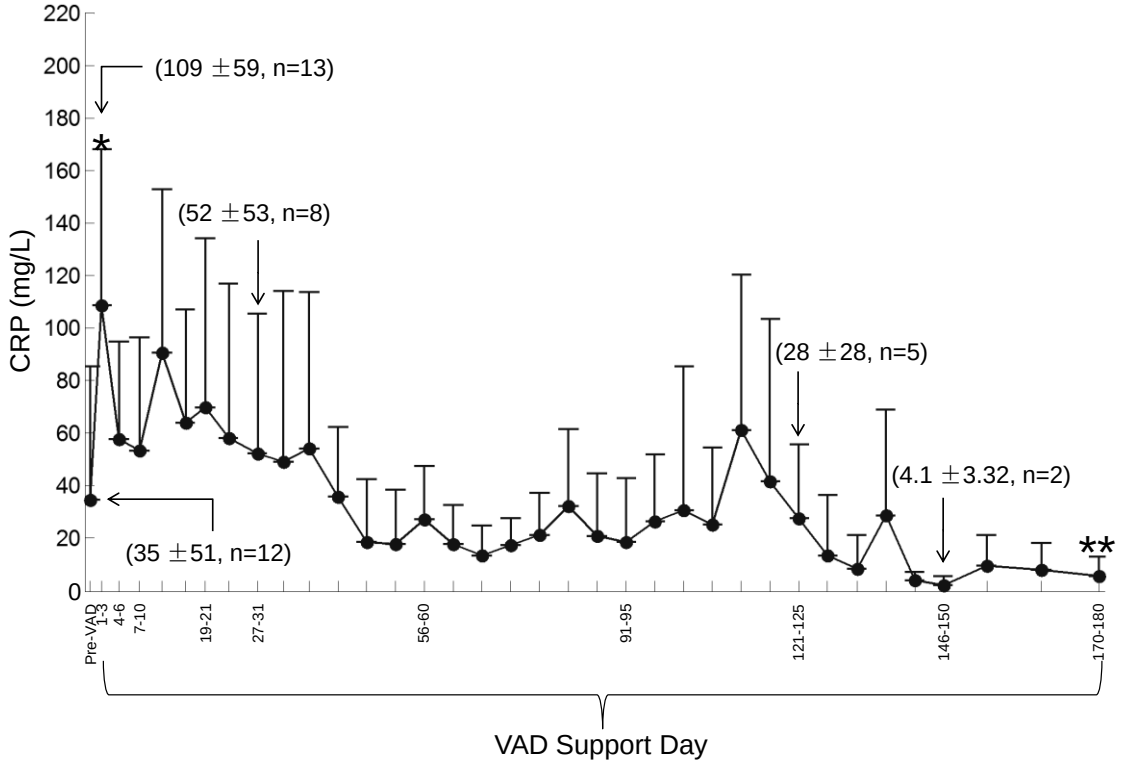
Table 2.2 Correlations of CRP with other clinical variables during VAD support

	Parameter estimate*	p value
Lymphocytes	-8.5	0.02
Monocytes	13.4	0.02
Neutrophils	1.1	NS
Device mode	43.3	0.003
Norepinephrine	456	0.002
Epinephrine	821	<0.0001
Age	-1.2	NS
Weight	-4.7	NS
CPB	-0.09	NS
Culture	3.6	NS
Steroid	-25	NS
glucose	4.9	NS
Other Inotropic drugs	-9.3	NS
ICU stay	0.3	NS
Hospital stay	0.3	NS
Death	50.8	NS

NS: Not significant.

*****Parameter estimate indicates the slope of correlation.

Figure 2.1 Changes of serum CRP concentrations prior to and during VAD support in 13 patients

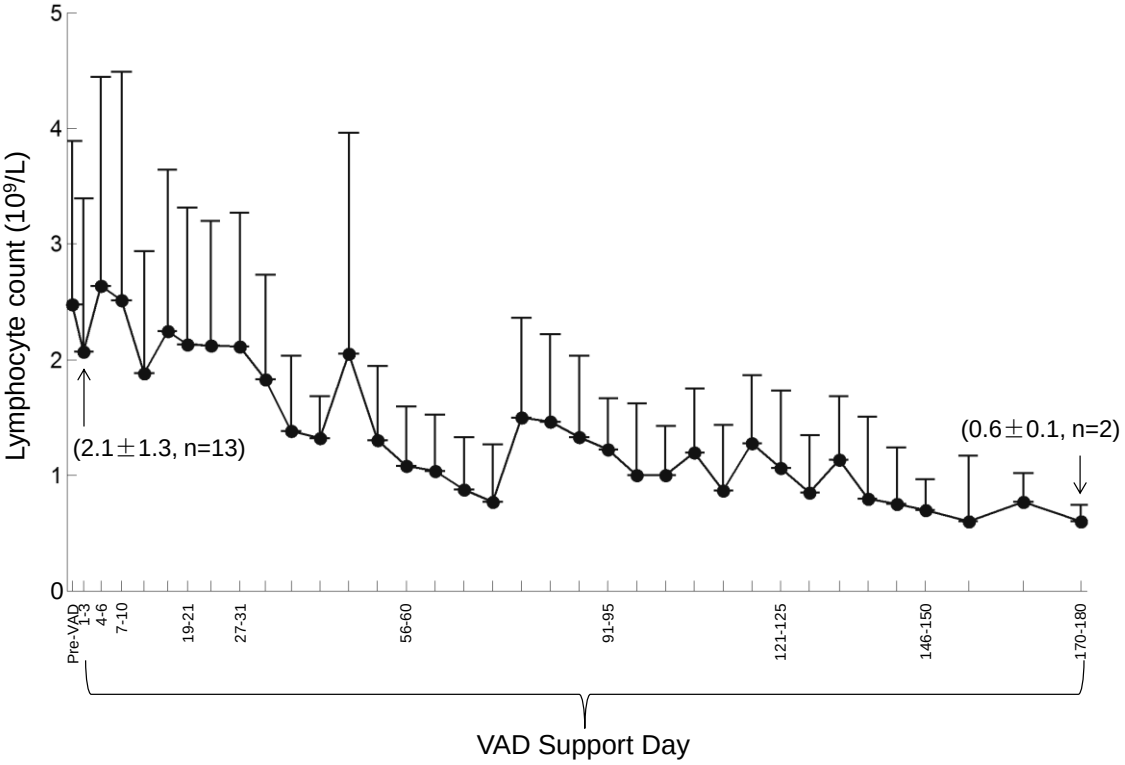


* indicates p<0.05 in the changes from pre-VAD to VAD support day 1--3

* * indicates p<0.0001in the changes during the VAD support period

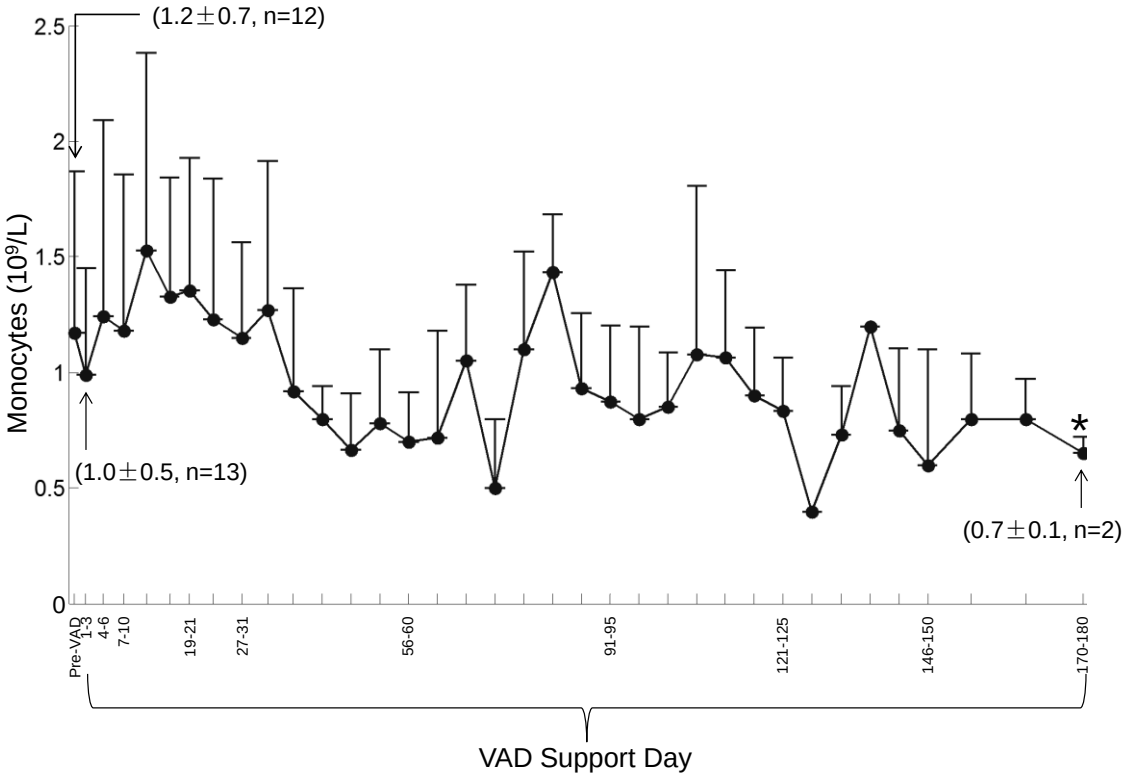
Values are Mean ±SD

Figure 2.2 Changes of lymphocyte prior to and during VAD support in 13 patients



Values are Mean ±SD

Figure 2.3 Changes of monocyte count prior to and during VAD support in 13 patients



* indicates p<0.001in the changes during the VAD support period

Figure 2.4 Comparison of CRP concentrations between LVAD (n=10) and BiVAD (n=3) patients prior to and during VAD support

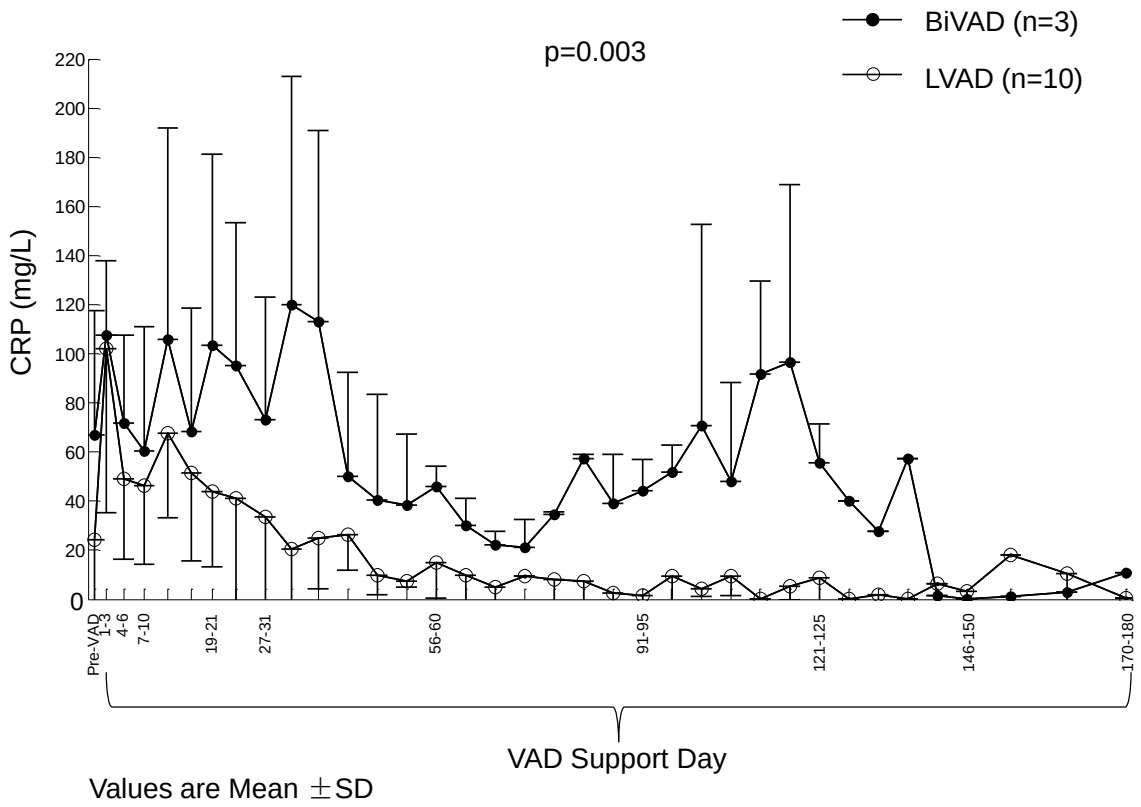
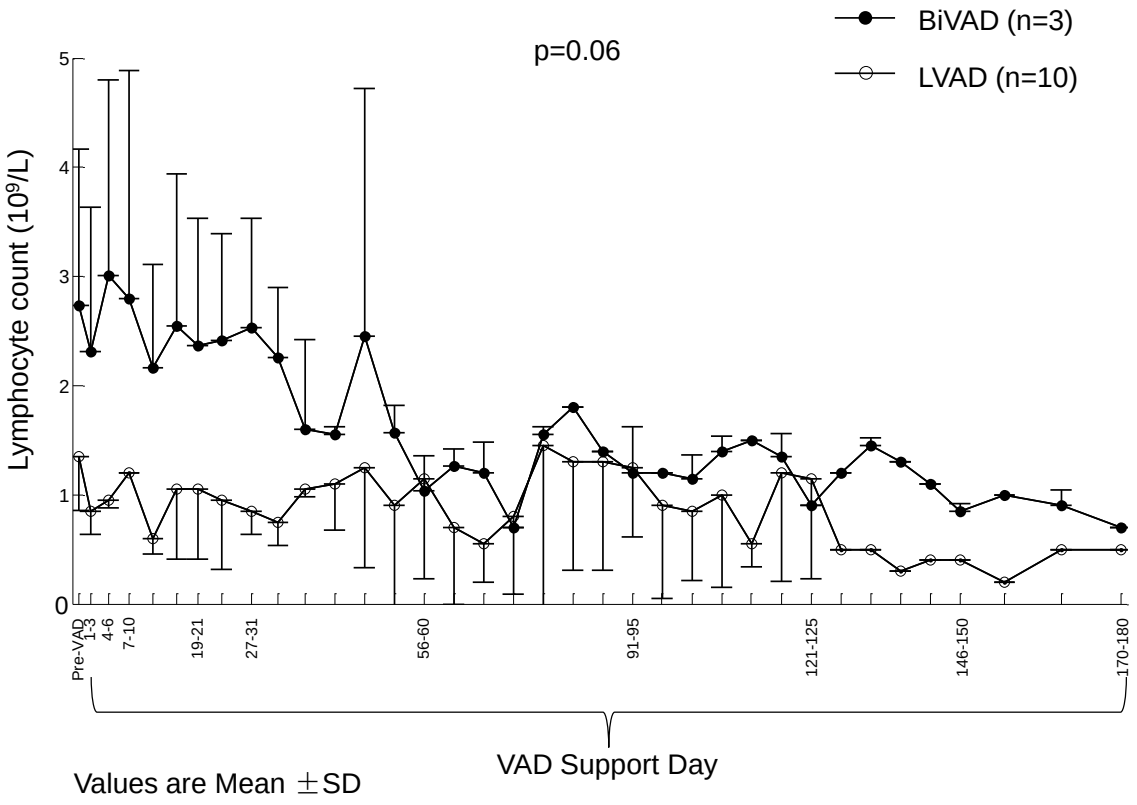


Figure 2.5 Comparison of lymphocyte counts between LVAD (n=10) and BiVAD (n=3) patients prior to and during VAD support



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Chapter 3 THE PROFILE OF SYSTEMIC INFLAMMATORY RESPONSE IN CHILDREN UNDERGOING HEART TRANSPLANTATION[2]

2 A version of this chapter has been published in *Clinical Transplantation 2012 Mar-Apr; 26 (2): E137-42* by Yu X, Larsen B, Urschel S, Cheung PY, Ross DB, Rebeyka IM, West LJ, Li J.

3.1 ABSTRACT

Objectives: Systemic inflammatory response (SIR) is an important factor to determine clinical outcomes. However, it remains unknown in children undergoing heart transplantation (HTx). We aimed to examine the perioperative changes of the SIR markers C-reactive protein (CRP) in children undergoing HTx.

Methods: Charts of 89 children undergoing HTx between 2002 and 2009 were review. Among them, 38 children had CRP measurements and were included. Data obtained prior to and within one month after HTx included CRP, total and differential white blood cell counts, doses of inotropes and immunosuppressants, cultures of blood and body fluids, duration of cardiopulmonary bypass (CPB), aortic cross clamp and donor heart ischemia, and the length of stay in the Intensive Care Unit and hospital.

Results: CRP was 32±49 mg/L before HTx, increased to 130±55 mg/L on postoperative day 1-2, and decreased to 21±31 mg/L by one month after HTx. Postoperative CRP positively correlated with epinephrine dosage and CPB duration.

Conclusions: SIR is present before HTx and acutely intensified after HTx. It may be mainly influenced by CPB duration and cardiovascular function status.

3.2 INTRODUCTION

Critical illness including surgical injury evokes systemic inflammatory response (SIR) [1, 2]. The response involves an alteration in protein metabolism in the acute phase response, characterized by an increased synthesis of acute-phase proteins by the liver to enhance host defence. Serum C-reactive protein (CRP), representing acute-phase proteins, has been used to stratify the SIR in patients with traumatic and surgical injury [3-6]. CRP level has been demonstrated to be an independent predictor of morbidity and mortality in children undergoing non-cardiac and cardiac surgery [7-10]. However, little is known about changes of CRP in children undergoing heart transplantation (HTx). This group of children have distinctive courses of critical illness. Before HTx, they frequently have experienced prolonged end-stage heart failure, and undergone ventricular assist device (VAD) or extracorporeal membrane oxygenation (ECMO) support. After HTx, they often have unstable hemodynamics as result of donor heart injury by sympathetic storm at brain death [11] and prolonged ischemia during transportation and cardiopulmonary bypass (CPB). As such, the SIR in children undergoing HTx might be different from other groups of critically ill children. Therefore, we aimed to examine the profile of SIR by evaluating perioperative changes of CRP, and to determine its clinical implications in children undergoing HTx.

3.3 METHODS

3.3.1 Patients

A retrospective chart review was conducted after approval by the Institutional Health Research Ethics Board (University of Alberta, Edmonton, Alberta). Clinical charts of the 85 consecutive children undergoing HTx at the Stollery Children's Hospital between 2002 and 2009 were reviewed, because it was since 2002 that CRP became routine measurements in clinical protocol at the Stollery Children's Hospital twice weekly (Monday and Thursday) during the stay in the Intensive Care Unit (ICU). Among them, 38 children had CRP measurements and were enrolled in the study. In the remaining 47 children, there were no CRP measurements and were excluded. The lack of measurements was due to varied practice among clinicians rather than the severity of the patient's illness. There was no statistical significance of age (68±74 and 59±63 months respectively, p=0.2) and the length of stay in the ICU (15±21 and 33±40 days respectively, p=0.1) between the excluded and enrolled patients. The clinical data of the 38 included patients were shown in Table 3.1.

3.3.2 Data Collection

Values of CRP concentrations were obtained prior to HTx (1 to 8 days, median 4 days), and within one month after HTx in the periods of postoperative day (POD) 1-2 (n=20), POD 3-5 (n=15), POD 6-9 (n=27), POD 10-15 (n=24), POD 16-19 (n=14) and POD 20-30 (n=14). The following data were also collected at the same time of CRP measurements: total white blood cell, neutrophil, lymphocyte, and monocyte counts, cultures of blood and body fluids (sputum, endotracheal tube suction, urine and

nasopharyngeal, throat and rectal swabs respectively), doses of inotropes (epinephrine, milrinone, nitroprusside), and immunosuppressants (anti-thymocyte globulin (ATG), prednisone, methylprednisolone, tacrolimus, mycophenolate). Demographic data included age, weight, gender, diagnosis, preoperative use of VAD or ECMO, and duration of CPB, aortic cross clamp and donor heart ischemia. Length of stay in ICU and hospital, clinical acute rejection and death were recorded to indicate clinical outcomes. Routine endomyocardial biopsies were not performed within the studied postoperative period. Immunosuppressants were used postoperatively according to institutional protocols: Induction with ATG i.v. for 5 to 7 days aiming for lymphocyte counts of 0.1 to 0.3/ml; Prednisone 30 mg/kg at release of aortic cross clamp followed by a slow weaning starting at 2 mg/kg daily to 0.3 mg/kg at 50 days post HTx; Mycophenolate 15 mg/kg twice a day; Tacrolimus starting at 0.05 mg/kg orally or 0.025 mg/kg intravenous infusion adjusted according to the blood tacrolimus level, target trough-levels 10-15 ng/ml. Inotropic and vasoactive agents were routinely used to maintain systolic blood pressure higher than 65 mmHg, including epinephrine (0.01-0.2 mcg/kg/min), milrinone (0.25-0.75 mcg/kg/min) and nitroprusside (0.5-5 mcg/kg/min), and occasional use of nor-epinephrine (0.01-0.2 mcg/kg/min). The glucose management protocol was to administer insulin when blood glucose exceeded 15 mmol/L. CRP and was measured using UniCel DxI 800 (Beckman Coulter, Brea, USA). Reference range of CRP is < 8mg/L.

3.3.3 Data Analysis

Data are expressed as mean ± SD and median with range. Mixed linear regression for repeated measures was used to compare the measurements prior to and the first time

after HTx, and also to determine the nature of any time trend of the variables during the postoperative period. Mixed linear regression for repeated measures was also used to analyze correlations of CRP with other clinical variables. The strength and trend of correlations were indicated by parameter estimate and p value. Parameter estimate indicates the magnitude of change in the dependent variable as a result of every unit change in the independent variable. Logistic regression was used to analyze the relation of CRP with death. All data analysis was performed with SAS statistical software version 9.2 (SAS Institute, Inc, Cary, NC). P values of less than 0.05 were considered significant.

3.4 RESULTS

Among the 38 children who had CRP measurements, eight patients received VAD support and 4 underwent ECMO. After HTx, blood culture was positive in 8 patients and culture of body fluids positive in 20 patients (sputum, endotracheal tube suction, urine and nasopharyngeal, throat and rectal swabs respectively). There were 2 hospital deaths on POD 27 and 38 from unidentified causes.

3.4.1 Changes of CRP and other clinical variables before and after HTx

Table 3.2 shows values of the variables during the study period expressed as mean±SD and median with range. CRP showed wide inter- and intra-individual variations. Prior to HTx, CRP in all the patients was 32±48 mg/L (ranged 0.5 to 178.9 mg/L). It was 33±42 mg/L in 8 patients undergoing VAD and 81±86 mg/L in 4 patients undergoing ECMO, whereas it was 12±11 mg/L in the remaining patients who did not receive circulatory mechanical support. There was no significant difference in preoperative CRP between VAD and ECMO patients (p=0.35), but there was significant difference between patients with and without circulatory mechanical support (including VAD and ECMO) (p=0.03).

After HTx, CRP in all the patients increased to 130±55 mg/L on POD 1-2 ($p<0.05$), and then continuously decreased to 21±31 mg/L by one month after HTx ($p<0.0001$) [Figure 3.1]. Neutrophil count was $6.5\pm2.9*10^9$/L before HTx, and increased to $10.7\pm6.3*10^9$/L on POD 1-2 (p=0.05), and remained at this level thereafter [Figure 3.2]. Lymphocyte count was $2.4\pm1.4*10^9$/L before HTx, and significantly decreased to

$0.8 \pm 0.9 * 10^9$/L in POD 1-2 (p=0.02), thereafter significantly increased to $1.2 \pm 0.8 * 10^9$/L by POD 20-30 (p=0.02) [Figure 3.3].

3.4.2 Correlations of CRP with other clinical variables after HTx

Table 3.3 shows the statistical results of correlations of CRP with other clinical variables after HTx. CRP positively correlated with epinephrine dosage (p=0.04) and trended towards a positive correlation with CPB duration (p=0.07). No significant correlations were found between CRP and blood glucose level, dosage of insulin, the results of cultures of blood and body fluids, and the length of ICU and hospital stay. There was no significant correlation of CRP with death (p=0.2). The results of cultures in blood and other body fluids were not significantly correlated with WBC and neutrophil count.

3.5 DISCUSSION

This study is the first to examine the profile of SIR in children undergoing HTx using the biomarker CRP. Our data showed that SIR increased before HTx, acutely intensified immediately after HTx, and remained above normal value one month later. It appears greater and more prolonged as compared to children after non-cardiac surgery [5, 10] and cardiac surgery for repair of congenital heart defects [9]. The preoperative SIR was mainly related to the use of VAD and ECMO, and postoperative SIR influenced by the duration of CPB and cardiovascular function status in heart-transplanted children.

The use of CRP as a marker of SIR to injury has been well established in critical illness [12-14]. Studies in children after non-cardiac surgery such as resection of necrotic bowel and the repair of gastroschisis have shown that preoperative CRP values were within normal range [5, 10]. Immediate postoperative CRP peaked to 80 mg/L and returned to normal within 5 to 8 days after surgery in survivors, whereas it peaked to 140 mg/L in non-survivors [4, 5, 10]. In children undergoing CPB for the repair of congenital heart defects [9, 15], CRP were also in normal range before surgery. In one study, postoperatively CRP increased to a mean of 88 mg/L on POD 2. It returned to normal values on average 10 days after surgery [9]. Another study showed a much less peak value of mean 38 mg/L after surgery, and none of these children had infection [15]. In our data, CRP prior to HTx was markedly higher than the normal range. This indicates an increased SIR in this special group of critically ill children with prolonged end-stage heart failure requiring HTx, who were apparently different from the relatively ‘healthy’ children before non-cardiac and other cardiac surgery. Interestingly, patients undergoing VAD or ECMO had a markedly higher CRP than those without circulatory mechanical

support, indicating the effect of the circulatory mechanical support to stimulate SIR [16, 17]. After HTx, CRP peaked in POD 1-2 and remained elevated by POD 20-30. The peak level of CRP appeared substantially higher than children after non-cardiac surgery and cardiac surgery [4, 9, 15]. This is readily plausible since CPB time in our patients was almost twice as long as compared to previous reports [9, 15], which may expectedly induce a much greater SIR [18, 19]. This is further supported by the trend of a positive correlation between CRP and CPB time in our data. In addition to the greater magnitude, SIR after HTx appears much more prolonged than non-cardiac and other cardiac surgery [4, 9, 10]. This may be attributed to more severe underlying pathophysiological condition, more complicated postoperative course in this particular group of children. Interestingly, although preoperative CRP was higher in patients undergoing ECMO and VAD, this difference disappeared after HTx, suggesting that the changes in CRP after HTx are mainly resulted from CPB and postoperative factors.

SIR is associated with alteration in the immune system [20, 21]. This is supported by the similar profile of neutrophil count and the reciprocal profile of lymphocyte counts after HTx. Clearly, the low lymphocyte levels in our patients after HTx owes largely to the routine use of immunosuppressants [22-24], in particular the induction treatment with polyclonal ATG that aims to deplete lymphocytes and is dose-adjusted to achieve 10-20% of the normal level.

This study also aimed to examine the clinical implications of the changes in CRP, by analyzing the correlations with the clinical variables. A higher level of CRP was associated with a higher dosage of epinephrine infusion indicating a poorer cardiovascular status. This supports the notion that the stress-induced SIR reflects

severity of injury and vice versa [12, 14]. CRP has been reported to independently predict adverse clinical outcomes, including length of ICU and hospital stay and mortality [7, 10, 25, 26]. This was not found in our data, most likely due to the complicated postoperative course in this high-risk group of patients requiring long post operative stays to adjust immune suppression and accompanying side effects.

3.6 SUMMARY

Prior to HTx, CRP was significantly higher in patients receiving ECMO and VAD than in patients not receiving mechanical circulatory support. After HTx, CRP significantly increased to 130 mg/L on POD 1-2 compared to pre-HTx, and decreased to 21 mg/L by 1 month. CRP positively correlated with the dose of epinephrine, and trended to correlate positively with the duration of CPB. CRP did not significantly correlate with the length of ICU or hospital or death.

3.7 CONCLUSIONS

CRP was elevated before HTx in patients receiving mechanical circulatory support. CRP was acutely increased after HTx, which may indicate an intensified SIR, and might be associated with long CPB duration and insufficient hemodynamics. CRP did not predict the length of stay in ICU or hospital or death in children undergoing HTx.

Table 3.1 Clinical data of children undergoing HTx (n=38)

Age	Median 16 months (ranged 1 day - 16 years)
Weight	Median 9.5 kg (ranged 2.6 - 70 kg)
Gender	Male (n=21), Female (n=17)
Diagnosis	Cardiomyopathy (n=16) HLHS without cardiac surgery (n=2) Post Norwood stage 1 (n=4) Post Norwood stage 2 (n=2) Failed Fontan (n=4) Other congenital heart defects after cardiac surgery* (n=6) and without cardiac surgery (n=2)
Donor heart ischemic time	294 ±117 min
CPB time	201 ±96 min
ACC time	62 ±27 min
ICU stay	33 ±40 days
Hospital stay	100 ±74 days
Clinical acute rejection	0
Death	2

Abbreviations: HLHS, hypoplastic left heart syndrome; CPB, cardiopulmonary bypass; ACC, aortic cross clamp; ICU, Intensive Care Unit.

*****Other congenital heart defects include: transposition of the great arteries, ventricular septal defect, aortic stenosis and tetralogy of Fallot.

Table 3.2 Mean ±SD values (median, range) of CRP and other clinical measurements in children undergoing HTx

	Pre-HTx	POD 1-2	POD 3-5	POD 6-9	POD 10-15	POD 16-19	POD 20-30
CRP (mg/L)	32.0 ±48.6 (15.9, 0.5~178.9)	130.1 ±55.2* (90, 14.0~200.0)	56.7 ±60.7 (50.4, 13.6~198.8)	38.2 ±41.7 (24.3, 4.9~197.3)	36.0 ±36.3 (26.2, 2.3~163.3)	41.9 ±47.1 (26.5, 5.0~170.0)	21.2 ±31.0† (10.1, 1.4~123.0)
Neutrophils (10^9/L)	6.5 ±2.9 (6.2, 2.0~13.0)	10.7 ±6.3* (10.4, 2.5~21.3)	11.3 ±7.7 (9.0, 2.0~30.0)	9.0 ±5.4 (7.6, 1.6~21.3)	12.5 ±8.7 (10.9, 0.2~27.3)	13.1 ±5.7 (12.6, 4.5~21.8)	9.8 ±6.6 (9.2, 1.4~25.8)
Lymphocytes (10^9/L)	2.4 ±1.4 (2.3, 0.7~5.6)	0.8 ±0.9* (0.4, 0.1~2.8)	0.8 ±0.8 (0.5, 0.0~2.3)	1.5 ±2.0 (0.7, 0.1~7.9)	2.5 ±2.6 (1.2, 0.1~9.5)	1.7 ±1.4 (0.9. 0.2~4.7)	1.2 ±0.8‡ (1.0, 0.3~2.8)

* indicates $p<0.05$ in the changes from pre-HTx to POD 1-2

† indicates $p<0.0001$ in the Changes during the first month after HTx

‡ indicates $p<0.05$ in the Changes during the first month after HTx

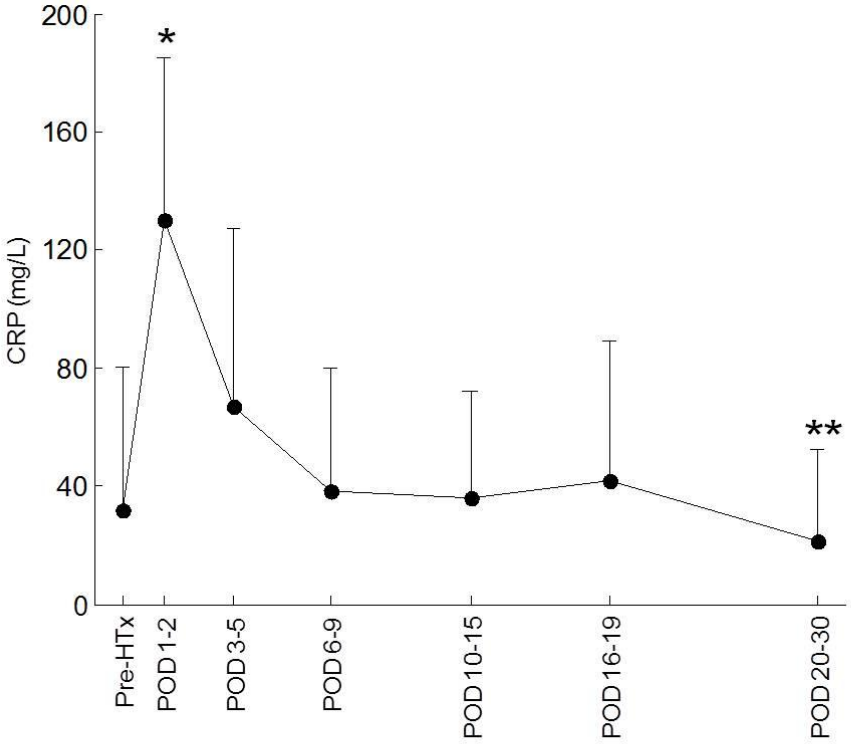
Table 3.3 Correlations of postoperative CRP with other clinical variables in children undergoing HTx

	Parameter estimate*	p value
Age	-0.13	NS
CPB time	0.09	0.07
Epinephrine	165	0.04
ACC time	0.06	NS
Donor heart ischemic time	0.03	NS
Weight	-0.03	NS
Culture	0.8	NS
Other Inotropic drugs	4.4	NS
Immunosuppressants	0.06	NS
ICU stay	0.9	NS
Hospital stay	0.4	NS
Death	14	NS

NS: Not significant.

*Parameter estimate indicates the slope of correlation.

Figure 3.1 Changes of CRP during study period (n=38)

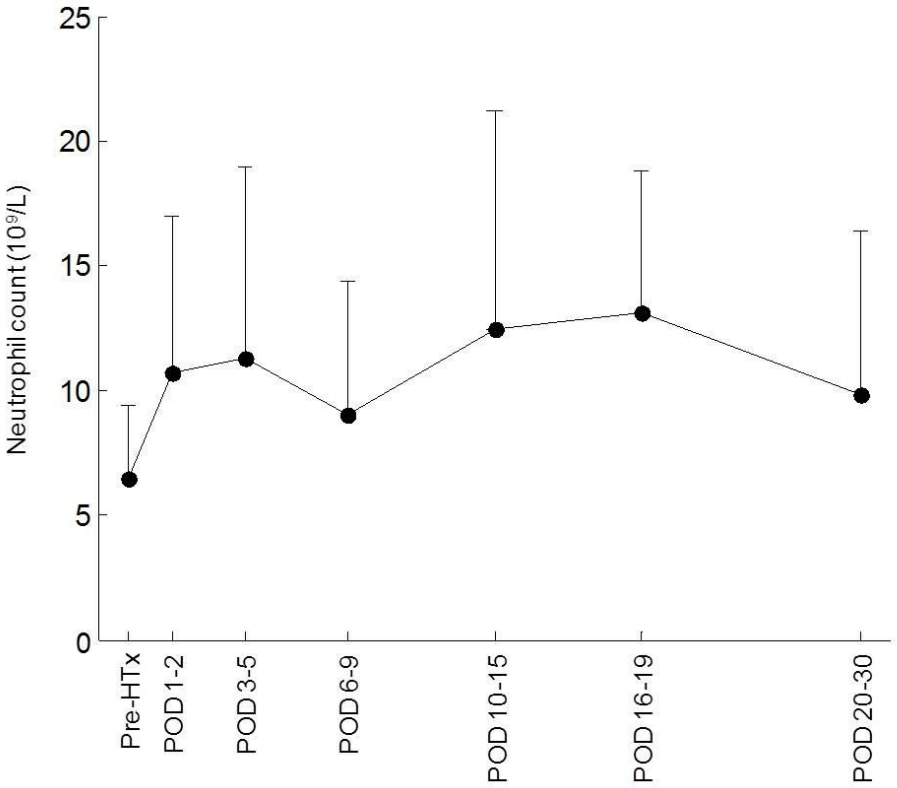


* indicates p<0.05 in the changes from pre-HTx to POD 1-2

** indicates p<0.0001 in the Changes during the first month after HTx

POD: postoperative day. Values are Mean ± SD

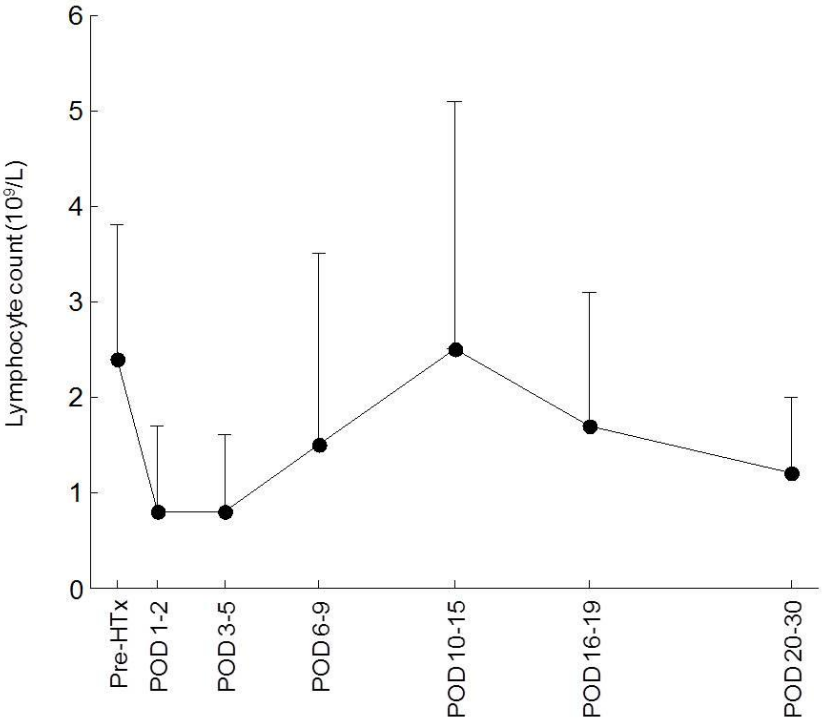
Figure 3.2 Changes of neutrophil count during study period (n=38)



* indicates p<0.05 in the changes from pre-HTx to POD 1-2

POD: postoperative day. Values are Mean ± SD

Figure 3.3 Changes of lymphocyte count during study period (n=38)



* indicates p<0.05 in the changes from pre-HTx to POD 1-2

** indicates p<0.05 in the Changes during the first month after HTx

POD: postoperative day. Values are Mean ± SD

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Chapter 4 CONCLUSIONS

4.1 SUMMARY

SIR is associated with the severity of illness and the outcomes of critical ill patients. Measurement of acute-phase proteins is a useful marker of SIR. CRP, as an acute-phase protein, has been extensively studied in critically ill patients. The perioperative changes of CRP and its relationship with clinical outcomes, such as the length of stays in ICU and hospital, have been well established in children undergoing non-cardiac and cardiac surgeries. However, the profile of SIR and the changes of CRP in children undergoing VAD support and HTx remain unknown. Given the characteristics of the two groups of patients, we hypothesized that the perioperative serum CRP level in children undergoing VAD implantation is elevated, and the elevation is prolong; the serum CRP level is elevated during perioperative period in children undergoing HTx, suggesting SIR; and the elevated CRP levels in this study setting may be associated with poor outcomes, such as longer stay in ICU, death. Therefore, we examined the perioperative measurements of serum CRP in children undergoing VAD support and HTx, as well as the correlation between CRP and other variables, including the length of stay in ICU and hospital and death, in children undergoing VAD support and HTx.

In the first study in children with VAD support, prior to VAD implantation, serum CRP was significantly higher, while lymphocyte count was lower, in patients undergoing ECMO support than in those not undergoing ECMO. After VAD implantation, CRP significantly increased in day 1-3 of support, and returned to normal by 5 months of support. Monocyte significantly decreased by 6 months of support. CRP was significantly higher in BiVAD patients than in LVAD patients. Lymphocyte count trended to be lower in BiVAD patients than in LVAD patients. CRP positively correlated with monocyte

count, doses of epinephrine and norepinephrine, and device mode; negatively correlated with lymphocyte count. CRP did not significantly correlate with the length of stay in ICU or hospital, or death.

We concluded from the first study that prior to VAD implantation, CRP was elevated in patients requiring ECMO support. VAD implantation was associated with a significant and prolonged increase in CRP. The elevated CRP level was associated with increased monocyte count and decreased lymphocyte count, which may suggest a prolonged SIR. The higher CRP levels in BiVAD patients may suggest that the SIR is more pronounced in children receiving BiVAD than LVAD. The correlation of CRP and the dose of epinephrine may suggest that elevated CRP levels are associated with insufficient hemodynamics. Contrary to our hypothesis, CRP did not predict the length of stay in ICU or hospital, or death in children undergoing VAD implantation.

In the second study in children undergoing HTx, prior to HTx, CRP was significantly higher in patients receiving ECMO and VAD than in patients not receiving mechanical circulatory support. After HTx, CRP significantly increased on POD 1-2 compared to pre-HTx, and decreased by 1 month. CRP positively correlated with the dose of epinephrine, and trended to correlate positively with the duration of CPB. CRP did not significantly correlate with the length of ICU or hospital or death.

We concluded from the second study that CRP was elevated before HTx in patients receiving mechanical circulatory support. CRP was acutely increased after HTx, which may indicate an intensified SIR, and might be associated with long CPB duration and insufficient hemodynamics. Contrary to our hypothesis, CRP did not predict the length of stay in ICU or hospital or death in children undergoing HTx.

4.2 LIMITATIONS

The two projects were both retrospective studies in a small number of patients with incomplete measurements of CRP at each time point as not uncommonly happens in clinical practice. The patients were heterogenous in age; however, no subgroups of younger or older patients were divided when analyzing data. In addition, the patient cohorts were also different in underlying disease and severity of illness, which cannot be acknowledged in the analysis. Moreover, the positive correlation of CRP with epinephrine doses may result from epinephrine itself that increase serum CRP level, but the mechanism is unclear.

4.3 FUTURE DIRECTIONS

Prospective studies to examine the perioperative changes of CRP should more accurately reflect the intensity and duration of SIR in children undergoing VAD support and HTx. Future studies could increase the number of patients and divide the patients into subgroups according to the age to further analyze the correlations between CRP and other variables. Additionally, research on the effect of epinephrine on CRP synthesis in human subjects would be helpful to test the positive correlation between the epinephrine dosage and the serum CRP level in this research, as well as to examine the mechanism of epinephrine increasing serum CRP.