ASSOCIATION OF THE STORAGE AGE OF TRANSFUSED RBC WITH ADVERSE OUTCOMES IN POST CABG PATIENTS AT HOSPITAL SERDANG

BY

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DECLARATION

I hereby declare that this dissertation has been sent to University Sains Malaysia for the degree of Master of Medicine in Transfusion Medicine. It is not to be sent to any other universities. With that, this research might be used for consultation and can be photocopied as reference.

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ABBREVIATION

AKI	Acute kidney injury
ATP	Adenine triphosphate
BMI	Body mass index
CAD	Coronary artery disease
CABG	Coronary artery bypass grafting
CHDW	Cardiac high dependency unit
CICU	Cardiac intensive care unit
CKD	Chronic kidney disease
COPD	Chronic obstructive pulmonary disease
CPDA	Citrate phosphate dextrose adenine
CPD	Citrate phosphate dextrose
DM	Diabetes mellitus
DPG	Disphosphoglycerate
FEV1	Force expiratory volume 1
FIFO	First in first out
Hb	Haemoglobin
ICU	Intensive care unit
MLogR	Multiple logistic regression
MODS	Multiple organ dysfunction score
NO	Nictric oxide
O2	Oxygen
PAD	Predeposit autologous donation
POLOS	Post-operative length of stay
RBCs	Red blood cells
RCT	Randomised control trial
RECESS	Red Cell Storage Duration Study
SLogR	Simple logistic regression

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ABSTRAK

Latar Belakang : Tempoh simpanan masa yang berpanjangan boleh membawa kepada perubahan kurang baik kepada sel darah merah (SDM) yang disimpan. Ini mungkin boleh menyebabkan kesan sampingan yang buruk kepada penerima darah. Walaubagaimanapun, setakat ini tiada lagi garis panduan bagi mencadangkan transfusi SDM yang lebih segar mempunyai kelebihan berbanding SDM yang disimpan lebih lama dalam kesan sampingan klinikalnya terhadap pesakit pasca pembedahan pintasan koronari cantuman. Kajian ini menganalisis kesan buruk klinikal bagi pesakit yang menjalani pembedahan pintasan koronari cantuman yang menerima berbeza-beza tempoh simpanan SDM.

Kaedah: Ini adalah satu kajian retrospektif pemerhatian yang dijalankan di Hospital Serdang. Data pesakit yang menjalani pembedahan pintasan koronari cantuman dalam tempoh setahun telah dikumpulkan. Subjek dibahagikan kepada tiga kumpulan mengikut usia simpanan SDM yang diterima; ≤ 14 hari, >14 hari dan mereka yang menerima campuran kedua-dua tempoh simpanan. Kesan sampingan bagi tempoh kumpulan simpanan SDM selepas pembedahan pintasan dianalisis menggunakan analisis pelbagai pembolehubah dengan nilai signifikan $p<0.05$. Perbandingan jumlah unit SDM yang ditransfusikan antara tiga kumpulan umur simpanan SDM di kalangan pesakit pembedahan pintasan koronari cantuman juga dikaji menggunakan ujian ANOVA-Satu arah.

Keputusan: Data dianalisis pada 253 pesakit yang menerima sejumlah 954 unit SDM. Subjek adalah; n = 62 dalam kumpulan SDM≤ 14 hari, n = 99 dalam kumpulan SDM> 14 hari dan n = 92 dalam kumpulan campuran. Tiada perkaitan antara mana-mana kumpulan umur simpanan SDM dengan risiko komplikasi pasca pembedahan pintasan koronari cantuman. Pesakit yang menerima lebih banyak unit SDM lebih berisiko menerima SDM yang disimpan dalam tempoh yang bercampur.

Kesimpulan: Tiada perbezaan signifikan di antara tempoh penyimpanan SDM dengan kesan buruk terhadap pesakit selepas pembedahan pintasan koronari cantuman.

ABSTRACT

Background: Extended storage time lead to storage lesion changes in red blood cells (RBCs) that can possibly cause harmful effects to recipients. Currently, there was no established guideline available to suggest transfusion of fresher RBCs had any advantages over older RBCs in clinical outcomes among cardiac surgical patients. In this study, adverse outcomes in post coronary artery bypass grafting (CABG) patients who received different storage age of RBCs were analysed

Methods: This was an observational retrospective study done in Hospital Selayang. Data from patients who underwent CABG within one year were collected. Subjects were allocated into three groups according to the storage age of RBCs that they received; ≤ 14 days, >14 days and mixed. The effect of RBCs storage age on various post-operative outcomes were analysed using multivariable analysis with significant $p<0.05$. Comparison total unit of RBCs transfused between the three storage ages of RBCs was also studied using One-way ANOVA test.

Results: A total of 253 patients who received 954 RBCs units were analysed. Subjects were; n = 62 in group of RBCs ≤ 14 days, n = 99 in group of RBCs >14 days and n = 92 in mixed group. There were no association of any of the groups of RBCs storage ages with post CABG adverse outcomes. Patients transfused with greater unit of RBCs more likely to receive mixed variance storage age of RBCs.

Conclusion: The duration of RBCs storage was not associated with significant differences in adverse outcomes among post CABG patients.

CHAPTER 1 INTRODUCTION

1.1 Introduction

Coronary artery disease (CAD) is the number one cause of death in Malaysia. World Health Organisation (WHO) reported that 20.1% of all death in Malaysia in 2012 was due to CAD which contributed for 98.9 deaths per 100,000 (WHO, 2015). One of the surgical interventions for revascularization management of CAD is coronary artery bypass grafting (CABG). Compared to medical therapy, CABG is well known to provide better result for patients with left main or triple vessel disease. Therefore, CABG is a fairly common surgical intervention among CAD patients.

CABG is a surgical intervention associated with one of the highest rates of transfusion compared to other type of surgeries (Gauvin *et al.*, 2010). However, among cardiac surgical patients, red blood cells (RBCs) transfusion itself may contribute to increased post-operative adverse outcomes such as increased morbidity and mortality (Koch *et al.*, 2008; Willems, 2016).

Extended storage time lead to storage lesion changes in red blood cells (RBCs) that can possibly cause harmful effects to the recipients. Patients with coronary artery disease are an important subgroup that may need to be treated differently, as oxygen delivery from RBCs to the heart is critical and may be reduced by obstructed coronary arteries or anaemia (García-Roa *et al.*, 2017). Current regulation allows storage of red RBCs of up to 35 to 42 days according to their additive solution. However, the standard parameters that use to justify the permissible limit for storage age of RBCs do not reflect the ability

and effectiveness of RBCs to transport oxygen to tissues which is the main purpose of RBCs transfusion (Lee and Kim-Shapiro, 2017).

There is no standard definition for fresh or old blood to clearly demarcate significant storage lesion clinically. However, many studies that investigated effects of RBCs storage age generally categorized fresh blood or new blood as storage age less than 7 or 14 days while more than 14 or 21 days storage age of RBCs were categorized into older blood (Eikelboom *et al.*, 2016; Steiner *et al.*, 2015).

In our study, storage age of RBCs was analysed as a categorical variable according to the days of stored RBCs that each patient received. The patients were divided into three storage RBCs age group. Patients who received exclusively RBCs stored for 14 days or less (RBCs stored ≤ 14 days) represent the fresh group, those who received exclusively RBCs stored for 14 days or more (RBCs stored > 14 days) represent old group and those who received mixture of both ages of RBCs storage represent mixed group. Cut off 14 days was used to categorise the age of RBCs was based on several studies which reported storage lesion started as early as two weeks of storage (Glynn *et al.*, 2016; Rafael Obrador *et al.*, 2015).

There is no established guideline available for any firm conclusion to suggest transfusion of fresher RBCs have advantages compared to older RBCs units in terms of clinical outcomes which include morbidity or mortality. However, guidelines in some health care systems are more strict compared to the accepted standard of care. These guidelines require transfusing fresher RBCs units to vulnerable patients groups, such as neonatal (National Blood Center Malaysia, 2016), patients undergoing cardiac surgery

(Gounder, 2006) or any patient with chronic transfusion such as thalassaemia major patient (M.O.H, 2009).

This was an observational retrospective study that specific for patients who had underwent CABG type of cardiothoracic surgery as it was the most common cardiac surgery done in Malaysia. Hospital Serdang is one of the local cardiothoracic centres in this country and had highest number of CABG surgeries as compared to other government cardiothoracic centres in Malaysia. Hospital Serdang was selected for this study based on adequate number of cases for this research.

1.2 Storage Lesion in stored RBCs

During storage, there are biochemical and physical changes which occurred within the RBCS referred as a 'storage lesion'. As storage time increased, intracellular ATP will be decreased. This will contribute to decrease deformable ability of RBCs to travel into small vessels and become more fragile (Kim□Shapiro *et al.*, 2011; Rafael Obrador *et al.*, 2015). Fragile RBCs that easily breakdown would release free haemoglobin and microparticles which can further decrease the nitric oxide bioavailability. Reduce in nitric oxide function will cause thrombosis, vasoconstriction and inflammation (Roback and D, 2011; Van de Watering and Brand, 2008; Van Straten *et al.*, 2011). Over storage time, 2,3-DPG will also decrease in concentration as result from reduce ATP. Thus, it will lead to decreases oxygen delivery to tissues and organs (Rafael Obrador *et al.*, 2015; Van de Watering and Brand, 2008).

With all those evident of storage lesion, there are now become a real concern about the safety and efficacy of stored blood. Thus, several studies had been conducted to explore the association between the storage duration of transfused RBCs with clinical impacts among post cardiac surgery patients. Some studies found that increase duration of stored RBCs was associated with the unfavourable outcomes in post cardiac surgery patients (Koch *et al.*, 2008; Muszynski *et al.*, 2017). This finding supported reports that as storage duration of RBCs increase, there was increased risk for vasoconstrictive, thrombotic and inflammatory effects that can possibly contribute to adverse outcomes. (Rafael Obrador *et al.*, 2015; Roback and D, 2011; Sweeney *et al.*, 2009). However, reports from several studies found that there was no significant correlation of older storage of RBCs with adverse outcomes in post cardiac surgery (McKenny *et al.*, 2011; Van de Watering and Brand, 2008).

1.3 Research Justification and Benefits

Storage lesion that can lead to alteration of RBCs metabolism, oxidative stress and red cell membrane damage are critical in patient who is already having cardiopulmonary problem especially who is undergoing cardiac surgery. Despite great number of transfusions associated cardiac surgery and remaining hesitancy concerning the clinical relevance of RBCs storage lesions, effect of storage age of RBCs on cardiac surgery adverse outcomes has not been broadly explored. There is a general feeling among clinicians that transfusing fresher blood would offer benefit to the patients.

There are still remaining controversial and variable preference of RBCs storage duration among cardiothoracic centres. Some centres prefer to use fresher blood for cardiac surgery (Gounder, 2006; Jobes *et al.*, 2015) and others recommend the use of RBCs of all ages (Boer *et al.*, 2018). In this study, the association of transfusion with different aged of RBCs to post CABG patients with adverse clinical outcomes were determined. Association between aged RBCs with total units of blood transfused to CABG patients were also compared.

Hospital Serdang is the only hospital under the Ministry of Health in the Klang Valley which provides cardiothoracic surgeries services. There was no guideline on RBCs storage threshold for CABG patients in Hospital Serdang. Thus, practice of transfusing either fresher or older age of RBCs depends on the hospital blood bank policy of issuing the oldest RBCs unit first. However, in major paediatric cardiac surgery, Hospital Serdang required fresh RBCs that less than ten days of storage for their patients. This custom can probably disturb the flow of inventory for practising first in first out (FIFO) policy. Generally, Hospital Serdang blood bank supplied about thirteen thousands of blood bag per year for the whole hospital and Cardiothoracic Department received about two thousands RBCs unit which was the second highest blood users in Hospital Serdang after Obstetrics and Gynaecology Department.

There were only six observational studies done to examine the effects of RBCs storage age in cardiac surgeries patients so far (Koch *et al.*, 2008; McKenny *et al.*, 2011; Min *et al.*, 2014; Van de Watering and Brand, 2008; Van Straten *et al.*, 2011; Yap *et al.*, 2008). However there was no any similar study in Malaysia.

1.4 Conceptual framework

There are a few factors that may influence post CABG outcome as shown in Figure 1.1. Based on previous studies, 7 identified cofounders that independently contributed to post-operative cardiac surgery adverse outcome were selected; age > 65 years, pre-operative CKD, COPD, DM), obese, female gender and number unit of RBCs transfused. There are several post CABG adverse outcomes that include mortality and other morbidities. However, only factors highlighted in bold were studied.

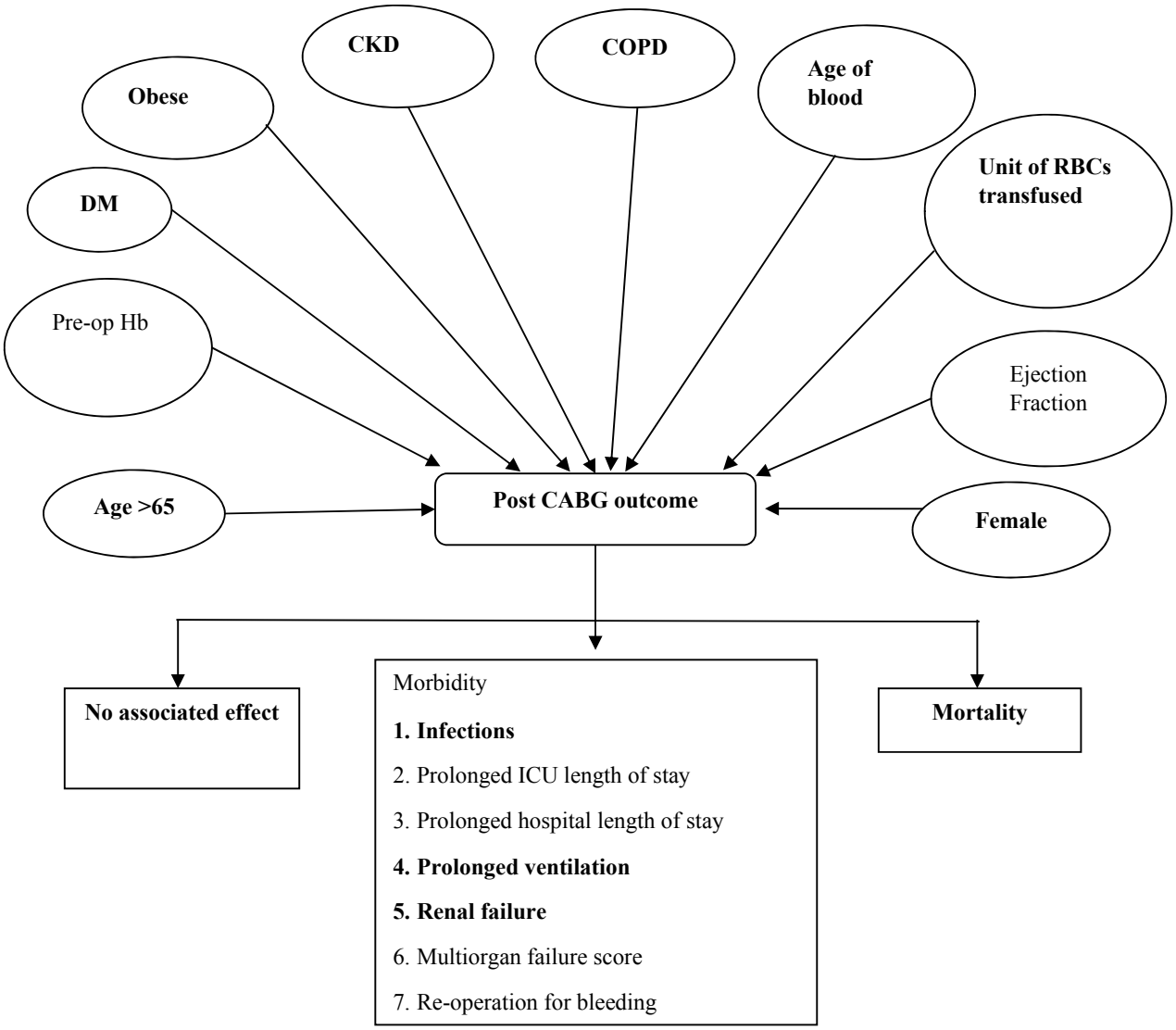

Figure 1.1 Factors influencing post CABG adverse outcome.

CHAPTER 2 RESEARCH OBJECTIVES

2.1 General Objective

To determine the association of storage age of transfused RBCs with the adverse clinical outcomes on post CABG patients in Hospital Serdang.

2.1.1 Specific Objectives

Objective 1

To correlate the associations of the age of RBCs storage transfusion with post CABG adverse clinical outcomes

Objective 2

To compare total unit of RBCs between the three groups of post CABG patients who received different storage age of RBCs.

2.2 Research Hypothesis

2.2.1 Hypothesis alternative

i. There is an association between storage age of transfused RBCs and post CABG adverse outcomes.

ii. CABG patients that transfused with RBCs stored > 14 days and mixed of both ages of RBCs storage have more RBCs unit transfusion compared to those who transfused with RBCs stored ≤ 14 days.

2.2.2 Hypothesis null

i. There is no association between storage age of transfused RBCs and post CABG adverse outcomes.

ii. There is no different in total unit of RBCs transfused between the three storage age groups of RBCs among CABG patients.

CHAPTER 3 LITERATURE REVIEW

3.1 Background

In cardiothoracic procedure, RBCs transfusion is one of the most frequent intervention. To minimised wastage, first in first out (FIFO) policy is currently practiced (*Transfusion Practice Guidelines* 2016). As a result, RBCs stored for longer periods are frequently supplied to users, and older RBCs tend to be used first.

Current regulation permit storage of RBCs is up to 35 to 42 days according to their additive solution. This is related with the characteristics of the RBCs product which are required based on 2 parameters, the level of haemolysis is less than 0.8% at the end of the storage time and a survival rate of the transfused RBCs is >75% at 24 hours after transfusion (Medicines, 2017). However, these standard parameters do not reflect the ability and effectiveness of red cells to transport oxygen to tissues which is the main purpose of RBCs transfusion.

During storage period, RBCs sustained many changes that known as storage lesion. This RBCs storage lesion is related with transfusion-related inflammatory reactions and decrease oxygen-carrying capacity. However, effects of these changes on patient's clinical outcomes in cardiothoracic setting is still unclear (Yap *et al.*, 2008).

3.2 RBCs changes during storage

Biochemical, biomechanical and immunologic changes that occurred in stored RBCs are known as storage lesion (Kor *et al.*, 2009; Rafael Obrador *et al.*, 2015). These changes involve decrease ability of RBCs deformity resulting in breakdown of RBCs and sluggish blood flow in microvascular circulation. Storage lesion also reduces intracellular adenosine triphosphate (ATP), pH and 2,3-DPG that shifts oxygen dissociation curve to the left which subsequently decrease oxygen delivery to tissue (Rafael Obrador *et al.*, 2015; Solheim *et al.*, 2004). RBCs changes can begins as early as one week of storage as RBCs started to show significantly higher stiffness than fresh RBCs (Xu *et al.*, 2018). However, many studies suggested that storage lesion involves changes begins after 2 to 3 weeks of storage, progress with duration of storage (Glynn *et al.*, 2016; Rafael Obrador *et al.*, 2015).

As storage time increase, intracellular ATP within the RBCs reduces. This changes lead to fragility of RBC membrane and less deformable (Kim□Shapiro *et al.*, 2011; Rafael Obrador *et al.*, 2015). RBCs storage lesion leads to loss of RBC membrane integrity, RBCs deformability and increased adhesiveness as well as RBCs aggregability (Ho *et al.*, 2003; Rafael Obrador *et al.*, 2015). This has also raised apprehensiveness over the mechanism that leads to tissue ischemic and occlusive microcirculation. Based on a risk analysis of cardiac surgery patients in 2008, there was noted that a significantly higher rate of ischemic events resulted from perioperative RBCs transfusion (Reeves and Murphy, 2008).

Free haemoglobin and microparticles are released due to the breakdown of unstable RBCs. This process also reduces nitric oxide bioavailability, which will cause vasoconstriction, inflammation and thrombosis. There was also a study found that older stored RBCs scavenge nitric oxide more than fresher cells (Neuman *et al.*, 2015). Decreased nitric oxide concentration together with endothelial dysfunction in blood recipients causing reductions in blood flow, and thus oxygen delivery to tissues and end organs. This changes subsequently may become potential caused for mortality and morbidity (Min *et al.*, 2014; Rafael Obrador *et al.*, 2015).

An association between RBCs transfusion and changes in immune systems was first reported in people almost 40 years ago by Opelz and his friends (Rafael Obrador *et al.*, 2015). Immunosuppressive effects include suppressed natural killer (NK) cell and macrophage function, alterations balance in T lymphocyte, impaired antigen presentation and suppression of lymphocyte proliferation (Remy *et al.*, 2018). Allogeneic RBCs transfusion downregulates cellular immunity and dysregulates innate immunity during inflammation. This effect happens by stimulation of Type 2 -T helper cell by allogenic RBCs as evidenced by increased in interleukin (IL)-10 and IL-4 cytokine secretion post transfusion of stored human RBCs. IL-10 and IL-4 are both subsequently downregulate the T helper 1 response, thus downregulating cellular immunity functions for antigen presentation (Rafael Obrador *et al.*, 2015). RBCs transfusion also results in proinflammatory effects by priming neutrophil function and increase inflammatory cytokine release (Muszynski *et al.*, 2017; Remy *et al.*, 2018). Both proinflammatory and immunosuppressive effects of RBC transfusion may be relevant for critically ill and surgical patients whom both excess inflammation and immune suppression are significantly associated with adverse outcomes (Muszynski *et al.*, 2017). Other studies

proved that accumulation of cytokines over RBCs storage time is related to transfusion-mediated systemic inflammatory reactions and increase likelihood of bacterial infections (Min *et al.*, 2014; Rafael Obrador *et al.*, 2015; Reeves and Murphy, 2008).

Over time, RBCs storage lesion cause drastic fall of 2,3-DPG and becoming below detection level within 2 weeks (Kor *et al.*, 2009). Decreasing level of 2,3-DPG will lead to deprive oxygen delivery to organs (Van de Watering and Brand, 2008). However, despite substantial drop in 2,3-DPG, multiple studies were unable to prove a clinically significant effect from the RBCs depleted 2,3-DPG transfusion (d'Almeida *et al.*, 2001). This finding can be explained by the level of 2,3-DPG has quick recovery level following transfusion and totally restored within 48 to 72 hours (Rafael Obrador *et al.*, 2015; Stan and Zsigmond, 2009).

3.3 Transfusion in cardiac surgery

In several studies, there were high rate of RBCs transfusion usage in cardiothoracic surgery which ranging from 40% to 90% (Rogers *et al.*, 2009). Blood transfusion itself can cause harm to the patients. There were more findings showed that RBCs transfusion has related with increased morbidity and mortality regardless the age of transfused RBCs (Galas *et al.*, 2013; Horvath *et al.*, 2013; Jakobsen *et al.*, 2012). In addition, a study in USA found that CABG patients who received RBCs transfusion had significantly prolonged intubation time, prolonged postoperative length of stay, higher morbidity and mortality (Scott *et al.*, 2008).

Several guidelines had been published regarding RBCs transfusion in cardiac surgery patients. Many of the guidelines recommended a restrictive transfusion strategy with most suggesting a haemoglobin threshold of 7 g/dL in asymptomatic patients (Carson *et al.*, 2016; Ferraris *et al.*, 2011; Retter *et al.*, 2013). Guidelines from the National Blood Authority of Australia recommended that the haemoglobin level solely should not be an indicator for RBCs transfusion instead it should also be based on the patient's clinical status. In one of the randomised controlled trials of cardiothoracic surgery patients, the odds ratio for mortality favoured a liberal RBCs transfusion practice rather than a restrictive transfusion strategy, but the difference between strategies was not statistically significant (Patel *et al.*, 2015).

Nonetheless, generally there is no standard guideline that determined age of storage RBCs that should be used in CABG. However, there is certain local guideline require transfusing fresher RBCs units for patients undergoing cardiac surgery such in New Zealand Blood Transfusion Service(Gounder, 2006). On the other hand, the American College of Critical Care Medicine and the British Committee for Standards in Haematology noted a lack of evidence to recommend fresher compared with standard issue RBCs.

Other blood component transfusion in cardiac surgery was based on the presence of ongoing bleeding and impairment in coagulation function. Blood product of non-RBCs transfusions were aimed toward the hemostatic abnormality as based on published guidelines and generally accepted practice (Shehata *et al.*, 2018). Plasma product with dosage of 15mL/kg was indicated in the presence of active hemorrhage with an international normalized (INR) ratio more than 1.5 time normal. In the presence of bleeding and a fibrinogen concentration less than 0.15 g/dL, cryoprecipitate was

indicated. In the other hand, platelet transfusion was justified when there was ongoing hemorrhage and platelet count less than 80,000 x 109 /L or presence of platelet dysfunction (Shehata *et al.*, 2018).

3.4 Post cardiac surgery adverse outcomes in regards of storage age RBCs.

To date, various observational research had done to investigate the association between storage duration of RBCs with clinical outcomes among post cardiac surgery patients (Koch *et al.*, 2008; Min *et al.*, 2014; Muszynski *et al.*, 2017). They reported that older storage age of RBCs was significantly contributed with the adverse outcomes which supported the harmful effects of storage lesion in stored RBCs. The dependent variables for adverse outcome were prolonged ventilation, renal failure, infection, multiple organ failure and longer hospital stay.

In a recent meta-analysis, only 2 observational studies that statistically adjusted for the number of red cell units transfused (Vamvakas, 2011). They were among patients who were diagnosed with cardiovascular disease (Eikelboom *et al.*, 2010) and the other one was in RBCs transfused patient (Edgren *et al.*, 2010). Both studies found that there was no effect between fresher and older storage age of RBCs in patient's outcome. This finding was similar with the only RCT done among patients undergoing cardiac surgery that investigate the effects age of blood storage (Steiner *et al.*, 2015). It was an international, multicentered RECESS trial that randomised 1098 cardiac surgical patients to transfuse with RBCs stored for ≤10 days or >21 days. Multiple Organ Dysfunction Score (MODS) was the primary outcome. They found that the older storage of RBCs was

not contributed to the higher risk for development of MODS. Other RCT trials done in different population which were among critical ill subjects and in-hospital patients requiring blood transfusion had also showed the replicate report (Eikelboom *et al.*, 2016; Lacroix *et al.*, 2011).

CHAPTER 4 METHODOLOGY

4.1 Introduction

Cardiothoracic Surgical Department in Hospital Serdang was established in January 2007 and started their first open cardiac surgery in Julai 2007. It provides 56 beds that includes Cardiothoracic Intensive Care Unit (CICU), Cardiothoracic High Dependency Unit (CHDW) and general cardiothoracic ward. Generally, this department had done about 270 CABG surgeries per year in 2015 and 2016. In Cardiothoracic Department of Hospital Serdang, there was no solid protocol exists for perioperative blood transfusion, but it was limited where possible. Generally, blood bank in Hospital Serdang practice standard policy of first in first out (FIFO) as is to issue the oldest available RBCs unit in the first instance. All patients were received crossmatch compatible, non-leucofiltered packed RBCs that were stored in citrate phosphate dextrose adenine (CPDA1) or CPD/Optisol with the storage temperature was 2–6°C.

4.2 Study design

This was an observational retrospective study that composed 292 data from patients who underwent primary and elective CABG from January to December 2015 in Hospital Serdang. Subjects were among patients who received at least one unit of RBCs transfusion during and post operatively. In this study, a total of 253 patients were studied that fulfil the inclusion and exclusion criteria.

The grouping of the patients were based according to the RBCs storage time (in days) of blood transfused. Each patient might receive more than one unit of RBCs. Thus, the grouping will be based on the oldest age of transfused RBCs. The first group were patients who received blood transfusion exclusively RBCs that had been stored for 14 days or less (RBCs stored ≤ 14 days), second group were patients who received exclusively RBCs stored more than 14 days (RBCs stored >14 days) and the third group were patients that received mixed both ages of RBCs storage (mixed) (McKenny *et al.*, 2011). The threshold of 14 days was chosen because storage changes becomes apparent after about 2 weeks of stored (McKenny *et al.*, 2011; Rafael Obrador *et al.*, 2015). Since RBCs stored > 14 days was postulated to has an impact on post CABG adverse outcome, group of stored RBCs > 14 days and mixed of both RBCs storage ages were put as a tested group while group of stored RBCs ≤ 14 days was put as a reference group. All subjects were chosen according to the inclusion and exclusion criteria stated in section 4.5. The demographic and clinical data for all the subjects were recorded in proforma form as in the Appendix 1.

4.3 Data Collection Method

Patient's data were collected using electronic database and the selection were focus on patients who underwent primary and elective CABG that received blood transfusion during and post-surgery at Hospital Serdang in year 2015. Electronic database from National Blood Centre was used to determined age of blood from the date of blood collected. The calculation of sample size as shown in section 4.6. The data gathered were filled in the proforma (Appendix 1).

4.4 Study duration

The sudy was conducted from January 2017 until December 2018 (24 months).

4.5 Inclusion and Exclusion criteria

4.5.1 Inclusion Criteria

Participants included in this study were Malaysian Citizen, aged from 18 years and above who had 1st time elective CABG surgery from January to December 2015 and received at least 1 unit of packed RBCs during or/and post operational hospital stay. All patient's medical records had complete results of all the data needed in this study.

4.5.2 Exclusion Criteria

i. Emergency CABG due to inadequate time to optimize preoperative patient variables. Emergency CABG procedures are indicated for patients with clinical condition requiring surgery within 72 hours of presentation.

ii. Previous heart surgery because this will render patient more susceptible to allogeneic blood transfusion.

iii. Patients who had performed Predeposit Autologous Blood Donation (PAD) for they may be transfused at higher haemoglobin level than the general population.

iv. Required re-exploration for surgical bleeding for such technical problems is unpredictable.

4.6 Sample size

Sample size was calculated using PS Software. Calculation was made based on objectives of the study.

For objective 1 : two-sample proportion was used ; α was set at 0.05, power = 80%, m ratio=1, proportion of patients received RBCs stored ≤ 14 days with adverse outcomes (P0) = 0.088, estimated proportion of patients received mixed ages of RBCs with adverse outcome (P1) = 0.254 (McKenny *et al.*, 2011). Final sample size needed was 240.

For objective 2, : two-sample mean was used ; α was set at 0.05, power = 80%, m ratio=1, SD = 10.3, mean difference = 6.6 (McKenny *et al.*, 2011). Final sample size needed was 117.

Since objective 1 require larger sample size, we need 240 samples. However, we managed to include all patients that had CABG done in 2015 in Hospital Serdang that fulfill the inclusion and exclusion criteria which were 253 patients.

4.7 Ethical approval

Ethical approval from Hospital Serdang, Jawatankuasa Etika Penyelidikan Manusia USM (JEPeM HUSM) & National Medical Research Register (NMRR) were obtained as all the data were retrieved from patient's medical record. The data were recorded with reference identification to prevent recognition of patient's identity. Thus all data collected will be anonymous. The use or disclosure of protected health information involves no more than a minimal risk to the privacy of individuals.

4.8 Statistical Analysis

Statistical analysis will be performed using IBM SPSS version 22 for window-software to present the descriptive, statistical and multivariate analysis.

4.8.1 Objective 1

The association between the eight factors (age group of RBCs storage, CKD, COPD, DM, obesity, gender, patient's age, total unit of RBCs transfused) with each four post CABG adverse outcomes (mortality, prolonged ventilation, renal failure and infection) were analysed individually using simple logistic regression (univariable analysis). Only significant independent variables with p-value < 0.25 and/or clinically significant factors were chosen as confounders and analysed using multiple logistic regression for each outcome. Multivariable logistic regression model was used for categorical variable of each adverse outcomes (mortality, prolonged ventilation, renal failure and infection) analysis. Significance level was set at $p < 0.05$. To correlate any association of RBCs storage age with each post CABG adverse outcomes, groups of storage age of transfused RBCs were put as a covariate in multiple regression analysis although they showed insignificant results in the univariate analysis.

4.8.2 Objective 2

One-way ANOVA test was used in analysing for comparing total unit of RBCs transfused between three groups of RBCs storage age (RBCs ≤ 14 days, RBCs > 14 days and mixed) with significance was set up at $p < 0.05$. Subsequent post-hoc analysis (Bonferroni procedure) was used to analysed which group of storage aged of RBCs showed the significant in total unit of RBCs transfused. Significance was set up at $p < 0.05$.

4.9 Operational Variable Definition

Independent potential confounders for post-operative CABG adverse outcome were adjusted using multivariable analysis. There were pre-existing chronic kidney disease (CKD), age more than 65 year old, chronic obstructive pulmonary disease (COPD), diabetes mellitus (DM), obese, female gender, total unit of RBCs transfused together with the patient's storage age groups of RBCs. The RBCs storage ages variable were divided into three groups. The first group were patients that received exclusively RBCs stored for 14 days or less (RBCs stored ≤ 14 days), second group were patients that received exclusively RBCs stored more than 14 days (RBCs stored >14 days) and the third group were patients that received mixed both ages of RBCs storage (mixed).

We choose to include group of mixed RBCs storage age as one of our variable group as we found a significant number of them were in our study population. Since mixed RBCs storage age group also consisted RBCs storage > 14 days, in that way we also can analyse if having received any number of units of older RBCs can also affect outcomes. To determine association of the age of RBCs storage with the post CABG adverse outcomes, group of RBCs ≤ 14 days was put as a reference while RBCs stored >14 days and mixed group as a tested group.

Patient's age > 65 years was categorized as old patients and 65 years or less as young patients. Old age was considered as a risk factors for mortality in patients with post coronary artery bypass graft surgery (Roback and D, 2011; Santos *et al.*, 2014). It also has a risk contributor for acute renal injury among post cardiac surgery patients (Yi *et al.*, 2016).

On the other hand, DM and female gender are predictive of sternal wound infection (Meszaros *et al.*, 2016). Diabetes includes both insulin dependent and non-

insulin dependent diabetes. Chronic obstructive pulmonary condition is defined as a requirement for pharmacology therapy for chronic pulmonary disease or FEV1 <75%. COPD was confirmed to be an independent predictor of AKI (Yi *et al.*, 2016).

Pre-existing CKD was one of independent predictor factor for mortality and prolonged ventilation among patients undergoing coronary artery bypass graft surgery (Santos *et al.*, 2014). Serum creatinine level >100 mmol/L in female and >110 mmol/L in male are grouped into pre-existing CKD (Karkouti *et al.*, 2001) as a binomial variable.

Obese was one of the predictive of sternal wound infection and prolonged ventilation in post CABG patient (Gürbüz *et al.*, 2014; Meszaros *et al.*, 2016). Obese is defined as body mass index (BMI) equal or greater than 30. There was a study that found that increased volume of RBCs transfused was significantly associated with increased morbidity of acute renal failure, sepsis, prolonged duration of ventilation and also mortality in cardiac surgery patients (Horvath *et al.*, 2013; Ranucci *et al.*, 2008). In this study, total unit of RBCs transfused was also include as one of the cofounder in our multivariable analysis.

4.10 End Points

We used definitions from the previous studies for each post CABG adverse outcome. All four post CABG adverse outcomes (mortality, prolonged ventilation, renal failure and infection) were examined as a binary endpoint. Prolonged ventilation, renal failure and infection adverse outcomes were observed within hospital stays. In the other hand, mortality was defined as death within 30 days or death in hospital (McKenny *et al.*, 2011). Prolonged ventilation was the ventilation of more than 72 hours post operation (McKenny *et al.*, 2011). Renal failure defined as a renal failure requiring new replacement therapy or increase of > 50% in serum creatinine from preoperative value and as for infection complications (Min *et al.*, 2014). Infection complication post CABG surgery by Society of Thoracic Surgeons includes septicaemia or sepsis, deep and/or superficial sternal wound infection. Sternal wound secretion that associated with clinical signs of infections (fever, inflammation of wound and localised chest pain) with positive secretions and blood culture was consider as sternal wound infection (Santos *et al.*, 2014).

4.11 Flow Chart of Study

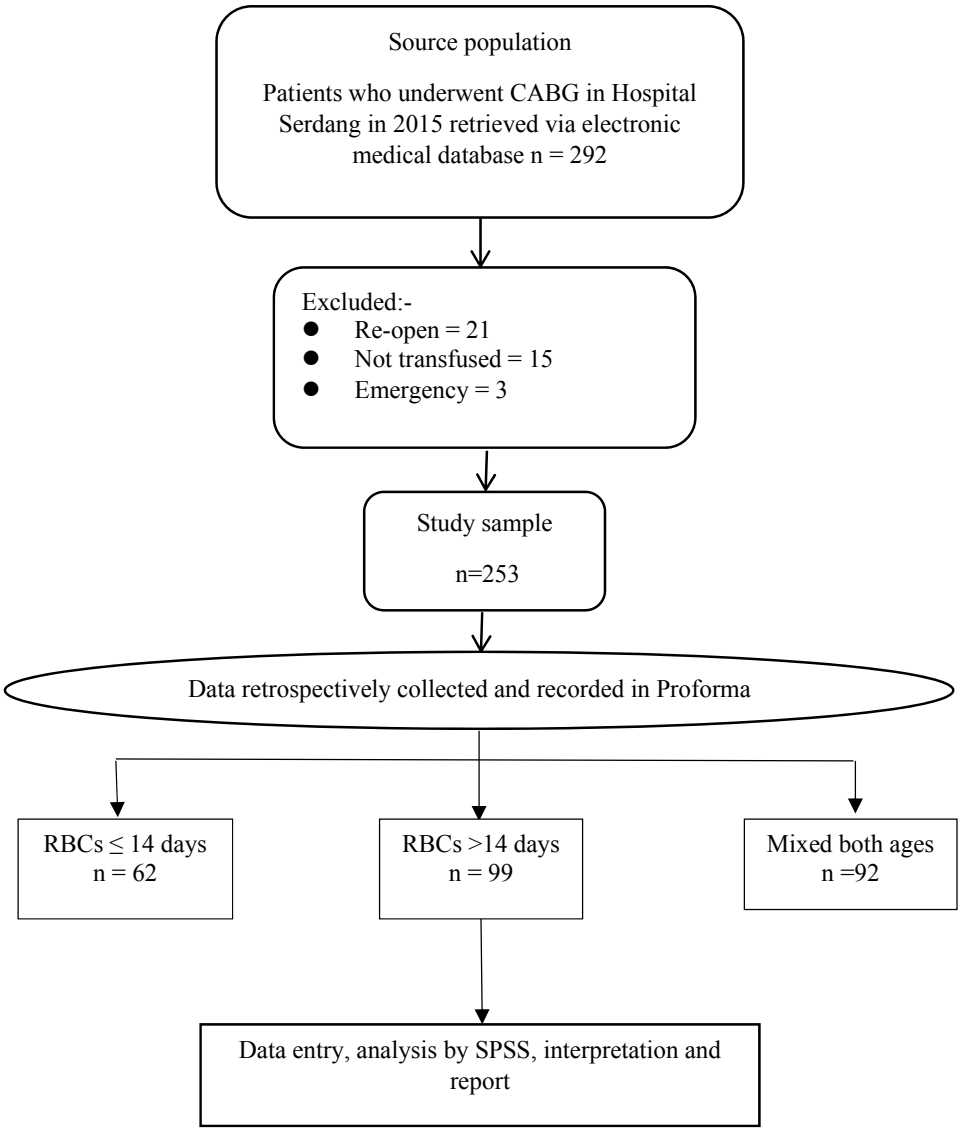

Figure 4.1 Flow chart of study

CHAPTER 5 RESULT