Cape Peninsula
University of Technology

An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services

by

ZEENAT ABDUL

Thesis submitted in fulfilment of the requirements for the degree
Master of Science: Biomedical Technology
In the Faculty of Health and Wellness Sciences

At the

Cape Peninsula University of Technology

Supervisor : **Dr. D.J. Bester and Dr. N. Naidoo**
Co-supervisor : **Dr. T. Farrar**

Bellville
July 2021

DECLARATION

I, Zeenat Abdul, declare that the contents of this dissertation/thesis represent my own unaided work, and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

06.07.2021

Signed **Date**

ABSTRACT

An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services

Background: Haemorrhagic shock patients are at risk of death, disseminated intravascular coagulation, hypoxic brain injury, global myocardial ischaemia and this risk is heightened among the young, old, or polytraumatised. The recommended definitive care is to stop the bleeding and to replace lost haemoglobin whilst providing supplemental oxygenation. Despite blood transfusion being a standard treatment of care for severe haemorrhagic shock, it is not used as a standard practice in the pre-hospital setting in South Africa. This could be due to a paucity in South African research regarding pre-hospital emergency blood transfusion (EBT), as many factors were to be considered.

Objective: This research study in the Western Cape, South Africa, determined the viability of packed red blood cells in the pre-hospital setting by placing blood on ambulance vehicles, this study also determined the self-efficacy of emergency care practitioners (ECP) to administer an EBT and lastly, estimated the burden of need for EBT in the pre-hospital setting.

Methods: The viability of Packed Red Blood Cells (PRBC) was determined by placing the blood in temperature-controlled fridges at the ambulance base as a Control, and the Treatment PRBC were transported. PRBC were transported in a cooler-box with ice-packs, and the cold-chain was monitored against the recommended transportation temperature range 1˚C – 10˚C. The PRBC were tested weekly for haemolysis. Questionnaires were given to EMS personnel to determine their self-efficacy about introducing blood as a resuscitation fluid. The estimation of the EBT need in the pre-hospital setting was done over a 2-month period by the advanced life support (ALS) ECP. This included patients who with penetrating injuries, displaying signs and symptoms of shock - class III or class IV (decompensated/irreversible).

Results: The PRBC that were placed on ambulance vehicles indicated that they were <0.8% threshold after day 35. However several packs in the treatment group were ≥0.8% on day 42, while all packs in the control group were <0.8% on day 42. PRBC transported remained between 1˚C – 10˚C, although slight fluctuations. Questionnaire results indicated that ECP were confident in administering an EBT if required.

A total of 12.8% of patients within the criteria used in this study was found, that could have been eligible for EBT.

Conclusion: The viability of blood on ambulance vehicles remained within the acceptable ≤0.8% haemolysis level until the PRBC expiry on day 42, as per the Council of Europe Guidelines. PRBC were able to remain within the transportation temperature range according to the Clinical Guidelines of South Africa. The self-efficacy questionnaires given to ECP displayed confidence levels in their ability to administer blood, provided training is received. A significant percentage of 12.8% of patients could have made use of ERBC in the prehospital field, as ECP would have had the ability to bridge the time critical gap of a life-threatening probable mortality, before reaching the emergency department where blood would be administered

We recommend that pre-hospital emergency blood transfusion be adopted as a standard practice in South Africa.

ACKNOWLEDGEMENTS

Firstly, I wish to thank the Almighty for granting me the strength and courage to complete my thesis.

Secondly, I wish to express my deepest gratitude to the following people for their assistance towards the success of this study:

- Dr. DJ. Bester, my supervisor for all the invaluable guidance, sincerity, motivation and continuous encouragement.
- Dr. N Naidoo, my supervisor for his insightful feedback, vision, support and continuous guidance throughout the study.
- Dr. T Farrar, my co-supervisor for his technical prominent ideas, and immense statistical knowledge.
- Dr. G.R.M Bellairs, CEO of the Western Cape Blood Service (WCBS), for granting me permission to conduct this study on site and providing me with all the resources I required to complete this study.
- Dr. S. de Vries, Head of the Emergency Medical Services (EMS) for the Western Cape Government - Health, for granting me permission to access the Western Cape EMS for the purpose of conducting this study.
- Gary Mc Cormick, for granting me approval to access the Air Mercy Service site.
- The Cape Peninsula University of Technology for giving me the privilege of being able to complete my research study at their tertiary institution and for providing me with funding to complete this study.
- HemoCue SA for the loaning of their equipment, and always being readily available to assist.
- WCBS and all the departments involved, inclusive but not limited to the Marketing and Public Relations department as well as the Learning and Development department (L&D) who allowed me their space to conduct my practical work. A special thank you to Faieqa Adams (L&D), who so willingly assisted me in getting started in the laboratory and constant motivation to complete my research study.
- A heartfelt thank you to all of the research assistants which are the ALS ECP for their hard work, dedication, valuable time and going over and above of what was required by them for the success of this study.
- All Western Cape Government EMS support staff, as well as Bradley Theron for his speedy assistance in obtaining statistics as requested and Binyamien Karriem for his valuable time, patience and always willing to help upon request.
- My Parents, for their constant motivation, love, support, words of encouragement, guidance and all their prayers.
- My sisters, Ghadijah and Sadiyah Abdul for their unconditional support, care, compassion and motivation which has guided me through.
- My husband, Taufeeq Ajam for sacrifices you made with me, being understanding and always ready to assist with any task required.
- Thank you to all who has assisted me, friends, family and colleagues. I appreciate each and every one of you!

DEDICATION

I dedicate this thesis to my Mother, Dhilnawaaz Abdul and my Father, Suffyaan Abdul. I cannot thank you enough for your constant support, guidance and encouragement throughout my education, so with this, I hope I've made you proud.

GLOSSARY

List of abbreviations

ALS	-	Advanced life support
BLS	-	Basic Life Support
CD	-	Conjugated Dienes
EBT	-	Emergency Blood Transfusion
ECP	-	Emergency Care Practitioners
EPRBC	-	Emergency Packed Red Blood Cells
Hb	-	Haemoglobin
HCT	-	Haematocrit
ILS	-	Intermediate Life Support
IV	-	Intravenous
LDH	-	Lactate dehydrogenase
MDA	-	Malondialdehyde
PRBC	-	Packed Red Blood Cells
TBARS	-	Thiobarbituric acid reactive substances
WB	-	Whole Blood
WHO	-	World Health Organisation
WPBTS or WCBS	-	Western Province Blood Transfusion Service or Western Cape Blood Service (Company name change in 2019)

Definitions of terms

Crossmatch: A test routinely used in the blood banks, as a final step of pre-transfusion compatibility testing. The test consists of using the donors PRBC and recipient's plasma (Tholpady and Bai, 2019).

Plasma: A straw-coloured liquid component in blood, which suspends all the other components of the blood. Plasma is the component left behind after PRBC, WBC, platelets are removed. It consists of various solutes, such as; salts, water, proteins, antibodies and enzymes.

Emergency Medical Services: A public or private out-of-hospital service that provides early care to injured and critically ill patients, and/or transportation by road ambulances or aircraft to the hospital or emergency unit.

Self-efficacy – Refers to having the confidence to apply control over one's own motivation and behaviour to carry out specific performance attainments (Locke and Bandura, 1987).

Shock – A life-threatening medical emergency whereby the circulatory system fails to maintain sufficient blood flow, thus decreasing oxygen delivery and nutrients to vital organs. Shock in medicine can also be described as failure of the circulatory system, resulting in insufficient cellular oxygen utilization (Tintinalli, 2010)

Vital signs – These are the objective measures of involuntary body functions determined during the physical assessment of a patient and includes pulse rate, rate of respiration, systolic and diastolic blood pressure, body temperature, blood glucose levels and in some cases, oxygen saturation ($Sp0^2$).

Volume – Refers to fluid volume in this study, of both red cells and plasma in the circulatory system of an individual.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE: INTRODUCTION

1.1. Introduction

According to the World Health Organisation trauma is said to be the third most common cause of mortality worldwide, with haemorrhage being responsible for 30%-40% of mortality. Of these deaths, 33%-56% occur during the prehospital period (Kauvar, Lefering and Wade, 2006). The burden of trauma is disproportionately borne to low- and middle-income countries (LMIC) who not only comprise of 90% of the world's population, but also account for the greater number of deaths (Chandran, Hyder and Peek-Asa, 2010). This is due to the lack of structured emergency medical services, prehospital delays, delays in treatment and inadequate resuscitation (Smith, 2019). The Global Burden of Disease data states that approximately 2 million lives could be saved annually in the LMICs, if the reported case fatalities among the seriously injured were to be reduced to those in high income countries (Mock et al., 2012). In the year 2000, intentional violent injuries resulted in the deaths of 311,000 people in Africa. This indicates a rate of 60.9 deaths per 100,000 people in Africa (Jamison, Feachem and Makgoba, 2006).

Against this global backdrop, Cape Town is referred to as the 'stab capital of South Africa' (Jooste, 2010). Later, Nyanga, a township in Cape Town, was given the title as the 'murder capital of South Africa' (Head, 2017). To date, Nyanga remains the "murder capital of South Africa". According to the 2018/19 crime statistics, Delft and Khayelitsha, both areas in Cape Town, follows immediately after Nyanga as having the highest murder rate in South Africa (SAPS, 2019). In addition, a high burden of penetrating trauma and road-traffic accidents may cause patients to experience exsanguinating haemorrhage on scene or in ambulance transit. Thus, there is a need to bridge the time-critical gap of a life-threatening insult and probable morbidity/mortality that could occur due to excessive blood loss before reaching the emergency department where blood transfusion may be administered.

There is no direct South African evidence for the burden of need, viability and EMS self-efficacy to safely enable prehospital emergency blood transfusion (EBT). Historically, the mainstay treatment strategy for volume resuscitation of trauma patients in South Africa, is large volumes of crystalloids which ultimately leads to clot dislodgements, dilutional coagulopathy, hypothermia, etc. (Nickson, 2015). According to the clinical guidelines of South Africa, limited volumes thereof should be used in resuscitation (SANBS and WPBTS, 2014).

This study seeks to appraise the need for EBT in the prehospital setting, and to investigate the viability of EBT as an advanced life support modality of care in the EMS. This will be investigated by placing blood on ambulance vehicles to determine their viability in-transit. Data will be collected from patient report forms fitting the criteria of class III/IV shock who could have been eligible for EBT. Questionnaires will be given to Emergency Care Practitioners (ECPs) to determine their self-efficacy about introducing blood as a resuscitation fluid.

1.2. Background to the research problem

Shock can be described as failure of the circulatory system, resulting in insufficient cellular oxygen utilization (Tintinalli, 2010). There are various types of shock which are classified according to the pathology, such as cardiogenic shock (from heart failure), hypovolaemic shock (from loss of circulating volume), septic shock (due to infections) anaphylactic shock (caused by an allergic reaction) and neurogenic shock (caused by damage to the nervous system).

The most common form of shock is hypovolaemic shock, due to haemorrhage (such as from trauma) or loss of circulating volume (which can include vomiting and/or diarrhoea common in the paediatric population) (Silverman and Wang, 2005). Haemorrhage is considered the most common cause of preventable trauma death worldwide (Cothren et al., 2007). The core pathophysiological aspects of haemorrhagic shock are caused by the failure of oxygen delivery to organs and tissues. Therefore, haemorrhage control and timely administration of PRBC are the most definitive aspects of resuscitation. Hypovolaemic/haemorrhagic shock is considered a medical emergency. It may occur in emergency cases when singular injury to blood vessels or when ‘solid’ vascular, fragile organs such as the kidney, spleen or liver is torn or ruptured (Henry, Stapleton and Edgerly, 2010). This form of shock that develops when the heart is unable to circulate adequate blood throughout the body, due to blood loss of one-fifth or more of the normal blood volume (Nall and Gotter, 2016). According to the same authors, preventing this shock leads to less complications than treating it. Therefore, treatment of the underlying cause (whether it is due to blood loss or fluid loss) should be attended to immediately as hypovolaemic shock could become life-threatening (Tisherman et al., 2015). The World Health Organisation (WHO) states that, traumatic injuries are one of the main causes of mortality in the world, with 90% of the injuries estimated to occur in low- and middle-income countries (Gosselin et al., 2009). Approximately 5 - 8 million people dies annually due to trauma worldwide (Spahn et al., 2013). Globally as stated by the WHO, road traffic injuries are now

the leading cause of death for ages 5 – 29 years and amongst the top three of mortality between the ages of 15 – 44 years (World Health Organization, 2015).

As previously mentioned, there is no direct South African evidence for burden of need for prehospital emergency blood transfusion, viability of prehospital emergency transfusion and EMS efficacy to safely and sustainably enable emergency blood transfusion. A study done by Mustafa et al. (2021) highlights the need for intervention for improved shock management by the ECP in the pre-hospital setting as current clinical recommendations are not being adhered to such as large volumes of crystalloid solutions have been used as prehospital treatment for volume resuscitation in trauma patients, for it to simulate an elevated blood pressure. However, limited volumes thereof should be used in volume resuscitation (SANBS and WPBTS, 2014). According to Lyon et al. (2017) prehospital transfusion of PRBC is associated with improved patient outcomes, in patients with major haemorrhage in the prehospital phase. PRBC are routinely used for hospital resuscitation as it provides an increased oxygen carrying capacity and more effective volume expansion (Doughty, Woolley and Thomas, 2011). PRBC are therefore stored at major trauma centres for immediate administration of EPRBC (group O), but not in the prehospital setting. It may therefore be prudent to implement the administration of EPRBC within the prehospital setting.

Before introducing emergency blood on EMS vehicles, the need of PRBC in-transit must be evaluated as blood is a very precious and scarce resource to be assigned and not be utilized. Storage temperatures and integrity of the PRBC must also be monitored for viability. Before the administration of blood, haemoglobin testing is routinely done in hospital. Currently, haemoglobin testing is not a standard test implemented by South African EMS and would need to be introduced on ambulances as a prerequisite to any prehospital blood transfusion implementations. Other measures, such as blood pressure, pulse, temperature, respiration rate, are all required before and during transfusion (SANBS and WPBTS, 2014), and are already a standard practice in the prehospital setting by ECP.

1.3. Statement of the research problem

Haemorrhagic/hypovolaemic shock patients are at risk of death, as well as disseminated intravascular coagulation, hypoxic brain injury, global myocardial ischaemia, with the risk of these conditions being heightened among the young, old, or in cases involving poly-trauma. The recommended definitive care is to stop the bleeding (surgically or using haemostatic agents) and to replace lost haemoglobin whilst providing supplemental oxygenation (Caroline,

2010). Despite blood transfusion being a standard treatment of care for severe haemorrhagic shock, it is not used as a standard practice in the prehospital setting in South Africa. In my opinion, this is possibly due to blood requiring a specific system to ensure it is stored at correct temperatures and remains viable in the prehospital setting. Quality control measures would also need to be included in this system to ensure patient safety. However, currently in South Africa prehospital emergency blood transfusions by the ECPs, are not administered despite their high skills set and the relative safety of group O administration.

Miller (2013), recommends a 1:1:1 ratio of PRBC:FFP:Platelets, (mimicking WB) for resuscitation (Miller, 2013). Logistically, issues such as maintaining the storage of Fresh Frozen Plasma (FFP) and platelets will be very difficult to maintain in the prehospital environment. FFP's will need to be stored at -18°C and thawed at 37°C in a water bath. Upon FFP being thawed, the product has 6 hours of expiry, if unused, it cannot be frozen again and would need to be discarded resulting in product loss. Platelets storage requires continuous agitation to prevent platelets from sticking to one another. Once platelets are removed from agitation, the product should be used within 6 hours (SANBS and WPBTS, 2014). PRBC storage requirements are easier to maintain, provided the PRBC remains within the range of 1°-6°C. This study seeks to appraise the relative burden of need for emergency blood transfusion (EBT[1]) in the prehospital setting (as a resuscitation fluid to increase oxygen delivery or as a start of a massive transfusion protocol[2]) and to investigate the viability of EBT as an advanced life support modality of care in the EMS.

1.4. Research rationale

Penetrating trauma, motor vehicle accidents as well as pedestrian vehicle accidents are common trauma cases presented to our large government tertiary hospitals, such as Tygerberg, Groote Schuur and Khayelitsha District Hospital, in Cape Town. Upon arrival at the emergency unit, EPRBC is given to patients experiencing exsanguinating haemorrhage. EPRBC are issued to patients for transfusion purposes, as the patient cannot wait for blood compatibility testing to be done, due to rapid blood loss. In my opinion having EMS vehicles

[1] Emergency blood transfusion of uncrossmatched, group O packed PRBC's, are routinely used to improve oxygen delivery. Packed PRBC's are routinely given as emergency blood to patients experiencing haemorrhage who are unable to wait for crossmatched blood (90 minutes).

[2] Fresh whole blood (WB) would be the gold prize treatment for massive/ongoing blood loss. Fresh WB is rarely possible. Packed PRBC's are routinely used containing no platelets or clotting factors. To prevent coagulopathy without waste a "massive transfusion protocol" (a set number PRBC concentrate, FFP's, platelets and cryoprecipitate in emergency cases) should be a practical plan of action (SANBS and WPBTS, 2014).

transport EPRBC on transit, and ECP administering the EPRBC to patients who fit the criteria for blood transfusion, may likely aid in prolonging stability of the patient, bridging the gap between life and mortality before reaching the emergency department.

ECPs are not trained to transfuse blood but are able to monitor patients' vital signs before and during blood administration, as per their scope of practice. For example, in instances where ECPs transport patients from hospital-to-hospital while a blood transfusion is occurring. All tests that would be required prior to administering blood are already routinely done on EMS vehicles except for the haemoglobin test. The haemoglobin test is a finger prick test, similar to the glucose test routinely done by ECPs.

Currently there is emergency blood on ambulance services in Texas (United States of America), Australia as well as in London. London is the first air mercy service in the United Kingdom to transport blood on board. Weaver (lead clinician of the London's Air Ambulance) believes carrying blood routinely is going to make a big difference for many patients, therefore blood is now also being carried by London's Air Ambulance's rapid response vehicles (Rehn, Davies, Smith and Lockey, 2017). Perkins, the trauma surgeon working on both air and ground with the ambulance service, says that the transfusion of blood on scene can transform prehospital care. He also mentions that about half of the patients die from bleeding due to traumatic injuries and often the bleeding is only really stopped once the patient reaches the hospital. However, one way to buy time is by replacing the blood lost. Therefore, he believes that carrying blood is a great step forward for ambulance services (Bowdler, 2012).

By conducting this study, we assessed the viability of EBT as an advanced life support modality of care in SA EMS. But before implementing a prehospital transfusion system, there must be a determination of need, in the Western Cape areas chosen to conduct the study.

1.5. Aims

This study aimed to address the contextual factors in a potential prehospital blood transfusion system. More specifically, it is imperative to ascertain whether blood as a scarce resource can retain its viability in the prehospital setting. This research study also aimed to estimate whether prehospital ALS practitioners have enough exposure to patients in shock (class III and IV haemorrhage) in need of blood transfusion, as well as their self-efficacy to administer EPRBC.

1.6. Objectives

The objectives of this research were as follows:

- To estimate the viability of packed red cells in the EMS setting, prior to the onset of haemolysis.
- To assess the practitioner self-efficacy for emergency blood transfusion.
- To estimate the burden of blood transfusion, need in the prehospital environment

1.7. Limitations and delimitations

- The study was kept in the prehospital setting
- Limitation to this study is that it was unable to address the confounding brought about by pain i.e. the physiological parameters that were used to measure shock could also be influenced by pain.
- Prehospital care does interface with hospital care, but due to the limited scope of this study, I have not included this. Therefore, the emergency department population was not included in this study.
- It would be highly desirable to conduct the experiments proposed in this study over an extended period, as opposed to the limited 2 months given to collect data.
- It would also be useful to determine the viability of blood on a larger scale as opposed to 24 units of blood.
- This MSc degree implies limited resources available, limited finances and limited time, being a maximum of 4 years part-time.

By conducting this study, we were able to determine if blood will remain viable in the prehospital setting, the self-efficacy of ALS practitioners to introduce EBT and lastly, to determine if there is a burden of need for EBT to patients presenting with class III or class IV haemorrhage and. This study may be beneficial as the viability of PRBC's as an advanced life support modality of care in the EMS was investigated. This study aimed to make the following recommendations to the HPCSA on completion:

- Conditions to manage temperature fluctuation and risk of haemolysis in blood on EMS vehicles
- Educative prerequisites to enhance practitioner efficacy in EBT
- The burden of need for EBT in the prehospital setting

There is no risk of therapeutic misconception[3] as no blood transfusion intervention was implemented (Henderson et al., 2007). This is due to blood being a scarce resource, therefore determining whether the need for ERBC in the prehospital environment is required. Furthermore, this study would also need to ascertain if ERBC will remain viable on EMS vehicles and determining the self-efficacy of ECPs to administer ERBC. Lastly ECPs should also be trained on blood administration if the need for ERBC in the prehospital environment becomes implemented.

[3] Therapeutic misconception is the false expectation that the study is intentioned to satisfy a therapeutic outcome. That may be the downstream consequence, but the proximal goal of scientific research is to generate new knowledge claims and contribute to the knowledge economy.
Therapeutic misconceptions exist when the defining purpose of the clinical research is misunderstood, which is to produce generalised knowledge, irrespective of whether or not the subject enrolled may benefit from the study or clinical trial (Henderson et al., 2007).

CHAPTER TWO: LITERATURE REVIEW

2.1. Introduction

Red blood cell transfusion is a life-saving intervention given to increase oxygenation directly to tissues in circumstances where Hb levels are low, in patients with exsanguinating haemorrhage or blood loss of any sort. There are four major blood groups; A, B, AB and O. Group O is the universal donor, in other words, it can be transfused to a patient of any blood group (Daniels and Withers, 2007). WCBS protects the health and well-being of both donors and recipients by providing donors with questionnaires about their health and lifestyle. This aids in eliminating donors who does not meet the donation criteria (WCBS, 2019). The donors who meets the donation criteria, with their consent, are screened for various viral markers, to ensure safe blood gets administered to the recipients. Blood is collected from donors into blood bags containing preservatives and anticoagulants to prevent PRBC's from clotting and to allow PRBC to survive storage. PRBC storage should be kept between 1°C and 6°C (Harmening, 2005).

Haemorrhagic shock in patients occur when there is severe blood loss internally or externally, thus causing a decrease of nutrients and oxygen to the tissues (Tintinalli, 2010). Various intravenous (IV) resuscitation solutions are available for use such as; crystalloids, colloids and blood products. The choice of the resuscitation fluid also depends on the cause of the deficit (Procter, 2018). Preventing shock leads to less complications than to treat it, therefore treatment of the underlying cause, whether it is due to blood loss or fluid loss should be attended to immediately as this could become life-threatening if untreated (Tisherman et al., 2015).

2.2. History of ABO and Rhesus blood grouping

The ABO blood group system was discovered and reported by Karl Landsteiner in 1900-1901 (Watkins, 2001) (Issitt and Anstee, 1985). To date it remains the most important blood group system in transfusion medicine. It is the only blood group system in which individuals predictably have antibodies in their serum to antigens that are absent from the red blood cells (Landsteiner's Law).

Landsteiner drew blood from co-workers in his laboratory, separated cells and plasma, and mixed cells and plasma from various people on glass tiles. He was able to identify three different patterns of reactivity which he termed A, B and C. These were later reclassified as A, B and O. Von Decastello and Sturli discovered group AB and reported this blood type in 1902 (Klein and Anstee, 1988). Group AB still remains the rarest of the common ABO types. The blood groups A, B, AB and O remains the four major groups in the ABO system. The Rhesus (Rh) blood group system, is the second most important blood group system following ABO. This blood group system was discovered in 1940 by Karl Landsteiner and Alexander Wiener (Schwarz and Dorner, 2003). The Rh factor determines whether an individual is Rh positive (presence of the D antigen on the red cell surface) or Rh negative (absence of D antigen). Group O Rh negative donors are called the “universal blood donors” (Rath, Mitra and Mishra, 2014) because their blood groups can be transfused into any patient of the ABO blood groups, their red cells lack both A and B antigens therefore they are unable to react with the anti-A and/or the anti-B that is present in the patients plasma and lastly, because their naturally occurring antibodies are not harmful to the PRBC of the patient. According to the Standards of Practice for Blood transfusion in South Africa, the selection of blood products for transfusion in times of emergency are: Rh negative recipients to receive Rh negative blood provided they are females of childbearing age, and males as well as post-menopausal females to receive Rh positive blood (Bellairs and Ingram, 2013).

2.3. Safety of blood products at WCBS

WCBS strives to provide safe and effective blood and blood products (WPBTS, 2013). To ensure safe blood supply, all donors are required to complete a donor questionnaire. In this manner it helps to defer people who are at greater risk of having transmissible disease infections due to their high-risk behaviour. A healthy person weighing over 50kg and is between the ages of 16-65 years may donate blood. A donation of 475ml of blood is made and it takes less than 30 minutes. Three to four hours before donating blood the donor must eat and drink lots of fluids. On arrival at the donation clinic the donors must fill out the health and lifestyle questionnaire honestly for the sake of the donors’ own health as well as the patient to which the blood will be given. A donor card or drivers licence or ID must be brought to the donation clinic for identification purposes. A finger prick test will be done to test the haemoglobin (iron) levels of the donor. For males the haemoglobin must be more than 13.5 g/dL and for females more than 12.5 g/dL. If the haemoglobin values are not within these ranges, the donor can be deferred for donation. Donors are given refreshments afterwards and are monitored to ensure that they do not collapse or suffer

adverse effects of the donation. Blood must not be donated if you are exposed to HIV or STD's and if you are in a new sexual relationship less than 6 months. If a person is pregnant, had exposure to needles, have a planned or recent surgery, history of cancer, severe heart or lung disease, epilepsy or bleeding disorders they are then deferred.

Donors who ultimately meet the donation criteria are bled and screened for disease markers. The donated blood is carefully screened firstly by deferral of donors by the health and lifestyle questionnaires and secondly by screening for hepatitis viruses and HIV, using the latest genomic amplification technology.

2.3.1. Virology testing at WCBS on all blood donations

The Virology Laboratory at WCBS screens all donors' blood every single time they donate (WCBS, 2019) for the following viral markers:

- HIV-1/2 antibodies (anti-HIV).
- Hepatitis C antibodies (anti-HCV).
- Hepatitis B surface antigen (HBsAg).
- HIV-1 RNA (Ribonucleic acid), HCV RNA and HBV DNA using Nucleic Acid Testing (NAT).
- Syphilis (TPHA)

Having confidence in results obtained is of utmost importance when screening blood. Enzyme-linked immunosorbent assay (ELISA) relies on the detection of serological markers which may not appear in blood up to 3 months after infection. Therefore, there is a risk of them not being detected and an increase in the risk of transfusion transmitted infection. Nucleic Acid Testing (NAT) reduces the window period by detecting viral RNA/DNA rather than waiting for the slower antibody response of the body. NAT is currently the most technological advanced blood testing system available commercially (Kleinman et al. 2006).

2.4. Blood products

After the blood donation process, the whole blood (WB) donated unit is processed into various blood components. Such as: Packed Red Blood Cells (PRBC), Fresh Frozen

Plasma (FFP), Infant Packed Red Blood Cells, Paediatric Red Blood Cells, Platelets and Cryoprecipitate. These are just the common blood products processed at the WCBS.

Various blood components are important for component therapy. Blood component therapy is required to achieve the desired effect of the product being administered, without letting the rest of the blood product go to waste (Hall and Murphy, 2015). What this means is, administer the blood product the patient requires. A patient could require blood due to: increase oxygen carrying capacity when impaired with haemorrhage as well as to correct anaemia (Tsai, Hofmann, Cabrales and Intaglietta, 2010), large blood volume haemorrhaging, coagulation factor deficiencies and general surgery.

According to the Clinical Guidelines for the use of Blood Products in South Africa (Bellairs and Ingram, 2014), the following indications is required:

2.4.1. Whole blood (WB)

WB is used when there is large blood volume haemorrhaging and for exchange transfusion in neonates.

2.4.2. Packed Red Blood Cells (PRBC)

PRBC is used when there is a need for an increase in oxygen carrying capacity, when 30% - 40% of blood loss has occurred, for general surgery, to correct anaemia and obstetric haemorrhage.

2.4.3. Fresh Frozen Plasma (FFP)

FFP is used in coagulation factor deficiency, vitamin K deficiency, reversal of warfarin overdose and treatment of Thrombotic Thrombocytopenic Purpura (TTP) (Pereira, 2009).

2.4.4. Pooled Random Donor platelets

Pooled Random Donor platelets is used for thrombocytopaenia and dilutional effects of massive transfusions.

2.4.5. Cryoprecipitate

Cryoprecipitate is a rich source of fibrinogen; therefore, it is used to control bleeding in trauma cases as well as for factor XIII deficiency and patients with hypofibrinogenemia (Holcomb et al., 2013).

Therefore, it is crucial to know what blood component is required to treat a patient, so that the patient is not necessarily exposed to a product not needed, thus reducing any associated risks. For instance, a patient that is treated for chronic anaemia, requires red blood cells and not any plasma, so the product of choice would be a PRBC. Plasma in this case would be a waste especially if multiple transfusions are given, allowing unnecessary exposure of plasma and can therefore open up the patient to risks of developing transfusion related lung injury (TRALI), acute transfusion reactions and transfusion-associated circulatory overload (Hall and Murphy, 2015).

2.5. Storage temperatures of PRBC and anticoagulants used in the blood packs

PRBC concentrate donations are able to remain viable between storage temperatures of 1°- 6°C for as long as 42 days and whole blood (WB) for 35 days, depending on the preservative solution used (Harmening, 2005). Anticoagulants and preservative solutions in blood packs are needed, to allow PRBC to survive in storage conditions for as long as possible and to prevent the PRBC's from clotting. There are numerous changes that occur in PRBC's during storage, collectively known as "storage lesion" (Bennett-Guerrero et al., 2007). A recent study suggested that "storage lesion" includes: Changes in PRBC morphology, a decrease in the concentration of ATP levels, acidosis with a decrease in the concentration of 2,3-DPG and loss of function of cation pumps (Choudhury and Mathur, 2011). During transportation of PRBC, storage temperatures of 1°-10°C must be maintained (SANBS and WPBTS, 2016).

2.5.1. Changes to PRBC during storage

PRBC haemolysis is an important quality indicator of blood manufacturing processes as it represents the breakdown of PRBC, causing structural and morphological changes. This release of free haemoglobin, lactate dehydrogenase (LDH) and potassium cations into the plasma causes osmotic fragility, leading to cell membrane deformity and this is due to oxidative damage caused by storage

lesions (Chaudhary & Katharia, 2012). Although some storage lesions can occur within days or weeks, increased levels of LDH and potassium levels may well be detected within hours of storage (D'Alessandro et al., 2010). Haemolysis in PRBC's can occur during blood collection, processing, handling of the PRBC unit, storage thereof and also during transportation (Zimmermann et al., 2003).

PRBC haemolysis is said to be an obvious marker of incorrect PRBC storage and bacterial contamination (Sawant et al., 2007). Haemolysis can be determined by visual inspection, spectrophotometric assays, photometeric method as well as a microplate technique. Visual inspection of the PRBC pack and segments attached to the pack are used in most blood banks as an easy and quick detection of haemolysis. This is observed by inspecting the PRBC pack and tubing for grossly pink discoloration of the plasma (Janatpour et al., 2004) as the normal colour of plasma in these segments can be described as yellowish or straw in colour (Choudhury and Mathur, 2011). An important factor affecting haemolysis of PRBC, is the storage of PRBC's as temperatures can cause membrane deformability and therefore affects membrane stability during processing. According to a study done by Zimmermann *et al.* (2003), haemolysis is also said to be a very important parameter for assessing the quality of stored PRBC, therefore it is crucial to monitor the temperatures of the PRBC regularly during storage and transportation. If haemolysis is suspected, the unit of PRBC should be allowed to settle for 24 hours or more to minimize red cell contamination of the supernatant before verifying haemolysis (Zimmermann et al., 2003).

According to the guidelines of the Council of Europe, the acceptable haemolysis level is 0.8%, and a recommended maximum of 1% haemolysis levels by the US Food and Drug Administration (Makroo et al., 2011). This study measured haemolysis levels of PRBC's against the 0.8% European Guidelines, to increase the sensitivity of results obtained.

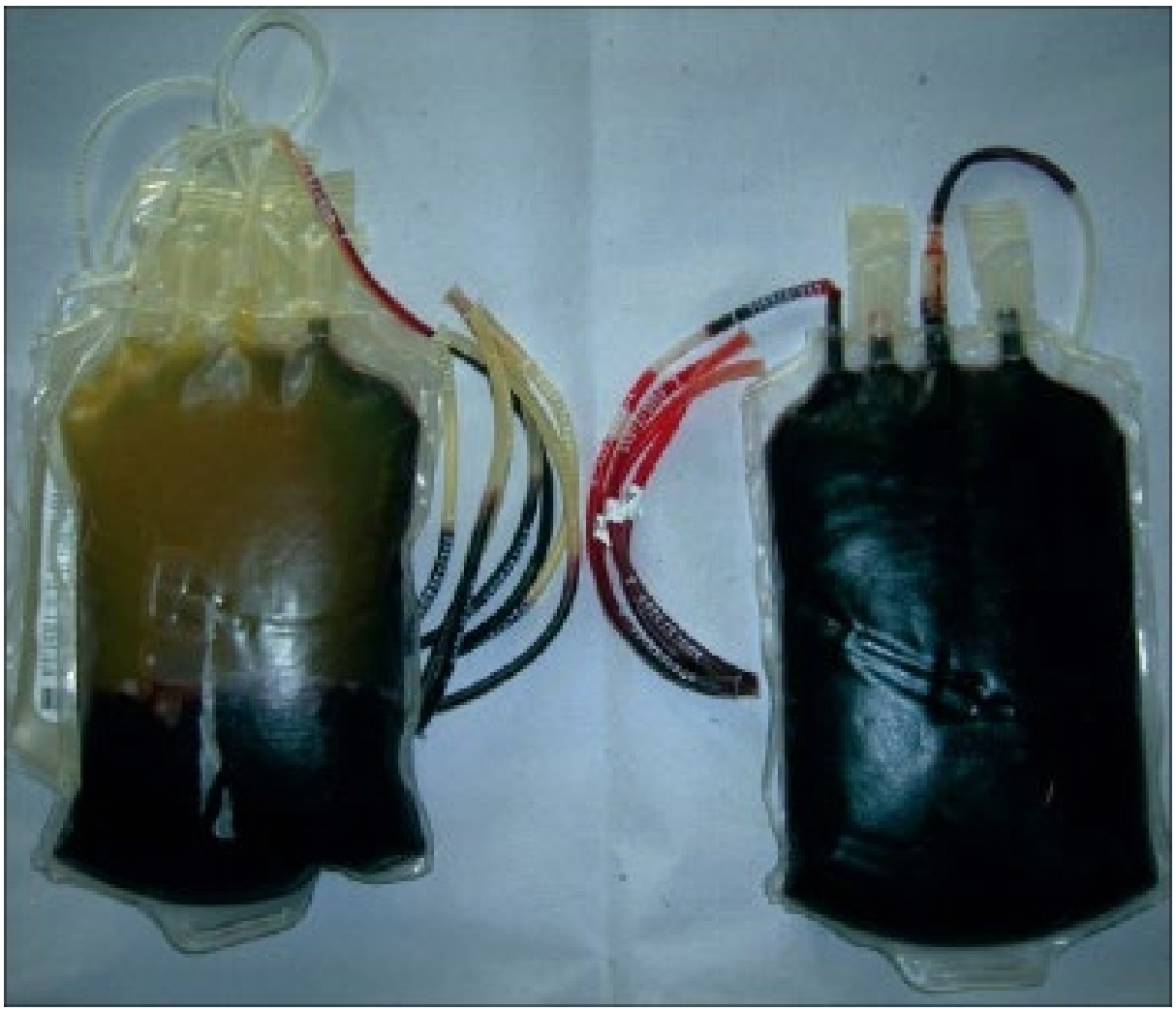

Figure 2.1: Colour of plasma in segments (Adapted from Choudhury and Marthur, 2011).

2.5.2. Oxidative markers on pre-stored blood

Pre-stored blood in the blood bank undergoes various changes, biochemically and mechanically, commonly known as 'red blood cell storage lesions', which will most likely affect their post-transfusion performance (Antosik et al., 2015). Some of the biochemical modifications to the RBC membrane includes lipid peroxidation[4] - injury to proteins, carbohydrates lipids, membrane haemoglobin association and oxidation, which are accompanied by metabolic depletion and intracellular calcium levels as well

[4] Lipid peroxidation is the oxidative break down of lipids. Lipid peroxidation is also referred to as a metabolic process which involves free radical attack on polyunsaturated fatty acids that may affect the cell membrane structure as well as the RBC function (Repetto, Semprine and Boveris, 2012).

as loss of shape, loss of membrane rigidity and decreased oxygen delivery (Hess, 2010).

Conjugated Dienes (CD) is a commonly used method to determine lipid peroxidation. During the lipid peroxidation process, the diene conjugations are formed which also absorbs ultraviolet light that can be read using a spectrophotometer between the range of 230-235nm. The extent of lipid peroxidation determined at this wavelength, can be related to the contents of the conjugated dienes in lipid extracts of tissues (Desai, Farris and Ray, 2014).

Thiobarbituric acid reactive substances (TBARS) however, is widely used as a generic measurement of lipid peroxidation in biological fluids. TBARS is seen as a good indicator for measuring oxidative stress levels in a biological sample, provided that the handling and storage of biological samples in testing has been done correctly (Aguilar Diaz De Leon and Borges, 2020). TBARS measurement yields a coloured chromogen fluorescent red-pink colour which can be read using a spectrophotometer at 532nm. This test involves the reactivity between thiobarbituric acid (TBA) and the lipid peroxidation products, malondialdehyde (MDA) (Ayala, Muñoz and Argüelles, 2014). This reactivity leads to the forming of MDA-TBA_2 adducts known as TBARS.

MDA is also known to be quite popular amongst the oxidative stress markers as it is said to give the most reliable results to determine oxidative stress in clinical situations (Giera, Lingeman and Niessen, 2012).

2.5.3. Prehospital blood storage

PRBC components were initially carried for transfusion by aircraft since World War II (Rous and Turner, 1916) but until recently, no reports have been found regarding whether or not storage of PRBC's is affected on a helicopter flight. Otani *et al*. (2012), determined the effects helicopter transportation has on PRBC products. In this study, PRBC's was transported by helicopter in a cooler box with ice packs, maintaining the temperature of 2°-6°C, even though the ambient temperatures ranged between 6.1°-27.8°C. The highest air pressure calculated was 755mmHg and the lowest air pressure calculated was 351mmHg that maintained the PRBC integrity. The PRBC's transported, and the PRBC's that remained in the refrigerator throughout the transportation period used as a control, was weekly screened over six weeks for: haemolysis percentage, haematocrit (HCT), pH, ATP, 2,3 DPG, glucose, supernatant potassium (mmol/L) and supernatant Hb (mg/dL).

In both the control and flight groups the percentage haemolysis and supernatant Hb concentration was increased. However, a statistically higher increase in percentage haemolysis and supernatant Hb was observed in the flight group, in comparison to the control on day 42 (end of shelf-life). The study concluded that there were no significant changes to PRBC's by helicopter transportation, except an increase in haemolysis at the end of shelf-life period but was still regarded as a small change and possibly having no clinical implications (Otani et al., 2012).

Another study was conducted whereby blood was stored and transported in an unconventional way, in the prehospital environment, using drones. This study was conducted in Rwanda and found that blood products transported by drones was found to be a viable option for blood product transportation as the blood products remained unaffected. The drone was flown 100 metres above the ground for 26.5 minutes, covering about 13-20km for each test. Approximately 2-3 units of blood products were flown in a cooler box and wet ice to keep the temperature constant. After each test, the PRBC's were checked for PRBC damage/haemolysis (Amukele et al., 2016). Rwanda is the first country to launch a national drone delivery service. This is to provide medical supplies quicker to the majority of the 11 million population of Rwanda, in the hard-to-reach rural areas. The drones are battery powered with a range of 150km and the drones can carry approximately 3 units of blood. The cargo weighs +/- 1.5kg and the drone +/- 13kg. The drones are fired into the air using a catapult, and the deliveries are made using a biodegradable parachute. The drones are able to deliver medication or blood to destinations within 15-30 minutes (Daily Nation, 2016). According to the president of Rwanda Paul Kagame, the drone delivery service is considered a milestone for Rwanda.

2.6. The EMS organization in the Western Cape

The EMS organization in the Western Cape provides a 24-hour medical response and prehospital service. Whether it is basic medical assistance that can be treated on scene or transportation to the nearest health care facility for further treatment. The EMS system is divided as follows:

- **Ambulance services -** There are 251 ambulance vehicles operational in the Western Cape (Western Cape Government, 2015). Each ambulance is fully equipped with a full medical kit consisting of medical equipment, and medication and each ambulance has a stretcher.
- **Rescue service -** These EMS technicians provide both medical and technical care on scene. Their specialized skills are removing patients from wrecked vehicles using Jaws of Life and other hydraulic extrication equipment. They assist with wilderness search and rescue as well as water rescues (Western Cape Government, 2015).
- **Healthnet -** Provides non-emergency transportation for patients between health care facilities.
- **Aeromedical Service (AMS) -** Contracting with the WCG, The Red Cross Air Mercy Service (AMS) also assists the EMS teams by providing air ambulance service to remote rural communities, rural health outreach areas. The AMS provides the service of airlifting injured sailors, swimmers, injured mountain climbers, injured motor vehicle crash victims as well as inter-facility transfers of high acuity patients (The SA Red Cross Air Mercy Service, 2014).

2.7. EMS practitioners

The EMS practitioners work in pairs in the ambulance and provides quality-based care on their levels of training. Below are some of the professions registered under the Professional Board for Emergency Care:

- Basic Life Support (BLS) registered with the HPCSA as a Basic Ambulance Assistant (BAA)
- Intermediate Life Support (ILS) registered with the HPCSA as an Ambulance Emergency Assistant
- Advanced Life Support (ALS) registered with the HPCSA as a Paramedic (HPCSA, 2017).

There are also other qualifications such as the 2-year diploma course where practitioners are registered with the HPCSA as Emergency Care Technicians (ECT). Lastly the recent university qualification that is the 4-year bachelor's degree in Emergency Medical Care (BEMC).

EC providers registered with a bachelor's degree have the highest clinical scope and are referred to as Emergency Care Practitioners (ECPs). The term 'Paramedic' is a protected term according to the Health Professions Act, Act 56 of 1974 for a particular registration category. However, emergency care provider or EMS personnel/worker can be used across all registration categories.

Only the ECPs were included in this study, due to time constraints for training as well as limited funding.

2.8. The EMS triage system

The triage[5] system commences as soon as the emergency call is made to the emergency call centre. The call agent will assess the incident based on the information provided by the caller and a colour code is assigned based on severity as in the emergency centres.

When a priority 1 (P1) medical emergency is established by the call agent, the emergency medical service will arrive within 15 minutes at 80% of the time, whereas with non-life-threatening emergencies which is priority 2 (P2) calls, will be seen to pending P1 emergencies (Western Cape Government, 2016).

2.9. Shock in trauma patients

Shock can be described as the clinical expression of circulatory failure due to insufficient cellular oxygen utilization. Shock is a common condition in critical care and therefore determining early signs of shock can be life-saving. It is crucial to provide sufficient

[5] Triage means 'to sort out' and is a way of discriminating patients on the basis of immediacy of the threat to health or life using colour codes: Red – life threatening injury; Yellow – potential life-threatening injury; Green – walking around; Blue – dead.

haemodynamic support early to patients in shock, to prevent worsening organ dysfunction and failure (Vincent and De Backer, 2013).

According to Bonanno (2011), there are various types of shock, including:

- Hypovolaemic shock can be divided into haemorrhagic (blood loss) and non-haemorrhagic (fluid loss) – this shock occurs when the total body volume of fluid/blood supply drops below normal levels due to internal or external bleeding, extensive burns, dehydration, etc. this results in the heart being unable to pump enough blood throughout the body, insufficient oxygen to the tissues and blood pressure may be decreased due to severe blood loss.
- Cardiogenic shock – occurs when the heart is severely damaged by heart failure or a myocardial infarction.
- Anaphylactic shock – occurs when having a severe allergic reaction.
- Septic shock – occurs when bacterial infection causes toxic shock syndrome and results in the drop of blood pressure.

There are 3 main goals that are required in the emergency setting when treating patients with hypovolaemic/haemorrhagic shock:

I. Control further blood loss
II. IV fluid resuscitation
III. Maximize oxygen delivery

The following classification is a mainstay of emergency medicine assessment of shock, due to urgent need for a diagnosis and severity. The classification of haemorrhage can also help guide volume replacement. The 4 classes of haemorrhage are as follows:

Table 2.1: 4 Classes of haemorrhage (Adapted from Gutierrez, Reines and Wulf-Gutierrez, 2004)

	Classification of haemorrhage			
Class	I	II	III	IV
Blood loss (ml)	<750	750–1500	1500–2000	>2000
Blood loss (%)	<15%	15–30%	30–40%	>40%
Pulse rate (beats/min)	<100	>100	>120	>140
Blood pressure	Normal	Decreased	Decreased	Decreased
Respiratory rate (breaths/min)	14–20	20–30	30–40	>35
Urine output (ml/hour)	>30	20–30	5–15	Negligible
CNS symptoms	Normal	Anxious	Confused	Lethargic

According to Gutierrez, Reines and Wulf-Gutierrez (2004), haemorrhagic shock is rapidly fatal and the use of IV crystalloids/colloids and blood products available can be lifesaving. When haemorrhage exceeds 30% of the estimated blood volume (EBV), which is class III haemorrhage, the use of blood and blood products is required. In critically ill patients, with no risk of tissue hypoxia, a haemoglobin level of 7-8g/dl is an appropriate threshold for blood transfusion.

Low haemoglobin concentrations in the acute setting may be a late sign of haemorrhage (Bruns et al., 2007), as an actively bleeding patient has dubious diagnostic value considering it takes time for the various intravascular compartments to equilibrate. Diagnosis of shock should rather be guided by the flow rate of blood loss as well as the changes in haemodynamic parameters, such as: increase or decrease of BP as well as heart beats per minute, cardiac output, central venous pressure, pulmonary artery occlusion pressure, and mixed venous oxygen saturation (Gutierrez, Reines and Wulf-Gutierrez, 2004).

Shock occurs in 3 successive stages namely: Compensated, decompensated and irreversible shock. The goal of EMS is to recognize the signs and clinical symptoms of these stages to commence with immediate treatment before permanent damage occurs. Compensated shock is the early phase of shock, which occurs when the body is still able to maintain adequate perfusion to the brain and vital organs, maintaining normal blood pressure. In haemorrhagic shock 15%-30% of blood loss can be estimated in the compensated phase. Decompensated shock/progressive shock is the next phase of shock, in which the body's compensatory mechanisms fail and blood pressure starts dropping. At this point, the body is unable to maintain adequate perfusion to brain and vital organs and patients' condition deteriorates (Nolan and Pullinger, 2014). In haemorrhagic shock 30%-40% blood loss can be estimated in the decompensated phase (Caroline, 2010). Lastly, irreversible shock is the terminal stage of shock that progressed due to cell injury beyond the stage where resuscitation is possible. In haemorrhagic shock >40% blood loss can be estimated in this stage.

2.10. Intravenous (IV) fluids used in resuscitation

Traumatic injury is a frequent occurrence throughout the world (Jabaley and Dudaryk, 2014). Haemorrhage within the first 24 hours of arriving to an emergency department is one of the most common causes of death (El Sayad and Noureddine, 2014). This is due to exsanguination and coagulopathy that could be prevented using the appropriate resuscitation medium.

Intravenous (IV) fluids are described as chemically prepared solutions that are given to patients to replace fluid loss based on what the body requires and serves as a delivery medium for IV medications. The choice of the resuscitation fluid depends on the cause of the deficit. IV fluids or resuscitation fluids can affect the body in various ways, as some are designed to increase

intravascular volume or volume of circulating blood, by remaining in the intravascular space; while others are designed so that the IV fluids are evenly distributed between the interstitial, intravascular and cellular spaces (NICE, 2013). IV solutions mainly consist of sterile water which is chemically referred to as the “solvent”. IV solutions also consist of “solutes” which refers to the electrolytes consisting of potassium (K^+), sodium (Na^+) and chloride (Cl^-) (Kayilioglu et al., 2015). The solvent and solutes together makes up the IV solution. There are several types of IV fluids such as: crystalloids, colloids and blood products.

2.10.1. Crystalloid solutions

Crystalloid solutions are solutions of ions commonly used in the prehospital setting as first line resuscitation therapy which includes concentrations of electrolytes Na^+ and Cl^- that determines their tonicity. Crystalloids can be subdivided based on their tonicity (Myburgh and Mythen, 2013). The tonicity of crystalloids describes the concentration of solutes dissolved in the solvent (sterile water) as compared to that of body plasma. A crystalloid solution can either be an isotonic, hypertonic or hypotonic solution. In prehospital IV therapy, isotonic solutions such as: Normal Saline Solution (NSS) and Lactated Ringers/Ringers lactate (RL) are commonly used for fluid replacement due to their ability to expand the volume of circulating blood.

About two-thirds of the infused volume moves into the tissues and the remaining volume stays in the intravascular space, thus more fluid administration is required for circulating volume. The increased volume hereof leads to side-effects such as oedema (NICE, 2017).

A large volume of approximately 2 litres of crystalloid solution (excessive amounts of sodium chloride 0.9%) is required for resuscitation according to the teachings of the Advanced Trauma Life Support. The disadvantages to this approach are: clot dislodgements, hypothermia, dilutional coagulopathy, impaired oxygen delivery possibly due to dilutional anaemia and worsening metabolic acidosis (Nickson, 2015).

A structured review on crystalloid solutions was done by Orbegozo Cortés *et al.* (2014), that included 28 studies investigating the physiological effects of crystalloids in various clinical situations. The review found that normal saline increased blood loss therefore a need for blood transfusion increased and Ringer's lactate solution caused an increase in lactate levels. This review concluded that overall the studies were inconclusive as to whether or not crystalloid solutions made any difference to patients' mortality or morbidity, as these 28 studies lacked definitive conclusions as the clinical settings was all different (Orbegozo Cortés, Rayo Bonor and Vincent, 2014).

2.10.2. Colloid solutions

Colloid solutions contain large proteins which are referred to as the solutes. Because the proteins and other molecules are too large to pass through the walls of the capillaries onto the cells, the colloids remain in the blood vessels for longer, thus increases the intravascular volume significantly. Colloids however, can cause cells to lose too much water and become dehydrated as they have the ability to attract water from cells into the bloodstream. Examples of colloids available include hydroxyethyl starch (HES), albumin, gelatin and dextran (Lira and Pinsky, 2014).

Caution should however be taken upon administering of HES, as the haemodilutional effects of this fluid can be exacerbated, negatively affecting the platelet count of the patient, which in turn could cause negative effects on coagulation and clotting times (Marx and Schuerholz, 2010).

Colloids are relatively expensive, has a very short shelf-life and very specific storage requirements, making them more suitable for use in the hospital setting. Colloids were also found to be associated with multi-organ failure (Hilbert-Carius et al., 2018).

2.10.3. Blood and blood products

While crystalloids replace blood volume, they do not contain clotting factors nor oxygen as blood does. Therefore, blood is said to be the most desirable fluid for resuscitation because of its haemoglobin that carries oxygen to the cells (Jooste, 2012), unlike crystalloids and colloids. According to the clinical guidelines of South Africa, the only

fluids that can be given concurrently through the same IV device as PRBC transfusions are; NSS, 4% albumin, plasma protein fractions, ABO compatible plasma and calcium-free balanced salt solutions.

Over the last 2 decades, both animal and human studies showed that large amounts of crystalloids in attempt to increase BP to normal, resulted in bleeding from injured vessels, haemodynamic decompensation and increased mortality (Heckbert et al., 1998) (Hahn, 2012). Large amounts of crystalloids and overzealous resuscitation could dilute the blood that is still present, causing dilutional-hyperchloraemic acidosis (any change in pH can have a detrimental effect on enzymatic function in the body that can result in organ dysfunction) and possible dislodgement of blood clots in the circulatory system (Levi and Jonge, 2007).

According to Takasu *et al*. (2010), if blood loss is the primitive derangement, then blood should be the corrective action replacement. The study consisted of uncontrolled haemorrhagic shock in rats. The resuscitation fluid used was crystalloids and it was found that crystalloids on its own is not ideal and even a brief delay in blood administration could worsen survival. Therefore, early blood transfusion combined with crystalloid solution seemed to be the ideal infusion solution.

In whole blood donations, platelets and other clotting factors deteriorate within hours of donation. Therefore, physically separating these components soon after donation to maintain their properties under different storage conditions is required. Different components can be used for different clinical uses. Clinical indications for whole blood is limited, since red cell concentrates is more appropriate in situations where an increase of oxygen carrying capacity is required (SANBS and WPBTS, 2014). The following blood products are derived from whole blood donations: Packed red blood cells (PRBC), Fresh frozen plasma (FFP), platelet concentrates and cryoprecipitate.

2.11. Haemovigilance

Haemovigilance can be described as ‘A set of surveillance procedures covering the whole transfusion chain (from the collection of blood and its components to the follow-up of

recipients), intended to collect and assess information on unexpected or undesirable effects resulting from the therapeutic use of labile blood products, and to prevent their occurrence or recurrence' (World Health Organization, 2016). The haemovigilance system is a continuous and standardized system for observing, recording, reporting, analysing, monitoring and intervention. This covers all processes throughout the transfusion chain, starting with blood donation, processing, transfusing of blood products to patients, monitoring the patient, reporting and investigating any non-conformances that could have occurred throughout the transfusion chain (Liang et al., 2018).

According to section 68 of the National Health Act 61 of 2003 read with regulation R179 published in Government Gazette, haemovigilance is a legal requirement for all organisations undertaking any part of the transfusion chain within South Africa.

The legal responsibilities of practitioners who prescribe and transfuse blood products are the following; transfusion to be given for clear clinical indications, educate patients about inherent risks, inform patients of other alternatives, obtain informed consent, ensure the correct identification of patient and blood products to be transfused, ensuring compatibility testing has been completed, products not expired, handled and stored correctly. Blood should also be transfused at a specified rate via an appropriate administration set, patient should be observed during transfusion, manage and report any transfusion reactions and deaths, lastly retaining blood packs as required by blood services (Wessels, 2017).

CHAPTER THREE: RESEARCH METHODOLOGY

3.1. Ethical consideration

Ethical approval from the Health and Wellness Sciences Research Ethics Committee was obtained (CPUT/HW-REC 2017/H32) (see Annexures A, B, C & D). An approval was also obtained from the Provincial Health Department, using the National Health Research Database for access to the EMS database on typology of cases (see Annexure E). Permission from the Professional Board for Emergency Care (PBEC), Health Professions Council of South Africa (HPCSA) was sought where any scope infringement or enhancement arises as the HPCSA is mandated to guide professionals and protect the public.

Ethical consideration was submitted to the WCBS ethics committee for ethical clearance, and a consent letter of approval was given by CEO: WCBS (see Annexure F). An application was also made to the Red Cross Air Mercy Service Research Committee and approval was granted (see Annexure G).

3.1.1. Morals, ethics and conduct

Morals are mainly driven by the individual's principle that indicates what we should do. Ethics are driven by informed guidelines, that informs us why and how we should do a specific task. Conduct - norms for conduct is mostly laid down in a profession or society, distinguishing acceptable from unacceptable behaviour. The first international document advocated voluntary and informed consent was the Nuremberg Code. The "Nuremberg Code" was established in 1948, stating that 'the voluntary consent of human participants is absolutely essential' (Parija, Mandal and Acharya, 2011).

3.1.2. Basic ethical principles

The core ethical principles in research are to do good (beneficence) and do not harm (non-maleficence). In practice, what this means to a researcher is that the following basic principles of ethics must be maintained: informed consent, minimizing the risk of harm to participants, protecting their anonymity and confidentiality, avoid using deceptive practices and giving participants the right to withdraw (Jahn, 2011).

3.1.3. Informed consent

The finger prick test is invasive insofar as the skin is breached and the sub-dermal capillary is punctured. Special permission was not needed as the target population for the Hb finger prick test was done on patients who were in shock that may have been unconscious or not at their full mental capacity to sign any consent forms. Finger prick testing is routinely done by emergency care personnel to determine blood glucose levels. If these patients were to be excluded, this would have meant excluding the group of patients who has the most beneficence from what is trying to be accomplished. On this basis, the patients' right to autonomy was protected by the practitioner appraising the shock state and conducting the haemoglobin finger prick test on the basis that it may yield some future value for patients who are unconscious. The only deviation from standard practice that this study required was the allocation of an additional blood droplet for haemoglobin analysis. As this analysis was used to only document the at-risk shock population and not actually base any clinical decision to administer or withhold blood or any other medication, the risk of false-positive or false-negative readings does not threaten patient safety. In this way, the risk of 'therapeutic misconception[6]', another ethical concern, was averted.

Informed consent was given by EMS personnel to partake in the study after briefing them about what the study is about and their role in this study. Oral consent was asked to patients who were conscious.

3.1.4. Protecting patient's confidentiality and anonymity

Identifiers such as patient names were removed and replaced by numeric codes, before data was provided for analysis. Password protected files were used to store all data obtained to protect the confidentiality and anonymity of patients.

[6] Therapeutic misconception is the false expectation that the study is intentioned to satisfy a therapeutic outcome. That may be the downstream consequence but the proximal goal of scientific research is to generate new knowledge claims and contribute to the knowledge economy.
Therapeutic misconceptions exist when the defining purpose of the clinical research is misunderstood, which is to produce generalised knowledge, irrespective of whether or not the subject enrolled may benefit from the study or clinical trial (Henderson et al., 2007).

3.1.5. Concern for the Public Interest

There are many reasons why adhering to ethical norms in research is required, for example; public money and building public support for research. Funding will most likely be given for a research project, if the public can trust the integrity and quality of the research conducted. Public money also promotes accountability to the public. For instance, national policies on research misconduct, the human subjects protections as well as animal care and use are needed in order to make sure that researchers who are funded by public money can be held accountable to the public (Resnik, 2015).

In relation to my study, the concern for public money and public interest is paramount to this research as the study is located in a public EMS system. This EMS system uses approximately R1 billion rand per annum which is the fourth largest expense in health in the Western Cape. This implies that the public has an interest in improving the system to get better value and quality out of tax spent (South African Government, 2017).

3.2. Paradigmatic choice

Research paradigms are a philosophical framework that influences, and guides research being conducted. The paradigm chosen is post-positivism (Bergman et al., 2012).

The rationale for choosing post-positivism is due to the study aiming to determine factors that may or may not be controlled, as this study is not in a laboratory environment. This study is in the ‘real’ world where it is difficult to control for set rules, human behaviour or environmental factors. In fact, it is the variation in these factors that will inform on the feasibility of blood transfusion in the EMS being the object of the study. On this basis, the positivist ideology would be a misfit as not all parameters can be controlled in the environment in which the study will take place. The ontological view of the researcher is that of critical realism. This implies that reality is assumed to exist however, evidence in research is open to error due complexity of the investigation. The critical realism approach will be captured in the study accepting that blood is able to remain viable in hospital-to-hospital transportation, but to determine if blood is able to remain viable in the prehospital setting under temperature extremities, varying transportation durations and environmental conditions. The epistemological considerations are

relative objectivism[7]. In the context of this study, we know that blood has value in the treatment of shock. However, we do not know whether the South African reality is conducive to this. The strategic approach to the methodology is that of using a deductive approach (Bergman et al., 2012).

3.3. Study Design

This study consisted of 3 parts and therefore all 3 study designs are discussed below:

3.3.1. The viability of blood in the prehospital setting

This research design is a quantitative experimental study. This study was experimental as testing of the PRBC was conducted on a weekly basis against controls, for various factors that determined the viability of blood. Thereafter, the results obtained was analysed and the quantifiable data was presented in graphs (Sousa, Driessnack and Mendes, 2007).

3.3.2. Self-efficacy questionnaire

This research study design is a quantitative descriptive study. This simply means that numerical data is transformed into usable statistics to quantify the problem. This type of research easily identified trends across data sets. The data obtained from questionnaires was measured, quantified then evaluated using statistical analysis. These represented trends found, was summarised and analysed (Goertzen, 2017).

3.3.3. The burden of need for EBT

This research design is a kwazi-experimental study, that dealt with the epidemiological description of shock patients presenting to a public EMS, and probable viability of emergency blood in prehospital transit. This research involved quantifying relationships between variables, as well as collecting, measuring and

[7] Knowledge is conjectural, based on a hypothesis that has not been manipulated. Objective knowledge about reality is the ideal which is unachieved.

analysing numerical data in order to get a result (Apuke, 2017). The dependant variable was the number of trauma patients and the independent variable was the measuring of criteria for blood transfusion eligibility of these patients (i.e. haemoglobin for a better clinical picture of the patient, pulse rate, blood pressure, temperature, respiration rate and blood loss estimation).

3.4. Statistical analysis

The statistical analysis was conducted by a statistician using a software programme called "R" (R: The R Project for Statistical Computing, 2020). The primary investigator used Microsoft Office® 2019 - Word® and Excel®. The statistical analysis of data was discussed between the statistician, primary investigator and supervisors from the Faculty of Health and Wellness Sciences supporting and guiding this research.

Predictions were computed using the following linear regression model:

$$y_i = \beta_0 + \beta_1 D_i + \beta_2 D_i^2 + \beta_3 D_i^3 + \beta_4 D_i G_i + \varepsilon_i ,$$

where the response *y* is the logit of % haemolysis, D is the number of days elapsed, G is the group (1 for treatment group, 0 for control group), and e is a random error assumed to be normally distributed with mean 0 and constant variance.

3.5. Introduction to study procedure

Once ethical consideration was granted by the WCBS, the blood obtained for research purposes were given as well as eutectics (ice-packs) and hampers (cooler boxes) in which the blood is usually transported in. The main aim of this blood-run[8] (transportation of blood) was to ensure that the units of blood remained within 1°-10°C transportation temperature, and that there was no haemolysis observed in the blood pack after transportation of the units.

The blood was distributed between 3 EMS organisations; Cape Peninsula University of Technology/College of Emergency Care, the Red Cross Air Mercy Services (AMS), and the Provincial Government of the Western Cape Emergency Medical Services (PGWC EMS). The 4 key divisions of the PGWC EMS included were Northern, Southern, Western and Eastern

[8] Blood-run: A term used in this study, pertaining to the transportation of blood on the ambulance vehicles.

divisions. Traceability of blood distributed was maintained using their unique serial numbers and discarded upon expiry. Blood was stored in temperature-controlled fridges to ensure the blood products remained within the storage temperatures of 1°-6°C. For every 2 units of blood transported in the hampers, 1 eutectic (ice-pack) stored at >-18°C was added

After every blood-run, the unit temperatures were taken and recorded. The eutectics were placed back in the freezer and the units of PRBC was hung up in storage. This was to visually inspect that the units of PRBC have not been haemolysed during handling or transportation. Not all units of PRBC were transported, as two units of PRBC at each ambulance base was used as a control by allowing it to remain in storage for comparison with units that have been transported.

Haemolysis levels was assessed once a week, visually and using a Hemocue® Plasma/Low Hb System. At the end of the study, day 42 of PRBC expiry, a conjugated dienes test was done and testing for thiobarbituric acid reactive substances (TBARS) was also conducted on all 24 units of blood.

Concurrently, all the ambulance bases mentioned were used to collect data, over the period of March 2019 – May 2019, of patients who could have possibly made use of emergency PRBC in the prehospital setting in the case of class III or class IV haemorrhage or simply in decompensated shock. Data was collected from patient report forms of patients who fitted the criteria of class III or class IV shock (decompensated/irreversible shock) and possible candidates for EBT consisting of; clinical symptoms consistent with haemorrhagic shock and the estimated amount of blood loss, low haemoglobin, elevated or reduced pulse rate, low blood pressure – systolic <90, low oxygen saturation and skin temperature. Haemoglobin finger prick testing was incorporated into the point of care testing, as haemoglobin is one of the transfusion triggers but not the sole deciding factor for transfusion.

Questionnaires were given to emergency care personnel (see Annexure J) who played a role in this study at all the ambulance bases to determine their self-efficacy about introducing blood as a resuscitation fluid.

3.6. The viability of blood in the prehospital setting

The 24 units of PRBC used for this study was obtained from WCBS. The blood used was freshly bled blood to make full use of the 42-day expiry it has; as the main aim of the blood-run was to observe the feasibility of blood, in terms of temperature stability and haemolysis. The units used should therefore not show haemolysis levels of more than the acceptable 0.8% level. Haemolysis was visually assessed in the segments of the PRBC. The different densities of the PRBC and plasma allowed for this visualization. Due to the plasma having a lower density than PRBC's, the plasma would settle above the PRBC's. The plasma colour should be straw-yellowish in colour. However, if the plasma colour in the segments were similar to that of the PRBC's, then it would be regarded as haemolysed. This would however be indicated as the breakdown of PRBC's, thus the integrity of the PRBC's has been disturbed.

Various EMS organisations were involved in this study: Cape Peninsula University of Technology/College of Emergency Care, the Red Cross Air Mercy Services (AMS), and the 4 key divisions of the PGWC EMS - Northern, Southern, Western and Eastern divisions. At each of these ambulance bases the advanced life support (ALS) was informed about the study, and consent forms were given to them as participants of this study (see template Annexure H). Training was provided to these participants about what was required of them in the study.

All ambulance bases stored blood in temperature-controlled refrigerators of 1°- 6°C. Refrigerator temperatures where blood was stored was written down in a temperature log book provided to each base. The eutectics at each base were stored at <-18°C. PRBC units were stored upright on hooks in the refrigerator to easily observe haemolysis in the segments of PRBC unit, before a blood-run.

The PRBC units were distributed to all abovementioned ambulance bases, each having a unique serial number, whereby the ALS practitioners indicated which units of PRBC's left the refrigerators, the temperature of the blood of the specified serial number, the time it has left the refrigerator, visually inspected the PRBC unit for haemolysis and the temperature of the unit before hanging it up back in storage and the time hereof.

Data sheets tabulating the above was provided to the ambulance bases for ease of data capturing (see template Annexure I).

The PRBC units was transported in hampers (cooler boxes). For every two units of PRBC's transported, one eutectic (ice pack) was used, as per WCBS operating procedures, to ensure that the blood was kept within transportation temperature of 1°-10°C. Blood was transported on the ambulance vehicles and helicopter (AMS).

On a weekly basis all PRBC units at all ambulance bases, were assessed for percentage haemolysis and the temperatures of the refrigerators in which the PRBC's are stored, was also evaluated. The temperatures of the blood in-transit were also assessed to determine if temperatures remained within temperature range.

Two units of PRBC's were kept in the refrigerator at all times. These units were used as the control against which the comparison of the degree of haemolysis occurred, due to haemolysis increasing slightly with storage too. Red cell concentrates have an expiry of 42 days from the date bled.

There is currently no specific threshold for the visual inspection level of acceptable haemolysis in PRBC before release of transfusion from the blood bank. The visual inspection is the easiest, fastest detection method for haemolysis in blood banks, but can be biased and inaccurate, leading to overzealous reading of haemolysis (Janatpour et al., 2004). Therefore, a standardized method determining percentage plasma haemolysis using the Hemocue® Plasma/Low Hb System, in addition to the visual inspection widely used at blood banks, was used in this study. A maximum acceptable haemolysis level of 0.8% was used as per the Council of Europe Guidelines.

3.6.1. Instrumentation for the determination of haemolysis

The Hemocue® Plasma/Low Hb System was the standardized method used to detect haemolysis once a week on all PRBC units, at all ambulance bases that was participating in this study. The Hemocue® Plasma/Low Hb system was used for quantitative determination of low levels of haemoglobin in plasma and serum specimens, aqueous solutions, or stored or banked PRBC using a specially designed photometer and microcuvettes. The Hemocue® Plasma/Low Hb Photometer should

only be used with Hemocue® Plasma/Low Hb Microcuvettes, which was adhered to in this study.

3.6.1.1. Principle of the method

The reaction in the microcuvette is a modified azidemethaemoglobin reaction. The PRBC are haemolysed to release Hb. The Hb is converted to methemoglobin and then combined with azide to form azidemethaemoglobin. The measurement takes place in the photometer in which the transmittance is measured and the absorbance and Hb is calculated. The absorbance is directly proportional to the Hb concentration.

A HemoSmart Gold Hb meter (refer to 3.8.3 for the principle of this system) was used to determine the weekly total haemoglobin as well as the haematocrit of the 24 packs of PRBC. The plasma haemoglobin was determined using the Hemocue® Plasma/Low Hb System.

The percentage of hemolysis in a PRBC unit was calculated as follows:

$$\text{Percentage Haemolysis} = \frac{(100 - \text{Haematocrit}) \times \text{Plasma Haemoglobin}}{\text{Total Haemoglobin}}$$

3.6.1.2. Haemolysis in PRBC donations can be caused by various factors such as:

- Rise in temperature during storage, therefore temperatures should be monitored regularly, or computerized central monitoring systems should be used (Zimmermann et al., 2003)
- Repeated handling of PRBC's during storage, issuing and transportation
- Oxidative stress on stored PRBC over time

3.6.2. Principle of the instrumentation for the determination of Conjugated Dienes (CD) method

The conjugated dienes levels in all the PRBC samples used in this study, was measured as described by Recknagel and Glende (1984), with modifications. The process was carried out as follows: 150 microlitres (µl) of sample and 150 µl of phosphate buffered saline was centrifuged. 200 µl of the supernatant was added to 450 µl chloroform-methanol and mixed for 60 minutes at room temperature (RT). The mixing continued for 20 minutes after adding 150 µl of chloroform again. 150 µl Hydrochloric acid was added to the mixture and mixed gently to wash the organic layer. The mixture was then centrifuged at 1500 x g for 10 minutes at 10˚C. After centrifugation, the mixture was separated based on their different densities, top layer being the aqueous layer, middle layer which is the protein layer and lastly the bottom layer being the lipid layer. A pipette was used to gently remove the lipid layer and placed in an Eppendorf tube, and left to air-dry overnight. The residual was then reconstituted the next day using 1 millilitre of cyclohexane and then vortexed for 30 seconds. Thereafter, 300 µl of this mixture (for all 24 PRBC) was transferred into a microtitre plate reader in triplicate. A wavelength scan was performed between 220 – 320 nanometers (nm) to determine absorbance at 234 nm with a Perkin Elmer spectrophotometer against a cyclohexane blank. Results were expressed Abs234/g wet weight of the tissue.

3.6.3. Principle of the instrumentation for the determination of Thiobarbituric acid reactive substances (TBARS) method

Lipid peroxidation products in tissues, cells and other bodily fluids is commonly measured by TBARS (Landau, Kodali, Malhotra and Kaufman, 2013). An end product of lipid peroxidation, being plasma malondialdehyde, was determined through a specific assay namely the HPLC-based thiobarbituric acid assay.

The process was carried out once again on all 24 PRBC as follows: 50 µl Plasma was added to 6.25 µl 4mM cold ETOH and 50 µl 0.2M ortho-phosphoric acid. The mixture was then vortexed for 10 seconds. 6.25 µl TBA reagent (0.11M in 0.1M NaOH) was added to the mixture and vortexed for another 10 seconds. Thereafter the microcentrifuge tubes lids were punctured and heated at 90˚C for 45 minutes. The tubes were then placed on ice for 2 minutes and then placed at RT for 5 minutes. One ml of n-butanol and 100 µl saturated NaCl was added to the tubes, and vortexed for 10 seconds. The tubes were then centrifuged at 12 000 rotations per minute (rpm) for 2 minutes at 4˚C. 300 µl of the top butanol phase of sample (all 24) was added into the

microtitre plate wells in triplicate. The first 3 wells consisted of n-butanol A1-A3 as it was used as a control. The absorbance of the microtitre plates was detected at 532nm and the result of lipid peroxidation was expressed as nmol malondialdehyde (MDA).

3.7. Self-efficacy questionnaire

Questionnaires were given before the study commenced and after, in light of self-perception of self-efficacy.

The questionnaires were given to all ALS EMS practitioners that were involved in this process to determine how comfortable they were with the study, how confident they were about this study, their willingness to undergo further training if blood is introduced as a resuscitation fluid. The data collected was analysed with the help of a statistician once all the questionnaires were completed. The data analysis was done to summarise the data.

3.7.1. Statistical analysis to determine questionnaire sampling

Based on a district total population of 763 (City of Cape Town and Cape Winelands) staff, the sample size for the staff questionnaire was easily analysed. For instance, if asked a Yes/No question on the questionnaire and 50% of the respondents answered "Yes," it could be 95% sure that the true proportion of "Yes's" in the whole population of 763 is between 46% and 54%. Whereas using a margin of error of 10% it would be 95% confident that it is between 40% and 60%.

Of the 763 staff members, only the ALS practitioners was included. Therefore, approximately 40 EMS staff operational during the study period in the Western Cape Metropole was given the questionnaires, thus a margin of error of 5%.

This calculation was based on a simple random sampling methodology which means one would randomly select the sampled individuals from the database of all staff. Randomly selected ALS EMS practitioners was chosen, regardless of which base they are in, to complete the questionnaire.

3.8. Determining the need burden of need for EBT in the prehospital setting and measuring haemoglobin using point of care testing

Point of care testing can be defined as a medical diagnostic testing that is performed outside the clinical laboratory or at the site of patient care (Turner and Hurley, 2015). The reason for point of care testing is to provide reliable and quick appropriate treatment for patients, especially in life-threatening situations. The ECPs performs various types of tests on patients which can determine if the patient is displaying signs of shock. Based on point of care testing, the results can well classify patients into their various classes using table 1 or classify patients indicating their stage of shock (compensated, decompensated and irreversible). The ECPs completed their patient report forms (PRF) as per usual. The only additional testing added to the PRF was the Hb finger prick test, as this could aid in providing a better clinical picture for possible blood administration of patients categorized (table 1) into the class III/IV shock (decompensated/irreversible) as Hb is a prerequisite for PRBC transfusions.

i. Hb finger prick testing was done additionally on actively bleeding trauma patients at the point of care testing by the ALS practitioners. The Hb finger prick testing are done in trauma units too. Haemoglobin testing was done using a finger prick test. The ECPs routinely performs glucose finger-prick tests that is based on the same principle. Although EMS practitioners are trained on how to perform finger prick testing, training was provided before commencement of Hb testing.

- Hb being tested prehospitally might aid in speeding up the treatment process in trauma units as well.
- PRF or electronic PRF from all the ambulance bases mentioned was assessed to filter the number of patients who fall into the category of class III/IV shock (decompensated/irreversible) that could have been eligible for PRBC transfusion.
- The data collected by the EMS practitioners on the PRF's was filtered and assessed on a weekly basis.

3.8.1. Sampling strategy

A purposive sampling scheme was used for a 2-month period, due to logistical and time limitations, and advanced emergency care providers. The employers or managers at the various bases selected an ALS practitioner based on eligibility and willingness to participate in the study. Purposive sampling from each of the 6 bases (Northern, Southern, Eastern, Western divisions, AMS and COEC/CPUT) screened Hb levels in all patients who fitted the inclusion criteria. The inclusion criteria consisted of: Penetrating or non-penetrating injuries/illness, in adults or children, who displayed signs and symptoms of shock class III or class IV (decompensated/irreversible). This

included victims of motor vehicle accidents, stabbings or gunshot wounds (strong interpersonal violence) and victims where rupture of internal organs was suspected such as: in the liver, kidney, spleen and lastly long bone and pelvic fractures.
The number of patients presenting to the EMS who fitted the inclusion criteria for this study was unpredictable.

A study done by Matthews et al. (2017) was done retrospectively for the same intended population and found 6% of priority 1 cases (Matthews, McCaul and Smith, 2017). Another study done by Newton et al. found <2% that had intervention needs, although the study was done in KwaZulu-Natal and over a short period of 72 hours (Newton, Naidoo and Brysiewicz, 2015). Blood transfusion need is likely to reside in this population sub-group. This sampling scheme would collect data from a proportion of the 2-6% priority 1 cases if available. The prospective number is highly variable given the ad hoc nature of emergencies, so whilst 80% power is aimed for, the power calculation will be done post facto.

3.8.2. Statistical analysis of sampling data

The analysis was done using descriptive statistics and some statistical relationships were tested (Hb, shock severity appraisal, BP, pulse rate, blood loss estimation, etc.).

3.8.3. Instrumentation for point of care haemoglobin testing

The chosen instrumentation used for the quantitative determination of Hb in capillary, venous or arterial blood was the HemoSmart Gold Hb meter. The system consisted of the portable hand-held meter and HemoSmart gold Hb test strips. The principle of the method used by this device was as follows: Sodium deoxycholate and cell lysis material haemolysis the PRBC and Hb is released. Ferricyanide converts the Hb2+ to Hb3+. An analytical chemistry method is used to analyse resistant value to obtain HCT% and Hb result.

3.9. Inclusion criteria

- Penetrating or non-penetrating injuries/illness, in adults or children, who displays signs and symptoms of shock - class III or class IV (decompensated/irreversible). This included victims of motor vehicle accidents, stabbings or gunshot wounds (strong

interpersonal violence) and victims where rupture of internal organs are suspected such as: in the liver, kidney, spleen and lastly long bone and pelvic fractures.

- ALS practitioners

3.10. Exclusion criteria

- Any medical presentation that includes internal haemorrhage such as: oesophageal varices, ruptured abdominal ulceration, rupture of uterine cysts, intravascular haemorrhage.
- BAA and AEA practitioners

CHAPTER FOUR: RESULTS

This study yielded unprecedented results in South Africa, demonstrating feasibility of blood in the prehospital setting and the self-efficacy of ALS practitioners. The results chapter consists of 3 parts namely: “The viability of blood in the prehospital setting” - particularly in terms of temperature stability, haemolysis and oxidative stress, the “Self-efficacy questionnaire” - regarding the self-efficacy of emergency care practitioners to perform emergency blood transfusions and “The burden of need for EBT” - determining the need for blood in the prehospital setting and measuring haemoglobin using point of care testing.

4.1. The viability of blood in the prehospital setting

Measuring percentage haemolysis for a 42-day period (shelf-life of PRBC)

A total of 12 units of PRBC was transported in ambulance vehicles over a 42-day period, and 12 PRBC remained at the various ambulance bases as a Control. On day 1 the blood was tested for haemolysis using the Hemocue® Plasma/Low Hb System, which was used to obtain baseline results, and weekly thereafter to determine any changes.

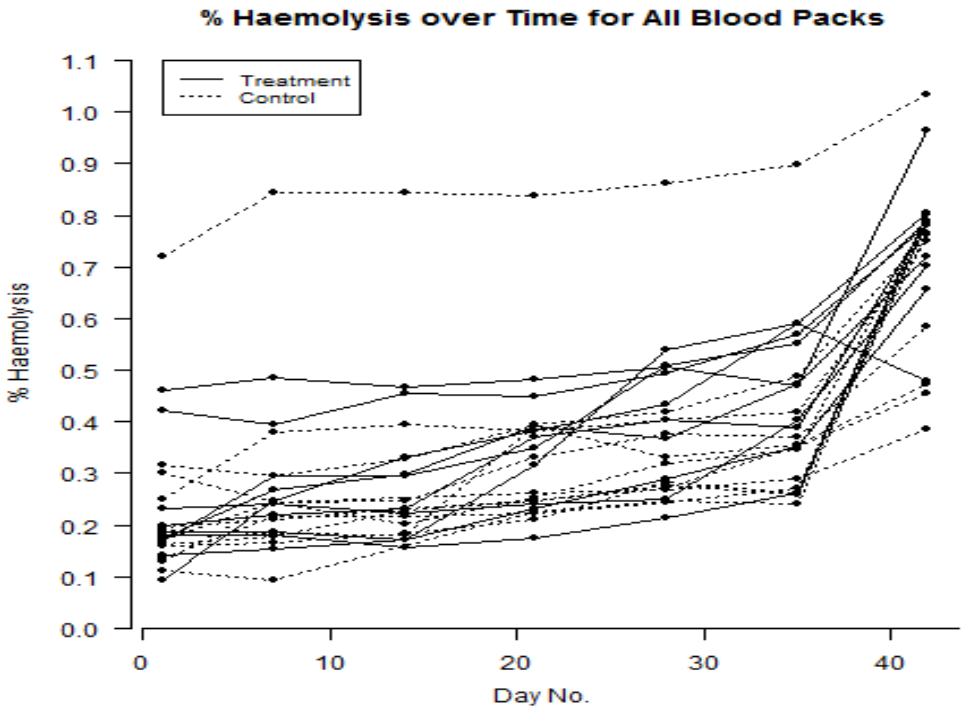

Figure 4.1: Percentage haemolysis over the 42 day PRBC shelf-life period

It is clear that the haemolysis percentage has predictably increased over the 42 day period, being the shelf-life of PRBC. This graph also indicates a clear outlier with a high haemolysis

percentage on day 1, in comparison to the other 23 PRBC, this could be due to many factors such as; processing of blood after donation (Zimmermann et al., 2003) or bacterial contamination (Sawant et al., 2007), etc.

All blood packs (Treatment[9] and Control) are below the 0.8% threshold after day 35, however several packs are at or around 0.8% on day 42 of the Treatment group, and below 0.8% for the Control group.

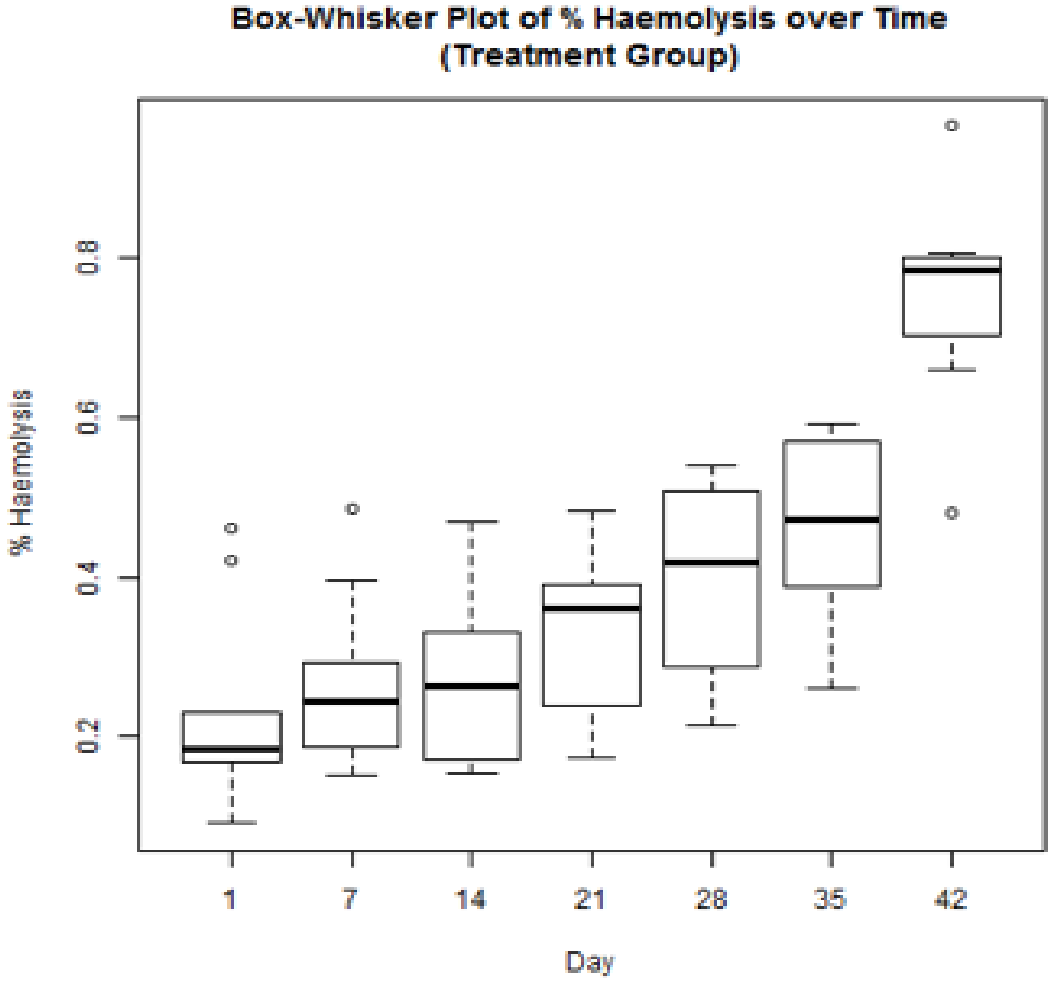

Figure 4.2: The minimum level of haemolysis for the treatment group

The minimum level of haemolysis for the treatment group was <0.2% on day 1 (baseline testing) and reached the maximum acceptable level of haemolysis which is 0.8%, on the last day, day 42.

[9] Treatment group – refers to the blood that was transported during the study as part of the "Blood-run".

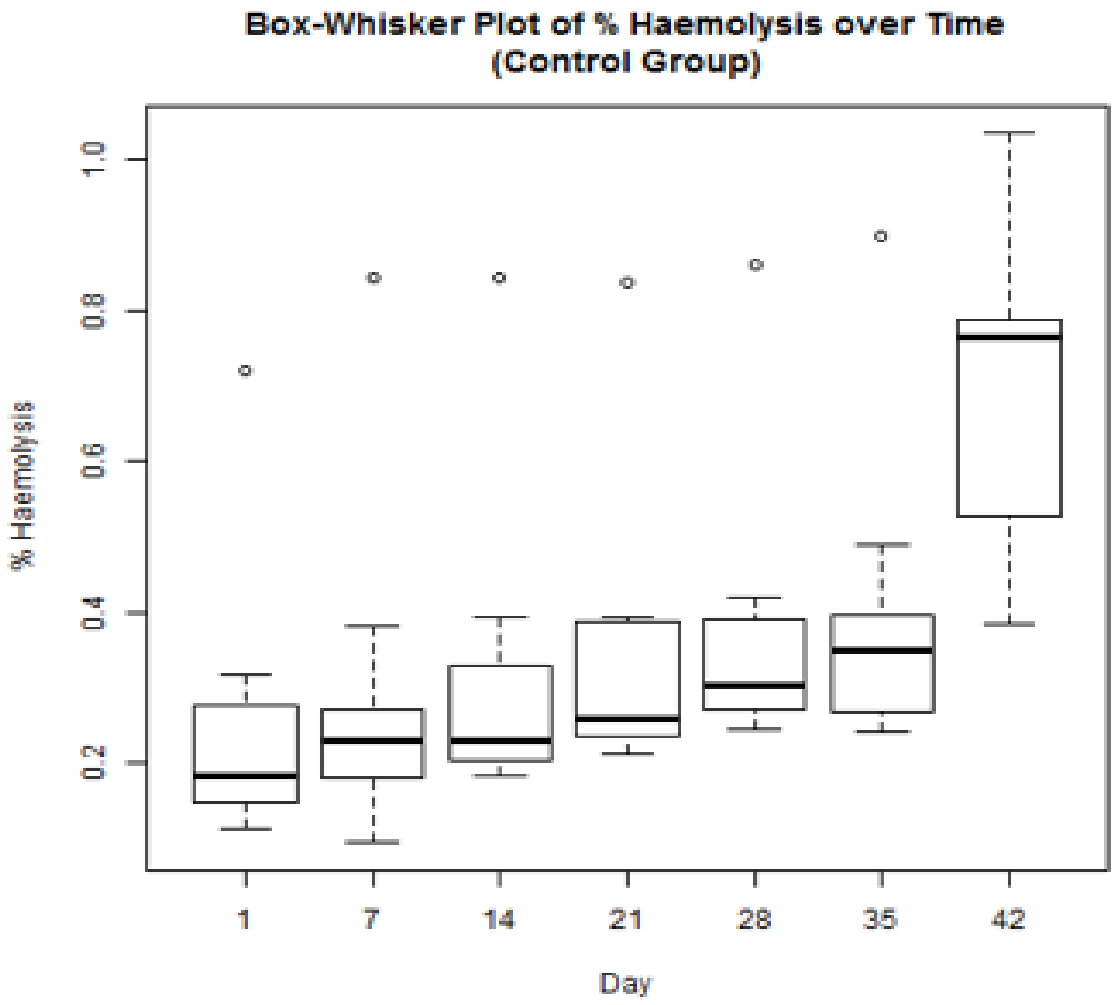

Figure 4.3: The minimum level of haemolysis for the control group

The minimum level of haemolysis for the control group was <0.2% on day 1 (baseline testing) and reached below the maximum acceptable level of haemolysis which is 0.8%, on the last day, day 42.

Frequency, Time and Temperature

The figure below illustrates that there were three instances with temperature increases of >6˚C, which is a cause for some alarm. Interestingly, the three blood packs that experienced a temperature change of >6˚C actually had among the lowest final haemolysis percentage values after 42 days has elapsed (SN 11457589: 0.48%; SN 11439130: 0.70%; SN 11453708: 0.72%).

At 5% significance level, no association was found between time elapsed and temperature change. This was done using Kendall's nonparametric correlation coefficient (p-value: 0.116), which suggests that the refrigeration method used for the blood-run, on the emergency vehicles was effective.

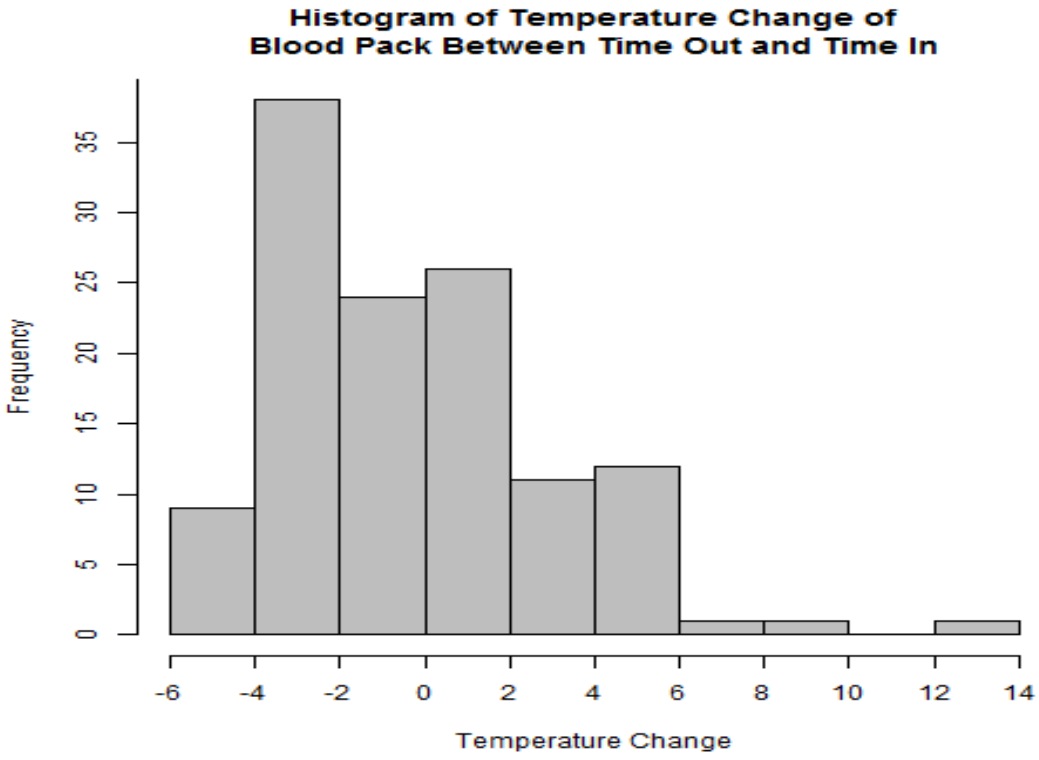

Figure 4.4: The blood transportation temperature range

The blood transportation range is between 1˚C-10˚C. The blood temperature returned to the fridge after a blood-run was below 0˚C in majority of cases (median = -0.8 degrees C) and exceeded temperatures of <10˚C in rare instances.

Time and Temperature

The blood-run lasted from the time the blood was taken out of the refrigerator at the ambulance base at the start of their shift, until it was returned to the refrigerator after their shift. The figure below illustrates the average duration that the blood was in-transit.

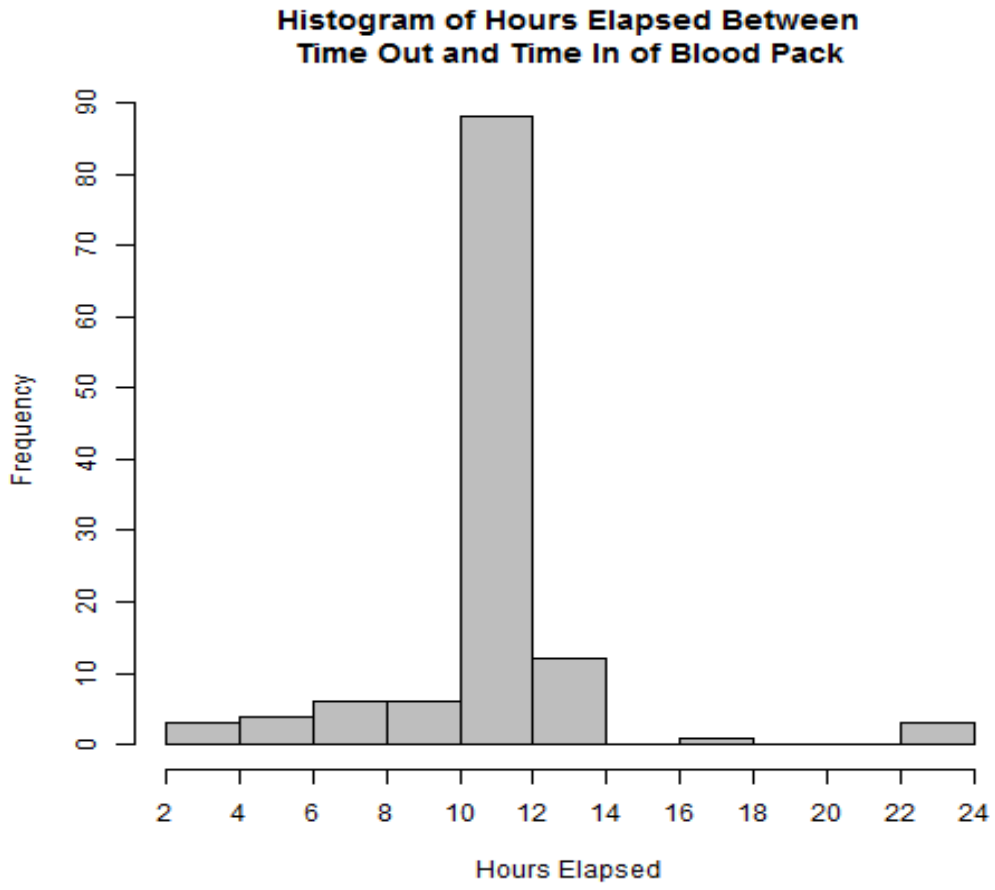

Figure 4.5: The number of hours elapsed from the time the blood was taken out of the refrigerator and returned

In most cases the blood pack was away from the base for between 10-12 hours.

Linear Modelling of Haemolysis Trajectory

A linear regression model was fit to model the haemolysis trajectory over time for both Treatment (PRBC in ambulance vehicle) and Control groups (stationary refrigerator). The response variable was:

$y_i = \log\left(\frac{p_i}{1-p_i}\right)$ where p_i is the % haemolysis for observation i. The logit transformation was used to transform the percentages onto an unbounded continuous scale to improve the fit to normal distribution; it should not be confused with logistic regression. The explanatory variables were:

Day_i, days elapsed from bleeding the blood pack (baseline measurements), which takes values of 1, 7, 14, 21, 28, 35, and 42 (days when haemolysis was measured), and

$Group_i$, a dummy variable that takes a value of 1 for the Treatment group and 0 for the Control group

The linear regression model equation was therefore:

$$y_i = \beta_0 + \beta_1 Day_i + \beta_2 Day_i^2 + \beta_3 Day_i^3 + \beta_4 Day_i Group_i + \varepsilon_i$$

where $\beta_0, \beta_1, \beta_2, \beta_3, \text{and } \beta_4$ are unknown parameters to be estimated and ε_i is a random error.

This model including quadratic and cubic terms for Day_i, a main effect for $Group_i$ and an interaction between Day_i and $Group_i$ was found to be the best fit to the data among candidate models considered, as judged by Akaike's Information Criterion (AIC), which for this model was 226.6576.

The multiple coefficient of determination (r^2) for the model was 0.6835 and the adjusted r^2 was 0.6744.

The table below gives the model's coefficient estimates with significance tests. It is evident from the p-values in the last column that the linear, quadratic and cubic terms for Day_i are all statistically significant at 5% level, as is the Day * Group interaction term. The interpretation of the interaction term (since the coefficient estimate is positive) is that haemolysis is expected to occur at a faster rate over time for the Treatment group than for the Control group.

Linear Regression Coefficient Estimates

Table 4.1: The model's coefficient estimates with significance tests

Variable	Coefficient Estimate	Standard Error	*t* Statistic	Significance *p*-value
Intercept	-1.547×10^{0}	1.275×10^{-1}	-12.134	$< 2 \times 10^{-16}$
Day[1]	8.722×10^{-2}	2.877×10^{-2}	3.031	2.901×10^{-3}
Day[2]	-5.309×10^{-3}	1.608×10^{-3}	-3.302	1.219×10^{-3}
Day[3]	1.064×10^{-4}	2.449×10^{-5}	4.344	2.670×10^{-5}
Day * Group	1.321×10^{-2}	3.384×10^{-3}	3.904	1.470×10^{-4}

The model was used to predict the haemolysis trajectory for the first 42 days. The predicted values are plotted below together with 90% prediction interval limits (thus the estimated probability of percentage haemolysis being above the upper limit is 5%). For the Treatment group, the predicted percentage haemolysis after 42 days is 0.77%, with 95% prediction interval (0.58%, 0.89%).

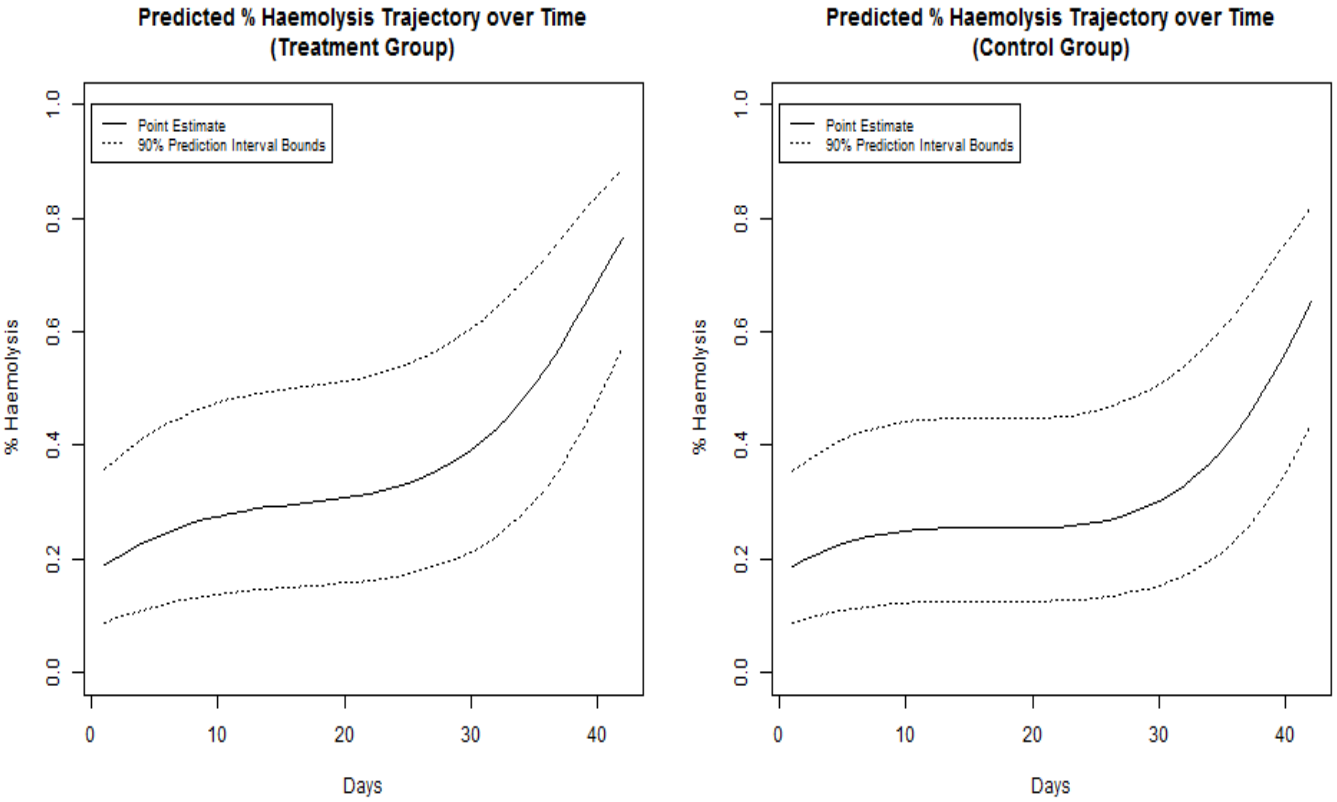

Figure 4.6 & 4.7: Predicted % Haemolysis Trajectory over Time

For the Treatment group, predicted haemolysis % after 42 days was 0.77%, with 95% prediction interval (0.58%, 0.89%). For the Control group, predicted haemolysis % after 42 days was 0.65%, with 95% prediction interval (0.44%, 0.82%). The group * day interaction in the regression model was statistically significant (p-value: 0.000147), which indicates that haemolysis did increase more quickly in the treatment group than in the control group, but only marginally.

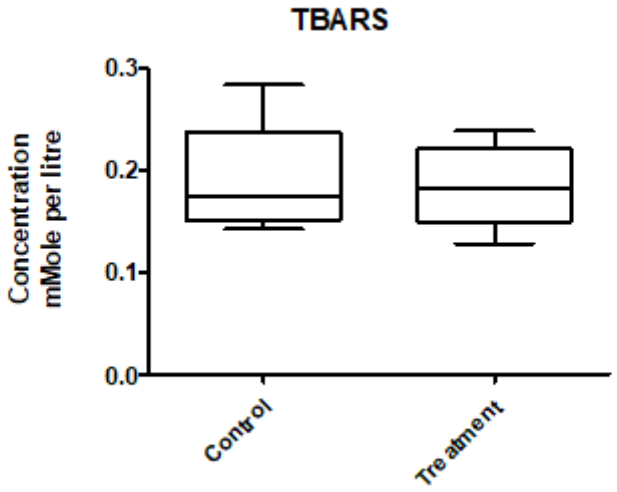

Figure 4.8: Comparing the TBARS Control group results to the Treatment group

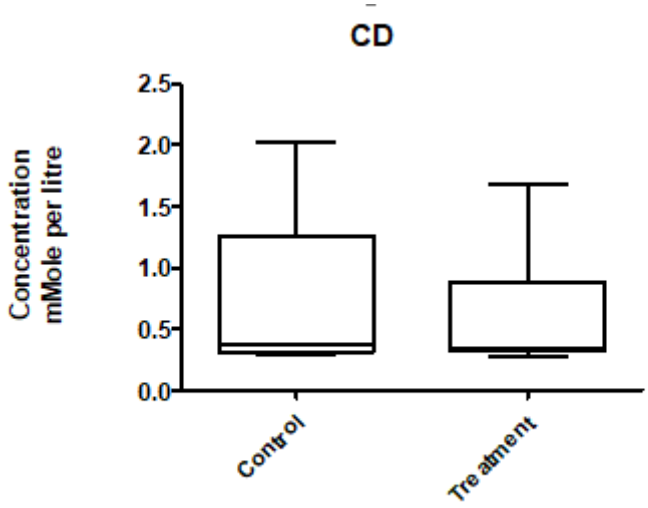

Figure 4.9: Comparing the CD Control group results to the Treatment group

A two-tailed *t*-test found no difference in mean TBARS between the Treatment and Control groups (p-value: 0.6768), and no difference in mean CD between the Treatment and Control groups (p-value: 0.2459).

4.2. Self-efficacy questionnaire

The questionnaire was self-administered among all 40 (n) participants (ALS practitioners) at the training of prehospital blood transfusion on emergency vehicles. This training was conducted at the Northern, Eastern, Western, Southern division amublance bases as well as at the Air Mercy Services, College of Emergency Care and the Cape Peninsula University of Technology.

The questionnaire was designed to assess the practitioner self-efficacy for emergency blood transfusion. The questionnaire consisted of 3 parts;

- Part I: Demographic information of participants
- Part II: Self-perception of self-efficacy
- Part iii: Experience and knowledge

In part I of the questionnaire, which is the demographic information of participants, it was found that majority of the participants were from the northern division ambulance base and the least from the Cape Peninsula University of Technology.

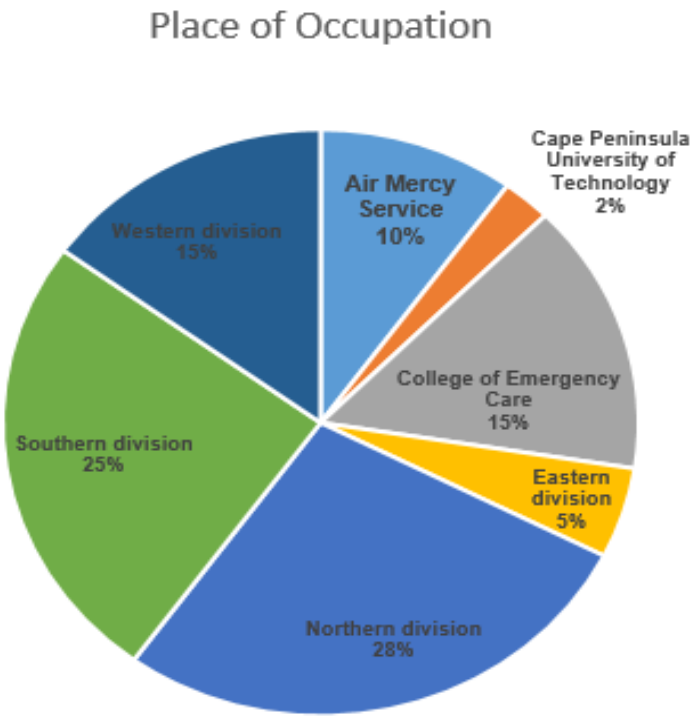

Figure 4.10: The distribution of participants based on their place of occupation

Participants n=40 from various ambulance bases

Participant's years of Experience as an ALS

Table 4.2: The participant's years of experience as an advanced life support practitioner

Years of experience	No. of participants
Less than 5 years	8
Between 5-10 years	23
More than 10 years	9

Out of n=40 participants, n=23; 57.5% has between 5-10 years of experience as a paramedic, n=9; 22.5% has more than 10 years of experience, and n=8; 20% has less than 5 years of experience.

Part ll of the questionnaire, which is the self-perception of self-efficacy, participants were asked to use a 7-point Likert scale to allow the participant to express their confidence level to a particular statement. Where 7 = extremely confident & 1 = not at all confident, as illustrated below:

Figure 4.11: 7-Point Likert scale used in the self-efficacy questionnaire

The Likert scale is the most widely used rating scales (McLeod, 2019), although various kinds of rating scales have been developed to measure an individual's attitudes, perceptions or opinions to something in particular.

In part ll of this questionnaire, majority of the participants (n=21; 52.5%) indicated that they will be extremely confident (Likert scale rating 7) in their ability to transfuse blood, provided they receive training on the risks and benefits of blood products and the transfusion.

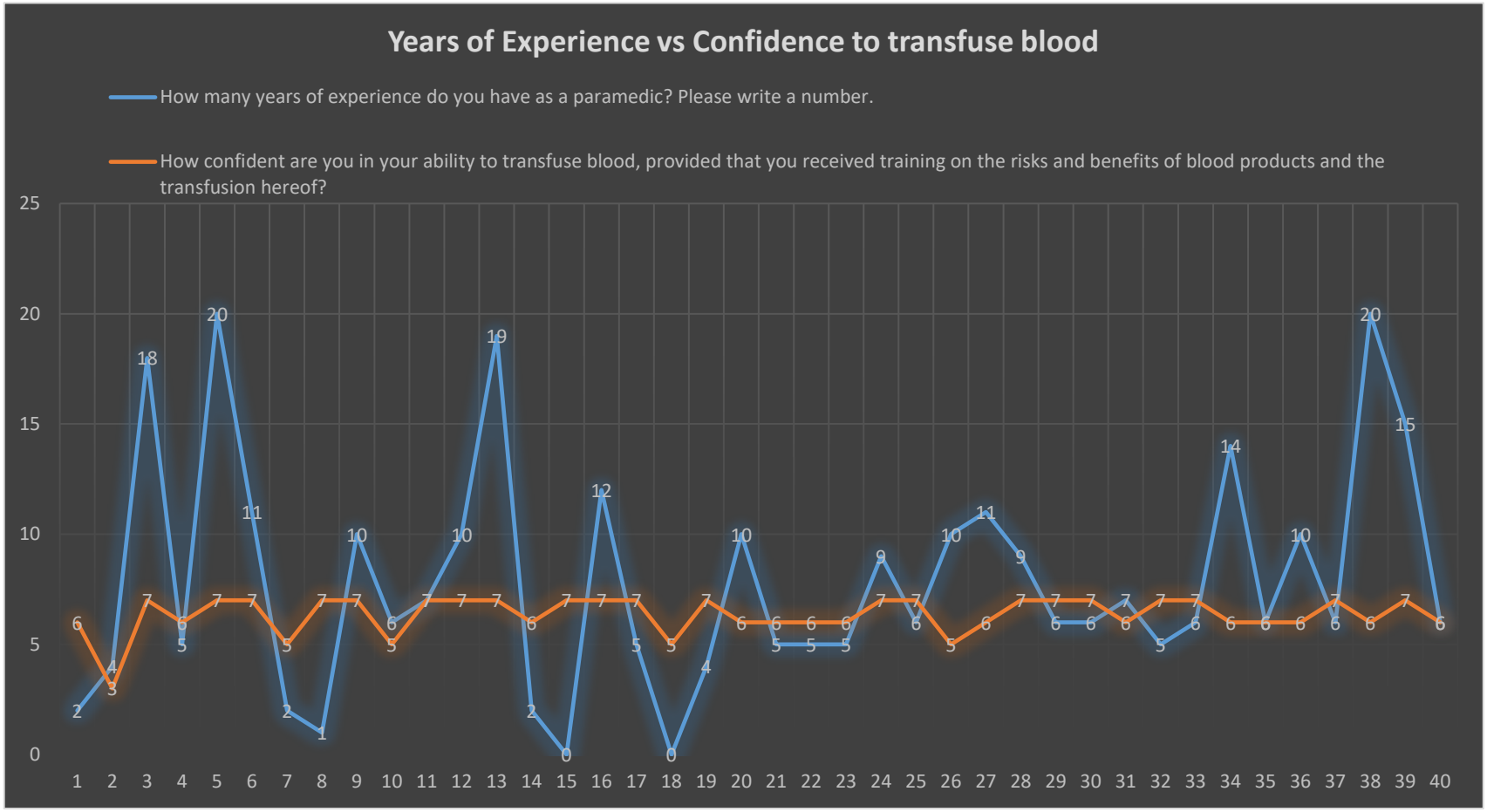

Figure 4.12: Correlation between the years of experience and the confidence of paramedics to transfuse blood

Majority of the participant's n=21; 52.5% chose 7 being extremely confident, following n=14; 35% chose 6 being confident as well. The years of experience played no major role in the self-efficacy determination to transfuse blood, provided more training is given.

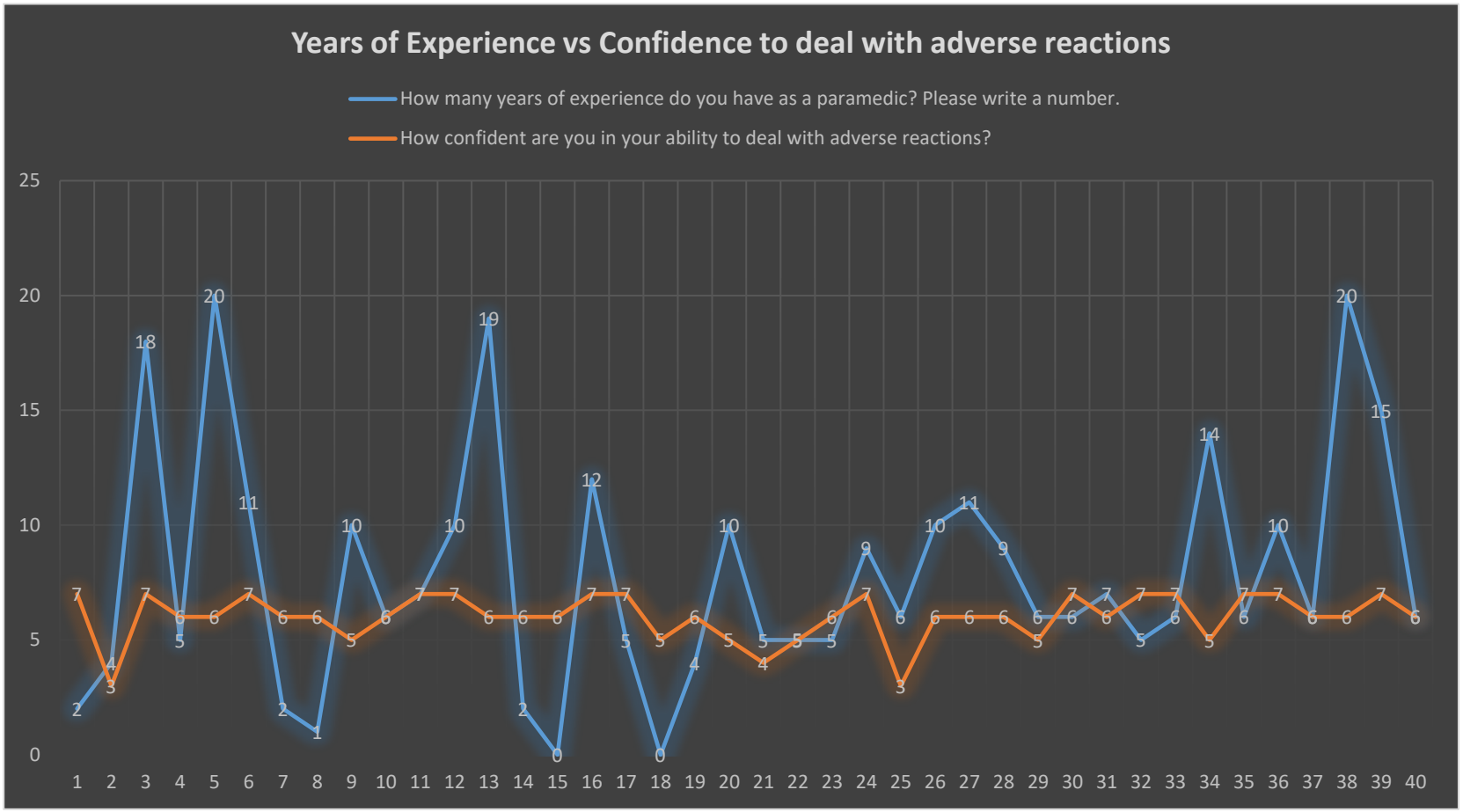

Figure 4.13: Correlation between years of experience and the confidence of paramedics when dealing with adverse reactions from patient.

Majority of the participant's n=17; 42.5% chose 6 being confident, following n=14; 35% chose 7 being extremely confident. The years of experience played no major role in the self-efficacy determination.

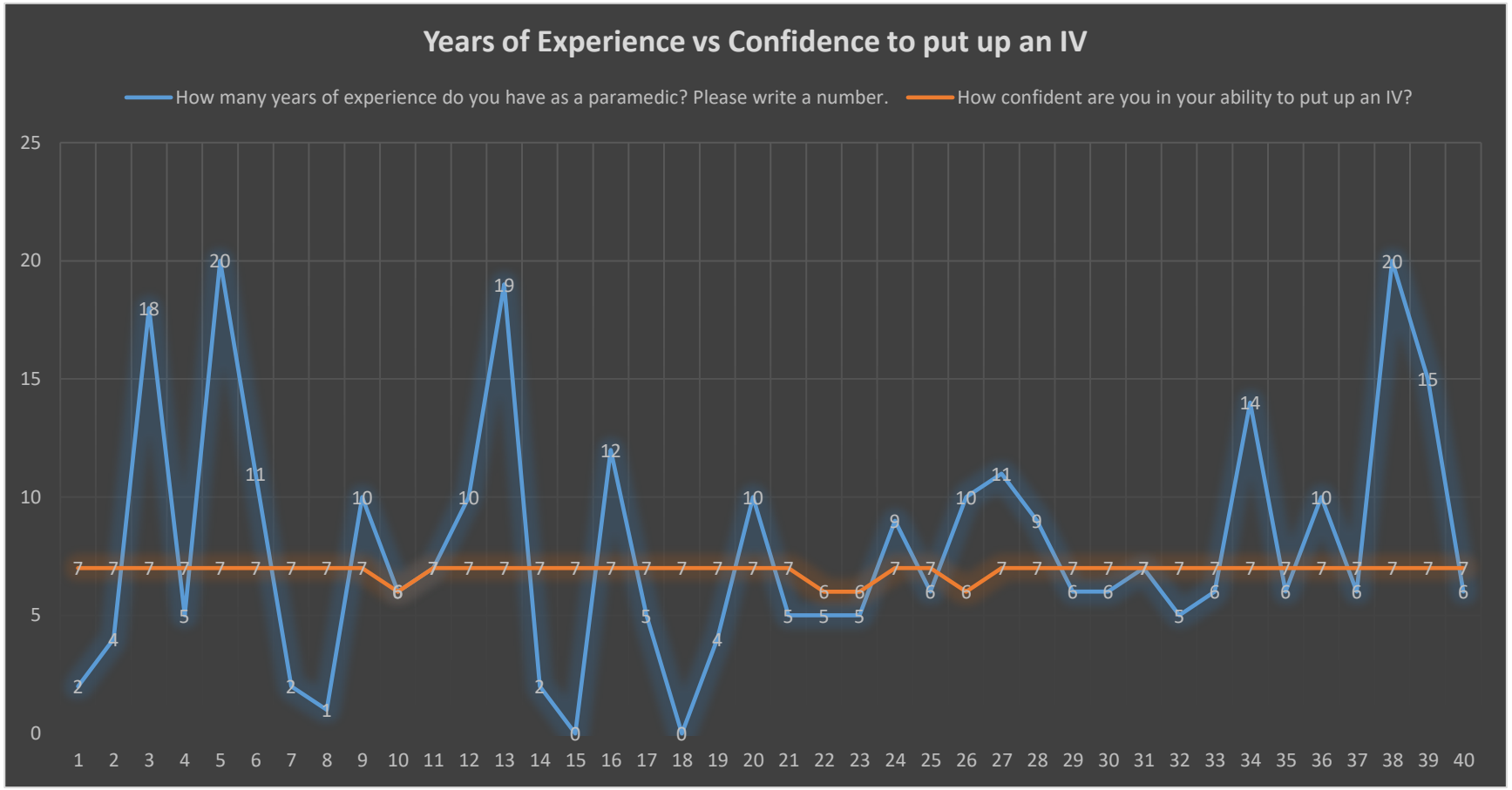

Figure 4.14: Correlation between years of experience and the confidence levels of paramedics with respect to putting up an IV.

Majority of the participant's n=36; 90% chose 7 being extremely confident, following n=4; 10% chose 6 being confident as well. The years of experience played no major role in the self-efficacy determination.

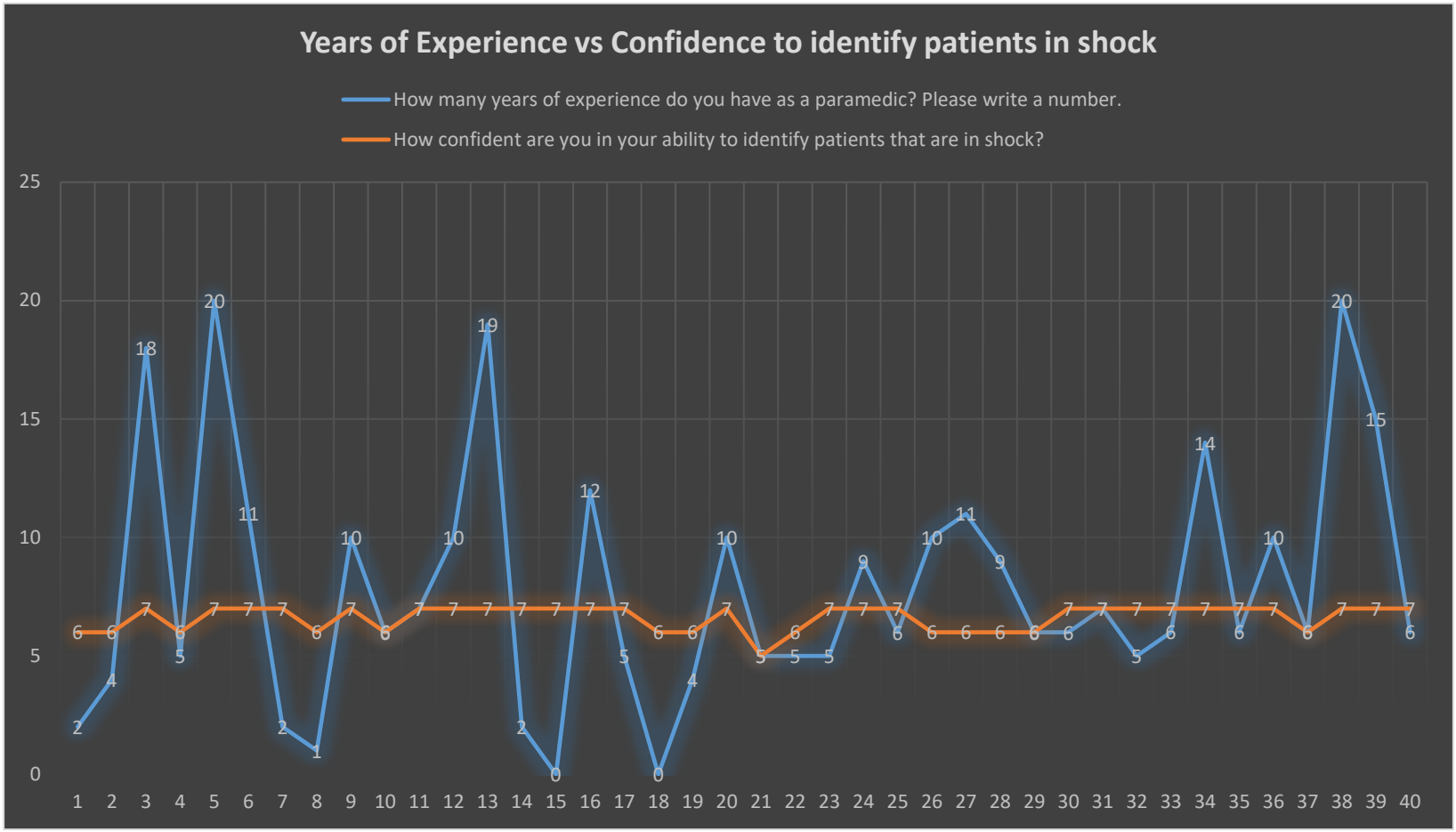

Figure 4.15: Correlation between years of experience and the confidence levels of paramedics to be able to identify patients that are in shock.

Majority of the participant's n=26; 65% chose 7 being extremely confident, following n=13; 32.5% chose 6 being confident as well. The years of experience played no major role in the self-efficacy determination.

In part iii of the questionnaire which is the experience and knowledge section, general knowledge questions about blood and blood transfusion was asked as multiple-choice questions and the responses were as follows:

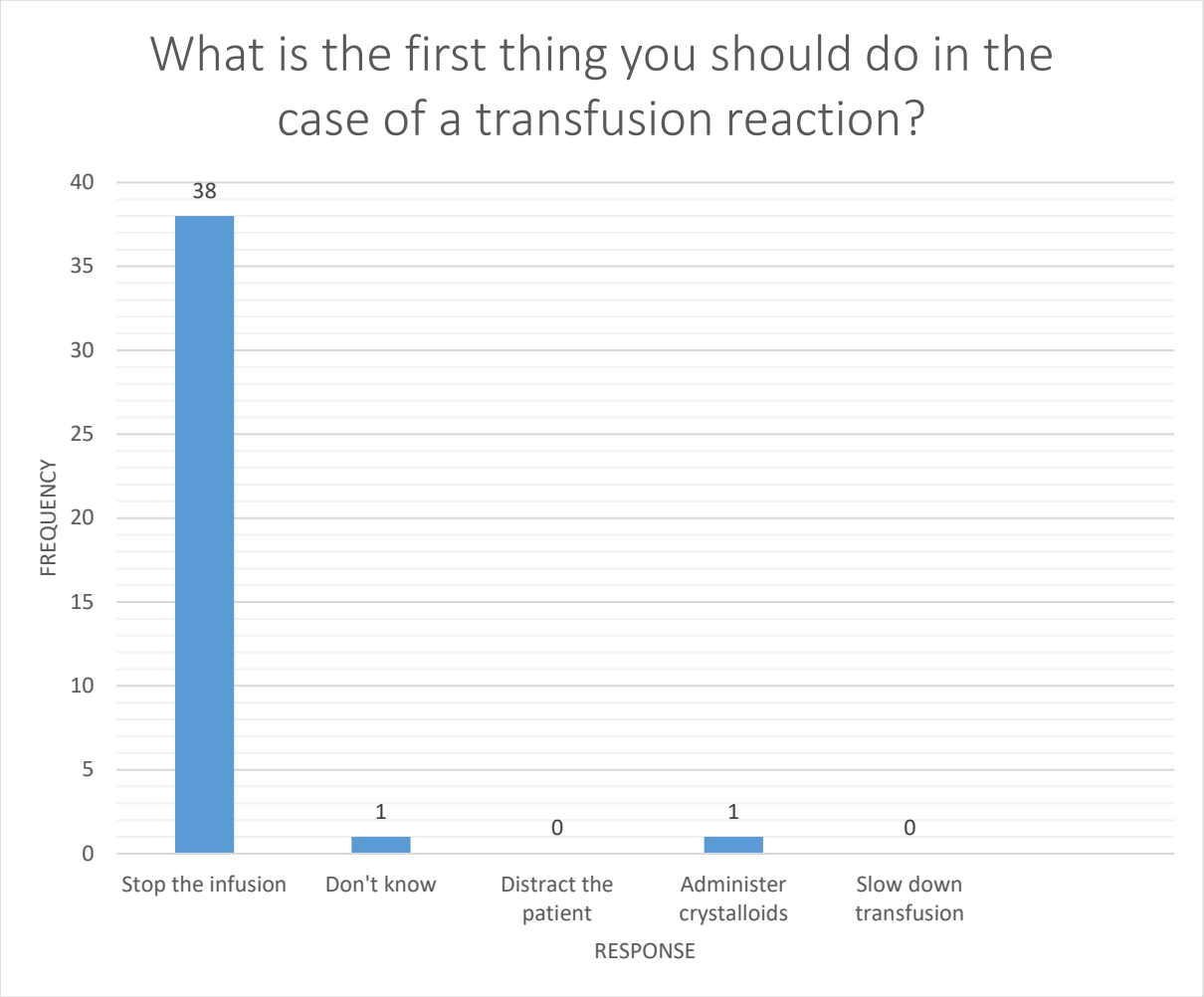

Figure 4.16: Paramedic's responses to a reaction upon transfusion.

Majority of participants answered correctly (n=38; 95%)

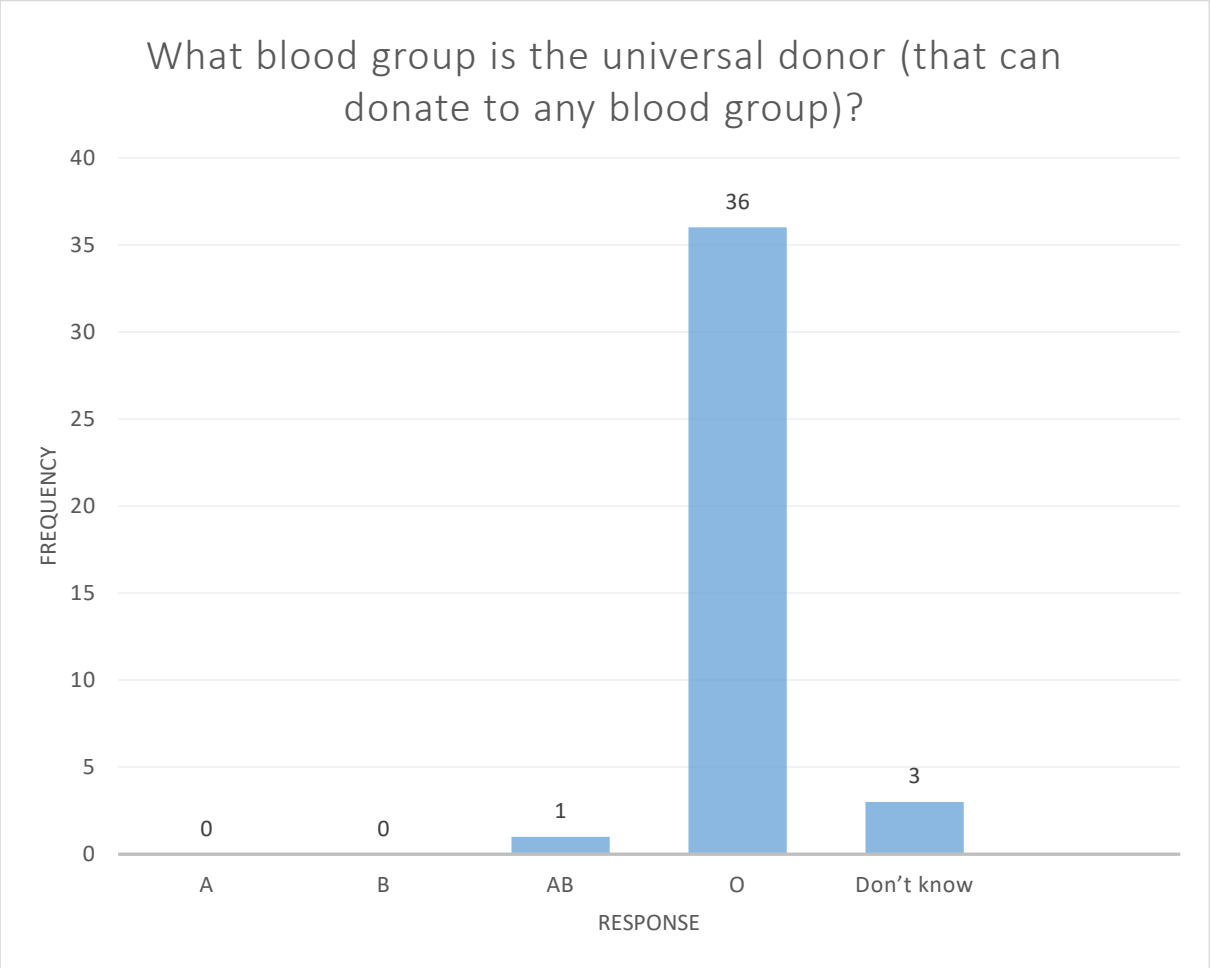

Figure 4.17: Paramedic's responses to their understanding of the universal donor blood group.

Majority of participants answered correctly (n=36; 90%)

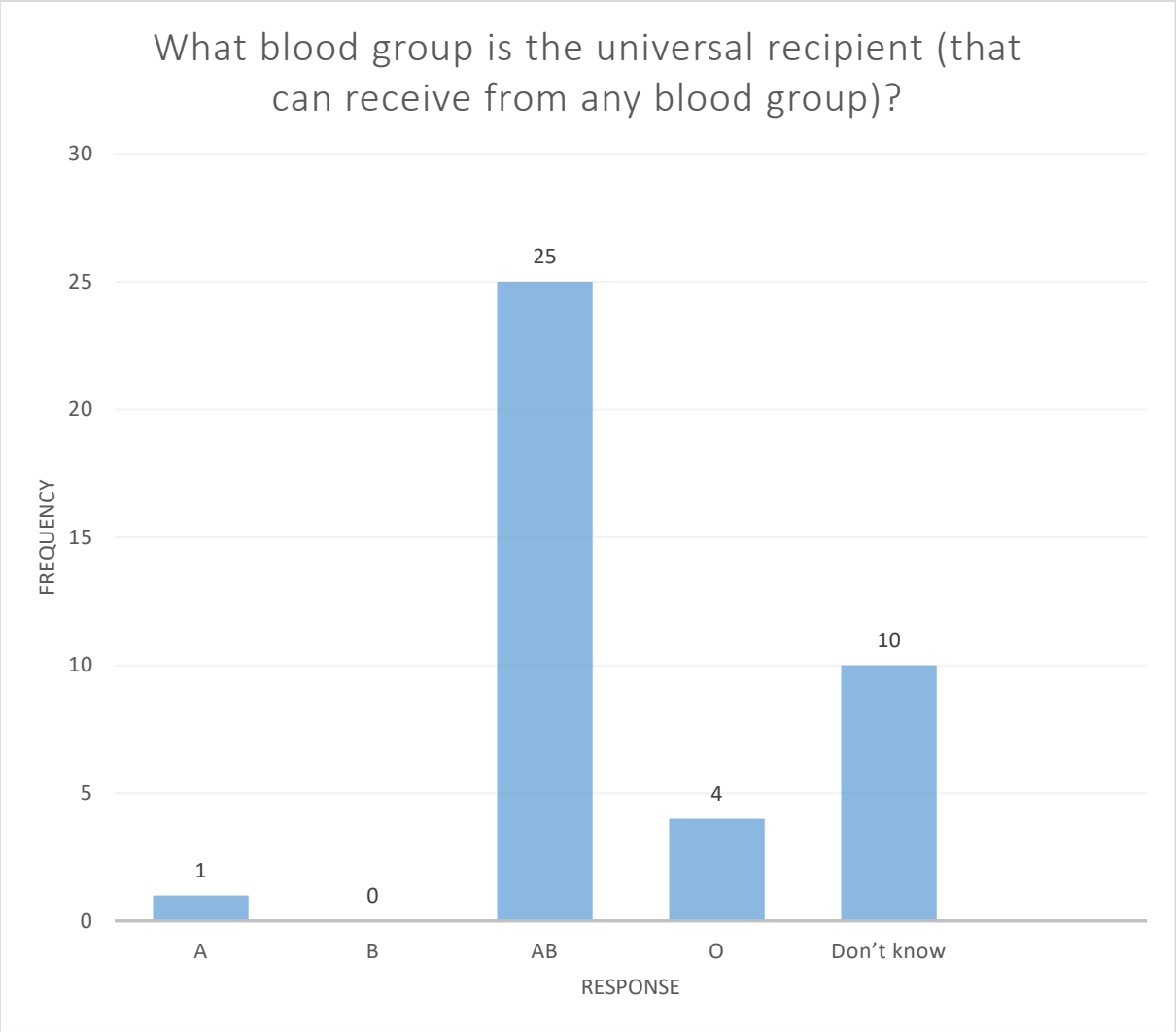

Figure 4.18: Paramedic's responses to their understanding of the universal recipient blood group.

Majority of participants answered correctly (n=25; 62.5%)

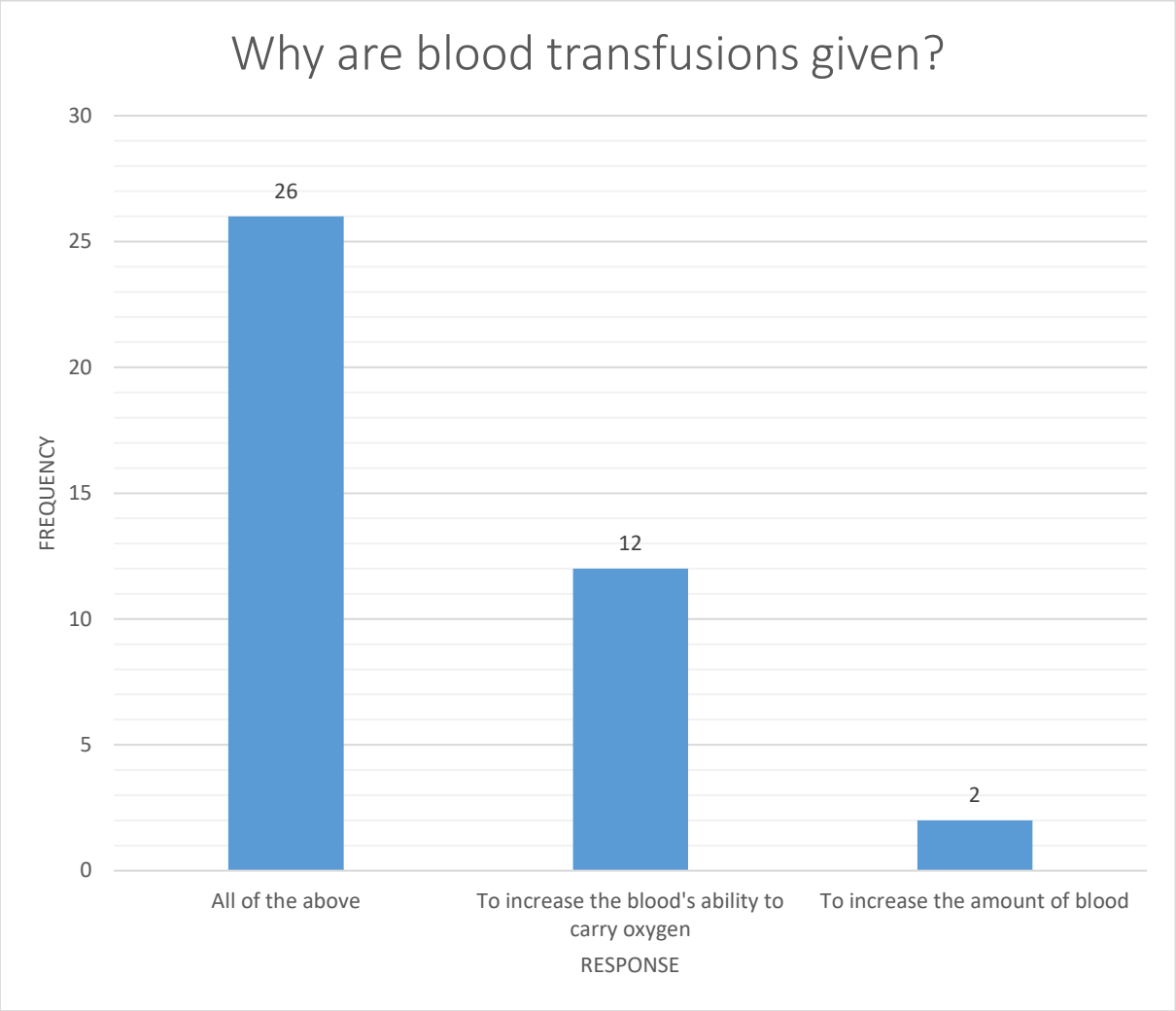

Figure 4.19: Paramedic's responses to the question: "Why are blood transfusions given?"

Majority of participants answered correctly (n=26; 65%). Thus, we can determine that majority of the respondents understands why blood transfusions are given.

The questions from the self-efficacy questionnaire that yielded no significant results, were not visually represented nor discussed.

4.3. The burden of need for EBT

Apart from the ECP's collecting data for patients fitting the criteria of class III/IV shock. Electronic patient report forms were obtained from the NHRD over the specified period of data collected by the ECPs.

The total amount of trauma and transport cases between March 2019 - May 2019 amounted to 987 and further filtered down to those patients that had uncontrolled haemorrhage, possible class III/IV shock, and in turn the number was reduced to 126.

$$x = \frac{Total\ no.\ of\ patients\ fitting\ the\ criteria\ \text{class III/IV shock}}{Total\ no.\ of\ trauma\ and\ transport\ cases}\ X\ 100$$

$$x = \frac{126}{987}\ X\ 100$$

$$x = 12.8\%$$

The percentage estimation fitting our inclusion criteria of patients that could have made use of ERBC in the prehospital setting is 12.8%.

CHAPTER FIVE: DISCUSSION

This is the first study to our knowledge in South Africa conducted whereby the feasibility of blood was tested in the prehospital setting. The results obtained in chapter 4 will be discussed into 3 parts namely; “the viability of blood in the prehospital setting”, “Self-efficacy questionnaire” and “The burden of need for EBT”.

5.1. The viability of blood in the prehospital setting

The primary objective of this study was to determine the viability of packed red blood cells (PRBC) in transit, prior to the onset of haemolysis. Factors such as temperature, processing, transportation and storage of PRBC were taken into consideration as these could exacerbate haemolysis (Zimmermann et al., 2003). Premature PRBC haemolysis is said to be an obvious marker of incorrect PRBC storage (Sawant et al., 2007).

The principle findings of this aspect of the study, indicated that the blood in-transit remained between the recommended transportation temperature range of 1˚C – 10˚C (SANBS and WPBTS, 2016). The storage temperature of the blood in stationary refrigerators (in this case at the ambulance base) needed to remain between 1˚C - 6˚C (Harmening, 2005). One eutectic per two units of PRBC as per the WCBS guidelines, were to be used as a standard in the study during the transportation of PRBC. However, one PRBC was transported at a time and it was found that using one frozen eutectic was insufficent. The ambient temperature of the ambulance vehicle was relatively high therefore two PRBC returned after the blood run, exceeded 10˚C, but has not skewed the data as all other parameters regarding the integrity of the PRBC were still intact. Amendments were made immediately due to the temperature spike of the PRBC, instead of using a single eutectic, recommendations were made to adjust the number of eutectics to two. Shortly therafter it was reported that blood was now returned below 1˚C, which brings to light the outliers found. Upon inquiry, we found that the participants were trying to be ‘extra cautious’ about blood exceeding 10˚C that they decided to add a third eutectic, which in turn caused the PRBC temperature to drop below the PRBC transportation range of <1 ˚C. However, when using the suggested ammendment method, i.e. 2 eutectics for 1 PRBC, the transportation temperature of the PRBC remained between 1˚C – 10˚C.

According to a study conducted in the United States of America by Conterato *et al*. (2016), PRBC remained within its’ temperature range using a similar prehospital blood transportation method. This included; housing the blood at the ambulance base, transporting it in a cooler

box with an ice pack, allowing blood to remain within temperature range for up to 18 hours, provided their ice packs were recharged 12-hourly (Conterato et al., 2016).

A study done by Zimmermann *et al.* (2003) indicated that haemolysis is a very important parameter for assessing the quality of PRBC. The haemolysis levels of both the Control and the Treatment (PRBC in transit) were tested on day 1, 7, 14, 21, 28, 35 and 42 (shelf-life of PRBC). This study has also shown that the percentage haemolysis increased from day 1 to day 42 of transportation and storage as per Arif et al. (2016). One of the control PRBC packs, showed a relatively high percentage haemolysis (0.2%) on day 1 of testing, i.e. before the blood was distributed to the various ambulance bases. This could have been due to many factors such as: the donor, having red cell membrance and enzyme defects; the enviroment, having high temperature range either during transportation of blood or while the blood donation is occuring; the material, possibly having bacterial growth; personnel, traumatic phlebotomy technique by untrained staff; process, rapid resuspension of blood with anticoagulant and the last factor being equipment, inner centrifuge temperature variation and type of spin (Dhawan et al., 2017).

Interestingly, it was found that both Treatment and Control blood packs were below the 0.8% (maximum acceptable limit of haemolysis as per the Council of Europe Guidelines) threshold after day 35. Additionally several packs were at or around 0.8% on day 42 of the Treatment group, and below 0.8% for the Control group. As such the viability of PRBC appear to not have been affected by the transport on ambulance vehicles. In order to allay any fear of wastage, it may be proposed that PRBC only be recommended for use in ambulance vehicles up until day 35 after collection.

A linear regression model was fit to model this haemolysis trajectory over time for both Treatment and Control groups. The model was used to predict the haemolysis trajectory for the first 42 days and the interpretation of the interaction term was that haemolysis is expected to occur at a faster rate over time for the Treatment group than for the Control group. With this in mind, based on figure 7, we can thus argue that the shelf-life of the PRBC that are being taken on emergency vehicles in the manner of the study will exceed 42 days over 95% of the time (if a 1% haemolysis threshold is used). If a 0.8% haemolysis threshold is used, the shelf life will exceed 38 days over 95% of the time. This confirms the feasibility of the procedure used in this experiment.

When looking at the temperature and time aspect of the blood-run, blood was transported in ambulance vehicles over an average of 10-12 hour shifts, and returned to the ambulance refrigerator at the end of the shift. Howerever, blood can generally be stored in the hampers (cooler boxes) provided to the EMS practitioners for up to 24 hours, with eutectics, maintaining blood transportation temperature of 1˚C - 10˚C (Hardwick, 2008). Additional factors can however override the mentioned temperature range due to heated ambient temperatures of ambulance vehicles, without air conditioners, as per this current study. This in turn can excaberate haemolysis.

Kendall's Nonparametric Correlation Coefficient method however, was used to check for a correlation between temperature change and the duration of time that the blood pack was in-transit, The correlation estimate was 0.0976 which is not statistically significant (p-value = 0.1176 > 0.05), hence there was no evidence of an association between the time elapsed and temperature change. Although not statistically significant, this is clinically significant as this interesting finding suggests that the "refrigeration" method of the PRBC on the vehicles was effective. The blood-run occurred at the start of the advanced emergency care providers shift, until they have returned to the ambulance base with the blood. Results indicates that the blood was in-transit on average for 12 hours and maintained its 1˚C – 10˚C temperature. This is a significant finding on the control of environmental conditions that favour blood transportation in an urban EMS.

Similarly, a study done in Texas transports blood in coolers and their blood needs to remain between a temperature range of 2˚C – 5˚C. The temperature range is to be maintained using ice packs that are changed 12 – 24 hours dependent on environmental conditions (Mehkri, Monroe, Escott and Bank, 2017). The study that we conducted required similar conditions for blood temperature to remain within a 1˚C – 10˚C range. It was found that when using 2 ice packs, and having them replaced after 12 hours, the temperature remained within range. This means the cold chain management of the blood was maintained in this study.

Therefore to summarize the recommendations to carry PRBC on ambulance vehicles in the prehospital setting should be: to use 2 ice-packs for 1 PRBC in a hamper, the blood should be transported for a 35 day period only, alternating blood packs and ice-packs after every 10-12 hour shifts as conducted in the study. This would take into account all possible external factors that can occur allowing the blood to still remain way below the 0.8% acceptable haemolysis percentage as shown in figure 4.1.

The paucity in local research however, makes it difficult for international comparibility with regards to the PRBC storage during transportation. According to the clinical guidelines of South Africa, the transportation temperature range for PRBC is 1˚C - 10˚C (SANBS and WPBTS, 2016), which makes this study demonstrate compliance with the desired temperature range.

5.2. Self-efficacy questionnaire

The aim of the questionnaire was to determine the self-efficacy of ALS practitioner's willingness to introduce blood transfusion as a resuscitation medium, and to enhance educational requirements if need be. A total of 40 (n) participants (ALS practitioners) from Metro EMS in the Western Cape participated in the study.

The questionnaire was designed to assess the practitioner self-efficacy for emergency blood transfusion, and the questionnaire was administered to participants before the prehospital emergency blood training. The training consisted of a detailed explanation to the ALS practitioners as to why, when and how the research will be done, training of ALS practitioners how to visually assess blood haemolysis, how to handle blood and the storage temperatures pertaining to blood in-transit as opposed to the blood stored at the ambulance base, the role of the ALS practitioners in this study was explained and informed consent was given in writing of those who were interested. The questionnaire consisted of 3 parts; part I: Demographic information of participants, part II: Self-perception of self-efficacy and part iii: Experience and knowledge.

All ALS practitioners participated in the study that were on duty for the period of March – May 2019. Out of the 40 (n) participants, the largest percentage was from Northern Division (28%), and the least participants were from CPUT (2%). The participation percentage were all based on the percentage ALS operational at the various ambulance bases. The training institutions, such as CPUT and the College of Emergency Care ambulance service are only operational during training periods and certain weekends.

The majority of the participants' (n=23; 57.5%) experiences ranged between 5-10 years, followed by (n=9; 22.5%) with more than 10 years and lastly, the minority (n=8; 20%) with less than 5 years of experience. When assessing part II: Self-perception of self-efficacy of the questionnaire, it was found that the years of experience played no role in the self-efficacy

participant's displayed for emergency blood transfusion. More than half of the participants (n=21; 52.5%) indicated that they will be extremely confident (Likert scale rating 7) in their ability to transfuse blood, provided that they receive training on the risks and benefits of blood products and the transfusion. Majority of the participant's (n=17; 42.5%) indicated that they are confident dealing with adverse reactions, following the second highest percentage (n=14; 35%) indicating being extremely confident. A large percentage of participant's (n=36; 90%) chose 7 being extremely confident, in their ability to put up an IV, and the rest (n=4; 10%) chose 6 being confident as well. Majority of the participant's (n=26; 65%) chose 7 being extremely confident, to identify patients in shock following the second highest response (n=13; 32.5%) being 6, which is confident as well. Based on all the "extremely confident" (7) and "confident" (6) indications on the self-efficacy of the questionnaire, participants, despite years of experience, definitely indicated a great level of confidence to transfuse blood as a resuscitation medium.

In part iii of the questionnaire, which is the experience and knowledge section of the questionnaire, multiple choice answers were used, to answer general knowledge questions. The one question posed was similar to that asked in part II, indicating one's ability to deal with adverse reactions vs in part iii; enquiring what should be done if a patient has a transfusion reaction, majority of participants (n=38; 95%) answered correctly. This indicates that they extremely confident in their ability to deal with adverse reactions and has the skills and knowledge to deal with transfusion/adverse reactions that they may encounter. More knowledge-based questions were asked such as what the universal blood group is, as that particular blood type will be administered as an emergency blood transfusion. Majority of the participants (n=36; 90%) answered this correctly. A question was posed to ascertain why they think that blood would be the chosen resuscitation fluid for patients experiencing exsanguinating haemorrhage. Once again majority of the participants (n=26; 65%) answered correctly. More information regarding knowledge-based questions were further discussed in the prehospital emergency blood training.

While perusing literature it is evident that there are highly skilled EMS practitioners that are able to treat high acuity patients; these include but are not limited to rapid sequence intubation (Gunning et al., 2013), artificial ventilation and the use of various pharmacotherapy. It is likely that the self-efficacy for the administration of blood would be appropriate as EMS practitioners in the study were able to confidently identify patients in the haemorrhagic shock stages and classes. According to Gutierrez, Reines and Wulf-Gutierrez (2004), haemorrhagic shock is rapidly fatal and the use of IV crystalloids/colloids and blood products available can be lifesaving. When haemorrhage exceeds 30% of the estimated blood volume (EBV), which is

class III or IV haemorrhage or decompensated shock (Caroline, 2010), the use of blood and blood products is required.

According to the National Occupational Competency Profile for Paramedics in Canada, all Critical Care Paramedics (CCP) are required to: be able to describe blood and its components, discuss the various blood types, perform the administration of blood, be able to safely handle blood products and can integrate volume expanders with blood and blood products (The Paramedic Association of Canada, 2011). Additionally, in Australia, one of the largest ambulance services in the world, Queensland Ambulance Service (QAS), also has a protocol dealing with the administration of packed red blood cells by paramedics, as it is part of their scope of practice (Queensland Ambulance Service, 2020). In various parts of the world, learning about blood transfusions and the administration hereof is part of the scope of practice for paramedics. In South Africa however, it is currently not part of the ALS scope of practice.

5.3. The burden of need for EBT

Apart from determining if blood will remain viable in the prehospital environment and ascertaining the self-efficacy of ECP to transfuse blood (should it be required), determining the need for PRBC on emergency vehicles is essential, as blood is a scarce resource.

The ECP reported on patients eligible for blood transfusion based on transfusion triggers, specifically trauma patients who were haemorrhaging and displayed signs of class III or IV haemorrhage or decompensated shock (Caroline, 2010). This criterion ties in with the protocol used by the Helicopter Emergency Medical Services. Inclusive of hypotension with systolic BP <90mmHg, change in skin colour (pallor), central nervous system (CNS) status, heart rate >120bmp, shock index and actively bleeding patients (Brown et al., 2015). This was also the largest study conducted to date, examining patient outcomes associated with ERBC in the prehospital environment. The outcomes of this study indicated that the patients who received ERBC before arriving at the emergency unit, had an increase probability of 24-hour survival, the patients had a decreased risk of shock upon admission. This study fully supports the use of ERBC in the prehospital environment as it can lead to improved outcomes to the severely injured patients.

Blood products were also found to be viable and safe to transfuse in the prehospital setting, in Great Britain (Lyon et al., 2017), Sweden (José-Gabriel et al., 2018), Netherlands (Peters et

al., 2019), Norway (Zielinski et al., 2017), as well as Australia (Heschl et al., 2017). According to Zielinski et al. (2017), it is estimated that 1-2% of Norwegian helicopter emergency medical service patients, require prehospital emergency blood transfusions, that is approximately 25 patients a month and 2.5% of patients from Sweden (José-Gabriel et al., 2018).

The percentage of trauma patients in this study who could have made use of EBT in the prehospital field over the period of March 2019 – May 2019 is 12.8%, who fitted the inclusion criteria. However, due to limitations such as; sample size, limited Hb meters, research constraints and only a selected few EMS practitioners being ALS (minority in the field), determining the burden of blood transfusion need in the prehospital environment was challenging.

There is currently no literature pertaining to the need of EBT in the prehospital setting in South Africa.

CHAPTER SIX: CONCLUSION & RECOMMENDATIONS

6.1. Conclusion

6.1.1. The viability of blood in the prehospital setting

The main objective of this study was to estimate the viability of PRBC in the EMS setting, prior to the onset of haemolysis.

The integrity of blood was well maintained based on the following observations: the cold chain of the PRBC was well maintained with a cooler box and 2 ice packs for one unit of PRBC, the percentage haemolysis at the end of the PRBC's expiry reached a maximum level of 0.8% which is in line with the acceptable haemolysis percentage according to the European Guidelines, and the CD and TBARS which are the markers for the indication of oxidative stress, showed no statistical significant changes.

As per the above parameters that was monitored and well-maintained, PRBC could remain viable in the EMS setting.

6.1.2. Self-efficacy questionnaire

The ECP's was given a questionnaire to determine their self-efficacy and willingness to introduce blood as a resuscitation medium and to enhance educational requirements if need be.

It was found that the ECP's were confident in their abilities to determine if a patient is in class III or class IV shock (decompensated/irreversible shock), as well as their ability to transfuse blood, provided they are aided with more training on this aspect. It was also found that the ECPS's generally have good embedded knowledge about adverse effects as well as blood as a resuscitation fluid.

The ECP's displayed willingness to learn more about blood and its products, to advance EMS care.

6.1.3. The burden of need for EBT

This study also estimated the incidence of shock in patients presenting to a public EMS.

A percentage of 12.8% of trauma patients was found that could have been eligible for EBT, this is a significant percentage of patients that could have made use of ERBC in the prehospital field. EMS would have had the ability to bridge the time critical gap of a life-threatening probable mortality, before reaching the emergency department where blood would be administered.

6.2. Recommendations

It is recommended that determining the need for EBT on a larger scale will yield better results, thus including the ILS to be a part of collecting statistics of possible trauma patients experiencing severe haemorrhage in class III or class IV shock (decompensated/irreversible shock). As it was brought to light in the study that the largest group of EMS practitioners in the Western Cape is ILS and they are exposed to much more trauma cases than the ALS.

Future studies could attempt using mobile refrigerators in ambulance vehicles, eliminating the process of having to switch blood packs from the refrigerator at the ambulance base, at the start or end of their shift, to maintain the cold-chain. Blood would remain in the refrigerator throughout its shelf-life and a segment on the blood pack can be removed weekly up until expiry to test for the viability of blood in the mobile refrigerator.

Incorporating blood transfusion theory in the ECP scope of practice, would be recommended as well. This would better equip the ECP for EBT should it be implemented in the prehospital setting, as majority of the ECP had indicated this as well in the self-perception of self-efficacy questionnaire of this study.

REFERENCES:

Aguilar Diaz De Leon, J. and Borges, C., 2020. Evaluation of Oxidative Stress in Biological Samples Using the Thiobarbituric Acid Reactive Substances Assay. *Journal of Visualized Experiments*, (159).

Amukele, T., Ness, P., Tobian, A., Boyd, J. and Street, J., 2016. Drone transportation of blood products. Transfusion, 57(3), pp.582-588.

Antosik, A., Czubak, K., Gajek, A., Marczak, A., Glowacki, R., Borowczyk, K. and Zbikowska, H., 2015. *Influence Of Pre-Storage Irradiation On The Oxidative Stress Markers, Membrane Integrity, Size And Shape Of The Cold Stored Red Blood Cells*.

Apuke, O., 2017. Quantitative Research Methods : A Synopsis Approach. *Kuwait Chapter of Arabian Journal of Business and Management Review*, 6(11), pp.40-47.

Arif, S., Yadav, N., Rehman, S. and Mehdi, G., 2016. Study of Hemolysis During Storage of Blood in the Blood Bank of a Tertiary Health Care Centre. *Indian Journal of Hematology and Blood Transfusion*, 33(4), pp.598-602.

Ayala, A., Muñoz, M. and Argüelles, S., 2014. Lipid Peroxidation: Production, Metabolism, and Signaling Mechanisms of Malondialdehyde and 4-Hydroxy-2-Nonenal. *Oxidative Medicine and Cellular Longevity*, 2014, pp.1-31.

Bellairs, G. and Ingram, C. ed., 2013. *Standards of Practice for Blood Transfusion in South Africa. 6th edition.*

Bellairs, G.R.M. and Ingram, C. 2014. *Clinical Guidelines for use of blood products in South Africa*. *5th ed.*

Bennett-Guerrero, E., Veldman, T., Doctor, A., Telen, M., Ortel, T., Reid, T., Mulherin, M., Zhu, H., Buck, R., Califf, R. and McMahon, T. 2007. Evolution of adverse changes in stored PRBCs. *Proceedings of the National Academy of Sciences*, 104(43), pp.17063-17068.

Bergman, E., de Feijter, J., Frambach, J., Godefrooij, M., Slootweg, I., Stalmeijer, R. and van der Zwet, J. 2012. AM Last Page. Academic Medicine, 87(4), p.545.

Bonanno, F. 2011. Clinical pathology of the shock syndromes. *Journal of Emergencies, Trauma, and Shock*, 4(2), p.233.

Bowdler, N., 2012. *Air ambulance first in UK to carry blood*. [online] Available at: http://www.bbc.com/news/health-16928621 [Accessed 28 Apr. 2017].

Brown, J., Sperry, J., Fombona, A., Billiar, T., Peitzman, A. and Guyette, F., 2015. Pre-Trauma Center Red Blood Cell Transfusion Is Associated with Improved Early Outcomes in Air Medical Trauma Patients. *Journal of the American College of Surgeons*, 220(5), pp.797-808.

Bruns, B., Lindsey, M., Rowe, K., Brown, S., Minei, J., Gentilello, L. and Shafi, S. 2007. Hemoglobin Drops Within Minutes of Injuries and Predicts Need for an Intervention to Stop Hemorrhage. *The Journal of Trauma: Injury, Infection, and Critical Care*, 63(2), pp.312-315.

Caroline, N. 2010. *Nancy Caroline's emergency care in the streets*. 6th ed. Sudbury, Mass: Jones & Bartlett Learning, pp.21-22.

Chandran, A., Hyder, A. and Peek-Asa, C. 2010. The Global Burden of Unintentional Injuries and an Agenda for Progress. *Epidemiologic Reviews*, 32(1), pp.110-120.

Chaudhary, R. and Katharia, R. 2012. Oxidative injury as contributory factor for red cells storage lesion during twenty eight days of storage. Blood Transfusion, 10(1):5962.

Choudhury, N. and Mathur, A. 2011. Visual detection of hemolysis in a blood bag before issue. *Asian Journal of Transfusion Science*, 5(1), p.61.

Conterato, M., Witthuhn, S., Cohen, S. and Trembley, A. 2016. Implementing Protocols to Administer Blood Products in the Prehospital Setting. *JEMS*, [online] 41(5). Available at: https://www.jems.com/2016/04/30/implementing-protocols-to-administer-blood-products-in-the-prehospital-setting/ [Accessed 7 Nov. 2019].

Cothren, C., Moore, E., Hedegaard, H. and Meng, K. 2007. Epidemiology of Urban Trauma Deaths: A Comprehensive Reassessment 10 Years Later. World Journal of Surgery, 31(7), pp.1507-1511.

D'Alessandro, A., Liumbruno, G., Grazzini, G. and Zolla, L. 2010. Red blood cell storage: the story so far. Blood Transfusion, 8(2): 82-88.
Daily Nation. 2016. Rwanda begins to deliver medical supplies using drones. [online] Available at: http://www.nation.co.ke/news/africa/Rwanda-begins-to-deliver-medical-supplies-using-drones/1066-3416798-iqgtlc/index.html [Accessed 22 Jul. 2017].

Daniels, G. and Withers, S. 2007. Towards universal red blood cells. Nature Biotechnology, 25(4), pp.427-428.

Desai, S., Farris, F. and Ray, S., 2014. Lipid Peroxidation. *Encyclopedia of Toxicology*, pp.89-93.

Dhawan, H., Sachdeva, P., Gupta, A., Sharma, R. and Marwaha, N., 2017. Hemolysis during component preparation: An inadvertent cause. *Asian Journal of Transfusion Science*, 11(2), p.79.

Doughty, H., Woolley, T. and Thomas, G. 2011. Massive Transfusion. *J R Army Med Corps*.

El Sayad, M. and Noureddine, H. 2014. Recent Advances of Hemorrhage Management in Severe Trauma. *Emergency Medicine International*, 2014, pp.1-5.

Giera, M., Lingeman, H. and Niessen, W., 2012. Recent Advancements in the LC- and GC-Based Analysis of Malondialdehyde (MDA): A Brief Overview. *Chromatographia*, 75(9-10), pp.433-440.

Goertzen, M., 2017. *Chapter 3. Introduction To Quantitative Research And Data*. [online] Journals.ala.org. Available at: <https://www.journals.ala.org/index.php/ltr/article/view/6325/8275> [Accessed 12 September 2020].

Gosselin, R., Spiegel, D., Coughlin, R. and Zirkle, L. 2009. Injuries: the neglected burden in developing countries. *Bulletin of the World Health Organization*, 87(4), pp.246-246.

Gunning, M., Perkins, Z., Crilly, J. and Von Rahden, R. 2013. Paramedic rapid sequence induction (RSI) in a South African emergency medical service: A retrospective observational study. *South African Medical Journal*, 103(9), p.632.

Gutierrez, G., Reines, H. D., & Wulf-Gutierrez, M. E. 2004. Clinical review: Hemorrhagic shock. *Critical Care*, *8*(5), 373–381.

Hahn, R., 2012. Fluid therapy in uncontrolled hemorrhage - what experimental models have taught us. *Acta Anaesthesiologica Scandinavica*, 57(1), pp.16-28.

Hall, S. and Murphy, M., 2015. Limitations of component therapy for massive haemorrhage: is whole blood the whole solution?. *Anaesthesia*, 70(5), pp.511-514.

Hardwick, J., 2008. Blood storage and transportation. *ISBT Science Series*, 3(2), pp.177-196.

Harmening, D. 2005. *Modern blood banking and transfusion practices*. 1st ed. Philadelphia: F.A. Davis.

Head, T. 2017. Nyanga, Western Cape, has a murder rate of one in every 206 people. *The South African*. [online] Available at: https://www.thesouthafrican.com/news/nyanga-highest-murder-rate-south-africa/ [Accessed 18 Jul. 2019].

Heckbert, S., Vedder, N., Hoffman, W., Winn, R., Hudson, L., Jurkovich, G., Copass, M., Harlan, J., Rice, C. and Maier, R. 1998. Outcome after Hemorrhagic Shock in Trauma Patients. *The Journal of Trauma: Injury, Infection, and Critical Care*, 45(3), pp.545-549.

Henderson, G., Churchill, L., Davis, A., Easter, M., Grady, C., Joffe, S., Kass, N., King, N., Lidz, C., Miller, F., Nelson, D., Peppercorn, J., Rothschild, B., Sankar, P., Wilfond, B. and Zimmer, C. 2007. Clinical Trials and Medical Care: Defining the Therapeutic Misconception. PLoS Medicine, 4(11), p.e324.

Henry, M., Stapleton, E. and Edgerly, D. 2010. EMT prehospital care. 4th ed. United States of America: Kimbery Brophy, p.524.

Heschl, S., Andrew, E., de Wit, A., Bernard, S., Kennedy, M. and Smith, K., 2017. Prehospital transfusion of red cell concentrates in a paramedic-staffed helicopter emergency medical service. *Emergency Medicine Australasia*, 30(2), pp.236-241.

Hess, J., 2010. Red cell changes during storage. *Transfusion and Apheresis Science*, 43(1), pp.51-59.

Hilbert-Carius, P., Schwarzkopf, D., Reinhart, K., Hartog, C., Lefering, R., Bernhard, M. and Struck, M. 2018. Synthetic colloid resuscitation in severely injured patients: analysis of a nationwide trauma registry (TraumaRegister DGU). *Scientific Reports*, 8(1).

Holcomb, J., Fox, E., Zhang, X., White, N., Wade, C., Cotton, B., del Junco, D., Bulger, E., Cohen, M., Schreiber, M., Myers, J., Brasel, K., Phelan, H., Alarcon, L., Muskat, P. and Rahbar, M., 2013. Cryoprecipitate use in the PROMMTT study. *Journal of Trauma and Acute Care Surgery*, 75, pp.S31-S39.

HPCSA. 2017. Professional Boards - HPCSA. [online] Available at: http://www.hpcsa.co.za/PBEmergencyCare [Accessed 12 Aug. 2017].

Issitt, P. and David J. Anstee 1985. *Applied blood group serology*. 3rd ed. Miami: Montgomery Scientific.

Jabaley, C. and Dudaryk, R. 2014. Fluid Resuscitation for Trauma Patients: Crystalloids Versus Colloids. *Current Anesthesiology Reports*, 4(3), pp.216-224.

Jahn, W. 2011. The 4 basic ethical principles that apply to forensic activities are respect for autonomy, beneficence, nonmaleficence, and justice. *Journal of Chiropractic Medicine*, 10(3), pp.225-226.

Jamison, D., Feachem, R. and Makgoba, M. 2006. *Disease and mortality in Sub-Saharan Africa*. Washington, D.C: World Bank.

Janatpour, K., Paglieroni, T., Crocker, V., DuBois, D. and Holland, P. 2004. Visual assessment of hemolysis in red blood cell units and segments can be deceptive. *Transfusion*, 44(7), pp.984-989.

Jooste, B., 2010. *Cape Town is stab capital of SA,* Cape Town: Cape Argus.

Jooste, E., 2012. Clinical Fluid Therapy in the Perioperative Setting. *Anesthesia & Analgesia*, 115(3), p.740.

José-Gabriel, S., Per, A., Mari, H., Kristina, J., Gabriel, S. and Sven-Erik, R., 2018. *[Introduction Of Prehospital Blood Transfusion Programme In Sweden: Experiences From A Physician Staffed Helicopter Emergency Medical Service]*. [online] PubMed. Available at: <https://pubmed.ncbi.nlm.nih.gov/30299527/> [Accessed 20 January 2021].

Kauvar, D., Lefering, R. and Wade, C. 2006. Impact of Hemorrhage on Trauma Outcome: An Overview of Epidemiology, Clinical Presentations, and Therapeutic Considerations. *The Journal of Trauma: Injury, Infection, and Critical Care*, 60(Supplement), pp.S3-S11

Kayilioglu, S., Dinc, T., Sozen, I., Bostanoglu, A., Cete, M. and Coskun, F. 2015. Postoperative fluid management. *World Journal of Critical Care Medicine*, 4(3), p.192.

Klein, H. and Anstee, D. 1988. *Mollison's blood transfusion in clinical medicine*. 1st ed.

Kleinman, SH, Busch MP. 2006. Assessing the impact of HIV NAT on window period reduction and residual risk. *Journal of Clinical Virology Suppl*. 1:23-29.

Landau, G., Kodali, V., Malhotra, J. and Kaufman, R., 2013. Detection of Oxidative Damage in Response to Protein Misfolding in the Endoplasmic Reticulum. *Methods in Enzymology*, pp.231-250.

Levi, M. and Jonge, E. 2007. Clinical Relevance of the Effects of Plasma Expanders on Coagulation. *Seminars in Thrombosis and Hemostasis*, 33(8), pp.810-815.

Liang, W., Zhang, R., Ye, X., Sun, J., Xu, J. and Tan, W. 2018. The haemovigilance: the best quality management system of the transfusion chain?. *ISBT, Vox Sanguinis*, [online] 13(3), pp.306-311. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/voxs.12419 [Accessed 28 Jun. 2019].

Lira, A. and Pinsky, M. 2014. Choices in fluid type and volume during resuscitation: impact on patient outcomes. Annals of Intensive Care, 4(1).

Locke, E. and Bandura, A. 1987. Social Foundations of Thought and Action: A Social-Cognitive View. *The Academy of Management Review*, 12(1), p.169.

Lyon, R., de Sausmarez, E., McWhirter, E., Wareham, G., Nelson, M., Matthies, A., Hudson, A., Curtis, L. and Russell, M. 2017. Prehospital transfusion of red cell concentrates in 147 patients from a UK helicopter emergency medical service. Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine, 25(1).

Makroo, R., Raina, V., Bhatia, A., Gupta, R., Majid, A., Thakur, U. and Rosamma, N., 2011. Evaluation of the red cell hemolysis in packed red cells during processing and storage. *Asian Journal of Transfusion Science*, 5(1), p.15.

Marx, G. and Schuerholz, T., 2010. Fluid-induced coagulopathy: does the type of fluid make a difference?. *Critical Care*, 14(1), p.118.

Matthews, R., McCaul, M. and Smith, W. 2017. A description of pharmacological analgesia administration by public sector advanced life support paramedics in the City of Cape Town. African Journal of Emergency Medicine, 7(1), pp.24-29.

McLeod, S. 2019. Likert Scale Definition, Examples and Analysis. *Simply Psychology*.

Mehkri, F., Monroe, B., Escott, M. and Bank, E., 2017. *Considerations For Using Blood Products For Prehospital Trauma Patients - JEMS*. [online] JEMS. Available at: <https://www.jems.com/patient-care/trauma/considerations-for-using-blood-products-for-prehospital-trauma-patients/> [Accessed 18 January 2021].

Miller, T. 2013. New evidence in trauma resuscitation - is 1:1:1 the answer?. *Perioperative Medicine*, 2(1).

Mock, C., Joshipura, M., Arreola-Risa, C. and Quansah, R. 2012. An Estimate of the Number of Lives that Could be Saved through Improvements in Trauma Care Globally. *World Journal of Surgery*, 36(5), pp.959-963.

Myburgh, J. and Mythen, M. 2013. Resuscitation Fluids. New England Journal of Medicine, 369(13), pp.1243-1251.

Nall, R. and Gotter, A. 2016. *Hypovolemic Shock*. [online] Healthline. Available at: http://www.healthline.com/health/hypovolemic-shock#overview1 [Accessed 13 May 2017].

Newton, P., Naidoo, R. and Brysiewicz, P. 2015. The appropriateness of emergency medical service responses in the eThekwini district of KwaZulu-Natal, South Africa. South African Medical Journal, 105(10), p.844.

NICE 2013. Intravenous fluid therapy for adults in hospital: summary of NICE guidance. *BMJ*, 347(dec23 1), pp.f7624-f7624.

NICE, 2017. NICE CG 174: Intravenous fluid therapy in adults in hospital. *British Journal of Nursing*, 23(Sup8), pp.S6-S8.

Nickson, C. 2015. *Major Haemorrhage in Trauma*. [online] LITFL • Life in the Fast Lane Medical Blog. Available at: https://lifeinthefastlane.com/ccc/major-haemorrhage-in-trauma/ [Accessed 8 Aug. 2017].

Nolan, J. and Pullinger, R. 2014. Hypovolaemic Shock. *BMJ*, 348(mar07 1), p1139.

Orbegozo Cortés, D., Rayo Bonor, A. and Vincent, J., 2014. Isotonic crystalloid solutions: a structured review of the literature. *British Journal of Anaesthesia*, 112(6), pp.968-981.

Otani, T., Oki, K., Akino, M., Tamura, S., Naito, Y., Homma, C., Ikeda, H. and Sumita, S. 2012. Effects of helicopter transport on red blood cell components. Blood Transfusion, 10(1), pp.78-86.

Parija, S., Mandal, J. and Acharya, S. 2011. Ethics in human research. *Tropical Parasitology*, 1(1), p.2.

Pereira, A., 2009. Efficacy of different plasma sources in the treatment of thrombotic thrombocytopenic purpura. *ISBT Science Series*, 4(1), pp.111-117.

Peters, J., Smulders, P., Moors, X., Bouman, S., Meijs, C., Hoogerwerf, N. and Edwards, M., 2019. Are on-scene blood transfusions by a helicopter emergency medical service useful and safe? A multicentre case–control study. *European Journal of Emergency Medicine*, 26(2), pp.128-132.

Procter, LD. 2018. Intravenous Fluid Resuscitation available at https://www.msdmanuals.com/professional/critical-care-medicine/shock-and-fluid-resuscitation/intravenous-fluid-resuscitation

Queensland Ambulance Service, 2020. *Digital Clinical Practice Manual*. Australia, p.1054.

R-project.org. 2020. *R: The R Project For Statistical Computing*. [online] Available at: <https://www.r-project.org/> [Accessed 13 June 2020].

Rath, G., Mitra, R. and Mishra, N. 2014. Blood groups systems. *Indian Journal of Anaesthesia*, 58(5), p.524.

Recknagel, R. and Glende, E., 1984. [40] Spectrophotometric detection of lipid conjugated dienes. *Methods in Enzymology*, pp.331-337.

Rehn, M., Davies, G., Smith, P. and Lockey, D., 2017. Emergency versus standard response: time efficacy of London's Air Ambulance rapid response vehicle. *Emergency Medicine Journal*, 34(12), pp.806-809.

Repetto, M., Semprine, J. and Boveris, A., 2012. *Lipid Peroxidation: Chemical Mechanism, Biological Implications And Analytical Determination*.

Rous, P. and Turner, J. 1916. The Preservation of Living Red Blood Cells In Vitro: I. Methods of Preservation. Journal of Experimental Medicine, 23(2), pp.219-237.

SAPS. 2019. *Crime situation in Republic of South Africa twelve (12) months (APRIL-MARCH 2018/19)*. [online] Available at: https://www.saps.gov.za/services/april_to_march 2018/19_presentation.pdf [Accessed 01 Oct. 2019].

Sawant, R., Jathar, S., Rajadhyaksha, S. and Kadam, P. 2007. Red cell hemolysis during processing and storage. *Asian Journal of Transfusion Science*, 1(2), p.47.

Schwarz, H. and Dorner, F. 2003. Karl Landsteiner and his major contributions to haematology. *British Journal of Haematology*, 121(4), pp.556-565.

Silverman, A. and Wang, V. 2005. Shock: A Common Pathway For Life-Threatening Pediatric Illnesses And Injuries. [online] Ebmedicine.net. Available at: http://www.ebmedicine.net/topics.php?paction=showTopic&topic_id=149 [Accessed Jul. 2017].

Smith, G. 2019. *Pre-Hospital Factors that Lead to Increased Mortality and Morbidity in Trauma Patients in Developing Countries: A Systematic Review*. Ph.D. The University of Arizona.

Sousa, V., Driessnack, M. and Mendes, I. 2007. An overview of research designs relevant to nursing: Part 1: quantitative research designs.

South African Government 2017. MEC Nomafrench Mbombo: Western Cape Health Prov Budget Vote 2016/17. Cape Town.

South African National Blood Service (SANBS) and the Western Province Blood Transfusion Service (WPBTS). 2014*. Clinical Guidelines for the Use of Blood Products in South Africa*. [5th edition]. Johannesburg: Adcock Ingram Critical Care.

South African National Blood Service (SANBS) and the Western Province Blood Transfusion Service (WPBTS). 2016. *Standards of Practice for Blood Transfusion in South Africa*. 7th ed. South Africa, p.28.

Spahn, D., Bouillon, B., Cerny, V., Coats, T., Duranteau, J., Fernández-Mondéjar, E., Filipescu, D., Hunt, B., Komadina, R., Nardi, G., Neugebauer, E., Ozier, Y., Riddez, L., Schultz, A., Vincent, J. and Rossaint, R. 2013. Management of bleeding and coagulopathy following major trauma: an updated European guideline. *Critical Care*, 17(2), p.R76.

Takasu, A., Minagawa, Y., Ando, S., Yamamoto, Y. and Sakamoto, T. 2010. Improved Survival Time With Combined Early Blood Transfusion and Fluid Administration in Uncontrolled Hemorrhagic Shock in Rats. *The Journal of Trauma: Injury, Infection, and Critical Care*, 68(2), pp.312-316.

The Paramedic Association of Canada, 2011. *National Occupational Competency Profile.* Canada: Paramedic Association of Canada, p.103.

The SA Red Cross Air Mercy Service. 2014. *AMS*. [online] Available at: https://www.ams.org.za/services

Tholpady, A. and Bai, Y. 2019. *Crossmatching: Overview, Clinical Indications/Applications, Test Performance*. [online] Emedicine.medscape.com. Available at: https://emedicine.medscape.com/article/1731279-overview

Tintinalli, J. 2010. *Tintinalli's emergency medicine: A Comprehensive Study Guide*. 7th ed. New York: McGraw-Hill Companies, pp.165-172.

Tisherman, S., Schmicker, R., Brasel, K., Bulger, E., Kerby, J., Minei, J., Powell, J., Reiff, D., Rizoli, S. and Schreiber, M., 2015. Detailed Description of All Deaths in Both the Shock and Traumatic Brain Injury Hypertonic Saline Trials of the Resuscitation Outcomes Consortium. *Annals of Surgery*, 261(3), pp.586-590.

Turner, H. and Hurley, J. 2015. The History and Practice of College Health. Lexington: The University Press of Kentucky, p.95.

Vincent, J. and De Backer, D. 2013. Circulatory Shock. New England Journal of Medicine, 369(18), pp.1726-1734.

Watkins, W., 2001. The ABO blood group system: Historical Background. *Transfus Med,* pp. 243-265.

WCBS 2019. *Who can donate?* | *WCBS*. [online] Wcbs.org.za. Available at: https://www.wcbs.org.za/?q=aboutdonation/becoming-donor/who-can-donate [Accessed 20 Jul. 2019].

Wessels, P. 2017. *Ethical and Legal considerations in Transfusion Medicine*. [online] Available at: https://www.google.com/url?sa=t&rct=j&q=&esrc=s&source=web&cd=10&ved=2ahUKEwiDl7ONpZHjAhVRoXEKHcOhASsQFjAJegQIBhAC&url=http%3A%2F%2Fwww.igazi.org.za%2 [Accessed 21 Jun. 2019].

Western Cape Government. 2016. Emergency medical care. Cape Town.

Western Cape Government. 2015. Medical Emergency Transport and Rescue. Cape Town.

World Health Organization. 2016. *WHO Global Database on Blood Safety*. Available at:http://apps.who.int/iris/bitstream/10665/254987/1/9789241565431-eng.pdf?ua=1 [Accessed 22 Jun. 2019].

World Health Organization. 2015. *Global status report on road safety*. Geneva: World Health Organization.

WPBTS. 2013. Blood Donation. Pamphlet. Western Province Blood Transfusion Service, Pinelands

Zalgaonker, M., Naidoo, N. and Christopher, L., 2021. Penetrating injury from interpersonal violence and related haemorrhagic shock resuscitation practices in an urban South African emergency medical service. *Australasian Journal of Paramedicine*, 18.

Zielinski, M., Stubbs, J., Berns, K., Glassberg, E., Murdock, A., Shinar, E., Sunde, G., Williams, S., Yazer, M., Zietlow, S. and Jenkins, D., 2017. Prehospital blood transfusion programs. *Journal of Trauma and Acute Care Surgery*, 82, pp.S70-S78.

Zimmermann, R., Heidenreich, D., Weisbach, V., Zingsem, J., Neidhardt, B. and Eckstein, R. 2003. In vitro quality control of red blood cell concentrates outdated in clinical practice. *Transfusion Clinique et Biologique*, 10(4), pp.275-283.

Annexure A: Ethical Clearance Certificate 1

Cape Peninsula
University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

20 November 2017
REC Approval Reference No:
CPUT/HW-REC 2017/H32

Dear Ms. Zeenat Abdul

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC on 14 September 2017 to Ms. Abdul, for ethical clearance. This approval is for research activities related to student research in the Department of Biomedical Technology.

TITLE: **An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services.**

Supervisor: Dr N Naidoo and Dr D Bester

Comment:

Approval will not extend beyond 21 November 2018. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Prof P Engel-Hills
Deputy Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Annexure B: Ethical Clearance Certificate 2

Cape Peninsula
University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

6 December 2018
REC Approval Reference No:
CPUT/HW-REC 2017/H32

Dear Ms. Zeenat Abdul

Re: APPLICATION TO THE HW-REC FOR ETHICS CLEARANCE

Approval was granted by the Health and Wellness Sciences-REC on 14 September 2017 to Ms. Abdul, for ethical clearance. This approval is for research activities related to student research in the Department of Biomedical Technology.

TITLE: **An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services.**

Supervisor: Dr N Naidoo and Dr D Bester

Comment:

Approval will not extend beyond 7 December 2019. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

Prof P Engel-Hills
Deputy Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Annexure C: Ethical Clearance Certificate 3

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HW-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

14 November 2019
REC Approval Reference No:
CPUT/HW-REC 2017/H32 (renewal)

Faculty of Health and Wellness Sciences – Biomedical Sciences

Dear Ms Zeenat Abdul,

Re: APPLICATION TO THE HW-REC FOR ETHICS RENEWAL

Approval was granted by the Health and Wellness Sciences-REC on 14 September 2017 to Ms Abdul for ethical clearance. This approval is for research activities related to student research in the Department of Biomedical Sciences.

TITLE: An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services.

Supervisors: Dr N Naidoo, Dr D Bester

Comment:

Approval will not extend beyond 07 December 2020. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards

M. le Roes-Hill

Dr Marilize Le Roes-Hill
Deputy Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Annexure D: Ethical Clearance Certificate 4

Cape Peninsula University of Technology

HEALTH AND WELLNESS SCIENCES RESEARCH ETHICS COMMITTEE (HWS-REC)
Registration Number NHREC: REC- 230408-014

P.O. Box 1906 • Bellville 7535 South Africa
Symphony Road Bellville 7535
Tel: +27 21 959 6917
Email: sethn@cput.ac.za

11 December 2020
REC Approval Reference No:
CPUT/HW-REC 2017/H32/Renewal

Dear Ms Zeenat Abdul,

Re: APPLICATION TO THE HWS-REC FOR ETHICS CLEARANCE RENEWAL

Approval was granted by the Health and Wellness Sciences-REC on 14 September 2017 to Ms Abdul for ethical clearance. This approval is for research activities related to student research in the Department of Biomedical Sciences at this Institution.

Title: An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services

Supervisor: Dr N Naidoo and Dr D Bester

Comment:

Approval will not extend beyond 12 December 2021. An extension should be applied for 6 weeks before this expiry date should data collection and use/analysis of data, information and/or samples for this study continue beyond this date.

The investigator(s) should understand the ethical conditions under which they are authorized to carry out this study and they should be compliant to these conditions. It is required that the investigator(s) complete an **annual progress report** that should be submitted to the HWS-REC in December of that particular year, for the HWS-REC to be kept informed of the progress and of any problems you may have encountered.

Kind Regards,

M. le Roes-Hill

Dr Marilize Le Roes-Hill
Deputy Chairperson – Research Ethics Committee
Faculty of Health and Wellness Sciences

Annexure E: Letter of consent Dr. S. de Vries

Western Cape Government
Health

DIRECTORATE: **EMERGENCY MEDICAL SERVICES**
ENQUIRIES: **Dr Shaheem de Vries**
✉: shaheem.devries@pgwc.gov.za
☎: +27 21 508 4523

ATTENTION: MS ZEENAT ABDUL

RE: PERMISION TO ACCESS THE WESTERN CAPE GOVERNMENT HEALTH EMS FOR THE PURPOSE OF STUDY

Dear Ms Abdul,

You email correspondence has reference.

Thank you for sharing the outline of your research proposal. Following our engagement, I am pleased to confirm my provisional support, both of the proposed site and the overall research objective.

It is my understanding that this study will require ethical approval from the departmental research committee prior to commencement.

It is with this understanding in mind that I have given my permission to access the Western Cape Emergency Medical Services for the purposes of conducting this study.

Sincerely

Dr Shaheem de Vries
Head: **Emergency Medical Services**
Western Cape Government Health

DATE: 1st December 2017

Annexure F: Letter of consent Dr. G.R.M Bellairs

Western Cape Blood Service
Do something remarkable

www.wcbs.org.za / info@wcbs.org.za

Head Office
Old Mill Road, Pinelands, 7405 • PO Box 79, Howard Place, 7450
T: 021 507 6300 / F: 021 531 0322

25 January 2018

Department of Biomedical Science
Cape Peninsula University of Technology

To Whom it may Concern

APPROVAL OF MASTER'S QUALIFICATION RESEARCH PROJECT

The research project titled "An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services", to be conducted by Zeenat Abdul, is approved.

Warm Regards

Dr Gregory Ralph Martin Bellairs
CEO/Medical Director

Paarl
26 Zandwyk Industrial Park, Old Paarl Road, Paarl, 7646
PO Box 422, Paarl, 7620
T: 021 871 1031 / F: 021 872 5945

Worcester
26 Napier Street, Worcester, 6850
PO Box 194, Worcester, 6849
T: 023 342 2450 / F: 023 342 7556

George
Medical Centre, Courtenay Street
PO Box 65, George, 6530
T: 044 874 2074 / F: 044 874 6097

Western Cape Blood Service NPC
Pr. No: 7800045 / Reg. No: 1943/016692/08

PBR19 (16 Mar 20)

Annexure G: Letter of consent G. Mc Cormick

11 November 2018

Dear Zeenat Abdul,

Re: Approval & Access to AMS patient information and records for research purposes.

The AMS Research Committee has received your AMS research application form and AMS ethics checklist.

Your request for access to the SA Red Cross Air Mercy Service (AMS) database information has been approved and granted.

The initial information sought is available based on clarifying some information with Garth Moys located at our regional office in the General Aviation Area - Cape Town International Airport.

Should you require access to patient records, we consider all patient care records to be property of the respective Provincial Department of Health and as such, you would need to apply for permission to the Provincial Department of Health research committee for access to the records and patient identifiable information.

Guidance to the use of the database and or record files will be advised on site by Garth Moys.

Standard Ethical Rules together with the organisation's restriction on removal of confidential information offsite and the patients' right to confidentiality, apply.

Thank You

Yours faithfully

Gary Mc Cormick
Quality & Technical Manager

Fax: (+27) 86 554 6313
E-mail: gary@ams.org.za

Annexure H: Informed consent form

Informed Consent form for research assistants

This informed consent form is for advanced life support emergency medical service practitioners who we are invited to participate in research on the estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services.

Name of Principal Investigator: Zeenat Abdul
Name of Institution: Cape Peninsula University of Technology
Research Study: An estimation of the burden of blood transfusion need and viability of red blood cells in the emergency medical services

PART I: Information Sheet

Introduction
My name is Zeenat Abdul, employed at the Western Province Blood Transfusion Services as a medical technologist and conducting my research at the Cape Peninsula University of Technology. We are doing a research on estimating the burden of blood transfusion need and viability of red blood cells in the emergency medical services. This study could lead to further studies which investigate the use of blood transfusions on EMS. You do not have to decide today whether or not you will participate in the research. Before you decide, you can speak to me if you have any questions or queries or to anyone you feel comfortable with about the research. If there are any words or terms you are unfamiliar with, please ask me to stop as we go through the information and I will take time to explain. If you have questions or queries at a later stage, my contact details will be provided.

Purpose of the research
According to the World Health Organisation trauma is said to be the third most common cause of mortality with haemorrhage being responsible for 30%-40% thereof. Occurrence of death during the pre-hospital period range from 33%-56%. Against this global backdrop, Cape Town is seen as the 'stab capital of South Africa'. In addition, a high burden of gunshot wounds and road-traffic accidents may cause patients to experience exsanguinating haemorrhage on scene or in ambulance transit. Thus, there is a need to bridge the time-critical gap of a life-threatening insult and probable morbidity/mortality that could occur due to excessive blood loss before reaching the emergency department where blood transfusion is administered.

There is no direct South African evidence for the burden of need, viability and EMS self-efficacy to safely enable pre-hospital emergency blood transfusion (EBT). Large volumes of crystalloid solutions have been used as pre-hospital treatment for volume resuscitation in trauma patients, for it to be effective. However, according to the clinical guidelines of South Africa, limited volumes thereof should be used in resuscitation.

This study seeks to appraise the relative burden of need for EBT in the pre-hospital environment and to investigate the viability of EBT as an advanced life support modality of care in the EMS. This will be investigated by placing blood on ambulance vehicles to determine their viability in-transit. Data will be collected from patient report forms fitting the criteria of class III/IV shock who could have been eligible for EBT. Questionnaires will be given to EMS personnel to determine their self-efficacy about introducing blood as a resuscitation fluid and undergoing training thereof.

Type of research intervention
This research will involve transporting blood in cooler boxes with ice-packs. Temperature of the blood should be recorded before and after transportation, as well as visually inspecting the blood for red blood cell destruction. This will be recorded on a data sheet provided. (Temperature and visual assessing of blood from you is required). On a weekly basis, I will visually assess the blood that have been transported and perform a haemolysis test on those units of blood. This will be for a period of one month.
At selected EMS bases, haemoglobin finger-prick testing will be done on all shock patients when glucose measurements are taken and recorded on to the patient report form (PRF). The shock stage (compensated, decompensated, and irreversible) for all haemorrhagic shock patients and an estimate of blood loss (mls) must be

indicated on the PRF. This will be for a period of 2-3 months, thus data collection required for a maximum of 5 of your shifts.
At the end of the study questionnaires will be given to research assistants to determine their self-efficacy. The questionnaire will not require lengthy answers, it will not take up too much of your time, you will only be required to mark the appropriate boxes. It will be randomized and anonymous.

Participant selection
We are inviting randomly selected advanced life support EMS practitioners, based on knowledge and seniority to help in the success of this research.

Voluntary participation
Your participation in this research study is entirely voluntary. It is your choice whether to participate or not. Although your participation will be greatly appreciated and will aid in improving future EMS developments. You may change your mind later at a later stage and stop participating even if you agreed earlier. This can be done by informing me directly.

Sharing the results
The feedback we receive from doing this research will be shared with you via email or printed report to each ambulance base that participated before it is made widely available to the public. Confidential information will not be shared.

Who to contact
If you have any questions you may ask me now or later: Zeenat Abdul 0716225807 or zeenatabdul1709@yahoo.com. Alternatively you may contact Dr Bester at 021 959 6760 or Dr Naidoo at 0219596534

Ethical clearance
This proposal has been reviewed and approved by CPUT EMS department, the Western Province Blood Transfusion Service, the Red Cross Air Mercy Service and the National/Provincial Health Research Committee (PHRC).

PART II: Certificate of Consent

I have read and understood the above information. I have had the opportunity to ask questions and the questions has been answered to my understanding. I consent voluntarily to participate as a research assistant in this study.

Print Name of Participant: _____

Signature of Participant: _____

Date: _____
Day/month/year

I confirm that the participant was given an opportunity to ask questions about the study, and I answered to the best of my ability. I confirm that the individual has not been coerced into giving consent, and the consent has been given freely and voluntarily.

Print Name of Researcher: _____

Signature of Researcher: _____

Date: _____
Day/month/year

Annexure I: Data collection sheet for temperature & haemolysis

Data Collection Sheet for Temperature and Haemolysis

Unit _____ **&** _____ *to remain in the refrigerator for the entire month as it will be used as a control*

Blood leaving the refrigerator:

Serial No.				
	Day		**Night**	
Tick (√)				
Time and date (out)				
Temp (°C) (out)				
	YES		**NO**	
Haemolysis (√)				
Ice Pack (√)				
Mode of transport	**Response car**	**Ambulance**	**Rotor wing**	**Fixed wing**
Tick (√)				
Initials				

Blood returned to the refrigerator:

Time and date (in)	
Temp (°C) (in)	
Initials	

Annexure J: Self-efficacy questionnaire

Questionnaire

*Required

1. By completing this questionnaire, I acknowledge that I understand what my involvement in the study means and that I voluntarily consent to participate. Please indicate your HPCSA No. below. *

_____

PART I: DEMOGRAPHIC INFORMATION OF PARTICIPANTS

2. Place of occupation *

Mark only one oval.

- ◯ Air Mercy Service
- ◯ College of Emergency Care
- ◯ Cape Peninsula University of Technology
- ◯ Eastern division
- ◯ Northern division
- ◯ Southern division
- ◯ Western division

3. Indicate your gender *

Mark only one oval.

- ◯ Male
- ◯ Female

4. What emergency care qualifications have you completed? Indicate all that applies *

Tick all that apply.

- ☐ BAA
- ☐ AEA
- ☐ CCA
- ☐ NCert: EMC
- ☐ NDip: EMC
- ☐ BTech: EMC

Other: ☐ _____

5. Where did you complete your medical qualifications? *

Tick all that apply.

- [ ] Cape Peninsula University of Technology
- [ ] College of Emergency Care

Other: [ ] _____

6. How many years of experience do you have as a paramedic? Please write a number. *

_____

PART II: SELF-PERCEPTION OF SELF-EFFICACY

Please use the 7-point scale of confidence to complete this section. Where 1 = not at all confident & 7 = extremely confident

7. How confident are you in your ability to identify patients that are in shock? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

8. How confident are you in your ability to classify patients into the 4 classes of haemorrhagic shock? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

9. How confident are you in your ability to classify patients into the 3 successive shock stages namely; compensated, decompensated and irreversible shock? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

10. How confident are you in your ability to deal with adverse reactions? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

11. Haemoglobin testing uses the same fingerprick testing principle. How confident are you in your ability to do fingerprick testing? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

12. How confident are you in your ability to put up an IV? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

13. How confident are you in your ability to estimate blood loss (ml)? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

14. How confident are you in your ability to stay calm in emergency situations? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

15. How confident are you in your ability to overcome anxiety when treating trauma patients? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

16. Blood transfusion is in the scope of practice for EMS practitioners, however there is no operational protocol in place yet. How confident are you in your ability to transfuse blood, provided that you received training on the risks and benefits of blood products and the transfusion hereof? *

Mark only one oval.

	1	2	3	4	5	6	7	
Not at all confident	◯	◯	◯	◯	◯	◯	◯	Extremely confident

PART III: KNOWLEDGE AND EXPERIENCE

Please answer these questions to the best of your ability and note that you will not be rewarded or penalized for your perfomance herein as these individual scores will be kept strictly confidential.

17. Approximately how many P1 calls, were you dispatched to in the last month? *

Mark only one oval.

- ◯ 0-25
- ◯ 26-50
- ◯ 51-75
- ◯ 76-100
- ◯ 101-125
- ◯ 126-150
- ◯ Can't remember

18. If you had to estimate, what percent of the calls mentioned above were patients experiencing exsanguinating haemorrhage (excessive blood loss)? *

Mark only one oval.

- 0-10%
- 10-20%
- 20-30%
- 30-40%
- 40-50%
- 50-60%
- 60-70%
- 70-80%
- 80-90%

19. What is the first thing you should do in the case of a transfusion reaction? *

Mark only one oval.

- Stop the infusion
- Distract the patient
- Slow down the transfusion
- Administer crystalloids
- Don't know

20. What blood group is the universal donor (that can donate to any blood group)? *

Mark only one oval.

- B
- O
- A
- AB
- Don't know

21. What blood group is the universal recipient (that can receive any blood group)? *

Mark only one oval.

- ◯ B
- ◯ O
- ◯ A
- ◯ AB
- ◯ Don't know

22. Haemolysis in RBC is caused by? *

Mark only one oval.

- ◯ Influenza
- ◯ Anaphylactic reaction
- ◯ Red blood cell destruction
- ◯ Insufficient calcium
- ◯ Don't know

23. Why are blood transfusions given? *

Mark only one oval.

- ◯ To increase the amount of blood
- ◯ To decrease the risk of bleeding
- ◯ To increase the blood's ability to carry oxygen
- ◯ All of the above
- ◯ Do not know

24. Which parts of the blood can be transfused? *

Mark only one oval.

- ◯ Whole blood
- ◯ Platelets
- ◯ Red blood cells
- ◯ All of the above
- ◯ Do not know

25. What is regarded as a normal reference range for haemoglobin in a full blood count? *

Mark only one oval.

- 10.5-15.5g/dL
- 15.6-20.6g/dL
- 11.7-16.7g/dL
- 8.4-13.4g/dL

26. Donated blood at Western Province blood transfusion services undergoes screening for which diseases? *

Mark only one oval.

- Hepatitis B and C + HIV + Syphillis
- Diabetes + Malaria + Influenza
- All of the above
- None of the above