Aspects of Transfusion Medicine

by

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TABLE OF CONTENTS

DECLARATION

I certify that this thesis does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text

David Roxby

Date: 1 February 2018

ACKNOWLEDGEMENTS

I am grateful to Flinders University for providing the opportunity to staff associated with the University with academic status and working full-time who could not formally undertake a research based Degree of Doctor of Philosophy to submit a thesis based on their peer-reviewed published works.

The thesis would not have been possible without the help and support of my principal supervisor, Associate Professor Bryone Kuss, research associate Dr Romi Sinha, other research associates and co-inventor of the portable fluid/blood warmer, Dr Mark McEwen.

I have enjoyed the support of the Flinders Medical Centre SA Pathology Transfusion laboratory staff and I extend sincere thanks to all those who assisted me with my research over the years.

The advice and support from Dr Magda Sobieraj-Teague and my colleagues has been extremely valuable for which I am extremely grateful to them.

Finally, I am indebted to my family and friends who have supported me throughout this work. Their encouragement helped me to finish my thesis.

1. ABSTRACT

My thesis is based upon twelve of my recently published works (1-12) and focuses on four areas of Transfusion Medicine: development of a non-electrically powered intravenous fluid/blood warmer , the effect of age of transfused red cells on patient mortality (2-5), critical bleeding and massive transfusion (6-11), and use of rotational viscoelastometric testing (ROTEM) to identify patients with sepsis at risk of developing multi-organ failure (12).

Development of a novel portable non-electrically powered intravenous fluid/blood warmer has the potential to provide significant benefits for the prevention of hypothermia in non-hospital settings in critically injured patients. *In vitro* studies of our patented prototype has proved successful in warming up to four units of red cells from 2-6°C to 35-37°C (1) and an *in vivo* clinical trial is nearing completion.

The INFORM randomised controlled trial (RCT), the largest of its kind and associated subsequent meta-analysis of other RCTs are pivotal pieces of research that have provided substantial critical evidence that the age of transfused red cells has no effect on patient mortality (2-5). This provides a benchmark for standard of care in the management of blood banking and of patients requiring transfusion.

Our on-going observational studies of transfusion practice in critical bleeding situations, introduction of a standardised massive transfusion response and ROTEM has seen changes in clinical practice and more appropriate use of different blood products at Flinders Medical Centre (6-11) during massive transfusions.

Severe sepsis and associated decreased fibrinolysis are major causes of multi-organ failure and mortality. Currently there are a limited number of routine diagnostic tests that are useful in identifying septic patients at risk of developing multi-organ failure. ROTEM was clinically evaluated to determine its efficacy in identifying patients at increased risk of sepsis related multi-organ failure. The results clearly showed a significant inverse relationship with fibrinolysis and the degree of sepsis and associated severity of organ failure (12).

My publications are either based on novel developmental work or prospective studies, while others are centred upon retrospective and observational studies.

Observational studies and RCTs both have strengths and limitations; observational studies are often seen to have limitations that are mostly avoided by RCTs (13). However, observational studies may permit for example investigation of incidence, causes, and outcomes which could not be achieved through a RCT. Mann (14) argued that 'qualitative studies can produce high quality information but all such studies can be influenced by known and unknown confounders'.

Several articles do not support the claim that observational studies are inferior to RCTs (15, 16). According to Trentino *et al* (13) 'the view that dismissing observational studies play no role in establishing causality is invalid' especially in Transfusion Medicine when it is impossible, unethical or logistically impossible to undertake RCTs.

The body of work covered by this thesis demonstrates how blood transfusion related research and critical evaluation of practices influences patient management and long-term outcomes.

2. PUBLICATIONS FORMING PART OF THIS THESIS

- McEwen MP, Roxby D. Can latent heat safely warm blood? In vitro testing of a portable prototype blood warmer. BMC Emerg Med 2007; 7:8-14

- Eikelboom JW, Cook RJ, Barty R, Liu Y**,** Arnold DM, Crowther MA, Devereaux PJ, Ellis M, Figueroa P, Gallus A, Hirsh J, Kurz A, Roxby D, Sessler DI, Sobieraj-Teague M, Warkentin TE, Heddle NM. Rationale and design of the Informing Fresh versus Old Red Cell Management (INFORM) Trial: An international pragmatic randomized trial. Transfusion Medicine Reviews. Trans Med Rev 2016; 30:25-29

- Heddle N, Cook R, Arnold D, Liu Y, Barty R, Crowther M, Devereaux P, Hirsh J, Warkentin T, Webert K, Roxby D, Sobieraj-Teague M, Kurz A, Sessler D, Figueroa P, Ellis M, Eikelboom J. Effect of short-term vs. long-term blood storage on mortality after transfusion. New Eng J Med 2016; 375:1937-1945

- Chai-Adisaksopha C, Alexander P, Guyatt G, Crowther M, Heddle N, Devereaux P, Ellis M, Roxby D, Sessler D, Eikelboom J. Mortality outcomes in patients transfused with fresher versus older red blood cells: A meta-analysis. Vox Sang. 2017; 112: 268-278

- Cook R, Heddle N, Lee, K, Arnold D, Crowther M, Devereaux P, Ellis M, Figueroa P, Kurz A, Roxby D, Sessler D, Sharon Y, Sobieraj-Teague M, Warkentin T, Webert K, Barty R, Liu Y, Eikelboom J. Red blood cell storage duration and in-hospital mortality: a secondary analysis of the INFORM study using time-dependent exposure. Lancet Haematology. 2017 Nov ;4(11): e544-e552. doi: 10.1016/S2352-3026(17)30169-2. Epub 2017 Oct 8

- Allden R, Sinha R, Roxby D, Ireland S, Hakendorf P, Robinson K. Red alert – a new perspective on patterns of blood use in the South Australian public sector. Aust Health Rev. 2011; 35: 327-333

- Sinha R, Roxby D, Seshadri R. Transfusion practice in massive haemorrhage in pre-intensive care and intensive care. Vox Sang. 2011; 101: 230-236

- Sinha R, Roxby D. Change in transfusion practice in massively bleeding patients. Transfus Aphers Sci 2011; 45:171-174
- Sinha R, Roxby D, Bersten A. Experience with a massive transfusion protocol in the management of massive haemorrhage. Transfusion Medicine 2013; 23:108-113
- Zatta AJ, McQuilten ZK, Mitra B, Roxby DJ, Sinha R, Whitehead S, Dunkley S, Kelleher S, Hurn C, Isbister J, Cameron P, Wood E, Phillips L. Elucidating the clinical characteristics of patients captured using different definitions of massive transfusion. Vox Sang. 2014; 107:60-70
- Sinha R, Roxby D. Any new changes in recent massive transfusion practices in a tertiary level institution? Transfusion & Apheres Sci DOI 10.1016/j.transci.2017.05.013
- Prakash S, Verghese S, Roxby D, Dixon D, Bihari S, Bersten A. Changes in fibrinolysis and severity of organ failure in sepsis: A prospective observational study using point-of-care-test-ROTEM. J Crit Care 2015; 30:264-270

3. CO-AUTHORSHIP STATEMENTS

- McEwen MP, Roxby D. Can latent heat safely warm blood? In vitro testing of a portable prototype blood warmer. BMC Emerg Med 2007; 7:8-14

Statement

DR first approached Flinders Biomedical Engineering (FBE) with the idea of developing a portable intravenous fluid warmer using battery power. This was shown to be impractical and other possible sources of heating (latent heat) were explored by MMcE from Flinders Biomedical Engineering and DR.

MMcE co-conceived the study, constructed the prototype fluid warmers, carried out the fluid warming and infusion flow rate test, complied biochemical test data and drafted the manuscript

DR co-conceived the study, arranged supply of blood from the Australian Red Cross Blood Service, extracted samples from blood units during storage, compiled biochemical test data and drafted the manuscript.

- Eikelboom JW, Cook RJ, Barty R, Liu Y**,** Arnold DM, Crowther MA, Devereaux PJ, Ellis M, Figueroa P, Gallus A, Hirsh J, Kurz A, Roxby D, Sessler DI, Sobieraj-Teague M, Warkentin TE, Heddle NM. Rationale and design of the Informing Fresh versus Old Red Cell Management (INFORM) Trial: An international pragmatic randomized trial. Transfusion Medicine Reviews. Trans Med Rev 2016; 30:25-29

Statement

DR was chief investigator at Flinders Medical Centre and a member of the INFORM Steering Committee. DR contributed equally to the design and conduct of the INFORM trial. The analyses for this study were reviewed by all authors and all authors contributed to edits and revisions.

- Heddle N, Cook R, Arnold D, Liu Y, Barty R, Crowther M, Devereaux P, Hirsh J, Warkentin T, Webert K, Roxby D, Sobieraj-Teague M, Kurz A, Sessler D, Figueroa P, Ellis M, Eikelboom J. Effect of short-term vs. long-term blood storage on mortality after transfusion. New Eng J Med 2016; 375:1937-1945

Statement

DR contributed equally to the design and conduct of the INFORM trial. The analyses for this study were reviewed by all authors and all authors contributed to edits and revisions. DR was chief investigator at Flinders Medical Centre and a member of the INFORM Steering Committee.

- Chai-Adisaksopha C, Alexander P, Guyatt G, Crowther M, Heddle N, Devereaux P, Ellis M, Roxby D, Sessler D, Eikelboom J. Mortality outcomes in patients transfused with fresher versus older red blood cells: A meta-analysis. Vox Sang. 2017; 112: 268-278

Statement

All authors including DR critically reviewed the data and revised the report.

- Cook R, Heddle N, Lee, K, Arnold D, Crowther M, Devereaux P, Ellis M, Figueroa P, Kurz A, Roxby D, Sessler D, Sharon Y, Sobieraj-Teague M, Warkentin T, Webert K, Barty R, Liu Y, Eikelboom J. Red blood cell storage duration and in-hospital mortality: a secondary analysis of the INFORM study using time-dependent exposure. Lancet Haematology. 2017 Nov; 4(11): e544-e552. doi: 10.1016/S2352-3026(17)30169-2. Epub 2017 Oct 8

Statement

DR contributed equally to the design and conduct of the INFORM trial whose data were used for this secondary analysis. The analyses for this study were performed by RJC and KAL and were reviewed by all authors. RJC wrote the initial draft of the manuscript and all authors contributed to edits and revisions. DR was chief investigator at Flinders Medical Centre and a member of the INFORM Steering Committee.

- Allden R, Sinha R, Roxby D, Ireland S, Hakendorf P, Robinson K. Red alert – a new perspective on patterns of blood use in the South Australian public sector. Aust Health Rev. 2011; 35: 327-333

Statement

DR helped design the study, assisted in establishing the electronic data collection from SA Pathology and interpretation of the data and review of the manuscript

- Sinha R, Roxby D, Seshadri R. Transfusion practice in massive haemorrhage in pre-intensive care and intensive care. Vox Sang. 2011; 101: 230-236

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Statement

DR helped design the study, assisted in establishing the electronic data collection from SA Pathology and interpretation of the data and writing and editing of the manuscript.

- Zatta A, Mitra B, Roxby D, Sinha R, Whitehead S, Dunkley S, Kelleher S, Hurn C, Isbister J, Cameron P, Wood E, Phillips L. Elucidating the clinical characteristics of patients captured using different definitions of massive transfusion. Vox Sang. 2014; 107:60-70

Statement

DR is a member of the Australian and New Zealand Massive Transfusion Registry Steering Committee. He assisted with establishing the electronic data collection from FMC and RAH and participated in the planning, interpretation of data and review of the manuscript

- Sinha R, Roxby D. Any new changes in recent massive transfusion practices in a tertiary level institution? Transfusion & Apheres Sci DOI 10.1016/j.transci.2017.05.013

Statement

DR interpreted and critically evaluated the paper and contributed to edits and revisions

- Prakash S, Verghese S, Roxby D, Dixon D, Bihari S, Bersten A. Changes in fibrinolysis and severity of organ failure in sepsis: A prospective observational study using point-of-care-test-ROTEM. J Crit Care 2015; 30:264-270

Statement

DR established the laboratory testing protocol, performed the ROTEM measurements, collected and interpreted the data and participated in the writing and review of the manuscript.

4. INTRODUCTION TO THE PUBLISHED WORKS

4.1. How I Have Set Up the Thesis

My thesis summarises a number of my recently published research and development manuscripts which focus on four areas of Transfusion Medicine: development of a non-electrically powered intravenous fluid/blood warmer (1), the effect of age of transfused red cells on patient mortality (2-5), critical bleeding and massive transfusion (6-11), and use of rotational viscoelastometric testing (ROTEM) to identify patients with sepsis at risk of developing multi-organ failure (12). Following the 'Introduction to the Published Works' section of this thesis, each of my four areas of research and development are discussed separately beginning with a literature review followed by a synopsis of the results and discussion. A copy of the associated publications follows each section.

4.2. Development of an Intravenous Fluid/Blood Warmer

Complications of massive transfusion include metabolic changes, including alkalosis, acidosis, hyperkalemia, hypocalcaemia and hypothermia. Hypothermia is exacerbated in critical bleeding and massive transfusion if resuscitating intravenous fluids or blood is not warmed to 37°C prior to infusion.

The coagulation process consists of multiple, sequential, enzymatic reactions that are temperature dependent and function optimally at 37°C. Both *in vivo* and *in vitro* studies have shown that hypothermia impairs platelet function as well as the formation of a platelet plug and activates fibrinolysis (17, 18). Hypothermia induces morphological changes in platelets during activation, and causes defects in platelet adhesion and aggregation, as well as thrombin production and leads to prolonged bleeding time. Mortality of patients under these circumstances is significant (19).

The effect of hypothermia on coagulopathy is difficult to assess using routine coagulation tests such as prothrombin time and activated partial thromboplastin time because these tests are routinely carried out at 37ºC (20). Platelet dysfunction and impaired coagulation enzyme activity are reversible once body temperature is normalized, which highlights the need to prevent hypothermia or treat it aggressively once it occurs.

In hospital, treatment of hypothermia is readily achievable with access to electrically powered in-line fluid/blood warmers. However, at this point in time there are limited portable non-electrically powered cost effective light-weight devices available to warm blood or intravenous fluids at accident sites or on medical retrieval vehicles, therefore the need for a cheap reliable portable, non-electrically powered fluid warmer (1).

I have co-invented and patented such a device that in proof of concept studies has shown that it can efficiently and effectively warm 4 units of blood from 2-6°C to 35-37°C. During the design phase of the project four prototypes were developed. In a recently completed clinical trial using the 4th prototype and involving 25 patients, preliminary evaluation of the data showed that the blood warmer performed as expected in a clinical environment with no adverse patient events or detrimental effects on the warmed transfused red cells. A further clinical trial in the medical retrieval situation is planned.

4.3. Age of Blood at Transfusion

Red cells for transfusion can be stored for up to 42 days at 2-6^{0}C following collection from blood donors. Standard-issue RCs are defined as the oldest available ABO Rh blood group compatible RCs issued from inventory for transfusion to patients.

Early observational studies on morbidity and mortality related to the age of blood at transfusion suggested increased risk of: infection, deep vein thrombosis, cancer recurrence, organ failure, ICU length of stay and mortality (21-25). More recent small randomised controlled trials (RCTs) (26-29) do not support the hypothesis that standard-issue RCs are associated with poorer outcomes.

As a member of the INforming Fresh versus Old RC Management Study (INFORM) multi-centre randomised controlled trial Steering Committee involving more than 32,000 patients we aimed to determine the effect on in-hospital death rates of transfusing the freshest available blood compared with standard-issue RCs (2, 3). The INFORM RCT showed that there was no significant difference in the rate of death among those who underwent transfusion with the freshest available blood and those who underwent transfusion according to the standard practice of transfusing the oldest available blood.

A further systematic review and meta-analysis of published RCTs by the INFORM Steering Committee comparing the mortality outcomes in patients transfused with fresher versus standard-issue RCs and including the INFORM data showed that transfusion of fresher red cells does not reduce overall or in-hospital mortality when compared with standard-issue RCs (4).

Further secondary analysis of the INFORM data has been undertaken to determine if there is a noticeable effect on morbidity and mortality by transfusing blood that is 35-42 days old (5). This analysis showed that there was no effect on in-hospital mortality when blood that was older than 35 days was used for transfusion.

4.4. Critical Bleeding and Massive Transfusion

The management of critical bleeding remains a major clinical challenge and concern, without randomised controlled trials to better inform management principles (30).

My publications in massive transfusion and critical bleeding are based upon retrospective studies undertaken over several years (6-11). The aims of these studies were to develop an evidence-based best practice approach (6) to the treatment of massively bleeding patients by understanding current and past clinical practices (7, 8) and associated patient outcomes which lead to development of a clinical pathway for massively bleeding patients (9).

Massive blood loss is usually defined as the loss of one blood volume within a 24-hour period, with the normal adult blood volume being approximately 7% of ideal body weight, 8% to 9% in children. Alternative definitions include 50% blood volume loss within 3 hours, or a rate of loss of 150ml per minute. Such definitions emphasize the importance of early recognition of major blood loss and the need for effective action to prevent shock and its consequences.

Massive transfusion has arbitrarily been defined as the replacement of a patient's total blood volume or transfusion of 10 units of RCs in less than 24 hours however my work with the Australian and New Zealand Massive Transfusion Registry suggests that a definition of a transfusion of 5 RCs in 4 hours is more inclusive and able to better identify other clinically important patients (10).

To better understand transfusion practices in Flinders Medical Centre, massive

transfusion patients were identified from Transfusion records. The causes of massive transfusion and the extent of coagulopathy in these patients were reviewed (7-9). Laboratory parameters (haemoglobin, PT (INR), aPTT, fibrinogen, platelet count, pH and base deficit) were also reviewed and evaluation of transfusion practice in these acutely multi-transfused patients was undertaken by reviewing the usage of blood products and patient outcome.

Management of abnormal bleeding in patients given massive transfusions has been the subject of debate. Early studies involved patients who received stored whole blood, whereas, currently, red cells (RCs) are used. An early approach was to transfuse fresh frozen plasma (FFP) and platelets prophylactically after a certain number of units of RCs had been transfused, ranging from 1:10 to 2:3 for FFP:RCs and from 6:10 to 12:10 for platelets:RCs (31). There is no conclusive evidence that such a practice prevents the development of coagulopathy or reduces the transfusion requirements. Accordingly, effective management of abnormal bleeding associated with massive transfusion depends on the rapid differential diagnosis of the cause of the haemostatic abnormalities followed by timely administration of appropriate blood components in proper amounts or ratios with the implementation of additional treatments such as use of tranexamic acid designed to correct other factors contributing to the bleeding (9).

Up until 2006, there was no formal massive transfusion policy at Flinders Medical Centre (FMC) and Repatriation General Hospital (RGH). RC and blood component replacement were based on pre-emptive or laboratory test driven replacement of coagulation factors (9, 32).

My studies (6-11) helped in on-going review and development of clinical pathways and guidelines at FMC and RGH for effective management of bleeding patients and development of a massive transfusion protocol.

4.5. Viscoelastometric Testing and Sepsis

Sepsis is a very common illness and ranges in severity from mild illness to the severest forms treated in intensive care units. The severest forms of sepsis remain a challenge to treat with relatively poor success rates. The major cause of poor outcomes in these patients is not the infection but multi-organ failure. That organ failure is associated with abnormalities in fibrinolysis associated with micro-circulatory fibrin deposition thus depriving organs of essential blood supply.

The ability to detect reduced fibrinolysis would not only allow monitoring but also the ability to predict the disease process earlier and take necessary preventive steps. At present, there is not a diagnostic test which accurately predicts the abnormal clotting process in septic patients.

Clinically we evaluated the prognostic value of rotational viscoelastometric (ROTEM) testing for its potential to provide information on impaired fibrinolysis and coagulopathy in sepsis.

The results of our present study (12) demonstrated that severe sepsis is associated with reduced fibrinolysis and increased multi-organ failure. The ROTEM Maximum Lysis (ML) parameter proved to be a better biomarker of sepsis than Prothrombin Fragments F1+2 (PF1.2) and Plasminogen Activator Inhibitor 1 (PAI-1). ML showed a significant inverse relationship with the degree of sepsis and associated severity of organ failure.

The observation that decreased fibrinolysis occurred to a degree across groups of patients with increasing sepsis related organ failure suggests an important role of the fibrinolytic system in the pathophysiology of severe sepsis.

Our study provides the basis for further evaluation of changes in fibrinolysis over time and the relevance of thromboelastometry for monitoring these changes in haemostasis in sepsis related multi-organ failure.

5. DEVELOPMENT OF AN INTRAVENOUS FLUID/BLOOD WARMER

5.1. Introduction

Intravenous administration of cold fluids and blood stored at 2-6°C causes conductive heat loss to the patient's circulating blood volume which then contributes to hypothermia in the trauma population (33). In the average trauma response, it is not uncommon to transfuse up to 4 units (≤1.2L) of blood at 2-10ºC (transport temperature) at flow rates of up to 50 mL/min and/or other fluids which have been stored at room temperature at flow rates up to 180 mL/min.

In clinical situations such as these it would be preferable for patients to receive intravenous fluids at close to body temperature to reduce the risk of hypothermia. The thermal stress of infusing cold fluids into a patient already in shock may result in considerable changes in mean body temperature. The larger the gradient between the temperature of the infused fluid and the core temperature, the greater the drop in mean body temperature. Also, the greater the fluid replacement relative to the body weight, the greater the potential drop in body temperature. The amount of heat loss this causes in patients is approximately 0.5ºC temperature drop per litre of 4ºC fluid infused into a 70Kg patient starting at 37ºC (34, 35).

Trauma and retrieval patients are often in shock, hypotensive (systolic blood pressure <90mm Hg) and at risk of hypothermia (<37^{0}C). Treatment requires warming and rapid stabilisation, restoration of blood volume with intravenous fluids such as crystalloids/colloids and or blood products to maintain tissue perfusion, and correction of any acidosis, electrolyte imbalance or coagulopathy. The intravenous fluids or blood products are stored at either 20-24^{0}C or 2-6^{0}C and infusion at these temperatures contributes to development or exacerbation of hypothermia (33, 34).

Flinders Medical Centre (FMC) is a tertiary care hospital and trauma centre which receives severely injured medical retrieval patients. In my clinical scientist role within the FMC Transfusion Service and in discussions with senior anaesthetists I was aware of the ongoing problems of retrieval patients arriving at the hospital who were severely hypothermic not only due to shock but also to the infusion of cold intravenous fluids.

Because of this knowledge I have co-invented a patented portable non-electrically powered intravenous fluid warmer which is currently in clinical trial.

5.2. Hypothermia

Hypothermia is induced by heat loss at the scene of a trauma and by treatment in hospital including resuscitation with fluids which are not pre-warmed (36). All victims of major trauma are at risk for hypothermia which most often results in harmful effects including coagulopathy, cardiac arrhythmias, peripheral vasoconstriction, metabolic acidosis, compensatory increased oxygen requirements during rewarming, and impaired immune response which in turn has been associated with increased mortality and morbidity (33, 35, 37-39). Herron (37) reported that 66% of trauma patients are hypothermic at the time of admission, those with a core temperature lower than 34^0C suffered a mortality rate up to 35% higher than euthermic patients (39-42).

The coagulation process consists of multiple enzymatic reactions, which are temperature dependent and function optimally at 37^0C. Both *in vivo* and *in vitro* studies have shown that hypothermia contributes to coagulopathy, significantly impairs platelet function and the formation of a platelet plug and activates fibrinolysis (1, 2). The combination of coagulopathy, metabolic acidosis and hypothermia is known as the 'lethal triad' (43). The mortality rate in these circumstances may reach up to 90% (20, 33, 43).

The effect of hypothermia on coagulopathy is difficult to identify by routine coagulation tests, such as prothrombin time and activated partial thromboplastin time because these tests are routinely carried out at 37^0C and this should be taken into consideration when interpreting the results and correcting coagulopathy (20, 44).

During critical bleeding and massive transfusions in hospitals blood products and other fluids are often warmed to 37^0C using electrically powered fluid warmers. Although there are numerous mains-powered blood warmers on the market (34), there is only one commercially available unit that can operate without mains power (Thermal Angel, Estill Medical Technologies). It is a portable disposable battery-powered, blood and IV fluid warming device capable of warming 1-3 units of blood at various flow rates to approximately 30-34^0C. However, the problems that remain with battery powered fluid warming devices are the efficiency of the

unit, the short battery life and the weight of the device which is relatively heavy and therefore less transportable.

Ideally in out of hospital settings such as trauma and retrievals, fluids should also be warmed to close to 37^0C to minimise the risk or exacerbation of hypothermia. However cheap, portable non-electrically powered fluid warmers are not currently available for use in the pre-hospital critical and acute settings.

5.3. Development of a Non-Electrically Powered Fluid Warmer

The idea for the development of a portable, non-electrically powered fluid warmer came from discussions between the author (DR) and Flinders Biomedical Engineering (1). Criteria for the development of such a fluid warmer included:

- No battery
  - Latent heat material, constant heat release and constant temperature
  - Stable chemical
  - Cannot overheat blood
  - Fast acting
  - Non-flammable
  - Environmentally friendly
- Low risk
- Easy to use
  - Minimal technical expertise required
- Inexpensive
- No accessories
- Reliable
- Small
- Portable

5.4. Supercooled Liquids

Latent heat storage materials are available which can store large amounts of heat in supercooled liquids, to be released on the transition of the material from the liquid phase to the solid phase thus generating energy for heating. Supercooled liquids will remain in their liquid state at temperatures below their crystallisation temperature only changing phase when activated.

Different phase change salts have different heat of crystallisation and crystallisation temperatures, but also have different characteristics such as volume of salt required, stability, ease of triggering crystallisation and cost. Some of these salts change phases in a temperature range between 35-43ºC which is an ideal temperature range for warming intravenous fluids.

The portable fluid warmer uses energy stored in the form of latent heat, in a supercooled liquid (calcium nitrate tetrahydrate) which surrounds intravenous tubing, to warm fluid as it passes through the tubing (Figure 5.4).

This form of warming is inherently safe because the temperature is limited to the freezing temperature of the supercooled liquid. The fluid warmer uses heat of crystallisation to generate energy for heating. Heat of crystallisation is heat liberated when a salt solution changes from a liquid to a solid state. The freezing temperature of calcium nitrate tetrahydrate is 42.8ºC (45, 46). Studies have shown that it is safe to warm blood to 42ºC (47-51).

Using a prototype of the non-electrically powered blood warmer, several units of blood were warmed with the device in laboratory tests. These showed that ‘a low-cost portable non-electrically powered fluid warmer using latent heat storage may be used to significantly warm cold blood (2-6ºC) or intravenous fluids to near body temperature with minimal risk of cellular damage and haemolysis (1).

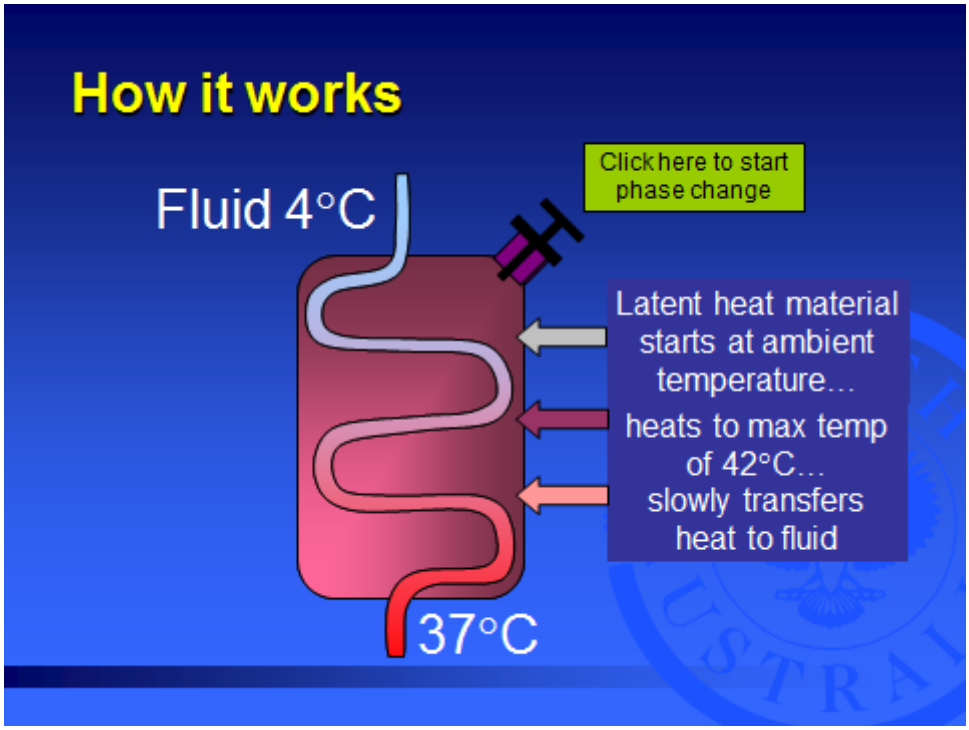

Figure 5.4. Intravenous fluid warmer pathway. Prior to use the portable blood warmer tubing is primed with intravenous fluid (i.e. saline) followed by blood, crystalloid or colloid solution. At the same time, the latent heat phase change salt (calcium nitrate tetrahydrate) is activated leading to release of heat and thus warming the fluid as it travels through the tubing surrounded by the phase change material. Cold intravenous fluids or blood (2-6ºC) enters the fluid warmer where the generated heat safely warms the fluid to 34-36ºC.

The heat of crystallisation technique has been proven to be a practical method of providing enough energy to heat blood or fluids from 4ºC to 30-42ºC at a flow rate of up to 200mL/min in a reasonably sized and weighted portable non-electrically powered blood warner for out of hospital use in critical situations (1).

Even though normal body temperature is 37^0C, blood for transfusion may be warmed to 42^0C without adverse effect (52). The American Association of Blood Banks guidelines (53) state that the maximum temperature that RCs can be safely warmed is 42ºC. This still allows traditional style fluid warmers to have a 2-3ºC safety margin for thermostat operation without danger of haemolysis. Studies in 1974 (47) where blood was warmed to 45ºC for 1 hour showed that no haemolysis occurred. Kruskall (54) compared blood warmed to 40ºC for prolonged exposure (> 4 hours) with unwarmed blood, these warmed units showed no significant changes in plasma haemoglobin, mean corpuscular

haemoglobin concentration, potassium, ATP, pH, and osmotic fragility. Similarly Uhl (50) found plasma haemoglobin and LDH was not increased over baseline between 37ºC and 44ºC, osmotic fragility curves were noted beginning at 46ºC. He concluded that subtle alterations in RC integrity are not apparent until 46ºC, and frank haemolysis did not occur until 48ºC. Van der Walt *et al* heated blood to 46ºC without affecting osmotic fragility (48). Herron (37) tested an in-line microwave blood warmer evaluating heated blood for changes in RC structure and function by measuring haemoglobin/haematocrit, potassium, lactate dehydrogenase, plasma haemoglobin, blood film, osmotic fragility and O_2 saturation. He concluded that an in-line microwave blood warmer may be used to heat blood safely to 49ºC for less than 9 seconds.

5.5. Blood Warmer Prototype Development

4 prototype fluid warmers were constructed, adopting the design principles outlined above in Sections 5.3 and 5.4.

5.5.1. First Blood Warmer Prototype

The first prototype was very bulky (Figure 5.5.1); it had a rigid 2.5L frame containing 1.75L of sodium carbonate decahydrate as the source of latent heat material and 20m of IV tubing. Using this bulky prototype, we confirmed that over 3L of water flowing at 100 mL/min could be heated from 4ºC to 30ºC. A provisional patent was submitted on 26th November 2004 (Number 2004906743).

Figure 5.5.1. Initial prototype blood warmer incorporating a rigid design

5.5.2. Second Blood Warmer Prototype

Improvements to the initial rigid design prototype were made to incorporate the following improvements (Figure 5.5.2 and Table 5.5.4):

- The latent heat storage material was changed from sodium carbonate decahydrate to calcium nitrate tetrahydrate which has a higher phase change temperature (39-42ºC) and is more stable at lower temperatures
- Size and weight were reduced by halving the length of tubing (to 10m) and reducing the quantity of latent heat storage material to 1.2L
- Flexible packaging was used to enable easy handling and storage of the fluid warmer

Figure 5.5.2. Second prototype incorporated into a flexible bag and weighing 3.1Kg

5.5.3. Third Blood Warmer Prototype

The third prototype's (Figure 5.5.3) weight was further reduced to a practical size (1.7Kg), holding 1.2L of latent heat storage material with 5m of IV tubing within a flexible polymer bag. This prototype was able to heat 4 units of blood from 4^0C to over 30^0C at 52 mL/min. The maximum temperature reached even when blood flow was stopped was less than 40^0C, well below the temperature at which haemolysis occurs (54).

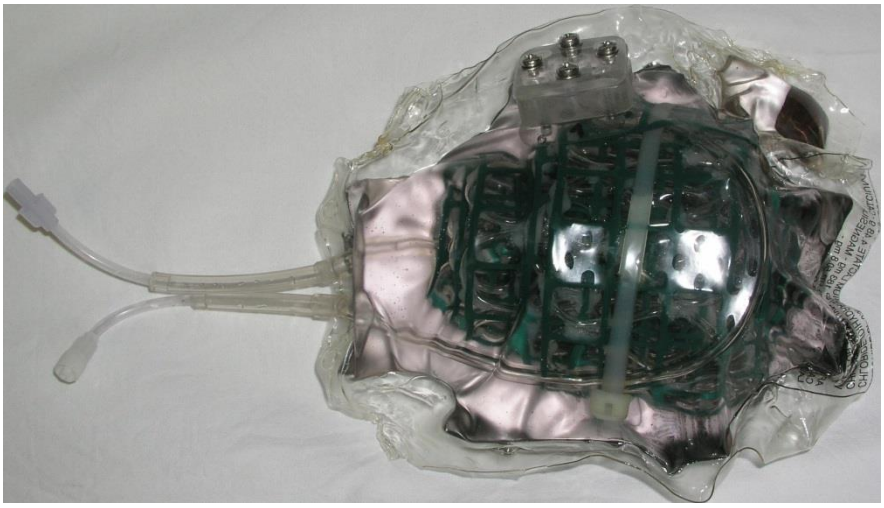

Figure 5.5.3. Third prototype in a flexible bag, weighing 1.7Kg

5.5.4. Fourth Blood Warmer Prototype

The fourth prototype was constructed to be reused during *in vitro* tests. It consisted of a serpentine pathway of IV tubing within a rigid casing packed with latent heat storage material. This reusable prototype (Figure 5.5.4a and Table 5.5.4) is bulkier than the disposable unit used in the clinical trial (Figure 5.8) with a total weight of 2.5 Kg. With prototype 4, six units of RCs could be heated from 4^0C to an average of 32.4^0C (Figure 5.5.4b)

Figure 5.5.4a. An example of the rigid fourth blood warmer prototype

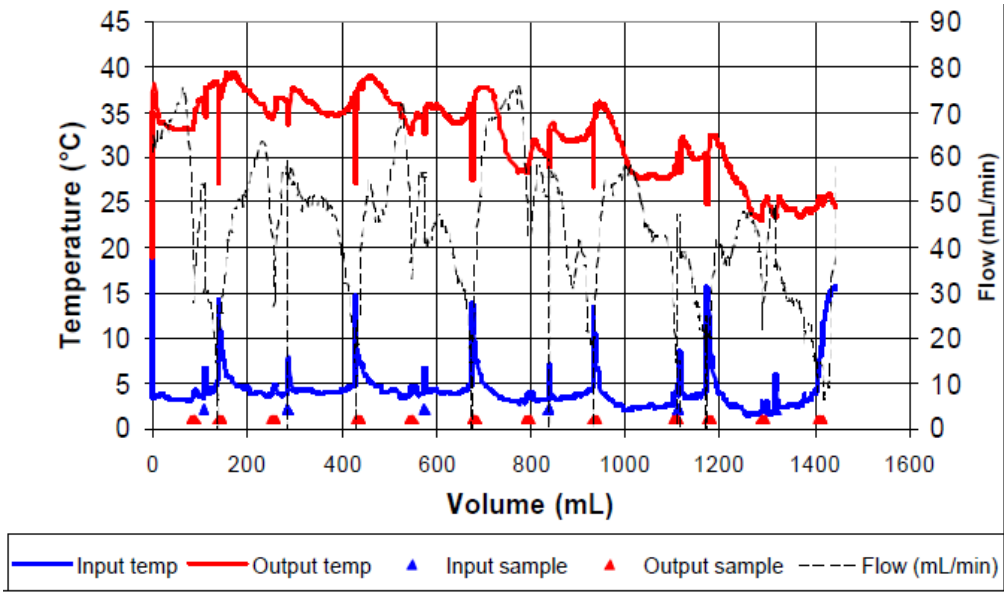

Figure 5.5.4b. *In vitro* results from heating of six units of RCs from 4.2^{0}C to an average of 32.4^{0}C at a flow rate of 36mL/min using the prototype 4 blood warmer.

Prototype	Latent Heat Material	L (mm)	W (mm)	D (mm)	Tube Length (m)	Empty Weight (Kg)	Chemical Vol (L)	Chemical Weight (Kg)	Overall Weight (Kg)
1	Sodium carbonate decahydrate	200	170	170	20	1.56	1.75	2.45	4.0
2	Calcium nitrate tetrahydrate	200	150	150	10	0.25	1.2	2.18	3.1
3	Calcium nitrate tetrahydrate	200	110	160	5	0.18	1.2	1.52	1.7
4	Calcium nitrate tetrahydrate	150	120	120	5	0.75	0.96	1.75	2.5
Clinical Trial (rolled up)	Calcium nitrate tetrahydrate	230	100	100	5	0.35	0.96	1.75	2.1
Clinical Trial (unrolled)	Calcium nitrate tetrahydrate	900	230	30	5	0.35	0.96	1.75	2.1

Table 5.5.4. Comparison of the size and weight of the different blood warmer prototypes.

5.6. *In Vitro* Prototype Testing

5.6.1. Overheating

To confirm the maximum temperature reached by fluid in our prototype fluid warmer, the flow was stopped for 3 minutes. If the fluid/blood warmer was unregulated, once flow resumed the temperature of the fluid would rise above and overshoot the upper safe temperature of 42ºC. No temperature overshoot occurred even after resumption of fluid flow. The maximum temperature was less than 40ºC. This is well below the temperature at which haemolysis occurs (50).

5.6.2. Haemolysis of Blood

Extra-cellular potassium and plasma haemoglobin were measured in unwarmed stored RCs and RCs that had passed through the prototype blood warmer (39-42ºC) and a dry heat electrical blood warmer. RCs that had passed through the prototype warmer showed no signs of increased cellular damage compared to non-warmed RCs or RCs passed through a clinically approved dry heat blood warmer (1).

5.6.3. Calcium nitrate tetrahydrate

The salt is classified as a strong oxidiser and may have special transport requirements in ambulances, planes or helicopters. We found it to be an inhibitor of fire rather than an accelerator.

Similarly, we found that the salt solution had no deleterious effect on the blood warmer tubing or caused splitting or brittleness of the plastic tubing. No changes were observed to the tubing in the months that the prototypes were tested.

5.7. Patents and Licence Agreements

Based on successful pre-clinical trials patents were awarded for a Portable Fluid Warmer:

- AU/2004/906743
- WO/2006/056015
- PCT/AU2011001369

A Licence Agreement was negotiated with Flinders Biomedical Engineering (FBE) Pty Ltd for the commercial development of the non-electrically powered fluid/blood warmer.

5.8. Clinical Trial

Because of the successful *in vitro* findings, a clinical trial of a prototype has now been completed. Although still fundamentally the same as the original prototype used for warming units of blood in initial experiments, the current design has now been changed to make it disposable, and simpler to use (Figure 5.8).

Figure 5.8. An example the clinical trial blood warmer prototype showing it rolled during storage and unrolled for clinical use.

The clinical effectiveness of the portable fluid warmer was determined, by using it to warm stored (2-6ºC) RCs prior to transfusion to a group of Haematology patients in a controlled environment. A further clinical trial is planned to test the prototype in emergency retrieval situations.

Ethics Approval was received from the Southern Adelaide Clinical Human Research Ethics Committee to undertake a Clinical Trial (SAC HREC EC00188)

of the prototype of the portable fluid/blood warmer. Associate Professor David Roxby, Dr Mark McEwen and Associate Professor Bryone Kuss are the co-investigators for the trial.

5.8.1. Objectives

- To determine how well the blood warmer performs in a controlled clinical setting in a group of Haematology patients who receive regular transfusions
- Identify any aspects of the blood warmer that should be changed

5.8.2. Study Design

A group of transfusion dependent Haematology patients who receive regular blood transfusions were recruited to the study. These patients were given the opportunity to learn about the study, and if they volunteered to participate, they would receive one transfusion with blood warmed by the portable fluid warmer.

The patient's normal treatment regime, involved transfusing one or more units of RCs (over several hours) whilst having the following parameters monitored and recorded every 30 minutes: oxygen saturation, temperature, blood pressure, pulse and, respiratory rate.

Patients who volunteered for the portable fluid warmer study underwent their normal treatment regime, with four additional steps:

At one visit to a transfusion clinic, participants received their transfusion via the portable fluid warmer.

- A small volume (10-20mL) was withdrawn from each unit of RCs, before it was warmed. This sample was sent for analysis to determine the concentration of: haptoglobin, plasma haemoglobin, total haemoglobin, potassium, lactate, lactate dehydrogenase, ionized calcium and total calcium.
- Prior to the transfusion commencing and at the end of transfusion (before and after all units of RCs have been delivered); a small volume (10-20mL) of blood was withdrawn from the participant, via the transfusion needle. These samples of blood were sent for analysis to determine the concentration of: haptoglobin, plasma haemoglobin, total haemoglobin, potassium, lactate, lactate dehydrogenase, ionic calcium and total calcium.

- 24 hours after transfusion (warmed transfusion and one cold transfusion) participants were visited by a nurse, who withdrew a small volume (10-20mL) of blood from the participant. These samples of blood were sent for analysis to determine the concentration of: haptoglobin, plasma haemoglobin, total haemoglobin, potassium, lactate, lactate dehydrogenase, ionic calcium and total calcium.

5.8.3. Preliminary Analysis of Blood Warmer Clinical Trial

The clinical trial ended in October 2017 with 25 patients enrolled. Detailed analysis of the data is being undertaken and once completed the data from the clinical trial will form the basis of another scientific paper. Preliminary review of the laboratory data and patient's clinical observations indicate no adverse patient events or *in vitro* or *in vivo* detrimental effects to the warmed transfused RCs compared to the non-warmed transfused RCs.

5.9. Summary

Following my original idea for a portable fluid/blood warmer for use in medical retrieval services, we have successfully developed a non-electrically powered portable blood warmer for heating of both blood products and intravenous fluids (1). Our device is ideal for use in medical retrievals, in remote locations, mass casualty incidents, military situations or third world countries.

The fluid/blood warmer uses latent heat of fusion as an energy source. Heating occurs at a constant temperature dependent upon the physical characteristics of the salt used and is not dependent on mains power electricity or battery power to operate. The risk of overheating of fluids is negligible as the warming is limited to the phase change temperature of the latent heat storage material.

The prototype is disposable, fast-acting and can warm up to four units of RCs from 4^0C to 34-36^0C at high flow rates. *In vitro* validation testing of the prototype has shown its practicality and safety (1). *In vitro* testing showed that heating of blood at high flow rates was adequate without causing any unwanted adverse effects such as haemolysis of the warmed RCs.

Preliminary analysis of the clinical trial data has shown that the current prototype blood warmer performs as required under in-hospital clinical conditions. The next planned phase of the project is clinical testing in medical retrieval situations outside the hospital environment.

5.10. Copy of BMC Emerg Med 2007; 7: 8-14

(DOI: https://doi.org/10.1186/1471-227X-7-8)

McEwen MP, Roxby D. Can latent heat safely warm blood? *In vitro* testing of a portable prototype blood warmer.

A copy of the reference has been removed due to copyright restrictions

6. AGE OF BLOOD AT TRANSFUSION – DOES IT MATTER?

6.1. Introduction

Even though blood transfusion is an integral part of life saving fluid resuscitation for anaemic, surgical, oncology and trauma patients, studies have suggested that RC transfusions may have harmful side effects (21-23, 55-64).

Transfusion Services typically provide the oldest compatible blood (first in first out) to minimise waste. This is amplified at larger centres where fresh blood usually goes to smaller centres where usage is low and frequently unused older cells are returned and redistributed to larger centres to minimise wastage. Therefore, some of the sickest patients are exposed to oldest available blood.

6.2. Red Cell Storage Lesion

It is well documented that there are physiological changes to blood during storage including biochemical changes, loss of RC function and the risk of bacterial growth (65). As such RCs undergo numerous, complex physical and chemical changes during refrigerated storage which impact on their function and survival, known collectively as the "RC storage lesion" (Table 6.2). Some of these changes are reversible; the clinical relevance of these changes is not clear, prompting significant research and debate in the area. This has led to concern regarding the efficacy and safety of older units of blood compared to younger units (generally taken to be less than 14 days in storage).

6.3. 2,3-DPG Levels of Stored Red Cells

Temperature and pH influences the metabolic rate in RCs. When stored at elevated temperatures (25-30°C) RCs produce more lactic acid than at 20°C and 4°C. This leads to a decrease in the pH, which activates 2,3-DPG phosphatase leading to a depletion of 2,3-DPG (66-68). The loss of 2,3-DPG occurs at an early stage during storage, increasing the affinity of haemoglobin for oxygen (69) (Figure 6.3).

If unseparated whole blood is kept at ambient temperature, without active cooling, within several hours an obvious decrease of 2,3-DPG can be observed. Even with active cooling less than 50% remains after 16hrs (68). Therefore, prolonged RC storage has been associated with changes that may render RCs

ineffective as oxygen carriers as demonstrated by the left shifted oxygen dissociation curve due to decreased 2,3-DPG levels associated with stored RCs (Figure 6.3).

The clinical importance of lowered 2,3-DPG levels in transfused RCs is not clear. It has been postulated that in patients with a very high oxygen affinity, it may cause ischemia sufficient to be detrimental to sensitive organs such as the brain and heart. On the other hand, 2,3-DPG can be restored *in vivo* within 24hrs and there are other mechanisms that may compensate for its loss (68, 70).

Changes occurring in the RC		Oxidative stress	
Increased	**Decreased**	**Increased**	**Decreased**
Echinocytes, reversible	CD47	Protein oxidation	
Sphero-echinocytes, irreversible	Deformability	Lipid perioxidation	
Osmotic fragility	O_2 delivery		
Micro-vesicles (procoagulant)	Na-K-ATPase		
Membrane rigidity			
O_2 affinity of haemoglobin			
Vascular endothelium adherence			
Metabolic changes		**Changes in the additive solution**	
Increased	**Decreased**	**Increased**	**Decreased**
Lactate	2,3-DPG	K+ and H+	pH
	Phosphate	Free haemoglobin	Complement
	ATP, ADP, AMP	Free haem & iron (redox injury)	Opsonizing ability
	Glutathione	Soluble lipids	Lytic ability
	S-nitrosohaemoglobin	Phospholipid vesicles	
	Nitric oxide (NO)	Cytokines (IL1, IL6, IL8, TNF)	
		Histamines	
		Complement split products	

Table 6.2. Red cell (RC) storage lesion (65). RCs are stored at 2-6^{0}C. During storage, they are exposed to oxidative stress and undergo metabolic changes leading to changes that affect the RC cytoplasm and membrane, parts of which are shed into the supernatant as microvesicles. These changes result in significantly altered deformability, integrity of the RCs and increased free haemoglobin in the plasma. Similarly decreases in 2,3-DPG levels alters oxygen carrying and release properties of the RCs. Some effects occur within hours of donation, while others accumulate over days and weeks of storage. (2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate; IL, interleukin; TNF, tumour necrosis factor).

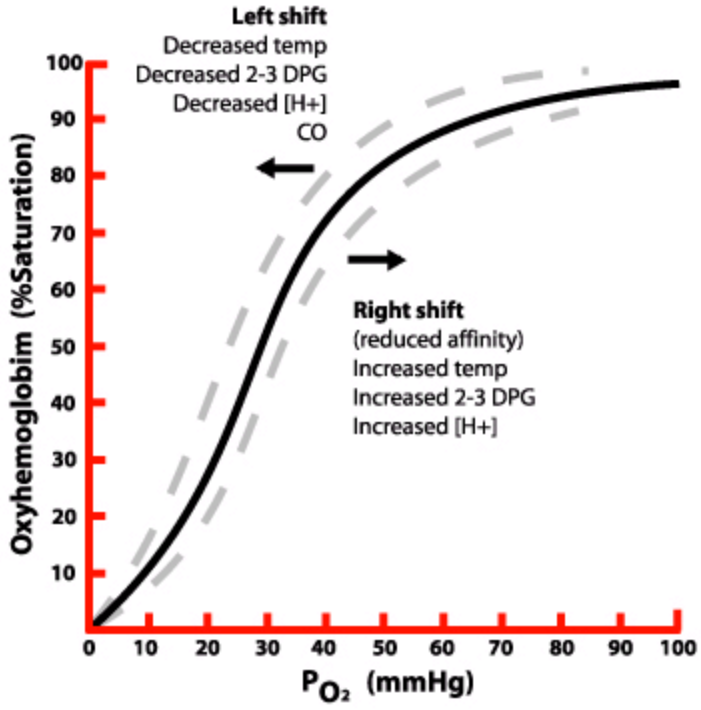

Figure 6.3. Oxygen dissociation curve (69). This curve describes the relationship of haemoglobin in its oxygen-laden saturated form (SaO_2) on the vertical axis against the partial pressure of oxygen on the horizontal axis (PaO_2) and is an important tool for understanding how haemoglobin acquires and releases oxygen to the tissues. For example, a RC with normal levels of 2,3-DPG will readily release oxygen to the tissues, however a RC deplete of 2.3-DPG will not release the oxygen as readily leading to a left shift of the curve

6.4. Storage of Red Cells for Transfusion

Beside the known changes that occur with the RC storage lesion (Table 6.2) one possible factor contributing to adverse effects of RC transfusions is the duration of RC storage prior to transfusion (4, 60, 63, 65, 71, 72). Current regulations permit storage of RCs for up to 42 days (73, 74).

RC concentrates prepared from CPD whole blood units are conventionally re-suspended and stored at 4°C in a plasma-free preservative solution containing saline, adenine and glucose. Some solutions contain mannitol, citrate and/or phosphate for additional support (73, 74).

During storage, there is a gradual deterioration in the morphology, membranous and metabolic integrity of the RCs. Storage also leads to accumulation of substances that have adverse biologic effects that may mean that RCs do not traverse the microcirculation as effectively.

This storage lesion is associated with reduced post transfusion recovery, which reaches 75-80% at 42 days. The metabolic storage lesion is related to a decreasing rate of glycolysis, which is associated with falling pH levels. Moreover, the decreased rate of glycolysis is related to metabolic depletion, and occurs when ATP levels drop over the storage time (75-77).

6.5. Membrane Damage

Approximately 25% of RC membrane phospholipid is lost during 42 days of storage. This loss leads to increase in the levels of extracellular haemoglobin, because micro-vesicles contain approximately 70% of the extracellular haemoglobin released during storage (57, 75, 76, 78-80).

6.6. Shape Change

Storage induces a shape change from the normal discoid shape to schiztocytes. This is important because the red blood cell shape is an important factor in diverse RC functions such as flow properties in capillaries and oxygen transport (81, 82).

6.7. Sodium - Potassium (Na^+/K^+) Pump

During storage, there is leakage of intracellular potassium ions from the RC and influx of extracellular sodium ions as the Na^+/K^+ pump is not functioning adequately at 4°C (63). Following transfusion of stored RCs, the Na^+/K^+ pump returns to normal and over a period of days the intra and extra cellular levels of these electrolytes return to normal. The high levels of extracellular potassium have led to recommendations that stored RCs should not be used for neonatal and paediatric patients, or cardiac surgery or renal dialysis patients (83-85).

6.8. Oxidative Injury to Lipids and Proteins

Blood is continually exposed to oxidative stress, and therefore it has a high antioxidant capacity. In the storage of blood, the demand for antioxidant capacity is increased. Consequently, damage to RCs by free radicals may occur. The total antioxidant capacity decreases during the formation of free radicals. Causes of free radicals in stored blood include high glucose concentration, exposure to light, as well as leakage of free radicals from blood leucocytes and macrophages (64, 86, 87).

6.9. Immune Activation and Vascular Adhesion

Several well described mechanisms can contribute to immune activation and vascular reactivity including release of haemoglobin, haem and iron from haemolysed RCs which can cause redox injuries and are toxic to mononuclear and endothelial cells. Activation of mononuclear cells leads to cytokine and chemokine release, endothelial cell coagulant responses and immune responses (88-90).

During storage shedding of micro-vesicles with negatively charged surfaces from intact cells occurs which is a way of shedding oxidized and polymerized lipids. These micro-vesicles are markedly procoagulant (91). Similarly, there is loss of lipid and protein products with biologic activities from damaged RCs (60).

Accumulation of these products/activities over time of blood storage means they are presented to the body in high activities/concentrates at the time of transfusion and are presented in abnormal concentrations to sick patients with less reserve to handle the extra physiologic or chemical loads.

6.10. Microvascular Function

The main aim of RC transfusion is the restoration of O_2 carrying capacity. However, transfusion also plays an important role in restoration of microvascular function. Adhesion of RCs to vascular endothelium has important consequences for RC blood rheology. In healthy people RCs do not adhere to vessel walls and therefore maintain a smooth blood flow (81).

In vitro stored RCs adhere to vascular endothelial cells and the number of adherent cells increases with storage duration. Adhesion can disrupt flow patterns, decrease O_2 delivery to peripheral tissues and in severe cases lead to occlusion of micro-vessels.

6.11. Immune Modulation

Transfusion-related immunomodulation (TRIM) and RC storage are thought to be due to adverse pro-inflammatory or immunosuppressive responses although it is not apparent immediately upon transfusion. It may manifest as poor outcome with increased morbidity or mortality (55, 56, 92). This concept was broadened to encompass the whole spectrum of haemodynamic control of blood and the effects of changes on organs and tissues. A two-insult model of post-transfusion injury was proposed (93-96) where the first insult (patient's underlying inflammatory condition) primes the patient's immune cells or endothelium and frank inflammation is triggered by a secondary inflammatory insult resulting in full scale activation whether immune and/or thrombotic.

Transfusion has been proposed as the second insult. The possible mechanisms by which transfusion has been implicated in TRIM as a risk factor for poor outcome include length of RC storage time, leucocyte content, RC processing method, storage solution composition and number of units transfused (55, 62, 72, 96).

6.12. Leucodepletion

Leucodepletion is a technique used to prevent potential complications of blood transfusion, non-haemolytic febrile reaction and alloimmunisation against leucocyte antigens. Leucodepletion can be performed both pre-storage and post-storage (72, 96, 97). Pre-storage filtration is the most effective leading to decreased cytokine levels and minimization of non-haemolytic febrile transfusion

reactions. Pre-storage leucodepletion of RC units decreases the number of adherent RCs however it does not eliminate effect. It has been suggested RC adhesion is at least in part induced by storage related changes.

6.13. Age of Red Cells

There is conflicting evidence regarding association of RC storage age and adverse clinical outcomes. Studies in critically ill patients reported independent association between RCs of increased storage age and increased risk of: infection, deep vein thrombosis, cancer recurrence, organ failure, ICU length of stay and mortality (21-25, 98, 99). RCs stored for greater than 14-28 days were associated with worse outcomes.

In the latter part of the 20th century, there was an attempt to prolong RC shelf life with additive solutions, to improve inventory and reduce outdating. Internationally, different preservative solutions are used, and different regulations exist for the shelf-life of RCs. RC shelf life has been determined based on *in vitro* haemolysis and *in vivo* survival studies (65, 100). In Europe and the U.S.A red cell storage systems have been based on post-transfusion RC viability and the acceptable degree of haemolysis following the approved RC storage period (77). Recovery of greater than 75% of transfused RCs is considered acceptable (77, 100). The Council of Europe (73) requires that haemolysis of stored RCs does not exceed 0.8% and in the US.A. less than 1% haemolysis at the end of the approved storage period is acceptable (77).

However, there is variation in international practice; some countries have voluntarily restricted the shelf-life of RCs despite use of additive solutions suitable for 42-day storage. Australia has a 42-day shelf-life, whereas the UK and Netherlands restrict RC shelf life to 35 days while Japan has a 21-day shelf life due to the use of universal irradiation of RCs to reduce the risk of transfusion associated graft versus host disease (TA-GvHD) (65).

6.13.1. Retrospective Study of Age of Transfused Red Cells

Goel *et al* (101) undertook a retrospective study to assess the outcome of 28,247 patients who were transfused with a total of 129,483 RC units. The authors evaluated the morbidity, mortality and length of stay (LOS) in patients transfused exclusively with RCs stored for 21 days, patients transfused exclusively with RCs

stored ~ 28 days and patients transfused with RCs stored ~35 days. The study revealed that when compared with the patients who were transfused with RCs that were stored for 28 days or less, patients who received RCs which were stored for 35 days or more were associated with increased morbidity but not mortality. Specifically, in older patients who were transfused with RCs which were stored for 35 days or more, there was also an increase in morbidity but not mortality. Of note was the group of critically ill patients, where increased morbidity and mortality was associated with the transfusion of RCs which were 35 days or older at age of issue. The authors concluded that RCs transfused in the last 7 days of their 42-day storage limit may be associated with adverse clinical outcomes in high-risk patients.

6.13.2. Meta-Analysis on Transfusion of Old and Fresh Red Cells

A meta-analysis was recently conducted by Remy *et al* (102). The authors searched five databases (PubMed, EMBASE, Web of Science, Scopus and Cochrane library) from May 2011 to 15 December 2014 and assessed both observational studies and RCTs studying the effect of RC storage age on mortality. Their aim was to examine the most updated and complete clinical evidence and compare results between two trial designs.

Analysis of six RCTs found no significant differences in survival comparing current practice (average storage age of 2 to 3 weeks) to transfusion of 1 to 10-day-old RCs. RC storage age was lower in RCTs as compared with observational studies. The 31 observational studies found an increased risk of death with increasing age of RCs, a different mortality effect than the RCTs.

The authors concluded that "RCTs have established that transfusion of 1 to 10-day-old stored RCs is not superior to current practice" and that "the apparent discrepancy in mortality between analyses of RCTs and observational studies may in part relate to differences in hypotheses tested and ages of stored RCs studied". They asserted that "further trials investigating 1 to 10-day-old stored RC benefits would seem of lower priority than studies to determine whether 4 to 6-week stored RCs have the safety and efficacy equivalent to the 2- to 3-week-old stored RCs commonly transfused today".

The meta-analysis by Wang *et al* (60) in 2012 of retrospective and prospective papers published assessing the age of RCs transfused and outcome suggested

that older stored blood was associated with increase in mortality where the primary endpoint was the effect of storage on mortality.

21 studies were included from 2001-2011 including 3 RCTs (Table 6.13.2) (60). There was wide variation in study sizes (from 17-57 for RCTs to 66-387 in 130 retrospective observational studies). Most of the studies were in cardiac surgery or trauma patients and most of the data in meta-analysis were from observational studies with larger cohorts (60). One of the variables between the different studies was the difference in definition of 'fresh' blood.

The mean RC transfused per patient in the studies ranged from 1 unit to 10 units and most of the studies used leucodepleted RC units. Transfusion of 'old blood' was associated with a significantly increased odds ratio of death (1.16; 95% confidence interval (1.07-1.24) compared with 'new blood' (60).

Analysis of secondary outcomes comparing transfusion of old and fresh RCs showed that old RCs were associated with increased multi-organ dysfunction (OR 2.26; 1.56-3.25) and pneumonia (1.17; 1.08-1.27) but no consistent effect on incidence of sepsis. In patient subgroup analysis, there was a higher risk of death if old RCs were transfused to cardiac surgery patients (OR 1.26; 1.04-1.53) and trauma patients (1.18; 1.02-1.35).

Most of the data in this meta-analysis were from observational studies and the three RCTs included in this meta-analysis showed heterogeneous results.

The issue of age of transfused RCs received considerable attention following the publication of a study by Koch *et al* (24) of 6002 cardiac surgery patients, which showed lower mortality (7.4% v 11.0% at 1 year) and lower risk of post-operative complications in patients transfused with blood 14 days or younger. 2364 patients who received a mixture of "newer or older" blood were excluded from the analysis and it is unknown whether a transfusion of newer or older RCs negates the effects of older RCs (24).

Confounding was a limitation in this observational study (24), many confounders such as emergency surgery, surgical blood loss and re-exploration for bleeding, use of leucodepleted or non-leucodepleted RCs, use of anti-fibrinolytic agents and others were not considered. Similarly, no allowances were made for patients

who received the oldest RCs and who are the sickest and often receive the most blood (103-105).

Whilst there have been numerous publications on this topic, many were observational studies, and many have been criticised due to confounding variables. The results of these studies have been inconsistent. For this reason, large prospective studies examining the safety, or otherwise, of stored RCs have been undertaken to further clarify this issue (2, 3, 26-29, 106-112).

First author (reference)	Year published	Years of enrollment	Study type	Study population	Number of patients in study	Number of patients used in meta-analysis*	Mean transfusion volume per patient (units of RBCs)	Age of stored blood compared (days)†		Blood storage solution‡	Leukoreduced	Mortality
								New	Old			
Van Straten[14]	2011	1998-2007	OBR	Cardiac surgery	3,597	3,141	New = 2.6, old = 2.4	<14	≥14	SAGM	Yes	30-day mortality
Pettila[15]	2011	2008	OBP	ICU patient	757	379	2	≤11	≥28	NR	Partially	Hospital mortality
Edgren[22]	2010	1995-2002	OBR	Transfused at least 1 RBC unit	387,130	387,130	2	<9	>30	SAGM	NR	Short-term mortality (1 week)
Eikelboom[21]	2010	2002-2006	OBP	Cardiac disease	4,933	4,933	3	<10	31-42	AS-3	Yes	Hospital mortality
Robinson[20]	2010	1999-2005	OBR	Cardiac surgery	909	712	NR	≤21	>21	NR	Yes	30-day mortality
Weinberg[13]	2010	2000-2009	OBR	Trauma	1,647	1,647	New = 2.9, old = 3.4	<14	≥14	NR	Yes	Hospital mortality
Karam[10]	2010	2004-2005	OBP	PICU patient	296	296	New = 2.6, old = 5.5	<14	≥14	NR	Partially	28-day mortality
Gauvin[24]	2010	2001-2005	OBP	PICU patient	455	224	New = 1.2, old = 2.3	≤7	>21	NR	Yes	28-day mortality
Van Buskirk[32]	2009	NR	OBR	ICU patient	298	298	NR	<8	>14	NR	NR	Hospital mortality
Spinella[17]	2009	2004-2007	OBR	Trauma	202	176	9	<21	≥21	NR	NR	Hospital mortality
Koch[16]	2008	1998-2006	OBR	Cardiac surgery	6,002	6,002	2	≤14	>14	NR	NR	Hospital mortality
Weinberg[26]	2008	2000-2007	OBR	Trauma	430	430	5.2	<14	≥14	NR	Yes	Hospital mortality
Yap[25]	2008	2001-2007	OBR	Cardiac surgery	670	670	3	8	19	NR	NR	Hospital mortality
Weinberg[19]	2008	2000-2007	OBR	Trauma	1,813	1,169	4.95	<14	≥14	NR	Yes	Hospital mortality
Leal-Noval[23]	2008	2004-2006	OBP	Anemia	66	34	NR	<10	>19	SAGM	Yes	ICU mortality
Van De Watering[18]	2006	1993-1999	OBR	Cardiac surgery	2,732	1,895	4.77	<18	≥18	SAGM	Yes	30-day mortality
Murrell[27]	2005	2001-2002	OBR	Trauma	275	275	3	NR	NR	NR	Yes	Hospital mortality
Fernandes Da Cunha[33]	2005	2002-2003	RCT	Premature infant	52	52	NR	1.6	9	CPDA-1	Yes	Hospital mortality
Hebert[34]	2005	1999-2001	RCT	Cardiac surgery	57	57	New = 3, old = 2		19	CPD-2, AS-3	Yes	Hospital mortality
Schulman[35]	2002	2000-2001	RCT	Trauma	17	17	New = 9.3, old = 10.6	<11	≥20	NR	Yes	Hospital mortality
Mynster[12]	2001	1991-1993	OBP	Colorectal surgery	740	429	3	<21	≥21	SAGM	Yes	Long-term mortality

* When multiple ages of stored blood were presented only the extremes were analyzed (see statistical method).
† See statistical method for how stored blood groups were determined for comparison.
‡ The numbers and letters indicate the type of storage solution: C = citrate, A = adenine, P = phosphate, D = dextrose, S = sodium chloride, M = mannitol, G = glucose.
ICU = intensive care unit; NR = not reported; OBP = observational prospective study; OBR = observational retrospective study; PICU = pediatric intensive care unit; RCT = randomized controlled trial.

Table 6.13.2. Characteristics of studies included in the meta-analysis by Wang *et al* (60)

Adapted from Wang D, Sun J, Solomon SB, Klein HG, Natanson C. Transfusion of older stored blood and risk of death: a meta-analysis. Transfusion. 2012;52(6):1184-95.

6.14. Randomised Controlled Trials

Randomised controlled trials are considered the standard to evaluate efficacy of a therapy or intervention. The strengths of an RCT is the development of a prospective study protocol with strict inclusion/exclusion criteria, a well-defined intervention, and predefined endpoints (113). Sometimes it is unethical to undertake a RCT and these will not be performed. Observational studies on the other hand have several advantages over RCTs including lower costs, broader range of patients and a cost-effective way of producing and investigating hypotheses before larger RCTs are commenced (14, 113). Observational studies may be used to identify risk factors and prognostic indicators and in situations in which RCTs would be impossible or unethical (114).

Both observational studies and RCTs could provide important information regarding the safety, efficacy and effectiveness of a clinical intervention. Concato *et al* (113) reported that 'the results of well-designed observational studies do not overestimate the magnitude of the effects of treatment as compared with those in RCTs on the same topic'

Until recently there has been a lack of robust RCT data on the clinical outcomes associated with transfusion of old versus new RCs (65). In earlier RCTs physiological parameters were the primary out comes rather than mortality (65). More recently the outcomes of the multi-centre ARIPI (26, 110), RECESS (115), ABLE (28, 111), TOTAL (29) and INFORM (3, 116) randomised controlled trials were published comparing "fresh blood" stored for approximately 7 days, with older standard-issue blood stored for 2 to 4 weeks and concluded that fresher blood afforded no advantage to critically ill adults, patients undergoing cardiac surgery, or premature infants. Results from the more recent larger INFORM RCT (3) similarly do not support the hypothesis that the transfusion of older standard-issue RCs is associated with poorer patient outcomes.

There are two RCTs, the ABC PICU (117) and TRANSFUSE (118) studies still underway comparing fresh Vs old RCs in paediatric intensive care and adult intensive care patients.

6.14.1. ABC PICU

The age of transfused RCs in children in the ABC PICU study (117) is a randomised clinical trial comparing the clinical consequences of RC storage duration in 1538 critically ill children transfused with either RCs stored 5-7 days or standard issue RCs (expected mean storage duration of 17-21 days). The primary outcome measure is development of New or Progressive Multiple Organ Dysfunction Syndrome (NPMODS) defined as the proportion of patients who die during the 28 days after randomisation or who develop NPMODS. Recruitment for the study commenced in January 2014 and the estimated completion date is August 2018.

6.14.2. TRANSFUSE

The TRANSFUSE Study is a randomised controlled trial being carried out in Australia and New Zealand (118). The study aim is to determine whether, compared with standard care, transfusion of the freshest available RCs decreases patient mortality. Recruitment commenced in October 2012 and was completed in late 2016. In the trial 5000 critically ill patients have been randomly assigned to receive either the freshest available RCs cells or standard issue RCs when they require a transfusion in Intensive Care Units.

6.14.3. ARIPI

In vulnerable patients such as critically ill premature infants, it has been suggested that transfusing older RCs may result in higher rates of organ dysfunction and morbidity because of the deleterious oxygen deficits or the pro-inflammatory effects of bioactive materials that accumulate during RC storage

Premature infants requiring multiple transfusions are routinely exposed to older standard-issue RCs because of a dedicated donor policy to decrease the risk of viral transmission through transfusions from multiple donors which leads to increased rates of transfusion of older RCs.

The ARIPI Study (26, 110) was designed to evaluate whether RCs stored for 7 days or less decreased serious neonatal morbidity and mortality compared with standard issue RCs. ARIPI was a double blind, randomised control trial undertaken in 6 Canadian tertiary NICUs. Infants were randomly assigned to receive either RCs stored for <7days (fresh) or current standard issue RCs

(standard – storage time ranging from 2-42 days) and were monitored for up to 90 days of their stay in NICU.

The study population included 377 infants with a birth weight <1250g and requiring 1 or more RC transfusions. Exclusion criteria included:

- Premature infants who had already received a RC transfusion
- Scheduled to undergo an exchange transfusion or receive directed donation
- Had rare blood type that would lead to difficulties with cross matching
- Moribund on admission
- Not expected to survive because of severe congenital anomaly
- Attending clinician specifically requested fresh RCs

Baseline demographic and clinical characteristics were similar in both groups with exception of more males in the fresh RC group. The mean and median volumes transfused were similar in both groups and the mean age of the RCs in the fresh group was 5.1 days and 14.6 in the standard group.

Primary outcomes were a composite outcome composed of mortality and major neonatal morbidities associated with acute organ dysfunction. Major morbidities comprising the composite outcome included:

- Death
- Bronchopulmonary dysplasia
- Retinopathy of prematurity
- Necrotizing enterocolitis
- Intraventricular haemorrhage
- Worsening of outcomes over the study duration

Secondary outcomes included

- Rates of individual complications comprising the composite outcome
- Rates of nosocomial infections

Tertiary outcomes included

- Length of mechanical ventilation and supplemental oxygen use
- Need for vasopressors

- Need for other blood products
- Need for invasive vascular access
- Length of stay in NICU
- Rates of major or minor interventions

In critically ill premature infants fresh RC transfusions compared with standard practice did not increase or decrease rates of complications or death. No effect of fresh RC transfusion on mortality, necrotizing enterocolitis, bronchopulmonary dysplasia, intra-ventricular haemorrhage (IVH) was evident. There was no significant difference in individual complications, secondary or tertiary outcomes or subgroup analyses. In the fresh RC group 52.7% of infants experienced a primary outcome and 52.9% in the standard group. Analysis of individual components of composite end-point showed no clinically significant difference between groups except a statistically non-significant increase in rates of grade III or IV intraventricular haemorrhage (IVH) in the fresh group (RR 1.65). Subgroup analyses by birth weight, gestational age and sex showed no differences.

With immature circulation, limited physiologic reserve, immature immune responses and enhanced susceptibility to oxygen damage in premature infants it was expected to find evidence for benefit if fresh RCs had favorable biological properties which was not obvious.

The many laboratory changes that occur with prolonged RC storage may not be as important as once thought or the mean storage time of 2 weeks in standard group may not have been sufficient to detect biological effects.

The ARIPI Study (26) shows no difference in complications such as necrotizing enterocolitis and mortality in a population of premature infants less than 37 weeks gestation who received either blood that was stored <7 days versus standard age.

6.14.4. ABLE

Fresh RCs may improve outcomes in critically ill patients by enhancing oxygen delivery while minimizing the risks of toxic effects from cellular changes and accumulation of bioactive materials in blood components during prolonged storage.

The Age of BLood Evaluation (ABLE) study was an international double-blind, multi-centre, parallel, randomised controlled trial that was conducted in 25 Canadian, 16 French, 9 British, 6 Dutch and 1 Belgian intensive care units.

The purpose of this study was to determine whether there was a difference in patient outcome in relation to receiving fresh or standard blood. Patients were randomised to receive either standard issue RCs or RCs stored less than 8 days. No transfusion trigger was stipulated, and patients were expected to require invasive or non-invasive mechanical ventilation for at least 48 hours. The primary outcome was 90-day all-cause mortality.

Over a five-year period from March 2009 to May 2014, 1211 patients were randomised to receive fresh RCs (6.1 +/- 4.9 days) and 1219 to receive standard issue RCs (>22.0 days). All RCs at the study sites were leucoreduced before storage and suspended in saline-adenine-glucose-mannitol additive solution.

90-day mortality was 35.3% (430 patients) in the standard group and 37.0% (448 patients) in the fresh group (Figure 6.14.4). There was no significant difference between the groups for all secondary outcomes (major morbidities, length of respiratory, haemodynamic, and renal support, length of stay, and RC transfusion reactions). The authors concluded that providing fresh RCs stored <7 days rather than standard issue RCs did not decrease the 90-day mortality among critically ill adults.

From this study, it could be inferred that changes to RCs or the storage medium that have been documented in laboratory studies may have limited clinical consequences.

The strengths of this study were that it was sufficiently large to detect clinically important differences in 90-day mortality with a wide spectrum of critically ill patients. Between group differences in the duration of RC storage was statistically and clinically significant. The authors identified several limitations including the potential that some groups of critically ill patients particularly vulnerable to adverse consequences of prolonged RC storage were under-represented. Most patients received transfusions according to a restrictive transfusion strategy – exposure to RCs less than would be expected at centres using a more liberal use of blood transfusions and only centres that used

leucoreduced RCs were included. The effect of leucocytes during prolonged storage is unclear.

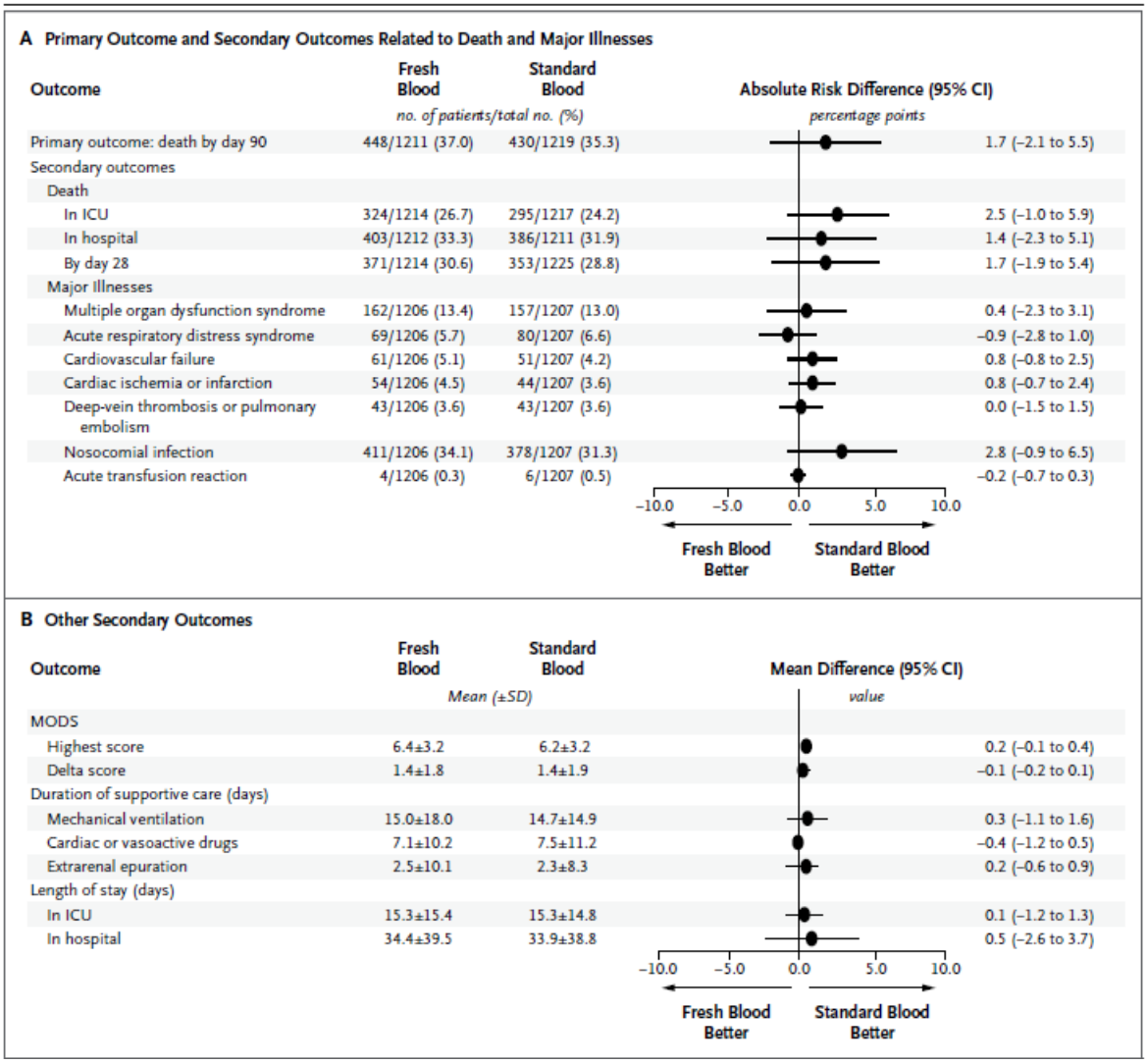

Figure 6.14.4. Primary and secondary outcomes from the ABLE randomized controlled trial (28, 112)

Adapted from Lacroix J, Hebert PC, Fergusson D, Tinmouth A, Capellier G, Tiberghien P, et al. [The ABLE study: A randomized controlled trial on the efficacy of fresh red cell units to improve the outcome of transfused critically ill adults]. Transfus Clin Biol. 2015;22(3):107-11 and Lacroix J, Hebert PC, Fergusson DA, Tinmouth A, Cook DJ, Marshall JC, et al. Age of transfused blood in critically ill adults. N Engl J Med. 2015;372(15):1410-8.

6.14.5. RECESS

Cardiac surgery patients often receive multiple units of RCs during their surgery or in the post-operative phase. These patients may be especially vulnerable to end organ injury because of compromised cardiac output or pro-inflammatory state post cardiac bypass. A previous retrospective study by Koch *et al* (24) of 6002 patients having cardiac surgery showed that those patients who received RCs stored >14 days had an increased incidence of several adverse outcomes.

The RECESS Study was a multi-centre, prospective, randomised clinical trial which compared the clinical outcome after cardiac surgery in 1098 patients 12 years of age or older who received RCs stored for 10 days or less or for 21 days or more for all intra-operative and postoperative transfusions through to hospital discharge, death or post-operative Day 28. The primary outcome was the change in Multiple Organ Dysfunction Score (MODS) from the preoperative score to the highest composite score through day 7 or the time of death or discharge. Exclusion criteria included planned use of autologous or directed donations, washed or volume reduced blood components or blood components where additive solution had been removed. Severe renal dysfunction, use of intra-aortic balloon pump for shock, planned deep hypothermic circulatory arrest and previous RC transfusion during admission were also exclusion criteria. All products were leucoreduced before storage.

The median storage time of RC units provided to the 1098 participants who received RC transfusion was 7 days in the shorter-term storage group and 28 days in the longer-term storage group. The duration of RC storage was not associated with significant differences in the change in MODS, the mean change was an increase of 8.5 and 8.7 points in the shorter-term and longer-term storage groups respectively (Table 6.14.5). The 7-day mortality was 2.8% in the shorter-term storage group and 2.0% in the longer-term storage group, and the 28-day mortality was 4.4% and 5.3% respectively. Adverse events did not differ significantly between the groups (27).

It was not feasible to power the study to detect differences in mortality or other infrequent clinical events nor was it designed to compare effectiveness of RCs transfused near the end of the allowed storage period with shorter-term storage.

Outcome	Red-Cell Storage ≤10 Days (N=538)	Red-Cell Storage ≥21 Days (N=560)	Estimated Treatment Effect (95% CI)	P Value
Primary outcome: ΔMODS at 7 days†	8.5±3.6	8.7±3.6	−0.2 (−0.6 to 0.3)	0.44
Secondary outcomes‡				
ΔMODS at 28 days	8.7±4.0	9.1±4.2	−0.3 (−0.8 to 0.2)	0.20
All-cause mortality — no. (%)				
7 Days	15 (2.8)	11 (2.0)	0.8 (−1.0 to 2.7)	0.43
28 Days	23 (4.4)	29 (5.3)	−0.9 (−3.4 to 1.7)	0.57
Median stay in ICU — days§	3	3	1.07 (0.95 to 1.21)	0.27
Median stay in hospital — days§	8	8	0.99 (0.88 to 1.13)	0.92

* Plus–minus values are unadjusted means ±SD. Unless otherwise noted, all outcomes were assessed through postoperative day 7, hospital discharge, study withdrawal, or death, whichever occurred first. The group receiving red cells stored for 21 days or more is the reference group. Analysis of covariance was adjusted for baseline value.
† For the change in MODS at 7 days, data were unavailable for four participants in the group assigned to receive red cells stored for 10 days or less and for seven in the group assigned to receive red cells stored for 21 days or more.
‡ Data on the change in MODS at 28 days were unavailable for 7 participants in the group assigned to receive red cells stored for 10 days or less and for 5 in the group assigned to receive red cells stored for 21 days or more. Data on all-cause mortality through 7 days were unavailable for 7 participants in the group assigned to receive red cells stored for 10 days or less and for 4 in the group assigned to receive red cells stored for 21 days or more; data on all-cause mortality through 28 days were unavailable for 14 participants in the group assigned to receive red cells stored for 10 days or less and for 9 in the group assigned to receive red cells stored for 21 days or more.
§ Length of stay was measured from date of surgery through day 28±3, death, hospital discharge, or the end of the study, whichever occurred first. For these outcomes, the estimated treatment effect was calculated as a hazard ratio with the use of a Cox model.

Table 6.14.5. Primary and secondary outcomes in the RECESS Trial (27)

Adapted from Steiner ME, Ness PM, Assmann SF, Triulzi DJ, Sloan SR, Delaney M, et al. Effects of red-cell storage duration on patients undergoing cardiac surgery. N Engl J Med. 2015;372(15):1419-29.

The results from the RECESS Study have shown that the standard practice for transfusing RCs, which may be stored for up to 42 days, is just as safe for patients undergoing complex cardiac surgery as transfusing them with RCs stored for 10 days or less.

6.14.6. TOTAL

The outcomes of the Tissue Oxygenation by Transfusion in Severe Anaemia with Lactic Acidosis (TOTAL) study were recently published (29).

The TOTAL study was a randomised non-inferiority trial which compared longer-storage (25 to 35 days old) versus shorter-storage (1 to 10 days old) RCs using reduction of blood lactate as an objective *in vivo* measure of transfusion efficacy.

The study population was Ugandan children, most with malaria or sickle cell disease, presenting to a national referral hospital in Kampala, Uganda between February 2013 and May 2015, with a mean haemoglobin level of 3.7g/dL and a mean lactate level of 9.3mmol/L. 290 children were randomly assigned to receive longer-storage (median age 32 days) or shorter-storage (median age 8 days) leucodepleted (pre-storage) RCs. The primary outcome was the proportion of patients with lactate levels of ~3mmol/L. The proportion of children achieving the primary end point was 0.61 in the longer-storage group and 0.58 in the shorter-storage group, meeting the pre- specified margin of non-inferiority. Mean lactate levels, clinical assessment, cerebral oxygen saturation (106), electrolyte abnormalities, adverse events, survival and 30-day recovery were not significantly different between the groups (29).

The authors concluded that, among children with lactic acidosis due to severe anaemia, transfusion of longer-storage compared with shorter-storage RC units did not result in inferior reduction of elevated blood lactate levels. These findings have relevance regarding the efficacy of stored RC transfusion for patients with critical tissue hypoxia and lactic acidosis due to anaemia. Additional analysis on a subset of patients from the study also showed that B-type natriuretic peptide (BNP), blood pressure, creatinine and plasma haemoglobin were not significantly different in the two groups (107). These findings are significant as prior studies have suggested that transfusion of stored RCs with increased levels of cell-free haemoglobin might reduce the bioavailability of recipient nitric oxide and cause myocardial strain.

6.14.7. INFORM

DR was chief investigator at Flinders Medical Centre and a member of the INFORM Steering Committee and contributed equally to the design and conduct of the INFORM trial. The analyses for this study were reviewed by all authors and all authors contributed to edits and revisions of the paper (3).

Following a pilot study (119) the INforming Fresh versus Old RC Management Study (INFORM) multi-centre randomised controlled trial involving more than 32,000 patients aimed to determine the effect on in-hospital death rates of transfusing the freshest available blood compared with standard-issue RCs (2, 3). Patients requiring RC transfusion were randomised to receive the freshest available RCs or standard issue (oldest product compatible in stock) RCs available (3).

The INFORM study was a large pragmatic randomised controlled superiority trial comparing the strategy of using shorter storage RCs with longer storage RCs for the prevention of in-hospital mortality in unselected patients requiring a blood transfusion. The trial was conducted in six international centres; Flinders Medical Centre in Australia, and hospitals in Canada, Israel and the United States. Using a web-based computer-generated randomisation program, the transfusion laboratory staff member allocated patients to receive shorter storage versus longer storage duration RCs in a 1:2 ratio to minimise the impact of randomisation on expiry rates of RCs and to maximise the separation in the exposure (storage duration) distribution of the arms. The aim was to achieve more than a 10-day difference in mean storage duration of the RCs between the two arms. The primary outcome was in-hospital mortality.

A total of 31,497 patients were randomised between April 2012 and October 2015. Of these patients, 6,761 did not meet all the enrollment criteria and were excluded. The primary analysis was conducted on 20,858 patients who were blood group A or O.

6,936 patients were assigned to the short-term storage arm (mean storage duration 13.0 days) and 13,922 to the long-term storage arm (mean storage duration 23.6 days). There were 634 deaths (9.1 %) in the short-term storage group and 1213 (8.7%) in the long-term storage group (odds ratio, 1.05; 95% confidence interval [CI], 0.95 to 1.16; P=0.34). The results were similar when the

analysis was expanded to include the 24,736 patients with any blood group, with rates of death of 9.1 % and 8.8%, respectively (odds ratio, 1.04; 95% CI, 0.95 to 1.14; P=0.38). Additional results were consistent in three pre-specified high-risk subgroups - patients undergoing cardiovascular surgery, those admitted to intensive care, and those with cancer.

We concluded that, among patients in a general hospital population, there was no significant difference in the rate of death among those who underwent transfusion with the freshest available blood and those who underwent transfusion according to the standard practice of transfusing the oldest available blood.

Previous RCTs (26, 27, 29, 112) were not powered to detect small differences in mortality and were restricted to specific patient groups whereas as our RCT was a large international multi-centre pragmatic RCT with broad patient enrolment. However, there were limitations to the study in that co-existing illnesses, interventions, reasons for transfusion and causes of death were not uniformly recorded by the various databases at the participating sites.

6.15. Patient's Mortality Transfused with Fresh Vs Standard-issue (Older) RCs

Following on from publication of our INFORM study (3) we reported on the results of a systematic review and meta-analysis of RCTs comparing mortality outcomes in patients transfused with fresher versus standard-issue red cells (4).

We conducted a systematic search between January 2015 and October 2016 using the Cochrane Library Central Register of Controlled Trials (CENTRAL), MEDLINE, EMBASE and CINAHL databases to identify RCTs of hospitalised surgical and non-surgical patients of any ages which compared mortality outcomes associated with transfusion of fresher versus standard-issue red cells (4). 2,220 citations were screened as well as 12 RCTs from a previous systematic review.

Review of the articles revealed that 14 RCTs comprising of 26,374 patients were eligible for analysis. The mean or median duration of storage of transfused red cells ranged from 1.6 to 13 days in patients who were allocated fresher red cells and 9.0 to 32 days in patients allocated to receive standard-issue red cells. Of the

14 selected RCTs, 5 trials investigated paediatric patients, 8 trials investigated adult patients and 1 trial did not report the participants' ages.

Our analysis revealed that 1,219 of the 9,531 patients (12.79%) who were transfused with fresh red cells died, compared to 1,810 of the 16,843 patients (10.74%) who were transfused with standard-issue red cells (RR: 1·04, 95% CI: 0·98–1·12, P = 0·90, I2 = 0%). Of the 6 RCTs which assessed in-hospital mortality, death occurred in 691 of the 7,479 patients (9.24%) transfused with fresher red cells and 1,291 of the 14,757 patients (8.75%) who were transfused with standard-issue red cells. The authors also assessed the certainty of evidence for all-cause mortality and in-hospital mortality according to the Grading of Recommendations Assessment, Development and Evaluation (GRADE). Our study found that the certainty of evidence was "high" for dismissing the benefit of transfusing fresher red cells versus standard-issue red cells. However, the certainty of evidence was "moderate" for dismissing the possibility of harm, as the upper limits of the 95% confidence intervals suggested that mortality could be increased by as much as 12% with fresher red cells. This is contrary to first thoughts and is further explored below in section 6.16.

Therefore, we concluded that the transfusion of fresher red cells does not reduce overall or in-hospital mortality when compared with standard-issue red cells and that our findings support the practice of transfusing patients with the oldest red cells available in the blood bank.

There is, however, still ongoing debate as to whether transfusion of RCs during the last week of storage (35 to 42 days) poses greater risk than transfusion of RCs stored for shorter periods. Recently, it has been shown that following transfusion of autologous RCs stored for 35 days or longer there was increased extravascular haemolysis, non-transferrin bound iron (NTBI) and saturated serum transferrin (120).

The original data analysis from the INFORM study (3) did not permit exclusion of the associated risk of transfusion of RCs stored for >35 days and up to 42 days. We recently published a manuscript 'Red blood cell storage duration and in-hospital mortality: a secondary analysis of the INFORM study (3, 109) using time-dependent exposure (5) examining this exact question.

This study was a detailed secondary analysis of the INFORM data. The importance of this secondary analysis was to assess the exposure to blood stored at the upper limit of current storage limits (35-42 days) where no randomized data exist. Cox regression modelling with time-dependent covariates was used to assess the exposure effect stratified on the total number of RCs received.

Conclusions from the secondary analysis of the INFORM data were that transfusion of RCs stored for more than 35 days was not associated with increased risk of in-hospital death compared to transfusion of blood stored for 7 days or less and that current systems for blood storage and inventory management are acceptable (5).

To compare the effect of RCs stored for more than 35 days to those stored for 7 days or less, ideally would be to randomly allocate patients to either arm in a RCT. However, such a trial would be logistically difficult and possibly raise ethical concerns because of the challenges of maintaining an adequate supply of RCs seven days or less in age and those greater than 35 days which could possibly lead to increased waste.

6.16. Effect of Whole Blood Processing

Prior to 2006 there appeared to be increased in-hospital mortality with standard-issue transfused blood, however after 2006 the effect disappeared but the trend changed to suggest fresher blood may be associated with greater harm. It was suggested that the method by which whole blood donations are processed could be associated with patient outcomes, specifically in-hospital mortality (72).

There is no consistency internationally in the way that whole blood is processed following donation. Since 2008 whole blood may be processed in two ways. For RC, platelet and plasma product processing, whole blood may be stored at room temperature (RT) up to 20hrs post collection prior to processing. Leucodepletion of RCs and platelets occurs separately within 24hrs of donation.

Whole blood not scheduled for platelet production blood is cooled to 4^0C within 10hrs of donation and processed into RCs and plasma products anytime within 72hrs of donation and is leuco-depleted just prior to processing (73, 121-127).

6.17. Red Cell Processing Methods and In-Hospital Mortality

A retrospective registry cohort study by Heddle *et al* (72) investigated whether the method of whole blood processing (RC filtration or whole blood filtration) and the duration of RC storage before transfusion, are associated with in-hospital mortality of transfused adults. Patients from three acute care hospitals in Hamilton, Ontario, Canada were identified from a Transfusion Registry for Utilization, Surveillance and Trafficking (TRUST) database. Information on each transfused RC unit (donation date/ processing method/ donor sex and age) was obtained from the Canadian Blood Services and linked to recipient data. The selection criteria included patients aged 18 years and over on the date of admission, who received one or more allogeneic RC transfusions during their admission. Data were analysed on the patient's first admission during the study period; patients for whom the hospital visit was not their first were excluded. The primary outcome was in-hospital mortality.

Between 1 April 2008 and 31 March 2014, 91,065 RC transfusions were given to 23,634 adult patients at first admission. Two randomised controlled trials (RCTs) investigating the effect of RC storage duration on in-hospital mortality were undertaken during the study period. Consequently, approximately one third of the cohort was assigned to receive fresh blood at the time of transfusion. Of the 91,065 RC transfusions, 47,629 (52%) were RC filtered units and 43,436 (48%) were whole blood filtered units. 6423 (27%) of the 23,634 patients received RC filtered units only, 5903 (25%) received whole blood filtered units only, and 11,308 (48%) received both RC types. The median time to leucodepletion was 19hrs for the RC filtration method and 21hrs for the whole blood filtration method. In addition, while the median age of the RCs on arrival at the hospital was 9 days for both the RC filtered units and whole blood filtered units, the median age of the RCs when transfused was 19 days. The transfused RC storage age was classified as fresh (1-7 days), mid (8-35 days) or old (36-42 days).

Upon examining an initial model on the RC processing method, the authors, found no significant effect on mortality when patients received whole blood filtered RCs exclusively, or in combination with RC filtered products. However, when storage duration was included in the model, in-hospital mortality was significantly higher with fresh whole blood filtered units, compared with the reference group of mid-age RC filtered

The authors concluded that RCs produced by whole blood filtration and stored for 7 days or fewer, were associated with higher in-hospital mortality when compared to the mid-age RCs. This finding suggests that the method of whole blood processing and the duration of storage for RCs could affect patient outcomes. Thus, adverse transfusion outcomes could potentially be reduced by minor changes to blood processing methods and inventory management practices.

A collaborative pilot study with Canada (Heddle *et al*) and Australia (Flinders Medical Centre) expanding on the recent publication by Heddle *et al* (72) to analyse our combined INFORM data in relation to RC processing and patient outcomes has been undertaken (data not shown). Plans are currently underway to further expand this retrospective study to review more than 300,000 RCs transfused over ten years at Flinders Medical Centre, the leucodepletion method and patient outcomes.

6.18. Effect on the Blood Supply

International blood services have needed to take a pragmatic approach to the issue of older or fresher blood to maintain adequate supplies of RCs and have adopted a 'first in first out' policy for supplying RCs. There is now considerably less concern following the publication of recent randomised controlled trials which concluded that fresher blood afforded no advantage to critically ill adults, patients undergoing cardiac surgery, premature infants or patients in general.

In practice in Australia (Figure 6.18) many of the RCs issued are fresher than 10 days at the time of issue from the Australian Red Cross Blood Service.

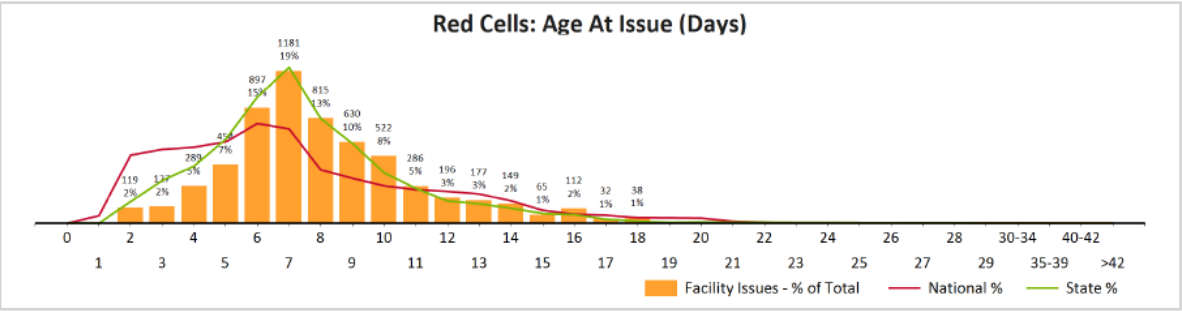

Figure 6.18. Typical age of RCs at the time of dispatch from the Australian Red Cross Blood Service to hospitals or pathology laboratories.

Based on the currently available data, the AABB (American Association of Blood Banks) (84) recently published clinical practice guidelines for RC transfusion with one recommendation stating that all patients (including neonates requiring transfusion) should receive RC units that are selected at any point within their licensed shelf life (standard issue) rather than limiting patients to transfusion of units that have been stored for less than 10 days (84) although the recommendation for RCs less than 5 days storage for intra-uterine or exchange transfusions remains.

The current recommendation was rated as a "strong recommendation" with "moderate quality evidence" using Grading of Recommendations Assessment, Development, and Evaluation (GRADE) method.

6.19. Summary

The aims of the RCTS were to understand the clinical impact and patient outcomes. However, one of the concerns with all these RCTs was the variability in defining what a ‘fresh’ RC is and what is an ‘old’ one.

The interpretation of ‘fresh’ and ‘old’ varies between institutions and research groups. For instance, the ABLE investigators (28, 111) took a pragmatic approach and set a limit of seven days for fresh blood whereas in the INFORM RCT a specific age for fresh or old RCs was not defined rather fresh was the freshest RC in inventory and standard issue RCs were the oldest in inventory (2, 3). Taking this approach allowed the RCs of any age to be included in the INFORM data and secondary analysis to occur (128).

How old is old in relation to a RC stored for clinical transfusion and what is the possibility of an adverse outcome following transfusion? This question remains unanswered. Undertaking an RCT comparing old and older RCs would be difficult and unethical, patients who require large numbers of RCs are usually the sickest and giving those patients only “old blood” may not be ethical and may in fact be dangerous.

6.20. Copy of Transfusion Medicine Reviews. Trans Med Rev 2016; 30:25-29

(DOI: https://doi.org/10.1016/j.tmrv.2015.11.002)

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A copy of the reference has been removed due to copyright restrictions

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A copy of the reference has been removed due to copyright restrictions

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7. CRITICAL BLEEDING AND MASSIVE TRANSFUSION

7.1. Introduction

The control of critical haemorrhage is vital to maintaining a patient's homeostasis. Massive loss of blood and electrolytes has systemic life-threatening effects, including hypovolemic shock and coagulopathy that require appropriate interventions and transfusion of blood and blood components to stabilise the patient. The aim being to control the haemorrhage, and to avoid hypothermia and acidosis, treat coagulopathy and minimise haemodilution.

Ongoing haemorrhage remains a major contributor to mortality (36). Haemorrhage is known to be the leading cause of death in the first hour of arrival in hospital (129) and is responsible for more than 80% of operating theatre deaths and accounts for nearly 50% of deaths within the first 24hrs of hospital admission (130). Morbidity and mortality associated with trauma-induced coagulopathy is up to four times higher than in patients without it (36).

7.2. Categories of Blood Loss

Blood loss can be categorized as follows:

- Category 1: 15% of the total blood volume (TBV) has been lost; no treatment required
- Category 2: 15% - 30% of TBV has been lost; usually requires IV fluid. Patient signs and symptoms include fatigue, light-headedness, and paleness.
- Category 3: 30% - 40% of TBV has been lost; IV fluid and blood transfusion required. Patient signs and symptoms include irritability, confused, weak, fatigue, and paleness.
- Category 4: More than 40% loss of TBV. Requires aggressive emergency treatment with IV fluids and blood transfusion. This is a life-threatening condition in which treatment must be immediately started to replace blood and fluids, as well as stop the haemorrhage.

Critical bleeding and massive transfusion may be seen in numerous different clinical settings including:

- Multiple trauma
- Industrial accidents
- Gastrointestinal bleeding
- Major cardiovascular surgery
- Obstetric emergencies
- Liver transplantation

Data from the Australian and New Zealand Massive Transfusion Registry shows that cardiothoracic surgery, other surgery and trauma are the leading causes of massive transfusion (131).

7.3. Australian and New Zealand Massive Transfusion Registry

The Australian and New Zealand Massive Transfusion Registry is a pre-existing database which provides an excellent source of data and enables large numbers of people to be entered into a study prospectively or retrospectively.

These types of databases can be used to identify patients or people with certain conditions or outcomes and produce a sample for a case controlled study. The data collected is usually independent of any specific hypothesis and thus observer bias is lessened and is ideally suited for testing various hypotheses (14).

7.3.1. Background

The Australian and New Zealand Massive Transfusion Registry (MTR) provides information to increase awareness and educate staff managing massive transfusion and help inform policies at government, blood services, specialist societies, specialist colleges and institutional levels. The MTR is not limited to trauma patients but captures all massive transfusion patients in those institutions enrolled. The MTR provides a unique tool for blood and blood product use in major bleeding. To date more than 5600 massive transfusion cases have been captured in the registry across 25 sites from Australia and New Zealand.

7.3.2. Aims of the Massive Transfusion Registry

The aims of the MTR are to collect data on transfusion practice and patient outcomes in the setting of critical bleeding and massive transfusion to:

- Systematically measure and monitor transfusion practice and outcomes in patients with critical bleeding and massive transfusions against national Patient Blood Management Guidelines (132).
- Provide data on critical bleeding and massive transfusion practice and outcomes to treating clinicians and hospitals
- Provide national data on blood utilisation to inform blood supply planning and inventory management
- Inform development of future clinical studies in critical bleeding to address evidence gaps in PBM Guidelines (132).

7.3.3. Structure of the Massive Transfusion Registry

The MTR is overseen by a national steering committee of nineteen clinicians, researchers, practice improvement experts and partnership representatives. The steering committee oversees MTR activities, provides advice on clinical matters and management, scope and funding of the registry, as well as collection and interpretation of data. Through the steering committee and various clinical networks investigating massive transfusion in a range of clinical contexts, the MTR has built an extensive network of engaged clinicians, medical scientists and transfusion nurses and hospital executives able to influence changes in transfusion practice.

7.3.4. Methods of Data Capture

The MTR has highly developed electronic data acquisition and linkage methodologies to various electronic databases to capture, import and link information for administrative datasets for patient demographics, admission data, transfusion history and laboratory results. MTR data linkages include the Australian and New Zealand Intensive Care Society (ANZICS) Adult Patient Database, National Death Data Registry, National Blood Authority BloodNet, National Trauma Registry and various laboratory information systems within Australia and New Zealand.

7.4. Massive Transfusion Definition

Massive transfusion has arbitrarily been defined as the replacement of a patient's blood volume or transfusion of 10 units of RCs in less than 24hs, or as the acute administration of more than half the patient's estimated blood volume per hour in response to massive and uncontrolled haemorrhage (133-135). However, more dynamic definitions such as replacement of 50% of the total blood volume within 3 hours or loss at >150mLs/hr is more appropriate in acute clinical settings (17, 43, 136).

Many authors have defined massive transfusion as transfusion for six hours for trauma patients, which reflects the time an acute haemorrhage lasts with the associated physiological consequences. This is perhaps the earliest time when clinicians can evaluate the benefits to the patient of aggressive component therapy (137, 138). The concept of massive transfusion in trauma patients has limited utility, and it is important to identify patients with massive haemorrhage and acute traumatic coagulopathy (139).

Because of the different definitions for massive transfusion used in the literature to identify patients and to compare those patient groups and outcomes it makes it difficult to compare studies and to ensure that all possible patients have been included. Therefore, and based on extensive analysis of data from the Australian and New Zealand Massive Transfusion Registry, we (10) have concluded that a definition of 5 RCs in 4 hours 'was the most inclusive' including capturing those patients whose deaths occurred early.

Such massive transfusions are often associated with both haemostatic and metabolic complications and involves the selection of the appropriate number and types of blood components to be administered as well as consideration of the associated issues of haemodynamics, tissue oxygenation, and management of haemorrhage and any associated coagulopathy as well as managing hypothermia and associated metabolic changes.

7.5. Coagulopathy

Coagulopathy, hypothermia, acidosis and the consequences of massive blood transfusion all lead to the development of a coagulopathy. Even if control of mechanical bleeding is achievable, patients may continue to experience

microvascular bleeding leading to a worsening of haemorrhagic shock and so worsening of hypothermia and acidosis, prolonging the problem.

Many major trauma patients are hypothermic on arrival in the emergency department due to environmental conditions at the scene. Inadequate protection, intravenous fluid administration and ongoing blood loss will worsen the hypothermic state. Haemorrhagic shock leads to decreased cellular perfusion and tissue oxygenation and so inadequate heat production. Hypothermia has dramatic systemic effects on the body's functions but most importantly in this context exacerbates coagulopathy and interferes with blood homeostatic mechanisms (133).

A considerable proportion of patients with ongoing bleeding and massive transfusion develop an associated coagulopathy which remains an important clinical problem (33, 140, 141). It is an intricate, multi-factorial, and multi-cellular event which contributes to an increased mortality rate compared to non-coagulopathic massive transfusion patients.

Coagulopathy will develop because of haemodilution, hypothermia, the extensive use of plasma poor-blood products and disseminated intravascular coagulation. The coagulation factors do not decrease homogeneously in severe bleeding (142). A decrease in fibrinogen is seen initially while thrombocytopenia is a late occurrence (142). The deficiency in fibrinogen level develops earlier than other coagulation factor deficiencies when plasma poor RCs and colloids or crystalloids are used for major blood loss and fluid replacement (133). Hiippala *et al* (143) showed that a fibrinogen concentration of less than 1.0g/L was reached when the blood loss was 142% of the estimated blood volume. And blood losses of more than two blood volumes caused deficiencies in coagulation factors II, V, VII and platelets (Table 7.5). Thrombocytopenia can occur earlier if disseminated intravascular coagulopathy occurs or if there is pre-existing thrombocytopenia (142).

Coagulation Factors	Critical Level	Estimated Blood Loss
Fibrinogen	<1g/l	142% (117-169)
Prothrombin	20%	201% (160-244)
Factor V	25%	229% (167-300)
Factor VII	20%	236% (198-277)
Platelets	50×10^9 /L	230% (169-294)

Table 7.5. The effect of estimated blood loss and critical clotting factor levels

Abnormalities of prothrombin time and activated partial thromboplastin time (Table 7.5.1) occur after transfusion of 12 units of RCs and thrombocytopenia develops after transfusion of 20 units of RCs (142-144).

Blood Volume Loss	% BV Exchanged	% Residual Coagulation Factors	PT/aPTT (x normal)	Fgn (g/L)	Plts ($\times 10^9$ /L)
One	70	30	<1.5	>1.0	>100
Two	85	15	>1.5	<1.0	50
Three	≥95	5	>1.8	<0.5	<50

Table 7.5.1. Blood volume loss and effect on prothrombin time, activated partial thromboplastin time, fibrinogen and platelet count

7.6. Acute Coagulopathy of Trauma

Many trauma patients present with a coagulopathy of trauma (43, 145) that does not meet the criteria for acute DIC or dilutional coagulopathy. This coagulopathy is caused by widespread tissue damage and shock and associated haemostatic, metabolic and physiological changes (Figure 7.6) (146, 147).

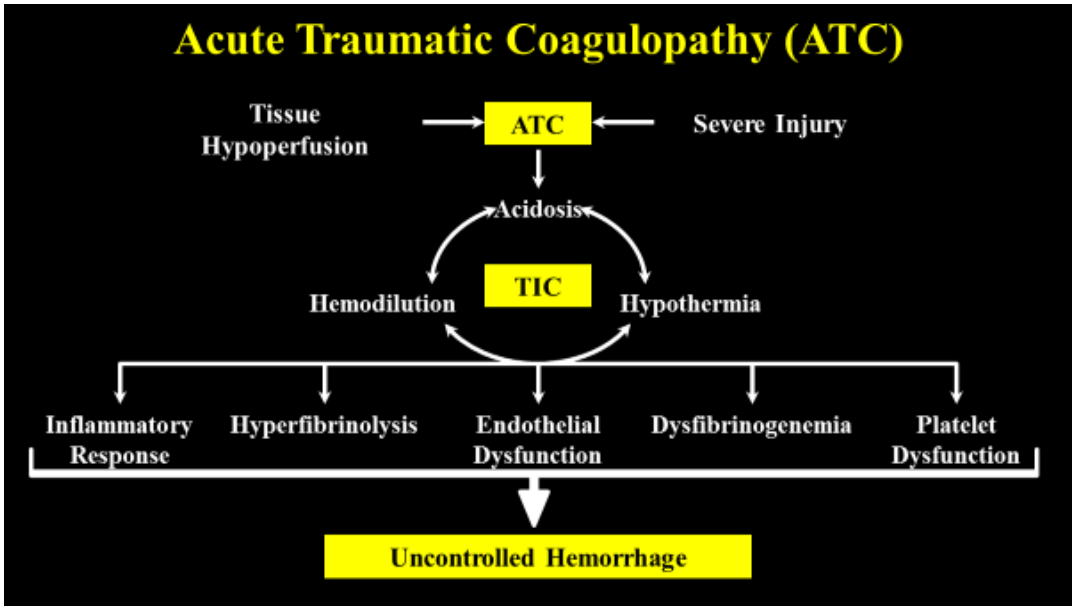


Figure 7.6. Schematic of acute traumatic coagulopathy

7.7. Coagulation Monitoring

Successful management of critical bleeding depends on timely monitoring of haemostatic processes. Development of coagulopathy needs to be detected and characterised in a timely manner to initiate the most optimal haemostatic intervention. Routine coagulation tests (prothrombin time and activated partial thromboplastin time) do not predict coagulopathy accurately in the acute patient, and likely do not to truly reflect real time *in vivo* haemostasis. These tests only provide information on initiation of clot formation. No information is available regarding properties of the clot, clot quality or stability, or the interactions of platelets and red cells, or the continuous development of the clot (148).

And secondly, these tests use platelet poor plasma which does not provide information about coagulation in whole blood. Thus, in cases of critical bleeding, the information provided by routine coagulation tests often lack specificity. In addition, the time to obtain the results from the laboratory is more than 45 minutes [local unpublished data] (36, 149). The incidence of abnormal tests of coagulation is high in trauma or critically bleeding patients. However, abnormal prothrombin PT (INR) or aPTT values have both a poor sensitivity and poor predictive value as indicators for potential bleeding tendencies (30).

Viscoelastic tests (rotational thromboelastometry [ROTEM] or thromboelastography [TEG]) provide an overall continuous functional view of *in vivo* haemostasis (150-152). ROTEM or TEG are being used more in critical bleeding situations (151, 152) providing faster more accurate means of detecting haemostatic defects and fibrinolysis. Because these viscoelastic tests can differentiate different haemostatic problems, they then may provide a means of guiding appropriate blood product replacement (36, 153, 154) and individualised directed patient management.

The role ROTEM/TEG in clinical practice is evolving and is not at this stage a substitute for routine coagulation tests but offers additional information that may reduce the need for blood products use and prevent inappropriate transfusions in non-coagulopathic patients (155, 156). In addition, ROTEM/TEG may assist in making decisions regarding the need for re-operation by excluding coagulopathy and suggesting a surgical cause of bleeding.

7.8. Critical Haemorrhage Treatment

The aims of treatment are the rapid and effective restoration of an adequate blood volume to maintain oxygen carrying capacity and tissue perfusion, to achieve haemostasis by treating any surgical source of bleeding and the use of haemostatic blood products to prevent or correct any coagulopathy and, possibly, the use of pharmacological agents, and to correct any electrolyte imbalances, acidosis and hypothermia. For massive uncontrolled traumatic haemorrhage, maintenance of full haemostatic ability is unrealistic (36, 153).

The need for transfusion of blood products in the severely injured trauma patient is based on clinical indications and associated laboratory tests. The complexity of treating massively bleeding patients is often empirical and may result in sub-optimal transfusion therapy further contributing to poor outcome. The recommendations of earlier guidelines were often based on clinical experience or the result of conventional coagulation assays (31, 157), although these assays poorly correlate with clinical coagulopathies (30). Those guidelines advocated early administration of crystalloids and colloids in conjunction with transfusion of RCs (31). According to those guidelines fresh frozen plasma (FFP) and platelets (Plts) should only be administered when a whole blood volume or more has been

substituted and then only in patients with excessive bleeding or microvascular bleeding and according to laboratory results (157).

The lack of randomised controlled trials in critical bleeding is obvious and most marked in the use of blood components to manage critical bleeding (30). The early recommendations were challenged based on retrospective observations and reported improved mortality that indicated that plasma and platelets should be administered in a 1:1:1 ratio (RC:FFP:Plts) (147, 158-160).

Much of the knowledge for the ratio of RC:FFP:Plts comes from retrospective military studies and more recent civilian studies which showed improved survival with use of higher ratios of RCs to FFP (149, 158, 161, 162). However, these studies were shown to be methodologically flawed due primarily to survival bias (30, 163). Further clinical studies have shown a benefit for the 1:1:1 approach to critically bleeding patients. In a retrospective study from Iraq of 246 patients who were stratified by a massive transfusion response showed that those patients who received a 1.4:1 ratio of RC:FFP had a higher survival rate (81%), compared with 66% and 35% who received ratios of 2.5:1 and 8:1 respectively (158). Another retrospective study by Holcomb *et al* (159) of 466 massively transfused trauma patients showed the 30-day survival was better in patients who received a high FFP:RC ratio (≥1:2) and a high RC:Plt ratio (≥2:1). Similarly, in another retrospective study by Perkins *et al* (164) of 694 massively transfused trauma patients, those who received a high ratio of apheresis platelets [equivalent to six units of pooled platelets]: RCs (≥1:8) had a 95% 24-hour survival compared to medium ratio (1:16 to 1:8, 87% survival) or low ratios (<1:16, 64% survival)

Following the publications of these studies the early use of RCs, FFP and platelets in ratios of 1:1 or 1:2 have been advocated in trauma and non-trauma critically bleeding patients. However, the optimal ratio of RC:FFP or RC:Plts and the sequence of component replacement remains to be determined.

More recently Holcomb *et al* (161, 162) in the PROPPR RCT have shown that early administration of RC:FFP:Plts in a ratio of 1:1:1 compared to 2:1:1 to severe trauma patients with critical bleeding did not result in significant mortality differences at 24 hours and 30 days. They did note that more patients in the 1:1:1 group achieved haemostasis with fewer deaths due to bleeding by 24 hours (162).

Overall the purpose of the 1:1:1 protocol is to facilitate the rapid availability and delivery of blood and blood-products when a massive transfusion critical bleeding situation occurs. More recently the use of ROTEM to guide component replacement is increasing with a move away from ratio based critical responses (150-152, 165).

7.9. Critical Bleeding and Massive Transfusion Studies

Up until 2006 Flinders Medical Centre did not have a Massive Transfusion Protocol (MTP). Responses to critical bleeding and massive transfusions were either empirical, or based on clinical experience or laboratory and clinical parameters. Because of the complexity of the clinical situation treatment may not have been optimal. Existing guidelines at the time (31) recommended administration of fresh frozen plasma and platelets only when a total blood volume had been replaced. There was little guidance on the replacement of fibrinogen.

Many observational studies suggested that patients with severe trauma, critical bleeding and coagulopathy have an improved survival when the ratio of transfused RC:FFP:Plts approaches 1:1:1 (158, 160, 166, 167).

A massive transfusion protocol was introduced at Flinders Medical Centre in April 2007. Activation of the MTP was at the discretion of the Emergency Department, the trauma team or Intensive Care. Upon activation of the MTP 5 RCs, 2-4 FFP and 1 adult therapeutic dose of platelets (prepared either as a pool of four platelet preparations obtained from four whole blood donations or a single apheresis platelet collection) were available in the first pack. One adult dose (10 whole blood or 5 apheresis cryoprecipitate units) of cryoprecipitate was included in the second and subsequent packs,

Based on my earlier involvement in the design and analysis of patterns of blood use in public hospitals in South Australia (6) and our ability to link electronic data bases using clinical (Diagnosis Related Group [DRG], Speciality Related Groups [SRG] and Major Diagnostic Categories [MDC], epidemiological and RC transfusion data within South Australian hospitals) I assisted in the design and development of a project to link a number of different electronic databases (Figure 7.9) to allow retrospective evaluation of laboratory and transfusion management of critically bleeding patients (7-9).

The independent electronic linked databases included:

- SA Trauma database
- Integrated South Australian Activity Collection (ISAAC)
- SA Pathology Laboratory database
- Australian & New Zealand Intensive Care Society database (ANZICS)
- Operating Room Management Information System (ORMIS)

Data collected for our studies included:

- Patient demographics and outcomes
- Laboratory parameters
  - Haemoglobin, platelet count
  - PT/INR, aPTT, fibrinogen
  - Arterial blood gas results
  - H, HCO_3, lactate, base deficit
- Blood components transfused
  - Red cells
  - Platelets
  - FFP
  - Cryoprecipitate
  - Recombinant FVIIa

7.10. Transfusion Practice in Massive Haemorrhage in pre-Intensive Care and Intensive Care

In our retrospective study of pre-Intensive Care and Intensive Care transfusion practice in massive haemorrhage (7) we reviewed all patients who received a massive transfusion (≥10 RCs in 24 hours). Patients with gastro-intestinal bleeding or direct admission to Intensive Care were excluded. The massive

transfusion episode was divided into pre-ICU and ICU and the patients were divided into three groups:

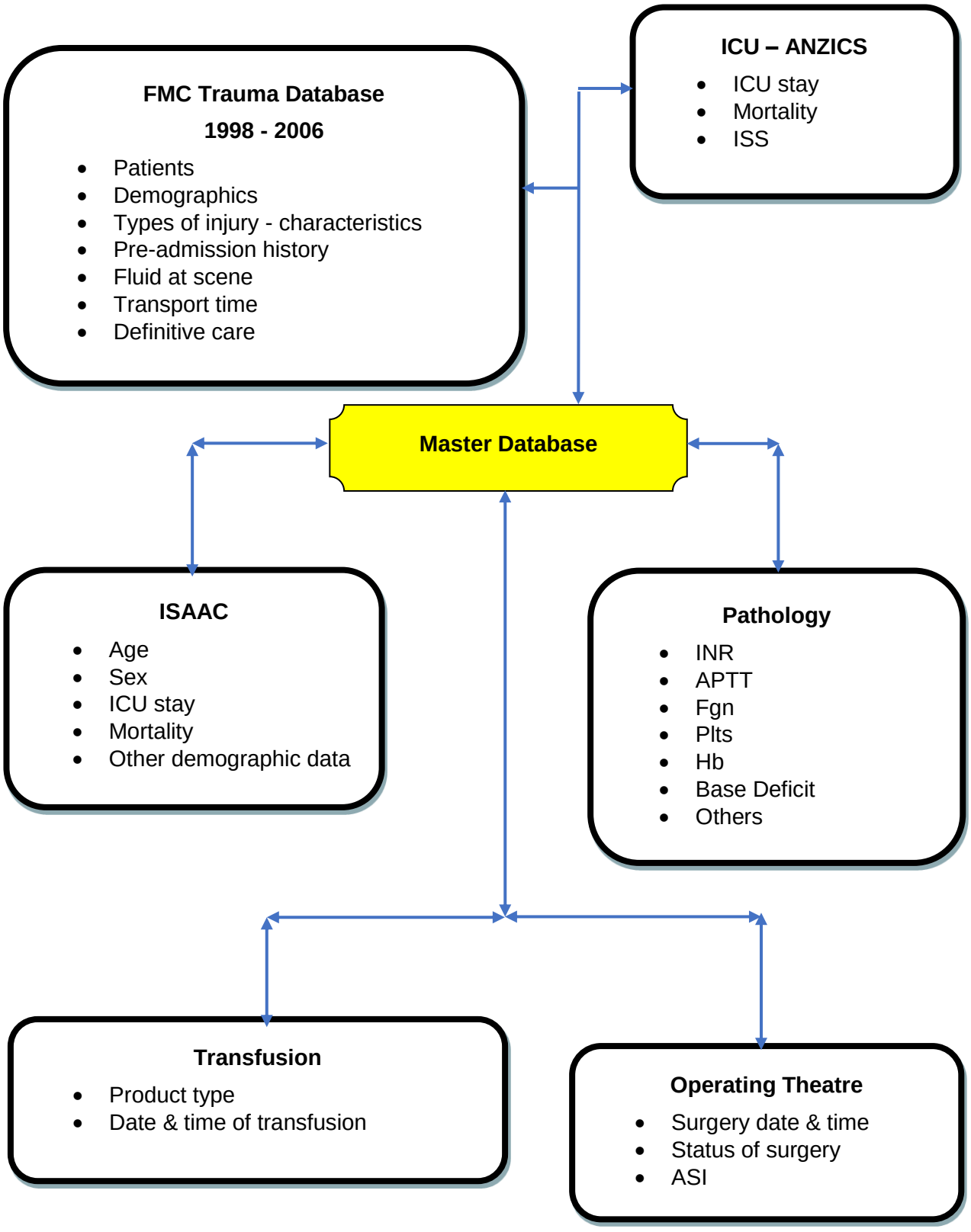
1. Survivors

2. Deceased within 24 hours of massive transfusion

3. Deceased 24 or more hours after massive transfusion

Significant differences in laboratory parameters and RCs and FFP used pre-Intensive Care and following admission to ICU (Table 7.10) were observed. The number of RCs transfused during the 24-hour period and the severity of coagulopathy present were associated with increased mortality.

Figure 7.9. Electronic data base linkage model



	24-h period							
	Pre-ICU				ICU			
	Group 1	Group 2	Group 3	*P* value	Group 1	Group 2	Group 3	*P* value
RBC	11 (8–17)	16·5 (11·3–27·5)	8·5 (5·3–14·8)	<0·001	4 (1–8)	9 (1–13·5)	6·5 (3·3–14·3)	0·007
FFP	4 (2–6)	6 (1·3–8·8)	2 (0–5·5)	0·09	2 (0–6)	4 (0–8)	4 (2–11·8)	0·03
Platelets	1 (0–2)	1 (0–2)	1 (0–1·8)	0·81	1 (0–2)	1 (0–2)	1 (0–2·8)	0·12
FFP/RBC	1:2·8 (2–3·8)	1:3·3 (2·3–3·8)	1:2·3 (1·8–3·4)	0·32	1:1 (0·8–1·7)	1:1·3 (0·7–2·2)	1:1·4 (1–2·4)	0·24
PLT/RBC	1:10 (8–13)	1:12 (9–20)	1:7·8 (6·8–9·0)	0·004	1:3·3 (2–6)	1:8 (3–12)	1:5 (4–7·6)	0·008

MT, massive transfusion; ICU, intensive care unit; RBC, red blood cells; FFP, fresh frozen plasma; PLT, platelets.
Data are median and interquartile range.
Kruskal–Wallis test.

Table 7.10. Transfusion of blood products by three massive transfusion groups during the pre-ICU and ICU phase (7).

Adapted from Sinha R, Roxby D. Transfusion practices in massive haemorrhage in pre-intensive and intensive care. Vox Sang. 2011;101(3):230-6.

We showed a significant difference in laboratory parameters and transfusion practices between pre-ICU and ICU admissions (7). Also, we found that more than 30% of patients were coagulopathic at hospital admission which was similar to that reported by others (30, 36, 140, 141).

Upon admission to the ICU, 69% of all patients were coagulopathic. Notably, resuscitation measures were unable to correct coagulopathy and acidosis in patients who died early (Group 2) in ICU. In pre-ICU, these patients received similar amounts of FFP and platelets and had similar RC:FFP ratios as compared with those of the other groups. Gonzalez *et al* (168) reported that coagulopathy was not corrected during ICU resuscitation, perhaps because of inadequate pre-ICU monitoring.

More aggressive pre-ICU intervention to correct coagulopathy and goal-directed haemostatic therapy using ROTEM is another option under scrutiny for managing these patients. Interestingly, even though in the absence of a massive transfusion protocol, the RC:FFP ratio was 1:1 in ICU. However the RC:FFP ratio in the pre-ICU phase was in the range of 1:2-1:3.

This study (7) showed that coagulopathy was present in more than one-third of the patients at the beginning of the pre-ICU phase, and that nearly half of the patients with coagulopathy were trauma patients. In addition, coagulopathy was a significant clinical issue at ICU admission and a predictor of mortality. Our findings are comparable to the findings of Gonzalez *et al* (168)where the severity of coagulopathy was predictive of mortality.

The retrospective design is a limitation of this study. In spite of these limitations, discrepancies in the management of the patients were identified. Fibrinogen levels were not measured when coagulation screens were ordered, which could be a reason for the limited use of cryoprecipitate in both pre-ICU and in ICU settings.

Fibrinogen constitutes an important component of the haemostatic process with roles in the formation of platelet aggregates and the generation of a sufficiently stable fibrin network. Often, the haemostatic role of fibrinogen is underestimated.

Another limitation of our study was lack of analysis of clinical sequelae of potential transfusion-related complications such as acute respiratory distress

syndrome, multiple organ failure, abdominal compartment syndrome, and damage control surgery. Finally, the three study groups were not well matched, and it is possible to introduce bias when comparing patients with massive bleeding who experience elective surgery with patients who experience emergency surgery.

In summary our study examined blood transfusion practices in patients with massive bleeding (7). Patients who died early were coagulopathic upon admission to ICU and emergency measures were unable to correct this condition, and our study demonstrated that coagulopathy is associated with increased risk of mortality. Early and aggressive treatment of massively transfused patients presenting with coagulopathy can be effective in reducing mortality.

7.11. Changes in Transfusion Practice in Massively Bleeding Patients

Following on from our initial study (7) we further retrospectively reviewed changes in massive transfusion practice at Flinders Medical Centre in critically bleeding patients (8) and the effect of introduction of a massive transfusion protocol (9).

During the period 1998 – 2008 we saw significant changes in transfusion practice and increases in the mean number of fresh frozen plasma and platelets transfused but no significant increase in RCs transfused (8). Similarly, we observed a significant increase in the amount of cryoprecipitate transfused. With these increases there were corresponding significant changes in the ratio of RC:FFP (1:2.6 to 1:1.4 $p<0.001$) and RC:Plts (1:10.5 to 1:6 $p = 0.002$) (8) even though a massive transfusion protocol was only introduced in 2007.

Overall there was improvement in mortality, however during the same time damage control techniques for trauma patients were introduced, the approach to rewarming was improved and anaesthetic techniques changed all of which may have contributed to improved outcomes and were beyond the scope of the study (8).

7.12. Experience with a Massive Transfusion Protocol in the Management of Massive Haemorrhage

In 2007 a massive transfusion protocol was introduced at Flinders Medical Centre. In this study we reviewed all activations from 2008 – 2011 and compared

them to the period 2004 - 2006 to determine if the change in practice was associated with improved outcomes (9). Following the introduction of the massive transfusion protocol the ratio of RC:FFP and RC:Plts was significantly higher than during 2004 – 2006 although no improvement in overall and 90-day mortality was observed. This may have been due to the fact that high ratios of RC:FFP and RC:Plts were already in use prior to introduction of a massive transfusion protocol.

The massive transfusion protocol was not targeted at any particular ratio of RC:FFP or RC:Plt ratios rather it was designed to minimise delays in availability of blood components. Others also found no differences in survival following implementation of massive transfusion protocols (135).

7.13. Changes in Recent Massive Transfusion Practices

Our most recent retrospective study of 190 massive transfusion episodes in 2008 and 2010 - 2014 compared blood component use, massive transfusion protocol activations and the use of thromboelastometry (11). Although there was no significant difference in median component use and high RC:FFP ratios over the study periods, there was a significant increase in the number of patients receiving cryoprecipitate.

Our previous study in 2011 (8) showed negligible cryoprecipitate use, however there is now increasing scientific evidence supporting the early use of fibrinogen or cryoprecipitate in critical bleeding (165, 169, 170).

Increasing evidence shows that ROTEM has a role in patient blood management in critical bleeding (152-154, 171, 172). From 2010 ROTEM was introduced into routine use at Flinders Medical Centre, and its routine clinical use increased steadily over the study period supporting the assumption that the increased cryoprecipitate use at Flinders Medical Centre was as a direct result of this change in practice. This observation of an associated increased use of cryoprecipitate following ROTEM introduction is supported by our unpublished data of intra-operative use of ROTEM to guide blood product replacement in liver transplant patients (172) where there was a significantly increased use of cryoprecipitate [2 (1-4) vs. 0(0-1) adult doses, $p<0.001$] between ROTEM and non-ROTEM groups respectively.

7.14. Summary

Management of massive transfusion is a challenging clinical problem. Debate continues on the use of empiric transfusion of blood products in pre-defined high ratios in critical bleeding or whether a goal-directed approach is more appropriate based on early viscoelastometric testing.

Recent studies based on retrospective observations indicate that altering the RC:FFP:Plt to 1:1:1 results in significant mortality reduction. The data show the importance of rapid product availability and emphasise that massive transfusion is a complex process in which product ratio and time to transfusion represent only the beginning of a better understanding. Therefore, to be successful, massive transfusion must be timely and directed at the needs of the patient.

Optimal management of critically bleeding, massively transfused patients remains unclear and requires careful review of numerous complex physiological and haemostatic interactions. What is lacking are RCTs and evidence based-guidelines and algorithms in trauma and non-trauma critical bleeding patients that clearly identify target values for coagulation and haematological parameters and that provide sound evidence to guide appropriate clinical management.

The management of critically bleeding patients remains a significant challenge clinically and for the Blood Bank.

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8. VISCOELASTOMETRIC TESTING AND SEPSIS

8.1. Introduction

Sepsis as defined by the Third International Consensus Definitions for Sepsis and Septic Shock (173) is life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is a subset of sepsis in which overwhelming circulatory, cellular and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone.

Previously severe sepsis was defined as sepsis complicated by organ failure and septic shock as sepsis-induced persistent hypotension even following adequate fluid resuscitation (173).

Sepsis and septic shock has significant morbidity and mortality with a reported mortality rate of >25% (174). Patients with severe sepsis typically die of multi-organ failure, with disseminated microvascular fibrin deposition as the underlying pathophysiology (175, 176). In Intensive Care, sepsis is the most common cause of disseminated intravascular coagulation although not all patients with sepsis can be regarded as having the same degree and type of coagulation abnormality (30).

Recognition of the role of the coagulation cascade particularly fibrinolysis in the pathogenesis of sepsis has recently led to evaluation of novel therapeutic options (177-183). However, the benefits are not without concomitant risks of haemorrhage and require suitable monitoring.

Severe sepsis leads to an inflammatory response resulting in synthesis of pro-inflammatory cytokines leading to the up-regulation of tissue factor, thrombin generation and dysfunctional physiologic anticoagulant mechanisms and fibrinolysis impairment resulting in systemic intravascular and extravascular fibrin deposition, and microvascular thromboses (30, 184). These manifest clinically as coagulation abnormalities that range from subtle activation that can only be detected by sensitive markers, through to increased coagulation activation that may be evident by a small decrease in platelet count and subclinical prolongation of global clotting times, to fulminant disseminated intravascular coagulation (DIC), characterized by simultaneous widespread microvascular thrombosis and profuse bleeding from various sites.

Routine coagulation tests are useful for detecting obvious DIC only, whereas a range of additional specific tests, which are not available in most hospitals, such as thrombin-anti-thrombin complex, protein C, and soluble fibrin are recommended to detect non-apparent DIC. These tests look at only a few aspects of the coagulation and inflammatory process, therefore often making interpretation difficult, especially when both qualitative and quantitative defects co-exist (30, 185-188). Hence it would be desirable to have a test which measures the result of overall haemostasis and inflammatory abnormalities in sepsis.

8.2. Rotational thromboelastometry (ROTEM)

- Rotational thromboelastometry (ROTEM) is a point of care test that provides real time functional global assessment of the viscoelastic properties of clot formation and subsequent lysis under low shear conditions. The principle advantage of ROTEM is its ability to quickly provide information regarding the overall haemostatic process compared to conventional coagulation tests such as PT, aPTT and fibrinogen, and its rapid assessment of clot characteristics such as clot formation, strength and fibrinolysis. Unlike standard coagulation tests that use platelet poor plasma, ROTEM uses whole blood. ROTEM measurement parameters include:
- Clotting time (CT) representing thrombin formation
- Clot formation time (CFT) representing fibrin polymerization
- α angle representing kinetics of clot formation
- Maximum clot firmness (MCF) representing the mechanical properties of the clot
- Clot lysis index at 60min (CLI60) represents the clot firmness at 60 mins after MCF is reached divided the MCF
- Maximum lysis (ML) represents the maximum amount of fibrinolysis at a given time

In a number of studies ROTEM has been shown to be a highly sensitive assay capable of detecting distinct, very early as well as late changes in coagulation when standard coagulation tests remain within the normal range (183, 189). Using clot lysis parameters such as Maximum Lysis (ML) and Clot Lysis Index

(CLI) represent the only bedside methods which provide information about the changes in the fibrinolytic system (190, 191).

ML has been well validated in a human model of endotoxemia as a marker of fibrinolysis (192). However, despite strong evidence for fibrinolytic shutdown contributing to intravascular thrombosis and organ failure in animal models of sepsis (193-197) there have been limited studies in septic patients evaluating ROTEM lysis parameters such as ML or CLI in the progression of sepsis and correlation with the degree of organ dysfunction.

Adamzik *et al* (198) in a recent study on 56 septic patients found that ROTEM parameters particularly CLI was abnormal very early in the episode. This study compared ROTEM parameters in septic patients versus post-operative controls and concluded that CLI proved to be a more reliable biomarker of severe sepsis in critically ill adults than procalcitonin, interleukin 6, and C-reactive protein. The calculated cut-off for the lysis index was > 96.5%, resulting in a sensitivity of 84.2%, and a specificity of 94.2%, with an odds ratio of 85.3 (CI 21.7 - 334.5).

Given the evidence so far it appears that ROTEM monitoring of coagulation could have the potential to not only detect sepsis early, when it is often difficult to differentiate from a systemic inflammatory response syndrome, but also could predict the onset of multi-organ failure thereby helping determine the prognosis and classification of septic patients.

8.3. Relationship between fibrinolysis and organ failure

Using the ROTEM we investigated the relationship between fibrinolysis as assessed by ML and organ failure in a prospective cohort of septic patients based on our hypothesis that fibrinolytic activity as assessed by ML in septic patients would be inversely related to the degree of organ failure and that the prospective changes in ML would correlate with disease progression in terms of organ dysfunction.

The prospective research project was designed to:

- Study differences in ROTEM NATEM ML, Prothrombin Fragments F1+2 (PF1.2, a marker of thrombin generation) and Plasminogen Activator Inhibitor 1 (PAI-1, a fibrinolysis modulator) across groups of septic patients

with increasing degrees of organ dysfunction (assessed using Sequential Organ Failure Assessment [SOFA] scores)

- Study the correlation of ML, PF1.2 and PAI-1 with SOFA scores, at baseline and prospectively in septic patients.

For the ROTEM NATEM analysis, 3.8% citrated whole blood samples were used for the non-activated ROTEM NATEM test. These samples were re-calcified with 20µL 0.2M $CaCl_2$ and the NATEM test run for 90 minutes. In order to adjust as much as possible to physiologic conditions and to quantify the intrinsic changes in TF-triggered coagulation no activators were added to the test. This approach increased the sensitivity of the test to endogenous tissue factor.

ROTEM NATEM parameters recorded included: clotting time (CT), clot formation time (CFT), α angle, maximum clot firmness (MCF), clot lysis index at 60min (CLI60) and maximum lysis (ML).

Over the study period of 2.5 years 77 Intensive Care patients were enrolled as were 20 healthy age matched controls. Sepsis-related organ failure was characterised using the Sequential Organ Failure Assessment (SOFA) scoring system (199, 200). Twenty, 37 and 20 patients respectively were enrolled in three groups of increasing organ failure (SOFA scores 0 to 1, 2 to ≤8 and ≥9) (Table 8.3). Patients with other diseases or drugs which significantly affected coagulation were excluded. Follow-up was for 72hrs unless the patient had been discharged from ICU (12).

Our findings showed that INR and aPTT increased significantly with increasing organ failure. Even though Prothrombin Fragment values between septic populations and controls were significantly different there was no correlation with SOFA scores.

ROTEM NATEM ML showed a significant negative correlation with the severity of organ failure in septic patients (Figure 8.3) (12). Also, we found that there was an increase in ML with an associated increase in fibrinolysis with improvement in organ failure related to sepsis.

	Without organ failure (SOFA 0-1; n = 20)	Mild to moderate organ failure (SOFA 2-8; n = 37)	Severe organ failure (SOFA >8; n = 20)	P
Characteristics				
Male sex, n (%)	7 (35)	21 (56.8)	13 (65)	.26
Age (y)	66 (46.5-78.8)	65 (55.5-79.5)	68.5 (55-82)	.77
Charleston index	4 (0-6)	4 (2-6)	6 (1.5-7)	.05
APACHE II	7 (4-10.5)	19 (14-23)[a]	21 (13-28)[a]	<.001
Duration of symptoms (d)	1.7 (1.0-4.7)	3 (1.5-7)	4 (2.3-7)	.12
Source				
Community	20	20	18	
Hospital	0	0	2	
Organ failure				
SOFA score	1 (0-1)	5 (3-6.5)	10 (9-11)	<.001
Respiratory failure, n (%)	5 (25)	11 (29.7)	7 (35)	.78
Acute renal failure, n (%)	4 (20)	21 (58.3)	18 (90)	.001
Hepatic dysfunction, n (%)	0	6 (16.7)	10 (50)	.001
CNS, n (%)	1 (5)	2 (5.4)	3 (15)	.37
Thrombocytopenia, n (%)	2 (10)	10 (27.8)	12 (60)	.002
Cardiovascular, n (%)	4 (20)	26 (70.3)	19 (95)	<.001
Laboratory parameters				
WBC (10^9/L)	13.8 (11.1-19.6)	14.5 (8.8-20.2)	17.7 (11.6-23.2)	.21
CRP (mg/L)	110 (31.5-190)	170 (76.5-295)	275 (202.5-315)[a]	.01
Lactate (mmol/L)	1.2 (0.7-1.8)	1.8 (1.1-2.7)	2.6 (1.6-4.9)[a]	.002
Bilirubin (μmol/L)	8.5 (6-13)	9.5 (6.2-13.2)	20 (11-35.3)[a]	.004
Creatinine (μmol/L)	66.5 (51.5-106.7)	127 (82.5-165.5)	225 (133.8-341)[a]	<.001
Site of infection				.22
Lungs, n (%)	9 (45)	21 (56.8)	11 (55)	
Uro-genital, n (%)	7 (35)	7 (18.9)	0	
Abdominal, n (%)	2 (10)	4 (10.8)	5 (25)	
Soft tissue, n (%)	2 (10)	3 (8.1)	3 (15)	
Other (heart, ENT), n (%)	0	2 (5.4)	1 (5)	
Microorganism				
Microorganism isolated, n (%)	12 (57.9)	20 (54.1)	14 (70)	.22
Gram-positive bacteria, n (%)	7 (58.33)	5 (25)	9 (64.3)	.07
Gram-negative bacteria, n (%)	4 (33.33)	13 (65)	4 (28.57)	
Fungi, n (%)	0	2 (10)	0	
Viral, n (%)	1 (8.33)	0	1 (7.14)	
Organ supports				
RRT, n (%)	0	4 (10.8)	5 (29.4)	.02
Vasopressors, n (%)	0	20 (55.6)	16 (88.9)	<.001
Mechnical ventilation, n (%)	0	12 (34.3)	8 (40)	.008
Outcomes				
ICU LOS (d)	0 (0-0)	5 (2.02-8.75)	5.5 (2.47-7.25)	<.001
Hospital LOS (d)	6.5 (4-11)	12 (7-20)	8 (5.25-18.75)	.06
Hospital mortality, n (%)	1 (5.3)	3 (8.1)	7 (35)	.01

CNS indicates central nervous system; WBC, white blood cell; ENT, ear nose throat; RRT, renal replacement therapy; LOS, length of stay.
[a] Significantly different from group I.

Table 8.3 Baseline demographics, comorbidities and clinical characteristics of patients with sepsis (12).

Adapted from Prakash S, Verghese S, Roxby D, Dixon D, Bihari S, Bersten A. Changes in fibrinolysis and severity of organ failure in sepsis: a prospective observational study using point-of-care test--ROTEM. J Crit Care. 2015;30(2):264-70.

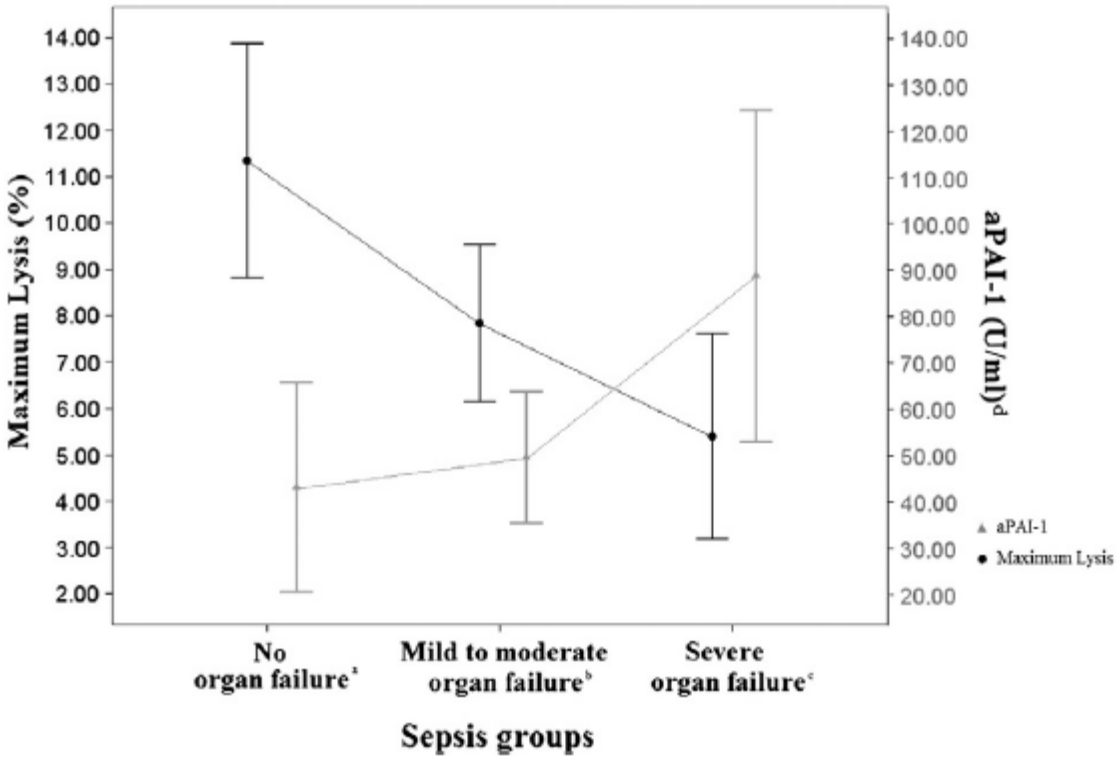

Figure 8.3. Trends in maximum lysis (ML) and plasminogen activator inhibitor (PAI) across groups of increasing severity of sepsis-related organ failure. p value for trend for ML = 0.001; p value for trend for aPAI = 0.01. Bars represent mean and 95%CI (12)

Adapted from Prakash S, Verghese S, Roxby D, Dixon D, Bihari S, Bersten A. Changes in fibrinolysis and severity of organ failure in sepsis: a prospective observational study using point-of-care test--ROTEM. J Crit Care. 2015;30(2):264-70.

8.4. Summary

ROTEM NATEM provides important information on clot strength and fibrinolysis and may reflect disease progression, however due to the limitations of our study the results currently are not suitable to base clinical decisions upon but may lead to a better understanding of sepsis related coagulopathy and clinical implications of therapeutic fibrinolysis that can be monitored at the bedside. Further studies are required based on a broader range of patients with varying degrees of sepsis related organ failure.

8.5. Copy of J Crit Care 2015; 30: 264-270

(DOI: https://doi.org/10.1016/j.jcrc.2014.10.014)

Prakash S, Verghese S, Roxby D, Dixon D, Bihari S, Bersten A. Changes in fibrinolysis and severity of organ failure in sepsis: A prospective observational study using point-of-care-test-ROTEM.

APPENDIX 1

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APPENDIX 2

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