Anaemia, Iron-Deficiency and Reducing Transfusion in Cardiac Surgery

and

A Novel Method for the Measurement of Total Haemoglobin Mass

This thesis is submitted for the degree of Doctor of Medicine (MD)

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Preface

This thesis my own work and includes methodology and results of various studies and publications, some which were performed individually and others in collaboration. The author's specific contributions to each study and publication are discussed in each chapter but outlined below:

The literature review in chapter 2 was undertaken and written entirely by the author. Chapter 3 describes a risk scoring system which was designed, and analysis performed prior to the authors involvement. The author wrote the draft manuscript for the resultant paper and coordinated the revision process and submission. He also developed the app integration for the score which was subsequently introduced by correspondence. Chapter 4 described the modification of this scoring system to a different population. The author coordinated this process and was involved in the concept, design and analysis and wrote, and coordinated revisions of the submitted paper. Other contributed to various stages and have been acknowledged specifically in the text. Chapter 5 describes a large multicentre trial for which the author was a site investigator and lead investigator for the sub-study arm exploring the effect of IV iron on total haemoglobin mass. The author was not involved in the initial broader trial design, he wrote the draft manuscript and coordinated the revision and submission process of the resulting paper for cardiac surgical patients. He also contributed to the published manuscript on the vascular patients in the trial which is included as an appendix but not explored in detail in this thesis. Chapter 6 describes various explorations of rates of anaemia and IV iron prescriptions in a number of populations. All work on this chapter was undertaken by the author. Chapter 7 describes a novel method for testing total haemoglobin mass. The concept, methodology, process, analysis and write-up of this study were all performed by the author.

This thesis is not substantially the same as any work that has already been submitted before for any degree or other qualification.

It does not exceed the prescribed word limit for the MD Degree Committee of 60,000 words

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Chapter 1: Introduction

Anaemia is an inadequacy of circulating haemoglobin (Hb), widely defined by a plasma concentration ([Hb]) of less than 130g/L in males and 120g/L in females. This results in limitation of oxygen carrying capacity of the blood and a resultant reduction in oxygen delivery to the cells which require it most. It is exceedingly common globally and has various causes including vitamin and mineral deficiencies, infection, chronic blood loss or inflammation. Anaemia can cause relative tissue hypoxia leading to organ dysfunction, damage and associated symptoms and signs. It provides a large contribution to the global disability burden and can lead to significant impacts on many quality-of-life indicators. The surgical population is predisposed to anaemia as they tend to be older, have a higher rate of concomitant disease, inflammation & nutritional deficiencies. Worsening this effect, the physiological stress of surgery places a higher demand on metabolic pathways and therefore requires an increase in tissue oxygen delivery from baseline, particularly during the post-operative period while metabolic demand is high. Accordingly, any process which limits tissue oxygen delivery during this period is frequently associated with an increase in the rate of complications such as organ dysfunction, poor tissue healing and infection. This is true of many types of surgery, but these effects are further exacerbated in cardiac surgery, where the tendency for blood loss is greater, the physiological stress often more profound, the haemodilution required for cardiopulmonary bypass (CPB) and fluid shifts from CPB and the ensuing inflammatory reaction are significant.

Anaemic patients having cardiac surgery have higher mortality rates and higher rates of almost all major complications. In addition, they tend to have longer stays in the intensive care unit (ICU) and spend more days in hospital overall, meaning their care often comes at a higher cost than others. While the most rapid and straightforward method of correcting a low circulating [Hb] is by directly increasing it using a transfusion of allogeneic red blood cells (RBCs), however the process of transfusion itself appears to be independently associated with higher rates of many complications and mortality and there is great uncertainty about transfusion trigger levels and what [Hb] should be targeted. Alternative, safe, and economical methods of transporting oxygen in the blood, such as a synthetic Hb substitute remain largely theoretical, and though alternative methods of producing RBCs *in vitro* using cord blood, monocytes, or even pluripotent stem cells are promising future techniques, they are not yet in

widespread use. This necessarily shifts the focus towards *in vivo* strategies to reduce anaemia by reducing blood loss and by stimulating existing haematopoietic processes to increase circulating red cell mass and therefore optimise tissue oxygen delivery, thereby reducing the need for allogeneic transfusion. It follows that this should result in an improvement in surgical outcomes, provided the measures taken do not themselves involve excessive risk.

Patient Blood Management (PBM) is an increasingly important concept, which has been particularly useful in the context of the peri-operative period. It involves a raft of measures that include pre-operative testing for anaemia and coagulopathy, pre-optimisation, intra-operative measures to minimise blood loss and peri-operative measures to conserve blood and minimise transfusion. PBM programs have been increasing in popularity and have been instituted at local, regional and, increasingly, national levels.

Intra-operative blood conservation strategies to reduce blood loss during cardiac surgery are diverse and include methods of reducing blood dilution during the CPB phase of surgery, changes in technique, topical haemostatic methods, fibrinolytic medications for minimising bleeding and RBC conservation using cell-salvage devices. While many of these methods are highly effective, most are associated with a significant cost and resource allocation which makes them difficult to justify their routine use.

It becomes important, therefore, to stratify risk and use these resources only in those considered high risk for transfusion. While cardiac surgery can be associated with unanticipated major bleeding and transfusion, many patient- and surgical factors can be identified that predict transfusion needs. The ability to stratify transfusion requirement risk allows for appropriate allocation of resource-intensive blood conservation strategies to those with the most to gain from the process. By utilising PBM strategies, it is possible to reduce transfusion requirements and improve surgical outcomes. These strategies are frequently costly, and to direct these strategies appropriately is important to limit the resource burden they place on already strained healthcare systems. There are several factors which allow us to predict the likelihood of various surgical patients requiring transfusions and target those at high risk to limit this need. Scoring systems have previously been described that provide an estimation of transfusion risk in cardiac surgery, but they have been either inadequately accurate, not easily generalisable, or difficult to practically calculate and therefore have been under-utilised. Our group therefore designed, externally validated, and published a new scoring system called the ACTA- PORT score based on a UK-wide audit by the Association of Cardiothoracic Anaesthetists (ACTA). It is a relatively simple, accurate, widely applicable, and easily calculated using online or app-based tools, which were developed in conjunction

with a globally available medical calculator application. The development of this scoring system is further discussed in Chapter 3. In order to broaden the applicability of this scoring system, it was subsequently modified and calibrated to Australian and New Zealand populations by way of data from a national database to form an antipodean equivalent, the AntiPORT score. Discussion of this process is contained in chapter 4.

There are many causes for anaemia. In the pre-operative population in higher-income parts of the world, the most common aetiology is absolute or functional iron deficiency, for which there are many underlying causes.

Iron deficiency is not a simple pathological process and can be challenging to correctly define and therefore diagnose. There are many predisposing factors: from impaired intake, to chronic blood loss, inflammation, infection, cancer, pregnancy, or even heavy exercise. While absolute iron deficiency is relatively easy concept to understand and diagnose based on a reduced serum ferritin, iron restriction can present with a normal [ferritin] but an inability to utilise the available iron stores for haematopoiesis, causing a reduction in [Hb] and impaired oxygen delivery.

The classic treatment for iron deficiency is oral iron supplementation. This, however, is hampered by several shortcomings. Firstly, replacing iron via the oral route is slow, taking weeks to months to restore depleted iron reserves. Secondly, oral iron is often poorly absorbed, which causes a high incidence of gastrointestinal side effects, which can lead to, and is then compounded by, a significant effect on compliance. Finally, in certain patient groups, such as those with anaemia of chronic disease (ACD), it has been shown that hepcidin, a protein produced by the liver in response to systemic inflammation, further impairs the ability of the gut to absorb iron, amplifying the aforementioned side-effects.

To avoid the limitations of oral iron replacement, experimentation with intravenous preparations began in the 1930's when the first infusions were described in the literature and since become safer and more widespread. The creation, and subsequent improvement of iron-carbohydrate complexes has allowed the increasingly safe infusion of iron, which given in its free state is highly toxic. Replacement of iron stores using older intravenous preparations was a laborious process, involving a 4-hour infusion, which carried a significant risk of adverse reactions including anaphylaxis. (Auerbach and Rodgers 2007) Newer IV Iron-carbohydrate preparations have been released over the last few decades which generally appear to have minimal risk of serious adverse side effects and can be given rapidly in hospital or outpatient settings. In the pre-operative setting, particularly in cardiovascular surgery with its relatively urgent nature, there is frequently little time to optimise a patient in the time

between referral for surgery and the surgery itself. For this reason, IV preparations and their ability to rapidly restore iron stores, could theoretically help treat anaemia in the short time available, where oral treatment is generally inadequate. In order to test this notion, the CAVIAR-UK study was formed. It was a multicentre UK study which aimed to establish if it was feasible to institute an iron infusion service for patients in the pre-operative setting, and then to explore whether the iron replacement was able to have a meaningful effect on anaemia and on patient outcomes after surgery. The author was a one of a larger group that undertook the investigation and then coordinated the writing and publication of the ensuing BJA paper. This study showed it was feasible to introduce pre-operative anaemia testing and treatment within the current NHS structure in cardiac surgery, but this was not mirrored in vascular surgery. In cardiac surgery it showed that by giving iron-deficient anaemic patients iron more than 10 days pre-operatively, it was possible to increase [Hb] meaningfully, and this effect was also seen to a lesser extent in vascular surgery patients. Despite the significant effect on [Hb], there was no statistically significant effect on outcomes, although the study was not powered to detect these. A detailed description of the CAVIAR-UK trial is provided in chapter 5.

The CAVIAR-UK trial additionally acted as a pilot study for a subsequent international randomised control trial (RCT) called the intravenous Iron for the Treatment of Anaemia before Cardiac Surgery trial (ITACS), which was designed to have adequate power to detect a treatment effect for pre-operative IV iron before cardiac surgery on a composite measure of morbidity and mortality. This study is still recruiting patients and the methodology and results are not included in this thesis, although the author continues to be involved as the primary investigator for this RCT at St Vincent's Hospital in Sydney.

Although the presence of anaemia is defined by a reduction in plasma [Hb] the measurement of [Hb] is quite unreliable. [Hb] as a sole indicator of the pathological process underlying anaemia, namely failure of adequate oxygen-carriage capacity of the blood, is a crude measure. While in healthy, normovolaemic subjects it can be a good indicator of oxygen carrying capacity, it is limited by inaccuracy in those with altered volume states, such as those with cardiac, hepatic, or renal impairment. This failure to adjust for fluid status of the tested subject can change the result significantly and cause missed- or misdiagnoses and potentially inappropriate treatment.

Measuring the total amount of circulating Hb should be a more reliable measure and should be a better indicator of oxygen delivery capacity. It is independent of plasma volume and therefore is also better placed to detect treatment effects from blood manipulation strategies,

as generally it remains quite stable. There is increasing evidence from sports medicine for this notion. The process of measuring total Hb mass (tHb-mass) or total blood volume (TBV) is historically quite challenging, requiring the use of radioisotope techniques to label and count red cells. Other techniques include inaccurate intravenous blood dilution methods, and impractical methods including the prolonged rebreathing of gas mixtures in a respiratory laboratory. Relatively recently, a new technique was developed by sports physiologists, Walter Schmidt & Nicole Prommer (University of Bayreuth, Germany) to more quickly and easily measure tHb-mass using a simple spirometer and a small amount of inhaled carbon monoxide (CO) and measuring the resultant known increase in carboxyhaemoglobin (%COHb). It was a modification of a classic method for tHb-mass calculation and retains its accuracy with a significantly quicker and more straight-forward technique. It was initially developed in conjunction with the World Anti-Doping Agency (WADA) to aid detection of covert blood manipulation such as "blood doping" and erythropoietin (EPO) use in competitive sport, but has also been shown to be useful in assessing the effects of legal blood manipulation strategies such as altitude training. (PROMMER et al. 2008) This technique had also shown a significant treatment effect on tHb-mass from IV iron in iron-deficient athletes, which was undetectable with [Hb] alone.

Some early work had been done using this technique in the hospital laboratory setting at UCLH (London, UK), so a sub-study was designed for the CAVIAR-UK trial to explore whether it was possible to detect such a treatment effect from intravenous iron in a small group of pre-operative patients at the Papworth site, which the author oversaw. The hope was that despite the small size of the group, the new testing technique may be able to detect a change in total Hb mass that may not be significant if using [Hb] as a measure. This would have implications for future studies in anaemia treatment allowing for smaller to studies to potentially detect a treatment effect.

The CAVIAR sub-study faced several technical and logistical challenges and ultimately failed to recruit enough patients by the time of the completion of recruitment for the parent trial, CAVIAR-UK. There were several factors involved; including the timeframe required to set up the testing technique, the difficulty in recruiting patients into a study requiring the inhalation of pure carbon monoxide and the imposition required to undergo the testing. As a result of these difficulties, the author designed a subsequent study, the PREFIX trial, which aimed to use a smaller group of similar patients to the CAVIAR-UK trial, and to test tHb-mass using the Schmidt and Prommer technique to test whether a detectable rise in tHb-mass was evident in patients receiving IV Iron, where a change in [Hb] may not be seen. If a

statistically significant rise was seen in such a small group of patients, it could have shown it to be a superior measure of effectiveness of pre-operative IV iron replacement. This study was approved by the research ethics department at St Vincent's Hospital (Sydney, Australia) where it was to be conducted but again has thus far not recruited enough patients for analysis. The reasons for this appear to be a combination of an inadequate supply of anaemic patients, competing recruitment from other trials such as ITACS, the complexity of the testing technique and the perceived risk or imposition of the tHb-mass testing technique to potential study recruits.

The observed, and anecdotally reported reduction in numbers of anaemic patients prompted further investigation into any changes in the rate of anaemia in the elective cardiac surgical population. Interrogation of the local database revealed that over a 5-year period to September 2019, the rate of anaemic patients had dropped significantly, from 27% in 2015-16 to just 14% in 2018-19. Furthermore, the proportion of these anaemic patients with iron deficiency was very small and those presenting had largely received iron already or were too close to the surgical date to meet the eligibility criteria for the studies. One theory amongst the investigators was that the rapidly increasing use of IV iron in primary care over the preceding years may have contributed to an improvement in iron status, and thus anaemia rates. To further explore this, the results of the local audit were compared to the national ANZCTS database, to establish whether there might be a larger trend in anaemia rates for those presenting for elective cardiac surgery. Additionally, data was obtained for Iron prescription trends from the Australian national prescribing service (PBS). Similar data from the UK was sought, though is not centrally recorded and was therefore not able to be interrogated, however limited data were obtained from the prescribing reports of the Medicare and Medicaid programs in the USA and included to provide a global comparison. The results of this audit are described in chapter 6 and demonstrate that there has been a significant increase in IV Iron prescription in those regions and, at least in the Australian population, a concomitant decrease in anaemia rates in our target population. Similar trends in IV iron prescription are seen in the US data. A more detailed description of this exploration is contained in chapter 6.

The technical challenges associated with the setting up and undertaking measurement of tHb-mass in the hospital setting led the author to consider alternative measurement methods. A component of routine lung function testing in standard hospital respiratory laboratories is a measure of gas exchange known as diffusing capacity of the lungs to carbon monoxide (DLCO), which also involves inhaling a diluted mixture of carbon monoxide and measuring

the rate at which it is absorbed. By modifying this process and adding a measurement of plasma %COHb before and after the technique was complete, it seemed reasonable that an estimation of tHb-mass and plasma volume could be obtained. If suitably accurate, this test could be easily established in any hospital with a respiratory function lab and with minimal training and no additional equipment or gas-mixtures. This could provide a very useful method of testing the effect of blood manipulation measures and could be useful in appropriate allocation of PBM resources to those who will benefit most from them. Measuring total-Hb mass also allows calculation of plasma volume, which is notoriously challenging value to measure directly. This could potentially have far broader applications in the hospital setting in various patient groups beyond the anaemic pre-operative patients that this thesis focuses on. The description of the development and testing of this modified DLCO process (MoDLCO) and the preceding audit of DLCO testing and resultant change in %COHb (ACADEMY audit) is contained in chapter 7.

Chapter 2 – General review of literature in anaemia, iron deficiency & iron replacement therapy, with a focus on cardiac surgery.

Anaemia

Oxygen is crucial to cellular metabolic pathways in order to resynthesise adenosine triphosphate (ATP), which provides energy to many crucial processes in the body and is commonly referred to as the "molecular unit of intracellular energy currency". For oxygen to be utilised in the mitochondria via the electron transfer chain, it must be delivered to cells. This delivery is the product of several factors including oxygen transfer by the lungs to the blood, carriage in the blood and delivery of that blood to the tissues via cardiac output. If delivery is compromised, then cellular processes begin to fail which results in organ dysfunction. With the normal human cardiac output of 4-8L/min, dissolved oxygen in the blood would only provide approximately 1.5% of the required total. Evolution has provided an elegant solution to this in the form of haemoglobin, a metalloprotein which provides a far more efficient transport medium for oxygen to the cells. The binding of oxygen to Hb allows for approximately 200mL of oxygen to be carried in each litre of blood(Dunn, Mythen, and Grocott 2016). A reduction in [Hb] in the blood can be compensated for by increasing either cardiac output or oxygen extraction, although these compensatory mechanisms have limits. At rest, and with a normal circulating volume, it is possible for a healthy person to compensate for a [Hb] as low as 50g/L, though beyond this, or in states of increased oxygen consumption, organ hypoxia becomes evident (Weiskopf et al. 1998). As even the most basic of activities of daily living increase oxygen demand, anaemia tends to become symptomatic in most healthy people at a [Hb] closer to 80-90g/L and in people with co-morbidities, this threshold is higher.

The globally accepted definition of Anaemia is currently a [Hb] of less than 120g/L in females and less than 130g/L in males, as endorsed by the World Health Organisation (WHO).

Prevalence of Anaemia

The prevalence of anaemia in the general population of higher-income countries is generally less than 10% (L. T. Goodnough and Schrier 2014) although there is still significant

variability within these populations with higher rates in women, minority ethnic groups (Le 2016) and indigenous populations(Khambalia, Aimone, and Zlotkin 2011). The WHO estimates 42% of children under 5 are anaemic globally(World Health Organization 2021a). It is also more common in women, particularly in pregnancy or in women of child-bearing age where it affects approximately a third of the this population globally(World Health Organization 2021b). The prevalence of anaemia reliably increases with age from around the age of 50 to over 20% in those older than 85 and can be as high as 48-63% in nursing home residents (Patel 2008). In the UK, as many as half of the elderly population is anaemic (Gaskell et al. 2008).

There is enormous disparity in the frequency of anaemia throughout the world, with the overall global prevalence estimated at 33% in 2010 (N. J. Kassebaum et al. 2019), largely due to the high rates in young children, women and the elderly. The high global prevalence is significantly increased by the very high rates of anaemia found in low- and middle-income countries due, in part, to the much higher rates of nutritional deficiencies. (Chaparro and Suchdev 2019) Chronic infections such as malaria or HIV and parasitic infections are also major contributing factors and cause as much as half of the cases of anaemia in some parts of the globe. (Mason et al. 2013) Higher rates of inherited haemoglobinopathies such as thalassaemia and sickle-cell disease also contribute to this disparity.(McLean et al. 2009)

Causes of anaemia

Anaemia is generally caused by three primary mechanisms: blood loss, impaired erythropoiesis, or RBC destruction, and often a combination of these. There are several well understood causes of increased RBC destruction such as haemolytic anaemias or hypersplenism although these remain rare in general. Blood loss and impaired erythrogenesis are the most common causes of anaemia and these can be further broken down into contributing factors,

A number of specific deficiencies can cause anaemia, notably: iron, folate, vitamin B12, vitamin A and copper. While all of these have the common theme of a low circulating concentration of various circulating molecules, the cause of this varies enormously. In most cases, vitamin B12-deficiency is caused by a reduced absorption due to a failure of intrinsic factor (IF), copper-deficiency is also commonly malabsorptive and is most commonly seen in patients who have has gastric bypass surgery or those with coeliac disease(Prodan et al. 2009), whereas reduced plasma [folate] is primarily a result of dietary deficiency and iron deficiency

is most commonly due to increased iron-loss (Carmel 2008). Many of these nutritional causes of anaemia are more common in elderly populations (Carmel 2008)(Andrès et al. 2008) and as discussed earlier, all are more frequent in low- and middle-income countries.(McLean et al. 2009)

In addition to the physiological stages, chronic infections and nutritional deficiencies discussed, there are a number of other pathological conditions that can increase the incidence of anaemia. Any pathological process leading to increased blood loss such as GI and gynaecological losses can lead quickly to the anaemic state, disorders of bone marrow function and many malignancies are also frequently associated with anaemia. As discussed, renal disease is well known to cause anaemia due to the contribution of the kidney to the haematopoietic process, particularly through underproduction of the protein erythropoietin (EPO).

Physiological consequences of Anaemia

The effect of anaemia on exercise capacity and symptomology can reduce quality of life, anaemia and can also have a significant effect on overall performance(Gardner et al. 1977). This is also well-shown in elderly populations where even mild anaemia can effect indicators of performance and mobility (Penninx et al. 2003; Chaves et al. 2002). Anaemia in children can impact their intellectual performance (Iqbal et al. 2015).

There are a number of physiological states in which anaemia has been well shown to increase mortality, for instance there is significant evidence that anaemia increases overall mortality in the elderly(Dong et al. 2008)(Chaves et al. 2004)(Izaks, Westendorp, and Knook 1999)(L. T. Goodnough and Schrier 2014) and an increase in maternal mortality in pregnant women with severe anaemia (Daru et al. 2018). The effect does not seem to be limited to older people, and may increase mortality in children, with one meta-analysis of health outcomes children from 6 African nations suggesting that a 10g/L increase in [Hb] could reduce risk of death by 24% (Scott et al. 2014) In the clinical setting, this effect may be even more significant, with one large study of over 400,000 hospitalised patients demonstrating an overall increase in mortality with an odds ratio of 3.28 (95% CI 2.90-3.72) in those with severe anaemia(Colleen G. Koch et al. 2013). The effect has also been demonstrated in a number of pathological sub-groups, such as cardiac failure patients, where anaemia is associated with increased mortality (McClellan et al. 2002) (Ezekowitz, McAlister, and Armstrong 2003)(Felker et al. 2004). A similar increase in mortality is seen in anaemic

patients presenting with acute coronary events (Wu et al. 2001)(Al Falluji et al. 2002) including those undergoing percutaneous coronary interventions (PCI) (Nikolsky et al. 2004) In chronic renal-failure patients, anaemia is very common, and again, associated with higher mortality (Parfrey, Foley, and N 1999), increasing with the severity of the anaemia (Foley et al. 1996). This effect is magnified in those with concomitant heart disease (Weiner et al. 2005), which it may itself contribute to in the form of left ventricular hypertrophy (LVH) (Astor et al. 2004). Survival has also been shown to be reduced in the presence of anaemia in a variety of cancer patients (Caro et al. 2001)(Dubray et al. 1996) with a reduction in treatment effect of chemoradiation (Robnett et al. 2002)(Berardi et al. 2006)
In addition to the association with increased mortality, anaemia can have specific effects on several organ systems.

Effects of Anaemia on organ systems

Cardiovascular System

Anaemia has significant effects on the cardiovascular system and results in a compensatory increase in cardiac output via heart rate and stroke volume (Varat, Adolph, and Fowler 1972) and decreased oxygen-Hb affinity via 2,3-DPG mediated changes(Macdonald 1977) . This increase in baseline cardiac output and oxygen delivery can lead to a reduced physiological reserve and thus a reduced exercise capacity in both healthy and pathological states(Ebner et al. 2016; Ferrari et al. 2015). This can be directly seen as a reduction in physical performance, especially in the elderly (Penninx et al. 2003). It may also result in cardiomyopathy and resultant heart failure (Hegde, Rich, and Gayomali 2006) and can increase infarct-size in patients with acute coronary syndrome (Maroko and Braunwald 1976). Cardiac failure patients are particularly affected by anaemia due to various contributing factors. The incidence of anaemia in these patients is around 30% in total but up to 50% in those that are hospitalised and as much as 80% in those with those with NYHA IV symptoms (Silverberg et al. 2000). Presence of anaemia in heart failure patients is associated with worse outcomes (Felker et al. 2004)

Neurological system

Anaemia can have a significant effect on the neurological system. This effect can be seen by in impaired cognitive and behavioural development in anaemic children (Jáuregui-Lobera 2014)(Larson, Phiri, and Pasricha 2017) and is also clear in other groups. Anaemia is associated with accelerated cognitive decline in the middle-aged and elderly (Qin et al.

2019)(Schneider et al. 2016) and an increase risk of falls (Penninx et al. 2005). In the context of traumatic brain injury, anaemia is well known to be associated with worse neurological outcomes in head injury.(Hare et al. 2008)(Stocchetti et al. 2015), and this effect is not reliably reversed by transfusion(Menon and Ercole 2017). This notion of the outcome effects of anaemia not being corrected by correcting [Hb] with transfusion is particularly relevant and will be explored later.

Immune system

Anaemia caused by iron-deficiency has significant effects on immune function in multiple ways including effects on t-lymphocytes, natural killer (NK) cells (Santos and Falcao 1990)and in children has been shown to reduce phagocytosis(Ekiz et al. 2005) and lower levels of interleukin-6, IgG (Hassan et al. 2016) and has also been shown to impair immunity in older adults.(Ahluwalia et al. 2004) The relative roles of anaemia, and iron-deficiency in this effect are difficult to separate.

Renal system

While chronic renal failure is known to be associated with anaemia due to the kidney's role in haematopoiesis, anaemia itself appears to contribute to ongoing kidney damage in these patients. The presence of anaemia may also increase the rate of renal decline to end-stage in patients with chronic kidney disease.(Mohanram et al. 2004) which has been postulated to be a result of low-grade renal ischaemia or a result of anaemia-exacerbated inflammation. Anaemia is also associated with an increased rate of acute kidney injury (AKI) in both the pre- and post-operative surgical population(Fowler et al. 2015; Arai et al. 2015; Gorla et al. 2017)

Surgical patients & Anaemia

It was estimated in 2008 that globally each year, 234.2 million surgical procedures are performed (Weiser et al. 2008). While anaemia is common in general, it is even more common amongst surgical candidates, such as in the USA where the NSQUIP audit demonstrated a rate of 30% (Musallam et al. 2011) in non-cardiac surgical candidates. A similar study in Europe demonstrated a similar prevalence of 29%, (Baron et al. 2014).

Effects of anaemia on surgical mortality

Anaemia has reliably been shown to be associated with an increase in post-operative mortality, both generally(Beattie et al. 2009)(J. Carson et al. 1988)(Baron et al. 2014) and in more specific groups such as patients with hip fractures (Gruson et al. 2002), colorectal (Leichtle et al. 2011) and vascular surgery (Gupta et al. 2013).

This effect has been shown to be independent of the risks from transfusion by a study of operative patients that refused transfusion on religious grounds. Amongst this group, those with anaemia had significantly higher mortality, worsening with increased severity and worse in the presence of cardiovascular disease (J. L. Carson et al. 1996)

Even the presence of mild anaemia is associated with increased risk of death in major non-cardiac surgery, with the NSQIP data review showing an odds ratio for mortality of 1.41 (95% CI 1.30-1.53) in these patients compared to 1.44 (1.29-1.60) in those with moderate-severe anaemia(Musallam et al. 2011)

Effects of anaemia on surgical morbidity

In addition to effects on mortality-outcomes, anaemia is associated with increased in other markers of morbidity in non-cardiac surgery. Anaemia is frequently associated with worse cardiovascular outcomes such as increased rates of post-operative cardiac events in major surgery (Wu et al. 2007) and increased evidence of myocardial ischaemia post-prostatectomy (Hogue Jr., Goodnough, and Monk 1998). These effects appear to worsen with increasing severity of anaemia.

The effect of anaemia has been well studied in hip surgery patients and although results are variable, anaemia has been shown to be associated with a variety of worse outcomes including infection rate (Myers, Grady, and Dolan 2004), length of hospital stay (Gruson et al. 2002)(Halm et al. 2004), quality of life (Conlon et al. 2008) and functional ability (Hagino et al. 2009)

A 2015 meta-analysis of 24 observational studies involving almost one million surgical patients concluded that anaemia was associated not only with increased mortality (OR 2.90[2.30-3.68]), but also AKI (OR 3.75[2.95-4.76]) and infection (1.93[1.17-3.18]) (Fowler et al. 2015)

Anaemia in cardiac surgery

Given that cardiac surgery patients are frequently and increasingly older (Friedrich et al. 2009) and have a relatively high incidence of cardiac failure and renal disease, it is unsurprising that the incidence of anaemia in the patients is also relatively high. Cardiac

surgery patients in the UK were studied in the national ACTA audit undertaken between 2010 and 2012. (Klein, Collier, Brae, Evans, Hallward, Fletcher, and Richards 2016) It demonstrated an overall prevalence of 31% in pre-operative patients with a significant range between centres of 23 to 45%. One earlier UK single centre audit from 2009 showed an anaemia rate of 54%(M. Hung et al. 2011). Overall, though, national data appears to be relatively consistent throughout higher-income countries. A global study published in 2007 (Kulier et al. 2007) of 70 centres across 17 countries, showed an overall prevalence of 29.7% in pre-operative CABG patients. These were similar to figures from Canada (Karkouti, Wijeysundera, and Beattie 2008) where the overall prevalence was 26% and in New Zealand(Kim et al. 2015) where the prevalence was 28.1%.

Effects of anaemia on mortality in cardiac surgery

Baseline mortality risk is relatively high in cardiac surgery compared to many other surgical specialities, on account of both the relatively high-risk patient cohort and the complexity and risks of the surgery itself. Studies have shown the overall mortality rate is generally around 3% (Siregar et al. 2013)(Rutten and Grobbee 2001)(Mazzeffi et al. 2014)(Clayton et al. 2005) both short term(Hari Padmanabhan, Siau, et al. 2019), which is similar to other higher risk surgical subspecialities such as colorectal surgery(Alves et al. 2005). As with other surgical specialties, mortality risk increases in certain patient groups, in certain comorbidities, and in certain types of surgery.

Patients presenting for cardiac surgery with anaemia appear to have a higher mortality rate than their non-anaemic counterparts(A. A. Klein, Collier, Brar, Evans, Hallward, Fletcher, Richards, et al. 2016)(M. Hung et al. 2011)(Kulier et al. 2007)(Karkouti, Wijeysundera, and Beattie 2008). This effect is apparent on both in-hospital mortality (Cladellas et al. 2006) and longer-term mortality (Van Straten et al. 2013)

Effects of anaemia on morbidity in cardiac surgery

In addition to mortality increases, anaemia is associated with higher surgical morbidity such as major adverse cardiac (Cladellas et al. 2006) and non-cardiac events (Kulier et al. 2007), stroke (M. L. Williams et al. 2013), increased length of ICU stay (M. Hung et al. 2011)(Marco Ranucci et al. 2012), acute kidney injury (AKI)(De Santo et al. 2009)(Karkouti, Wijeysundera, and Beattie 2008) prolonged time to discharge(A. A. Klein, Collier, Brar, Evans, Hallward, Fletcher, Richards, et al. 2016), and unsurprisingly increased transfusion requirements (Marco Ranucci et al. 2012)(M. Hung et al. 2011)(Boening et al. 2011). Iron-deficiency may

itself be associated with poorer outcomes after cardiac surgery as has been suggested in one trial, although further evidence is needed to clarify this.(L F Miles et al. 2017)

While the presence of anaemia according to the WHO definition is binary, the disease of anaemia has an inherent spectrum of severity, which correlates to its physiological effects. As such, the severity of the anaemic state also contributes to an observed worsening of post-operative outcomes and this effect appears to persist on long-term follow-up of surgical patients (Padmanabhan et al. 2019), with one large Dutch study (Van Straten et al. 2009) demonstrating that the significant impact of pre-operative anaemia on mortality can be demonstrated out to 9 years post CABG surgery. This effect was significantly worse in those with severe anaemia, for example, those with an [Hb] considered 'very-low" (<110g/L in females and <120g/L in males) had a 9-yr survival rate of 37.6% vs 56% in those with milder anaemia (110-120g/L in females and 120-130g/L in males). This is compared to 9-yr survival of 74.7% and 84.3% in those with normal or high-normal [Hb] respectively. A similar trend has been observed in an assessment of 3-year post-cardiac surgery survival in German university hospital, where mild anaemia demonstrated a hazard ratio of 1.441 (95% CI: 1.201-1.728) whereas this rose to 1.805 (95% C: 1.336-2.440) in those with severe anaemia. (von Heymann et al. 2016)

While those with severe disease are more likely to do poorly, even patients with low-normal [Hb], not considered anaemic by conventional definitions, appear to be affected. For example, females in the 120-129g/L range were shown to have both a higher transfusion rate and a longer hospital stay after cardiac surgery (Blaudszun et al. 2018)

Type of anaemia may also contribute to outcomes with a recent paper suggesting that macrocytic anaemia is associated with significantly worse outcomes than normocytic and microcytic variants. (Dai et al. 2018) Interestingly, when Hung et al investigated cardiac surgical outcomes in 165 anaemic patients, they found that plasma [hepcidin] was the only haematological parameter that was independently associated with outcome, highlighting both its physiological importance and potential value as a target for future research(Matthew Hung et al. 2015).

Iron deficiency is independently associated with poor outcomes in cardiac surgery also as seen in a recent prospective trial (Rössler et al. 2020) and there is increasing interest in the non-anaemic iron-deficient population as a group to target treatment aimed at improving outcomes. We can expect more research to be published in this sphere over the next decade.

Anaemia: [Hb] vs total Hb mass

Although anaemia would best be described as a limitation in the ability to deliver oxygen to tissues, it is historically defined only in terms of [Hb]. Widely accepted definitions of normal values for [Hb] of >120g/L in females and >130g/L have evolved from population studies and are relatively arbitrary. There are many common patient factors that may modify the 'normal' [Hb] such as BMI, fitness, alcohol and smoking habits, and even stress levels (J P Isbister 1997)

Measuring [Hb] is very simple, cheap and in most cases provides a reasonable estimate of oxygen carriage capacity of the blood. The fact that it is the measure used to define anaemia by the WHO is indicative of its widespread acceptance. Given that [Hb] is essentially a measure of total Hb-mass divided by total blood volume, the plasma component (PV) of the blood volume, as the denominator plays a significant role. Changes in PV can have a dramatic effect on [Hb] without any real change in the total oxygen carriage capacity of the blood. For this reason, various techniques to measure total Hb-mass have been developed. While [Hb] may correlate well with total Hb mass in healthy subjects, it can be very misleading in other groups such as those with liver or heart failure, where significant changes in circulating volume can exist. (James M. Otto et al. 2017) As a result, a patient with a low [Hb] may be diagnosed as being anaemic where the actual pathology is an expanded plasma volume, thus diluting the circulating Hb, rather than a genuinely low red blood cell mass (RBCM) or total-Hb mass. This effect has been observed in a clinical setting with heart failure patients, for example in ones study of 19 out of 32 heart failure patients diagnosed with anaemia, only 4 were shown to actually have a low RBCM and 9 were actually considered to have excessively high RBCM (Miller and Mullan 2015). Another study of 99 patients showed that while [Hb] was frequently low in heart failure patients, it was the only value of blood cell count that was demonstrably low and red cell volumes remained normal (Adlbrecht et al. 2008) causing a "pseudo-anaemia". The converse is also true and may result in false negative tests for anaemia in those with constricted blood volume.

As we can see, while [Hb] provides some information about a subject's capacity for oxygen delivery, reliance on it as a sole indicator of disease severity has significant limitations.

The best-known method to assess the body's ability to deliver oxygen to tissues is VO2 max, or maximal oxygen uptake. It correlates well with cardiovascular fitness and is used frequently in sports medicine and has also been used to predict outcomes in certain surgical procedures. Increasing [Hb] has been shown to increase VO2 max in healthy patients(Ekblom, Goldbarg, and Gullbring 1972)(Buick et al. 1980)(M. H. Williams et al. 1981)(Brien and Simon 1987)

with an estimated increase of 1% for every 3g/L rise in [Hb] (Gledhill, Warburton, and Jamnik 1999). Similarly, while there is clearly some correlation between [Hb] and exercise capacity(Woodson 1984; Calbet et al. 2006) in healthy volunteers, this correlation in surgical candidates has been shown to be a relatively weak association(James M Otto et al. 2013). Improving VO2 max and exercise capacity has been achievable using autologous blood transfusion, (Solheim et al. 2019), which helped the rise of "blood doping" in competitive sport. The same effect has been seen in stable haematology patients with allogenic blood products (Wright et al. 2014).

In the context of cardiac surgery, where the presence of heart failure-related fluid disturbances is very common, it becomes clear that [Hb] itself has significant limitations as an indicator of the true oxygen delivery status and is perhaps not the ideal measure to define anaemia at all.

In addition to physiological reasons for the limitations of [Hb] as a marker of oxygen delivery, measurement of [Hb] itself is prone to error. [Hb] measurement can be performed using various techniques. Laboratory-based testing processes are more accurate, but slower and involve significantly more processing. Increasingly 'bedside' techniques are being utilised which can range from smaller blood gas analysers in an operating theatre or ICU setting, to finger-prick portable analysers such as Hemocue® and the emerging category of non-invasive real-time monitors using pulse oximetry techniques. Accuracy of these methods is variable, with a trend towards inferior accuracy with increased convenience.

It was demonstrated by Heinicke and colleagues that in elite endurance athletes, while total-Hb mass was 35% higher, the commensurate increase in PV meant that [Hb] was not significantly higher, suggesting that [Hb] may be a poor indicator of oxygen delivery(Heinicke et al. 2001)

Compared to [Hb], total Hb-mass testing has been shown to correlate much better with aerobic capacity on CPET testing and should provide a far more accurate estimate or oxygen carrying capacity (J M Otto et al. 2017).

As JP Isbister suggests, methods for measuring total red cell mass or total Hb mass are more laborious than measuring [Hb] and as such are generally not practical in a clinical setting(J. P. Isbister 2015). These techniques have progressed significantly however and in chapter 7, an exploration of the development of these techniques is described further. Following from this is a description of the development of a new total Hb-mass testing technique that may constitute a significant step in the slow march towards a practical, and easily established method of testing total Hb mass.

Iron Deficiency

Iron is utilised in a vast array of physiological processes and is crucial to many body systems in humans and indeed most species. It is best known for its crucial role in haemoglobin synthesis for erythropoiesis but is also required in cell division, gene expression and the synthesis of DNA in many cell lines (Furuyama and Kaneko 2007). It is fundamental to the function of all aerobic cells via the electron transport chain and in addition to haemoglobin and myoglobin synthesis is required in production of essential enzymes such as cytochromes, catalases, peroxidases, guanylate cyclase and NO-synthase. (Ponka 1999) Systemic iron homeostasis is a highly regulated balance between gut absorption and iron-loss involving proteins to facilitate transfer such as ferroportin and transferrin, and the regulatory proteins hepcidin, hephaestin and ceruloplasmin(Zhou and Tan 2017). Intracellular iron homeostasis is regulated by the iron-regulatory protein/iron responsive element (IRE/IRP) system which effects gene expression via mRNA effects. Iron deficiency (ID), as with most molecular deficiencies is caused by either inadequate intake, failure of absorption, increased loss, or a combination of these. It is estimated that over 2 billion people globally are iron-deficient with approximately 12% of the total population and 20% of females suffering from anaemia caused by iron-deficiency (N. J. et al. Kassebaum 2019). In higher income countries, the prevalence is lower, but remains significant.

Pathophysiology of Iron Deficiency

In the advanced stages of the disease, iron deficiency leads to anaemia which is associated with the detrimental physiological effects outlined earlier in this chapter, although even in the pre-anaemic stages it has widespread physiological sequelae demonstrating iron's crucial role in homeostasis, beyond its importance to haemoglobin production.

Non- (or pre-) anaemic iron deficiency (NAID) is estimated to affect 1-2 billion people globally. As iron-deficiency and anaemia are often studied together, it is difficult to separate the pathological processes caused by each, although it is clear there are significant effects on cognition, fatigue and mental health(Greig et al. 2010) with ID, even in the absence of anaemia. There are demonstrable effects on neural function (Beard and Connor 2003), neurotransmitter production and function (Beard 2001) and significant impacts on brain and spinal cord development (Dobbing 2013).

Iron plays a vital role in many aspects of Immune functions such as lymphocyte and natural killer cell function(Santos and Falcao 1990; Kemp 1993), thymus function (Kuvibidila et al.

2001)production of the microbiocidal hypochloric acid by peroxidases (Hampton, Kettle, and Winterbourn 1998). Iron deficiency in mononuclear cells seems to directly affect the quality of the cell-mediated immune response(C. Muñoz et al. 2007)

Athletic function is closely linked to anaemia, but endurance performance seems to be independently linked to iron status, independent of [Hb](Beard 2001)

In heart failure (HF) patients, iron deficiency is very common with almost half of these patients being iron deficient, but 30% of those who are non-anaemic demonstrating absolute iron-deficiency, and 22% having functional iron deficiency(Okonko et al. 2011b). Another study of 546 HF patients from Poland demonstrated a prevalence of ID of 37±4% (Jankowska et al. 2011) Myocyte iron-deficiency (independently of anaemia) in HF patients has been shown to be associated with more extensive coronary disease and reduced enzymatic function in the Krebs cycle and reduction in reactive oxygen species protective enzymes, which may lead to exacerbation of mitochondrial dysfunction (Melenovsky et al. 2017). This has been shown to translate to function and outcomes with studies demonstrating a tendency to lower aerobic capacity and increased risk of death(Okonko et al. 2011a) The negative effect on outcomes in these patients has been demonstrated to be independent of anaemia.(Rangel et al. 2014)

Although ID with anaemia has been clearly linked to surgical outcomes, there is a relative lack of research into this effect in the earlier stages of ID. One trial in colorectal cancer patients, in which ID is very common, showed that those with NAID had increased rates of readmission, infection and a lower 90-DAH, although it was a relatively small group of 141 patients(Lachlan F. Miles et al. 2019).

In cardiac surgery, there is a growing body of evidence that suggests that iron-deficiency is associated with higher mortality and morbidity, and that this effect appears to be synergistic, but independent of anaemia. One study of 730 patients demonstrated that in non-anaemic patients, 90-day mortality was 5% in those with iron deficiency (ferritin < 100ug/L) compared to 2% in controls, this effect was even more marked in those with anaemia with the presence of ID increasing the mortality from 4% to 14% (OR = 3.5(95% CI 1.5-8.4)). (Rössler et al. 2020). Another study linked iron deficiency to increased blood transfusion and post-operative fatigue scores(Piednoir et al. 2011). Yet another study of 277 Australian cardiac surgical patients failed to demonstrate an effect on mortality or transfusion, but found increased length of stay and reduction in the composite (90-DAH) in NAID patients, although the effect was not dramatic(L F Miles et al. 2017)

Causes of Iron Deficiency

Iron loss or increased demand

Normal iron loss occurs due to various sources including blood loss, sweat, intestinal cell loss, and desquamation of cells in the skin and urinary tract(Hentze et al. 2010). Normal levels of iron loss are compensated for by absorption, however some physiological and pathological states cause an increase in iron loss that overwhelms the absorptive reserve. Infancy, adolescence and late pregnancy are times of markedly increased erythropoiesis and thus iron requirements increase significantly from baseline explaining the increase in prevalence of iron-deficiency in these populations(Camaschella 2015).

Iron absorption

Iron is absorbed in the small intestine, specifically the by duodenal enterocytes (Camaschella 2015). Absorption may be impaired by conditions affecting gut function such as inflammatory bowel disease(Kaitha, Bashir, and Ali 2015), coeliac disease(Ditah et al. 2015), *Helicobacter pylori* infection(Annibale et al. 2001) and autoimmune conditions(Bini 2001). Bariatric surgery has been linked to reduced iron absorption(Khanbhai et al. 2015). There is also limited iron absorption in many chronic diseases involving inflammation which leads to the relatively new concept in iron-metabolism known as iron restriction.

Iron Restriction

While failure of iron-absorption has classically been considered as the rarer of these causes, there is increased focus on this over the last 20 years the concept of iron-restriction has become increasingly studied. The understanding of this process was progressed significantly with the discovery of a protein in 2000 that was named hepcidin as it was secreted by hepatocytes in the liver and appeared to have anti-bacterial properties. (Park et al. 2001) It was found that by removing gene expression for hepcidin in experimental mice, they developed severe iron-overload (Nicolas et al. 2001) which suggested that it played a crucial role in iron metabolism. Hepcidin has since been shown to act as an acute phase reactant and is elevated in patients with iron overload, infection or inflammation(Nemeth et al. 2003) and rises in a similar manner to ferritin in these pathological states. It prevents iron transportation by enterocytes (Fleming and Sly 2001) via an interaction with ferroportin (Bergamaschi and Villani 2009) and thus reduces absorption of dietary iron. This interaction also impairs the release of recycled iron by macrophages (D'Angelo 2013), presumably with the strategic evolutionary intent of reducing available iron to invading micro-organisms. It is elevated in

many conditions that are associated with anaemia of chronic disease such as inflammatory bowel disease (Semrin et al. 2006) whereas in pure iron deficiency anaemia, without an inflammatory component, hepcidin levels are generally low.

The cause of anaemia in cardiac failure patients is largely iron deficiency (approximately 70%), although ACD haemodilution and drug effects also contribute (Nanas et al. 2006). In cardiac surgery patients, the most common cause of anaemia is iron deficiency (either absolute or functional) followed by folate and vitamin B12 deficiency.(Matthew Hung et al. 2015) and studies have demonstrated that up to 39% of cardiac surgical patients are iron-deficient (L F Miles et al. 2017).

Treatment of Iron deficiency

Of all the principles contained within the PBM concept, restoring iron stores is arguably the most simple, effective, and economical. There are many ways in which iron stores can be restored, by reducing iron loss due to bleeding or chronic infection or by increasing iron intake by dietary modification or supplementation. Dietary modification alone can improve iron-stores significantly, although the timeframe is generally long. On a population level, there is some evidence that various public health measures can improve quality of nutrition, which should have a follow-on effect on nutritional-deficiency anaemias(Ruel and Alderman 2013)(Sharifirad et al. 2011). Iron is generally better absorbed from animal products than vegetable sources(Bothwell, Pirzio-Biroli, and Finch 1958). Increasing meat intake therefore has an obvious impact on iron intake, though factors such religion, personal preference or poverty prevent this from being a viable solution in many cases. In regions where oral iron intake is poor, supplementation of staple grains with iron, and/or agents to improve iron absorption is a commonly used alternative.

The process of dephytinising foods by the addition of phytase (an enzymatic supplement which increases phosphorous and zinc absorption from grains) appears to improve iron availability in many grains. (Cercamondi et al. 2013)(Frontela et al. 2009) The chelating agent ethylene diamine tetra-acetic acid (EDTA) appears to also have some benefit to iron absorption, especially when given in the form of NaFeEDTA. (Bothwell and MacPhail 2004) and ferrous bisglycinate may also be an effective alternative to common iron salts with some foods. (Bovell-Benjamin, Viteri, and Allen 2000) .

Even variations in agricultural practices can have a significant role in the prevalence of iron-deficiency. The iron concentration of many crops is variable depending on soil types, the specific cultivar or fertiliser use (Rengel, Batten, and Crowley 1999) and there is some broad

evidence that the mineral concentration of some grains has been dropping since the 1960s as a result of more intensive farming practices. (Fan et al. 2008). Interestingly, it has been demonstrated that when crops such as wheat, rice and barley are grown in atmospheric carbon dioxide concentration above 550ppm there is an associated decrease in the iron concentration in the grain. It is hypothesised that if climate change continues and CO2 reaches these levels, this may pose a significant risk to the anaemia burden in countries that are most reliant on these staple grains for food. (Smith, Golden, and Myers 2017)

While dietary modification or population-level food supplementation may be effective, oral iron supplementation at an individual level is more effective than these interventions alone, though restoring depleted iron stores remains a relatively slow process. One of the major factors responsible for this is the limited oral bioavailability of common iron preparations and, depending on the deficit, restoring iron stores to normal often takes months. Furthermore, ingesting iron (usually as ferrous sulfate) by the oral route is frequently associated with gastrointestinal (GI) side effects such as nausea, abdominal pain, bloating, diarrhoea, constipation and black or tarry stools. A meta-analysis from 2015 confirmed this, showing that oral iron supplements are 2.3 times more likely to cause GI side effects than placebo (3.3 times more likely in pregnant women) although they were unable to detect a relationship to dose.(Tolkien et al. 2015) High-dose oral iron may also increase gut inflammation(Jaeggi et al. 2015) and affect the important gastrointestinal microbiome.(Zimmermann et al. 2010). While GI side-effects are often mild, they have the knock-on effect of reducing compliance with supplementation, which significantly undermines their effectiveness. Non-compliance has been shown to be as high as 40% in some studies(Gereklioglu et al. 2016), which has been largely attributed to both GI upset and weight gain.

Various strategies have been shown to improve the effectiveness and tolerance to oral iron supplementation. (Hurrell et al. 2004) Ingesting oral iron with food is often said to reduce GI side effects, but appears to decrease the absorption rate significantly, in one paper reducing bioavailability from 8.5% to 2.3% (Cook and Reddy 1995). If iron is taken with food, the composition of the food is also important. Co-supplementing with ascorbic acid (vitamin C), due to its capacity to reduce ferric iron to ferrous iron, thus increasing its bioavailability more than other organic acids. (Teucher, Olivares, and Cori 2004)

Dosing strategies can have a significant effect on oral absorption of iron. The mechanism whereby this occurs appears to be closely linked to hepcidin, and it has been shown that daily or twice daily doses of iron increase hepcidin and may actually decrease iron absorption.(Moretti et al. 2015). A subsequent trial by the same group demonstrated that

giving oral iron on alternate days increased fractional and total iron absorption, despite the lower total dose. This effect may be magnified in clinical practice by the reduced amount of non-absorbed iron present in the gut, which is thought to be the principal cause of many of the GI side-effects which impact on compliance.

Intravenous iron replacement

Due to the general challenges and limitations of restoring iron stores successfully and in a timely manner using oral supplements, more advanced delivery techniques have been sought. The first-available parenteral iron preparation was iron hydroxide, developed in the 1930s, which was given intramuscularly or subcutaneously and was highly toxic due to its lack of carbohydrate shell and subsequent immediate release of free iron, which can catalyse the production of free-radical ions that damage cellular membranes, proteins and DNA.(Emerit, Beaumont, and Trivin 2001) Reports from the time suggest that the adverse effects were common and severe enough to recommend its use only in "rare instances".(Heath, Strauss, and Castle 1932) Subsequently it was found that by adding a carbohydrate component to the iron molecule, such as saccharide (Nissim 1947), the preparation was much better tolerated. In the 1950s, a high molecular weight iron dextran called Imferon was described (Baird and Podmore 1954), which remained highly effective at improving haemoglobin responsiveness (Adamson and Eschbach 1998), but was still associated with a very high rate of anaphylaxis/anaphylactoid reactions of up to 1 in 170 (Fishbane and Kowalski 2000) This preparation was found to be responsible for 31 deaths in the USA between 1976 and 1996, ultimately resulting in it being removed from the market.(Auerbach and Rodgers 2007) Later in the1990s, newer preparations such as low-molecular weight iron dextran (Cosmofer), ferric gluconate (Ferrlecit) and iron sucrose (Venofer) were developed and found to be safer, with an incidence of serious adverse events of around 1 in 200,000(Chertow et al. 2006). Although the lower-molecular weight dextran preparations were associated with lower rates of serious reactions than their higher-molecular weight counterparts, they still require a slow infusion process over 4-6hrs, which represents a significant burden on workforce costs and resource allocation. Ferric gluconate significantly improved this time to a 1-hour infusion and ferric sucrose can be given over 15-minutes, though for a full dose of 1000mg, it needs to be spread over 5 injections in 2 weeks. Much of the early use of these preparations was in end-stage renal failure (ESRF) patients, where a slow, or staged infusion is practical for those on intermittent haemodialysis in the outpatient setting, however it is far less practical for other groups of patients for whom an iron infusion would require a dedicated hospital presentation.

This, combined with more research into IV iron's use in non-ESRF patient groups, meant that these preparations were logistically and thus also financially challenging to administer. Other newer iron-carbohydrate molecules have been developed since, including iron polymaltose, ferric carboxymaltose (Ferrinject), iron isomaltoside (Monofer) and ferumoxytol (Feraheme). These are considered to be generally safer, and in most cases, a full replenishment of iron stores can be given in 15 mins or less with a much better safety profile. The different available IV iron preparations have distinguishing characteristics which make them unique and may significantly affect their clinical effects and potential for toxicity. Firstly, the rate at which they are able to make free iron available to combine with transferrin molecule varies significantly. In vitro studies have shown that the iron dextran molecules appear to saturate transferrin far more slowly than iron sucrose and iron gluconate as seen in the figure below.

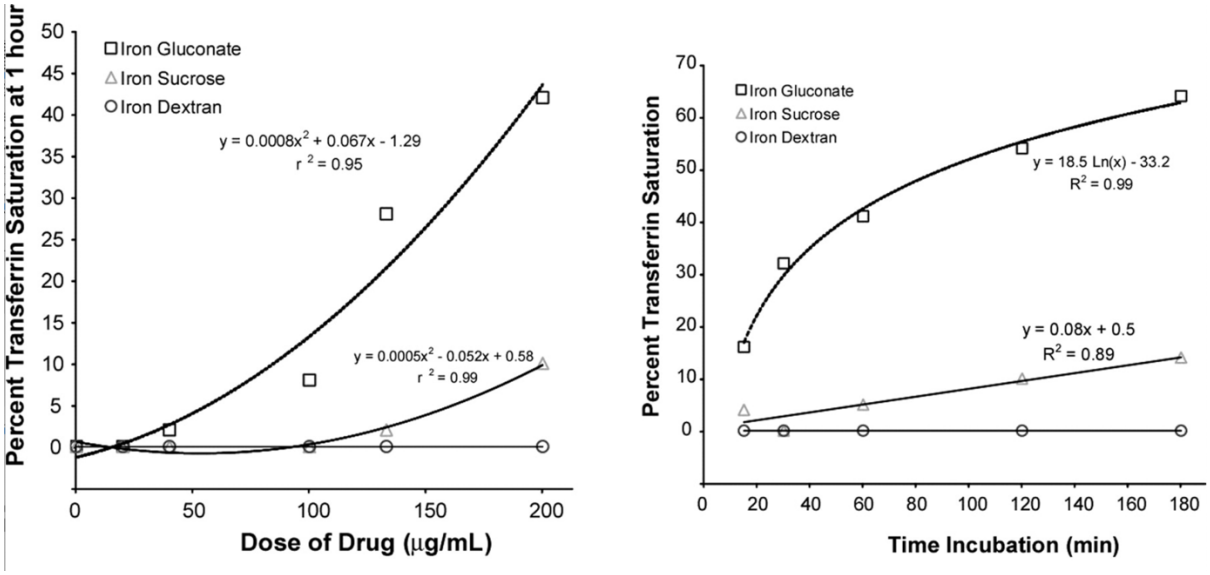

Figure 2.1 The effect of various iron preparations on transferring saturations. Reproduced from (Agarwal 2004)

While this increased rate of iron transfer to the biologically useful transferrin-bound form may imply a more rapid effectiveness, it may also contribute to a theorised increase in toxicity. There is also a significant variation in the amount of non-transferrin-bound iron (NTBI) in the plasma after infusions of various preparations. This NTBI has been shown to act as a catalyst for production of toxic oxygen radicals in vitro (Gutteridge, Rowley, and Halliwell 1982) and may contribute to adverse clinical effects.

Endothelium, and in particular nitric oxide (NO) that it produces appears to play a significant role in primary defence against atherosclerotic changes (Moncada and Higgs 1993)(Gimbrone Jr. 1995) that are the basis for ischaemic heart disease (IHD), cerebrovascular disease (CVD) and peripheral vascular disease (PVD). This is demonstrated clinically by the fact that those with increased cardiovascular risk appear to have lower NO availability (LÜSCHER 1993) which may be a result of oxidation by superoxide radicals. (Wever, Stroes, and Rabelink 1998) Various studies have shown that iron preparations may have toxic effects on endothelium in vitro and in vivo (Rooyakkers et al. 2002). One study by Zager et al (Zager et al. 2002) explored the effect of different iron preparations on cell cultures of renal tubular cells from mice and humans and human aortic endothelium. They showed that markers of cell death were highest in iron sucrose, then iron gluconate and lowest with iron dextran over a range of time periods. Given that the plasma half-lives of these drugs are markedly different (1-hr for Iron Gluconate, 6-hrs for iron sucrose and 57 hrs for the iron dextran) the effectiveness and side-effect profile of each is likely to be heterogenous. For example, while the transfer rate of iron-dextran preparations is slow, their prolonged half-life would likely prolong the transfer time-period, but also theoretically increase the period over which adverse cytotoxic effects may occur, potentially causing delayed cell-injury.

Benefits of IV iron replacement

IV iron treatment and the subsequent rapid restoration of iron stores has been shown to be beneficial in various contexts. In patients with heart failure and iron-deficiency, it has been shown to significantly improve quality of life, functional capacity and improves symptoms. (Anker et al. 2009) It has been shown to improve cardiac function, renal function and reduce hospitalisation rates if given with EPO (Silverberg et al. 2000), although IV iron alone appears to be as effective. (Terrovitis et al. 2012) Interestingly, a similar study with oral iron replacement in these patients failed to demonstrate an improvement in exercise capacity (Lewis et al. 2017). Improvement in quality-of-life indicators is a common theme in the literature with similar improvements seen in patients after colorectal cancer surgery, (Keeler et al. 2019) and multiple trials showing improvements in functional capacity (Vadhan-Raj et al. 2014). One systematic review has suggested that fatigue can be improved by replacing iron in iron-deficient patients even in the absence of anaemia. (Pratt and Khan 2016) and another showed that improvements in peak oxygen consumption were related to increases in transferrin saturation, but not [Hb].(Okonko et al. 2008)

There are a number of issues still related with iron infusions including a reduction in serum phosphate levels (M. Wolf et al. 2018)(Musgrove and Wolf 2019) and an effect on platelet count (Yessayan et al. 2014). Iron has some other theoretical negative effects such as a potentially increased risk of infection, given that many infectious agents require iron as a growth-factor. The association between genetic iron overload caused by hemochromatosis and increased rates of various infections supports this theory(Khan, Fisher, and Khakoo 2007) Although evidence of this effect has been sought extensively since the introduction of IV iron preparations, there remains no strong evidence that their use results in an increase in infections, as demonstrated in a 2015 meta-analysis of over 10,000 subjects in 103 trials (Avni et al. 2015).

As demonstrated, in the pre-operative setting, anaemia has been clearly shown to worsen outcomes and peri-operative transfusion does not mitigate this effect and indeed may worsen outcomes further. While blood conservation strategies should reduce transfusion needs to some extent, there remains a significant opportunity to identify anaemia and treat it in the pre-operative period. While it seems intuitive that this would thus improve outcomes, there is a paucity of high-quality evidence that demonstrates this. The concept of treating anaemia pre-operatively has become increasingly present in guidelines, although the evidence for such recommendations remains relatively limited, particularly in cardiac surgery.

Iron optimisation before surgery

The National Institute for Health and Care Excellence (NICE) reviewed transfusion practices and recommended identifying patients with anaemia pre-operatively and to consider methods to avoid transfusion. ("National Institute for Health and Care Excellence. Blood Transfusion. NICE Guideline (NG24)." 2017). Similar consensus statements, guidelines and protocols exist worldwide(Munoz et al. 2017; Mueller et al. 2019), despite there being limited high-quality evidence that intervention at this stage results in a change in outcomes for patients. In fact, the group that developed the NICE guidelines openly state that the guidance to offer oral or IV iron pre-operatively to iron-deficient patients is based on very low to low quality evidence (Padhi et al. 2015). Newer IV iron preparations have been shown to be a safe, rapid alternative to oral replacement, with a lower incidence of side-effects(Tomer et al. 2015) and appear to be more effective at increasing [Hb] (Clevenger et al. 2016)

While IV iron has been observed to reduce transfusion in many surgical specialties.(Manuel Muñoz et al. 2012), it is better studied in certain specialties such as orthopaedics, where there appears to be a significant treatment effect. A 2019 meta-analysis of IV Iron before acute

major non-cardiac surgery showed a decrease in blood transfusion, post-operative infection and 30-day mortality, and the majority of those studied were hip-fracture patients (2582 of 3044 patients)(Schack et al. 2019). A more recent meta-analysis in those undergoing elective total hip- or knee-arthroplasty suggested a significant decrease in transfusion and a corresponding decrease in length of hospital stay for patients.(Scrimshire et al. 2020)

Outside of orthopaedic surgery, here are other smaller trials which have attempted to establish whether IV iron can reduce transfusion rate in major general surgery including a small Australian trial which demonstrated a 60% reduction in transfusion rate with IV iron prior to major abdominal surgery in 72 patients (Froessler et al. 2016). The impact of this evidence has been limited however by contradictory studies showing little or no benefit of IV iron over oral iron in 116 in patients undergoing major colorectal cancer surgery (Keeler et al. 2017). In this study, it was again found that while pre-operative IV iron improves anaemia, more effectively than oral replacement, no significant effect on transfusion rates was detectable.

A 2018 Cochrane review found only 6 RCTs with a total of 372 patients evaluating the effectiveness of iron replacement before surgery which was an inadequate number to power an evaluation of transfusion reduction. They did conclude that iron replacement appeared to improve both iron studies and [Hb](Ng et al. 2019) but without a statistically-significant improvement in outcomes. A more recent meta-analysis of 10 RCTs with 1039 participants agrees with the positive effects on IV Iron on [Hb] when compared to both placebo and oral iron, as seen in the tables below:

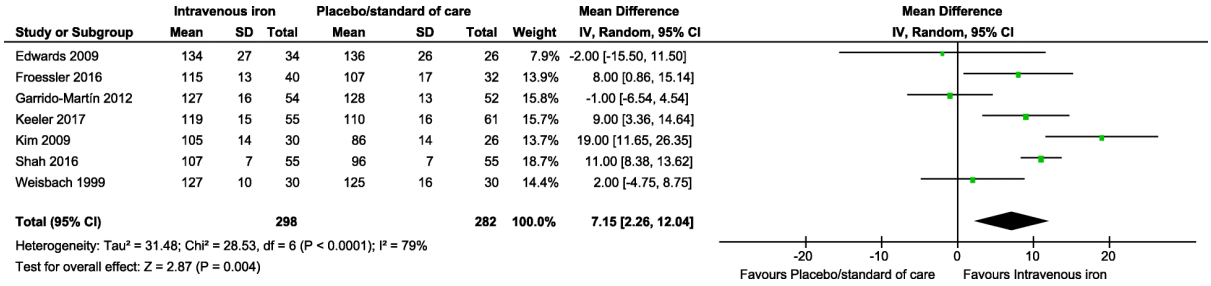

Figure 2.2: Forest plot comparison shows the pooled comparison effect of intravenous iron therapy versus placebo/standard of care groups on the change of haemoglobin level (g/L) at the post-treatment (pre-surgery) time (random effects model). Reproduced from (Elhenawy et al. 2021)

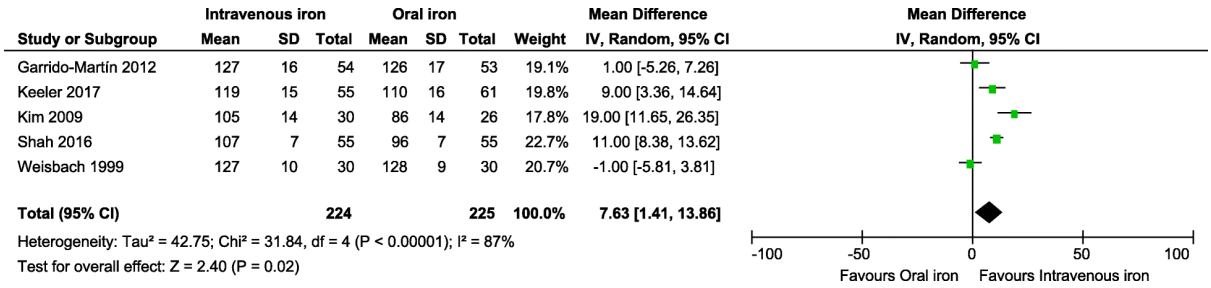

Figure 2.3: Forest plot comparison shows the pooled comparison effect of intravenous iron therapy versus oral iron groups on the change of haemoglobin level (g/L) at the post-treatment (pre-surgery) time (random effects model). Reproduced from (Elhenawy et al. 2021)

The same meta-analysis was also able to demonstrate a statistically significant 16% reduction in allogenic blood transfusion. (Elhenawy et al. 2021) This metanalysis was performed before the publication of, and thus did not include the data from the PREVENTT trial, a relatively large RCT published in 2020, by members of our collaborative group and others. This study attempted to demonstrate an outcome-benefit of IV iron before major abdominal surgery showed that while it was possible to increase [Hb], there was no significant improvement in transfusion outcomes (Richards et al. 2020), which may affect the composite outcomes of future meta-analyses.

Although guidelines regarding transfusion-prevention strategies, including peri-operative iron replacement, are now widespread, it seems current practice may be not yet be keeping to these recommended standards. Audits performed within the NHS have repeatedly shown that there are shortcomings in PBM practices and that pre-operative anaemia is not adequately managed.(NHS Blood and Transplant 2015, 2016) Similar limitations exist in Europe where standards in detection and management of pre-operative anaemia vary significantly between countries. (A Shander et al. 2012)

Peri-operative Iron replacement in cardiac surgery

As much as one-third of cardiac surgical patients suffer from iron-deficiency, even though many of these will not be in the anaemic stage of the disease.(Hubert et al. 2019) The optimisation of iron stores in cardiac surgery is being researched as a potential method of improving surgical and patient-based outcomes.

While there are many smaller observational trials exploring the effectiveness of iron replacement in cardiac surgery patients, the quality of evidence is mixed. A recent systematic review undertook to assess the quality of this evidence and provide some firmer

conclusions(Tankard et al. 2020). They established six trials that met inclusion criteria, totalling 1038 cardiac surgery patients receiving IV iron prior to cardiac surgery. The trials included 4 randomised double-blind prospective cohort studies, a randomized non-blinded prospective study, and a non-randomised non-blinded prospective study with historical control. They concluded there was insufficient data to adequately perform meta-analysis. Only one of these trials (Cladellas et al. 2012), which was considered to be of fair quality, showed a mortality improvement from 23% to 9%. Two trials (Cladellas et al. 2012; Spahn et al. 2019) showed an improvement in [Hb], the former pre-operatively (although this trial used historical controls) and the latter post-operatively as IV iron was given on the day of surgery. One showed an improvement in length of stay. A summary table from this systematic review is shown below.

Other Outcomes

Author	Surgery	Significant AE[a]	QOL	Infections	LOS, median (IQR)	Mortality
Cladellas et al, 2012 [32]	VR	None	NR	Decreased: 8% vs. 24%, P = 0.01	Shorter: 10 days (8 - 14) vs. 15 days (10 - 27), P < 0.01	Decrease: 9% vs. 23%, P = 0.04
Urena et al, 2017 [34]	VR (TAVI)	No diff	NR	No diff	No diff	No diff
Johansson et al, 2015 [36]	CABG, VR	No diff	NR	No diff	NR	No diff
Padmanabhan et al, 2018 [35]	CABG, VR	None	No diff	No diff	No diff	No diff
Garrido-Martin et al, 2012 [33]	CABG, VR	None	NR	Not increased	NR	NR
Spahn et al, 2019 [37]	CABG, VR	No diff	NR	No diff	No diff	No diff

[a]As defined by study. AE: adverse event; CABG: coronary artery bypass grafting; diff: difference; IQR: interquartile range; LOS: length of stay; NR: not reported; QOL: quality of life; TAVI: trans-aortic valve implantation; VR: valve replacement.

Table 2.1: Effects of IV Iron on surgical outcomes in cardiac surgical patients (reproduced from systemic review by Tankard et al(Tankard et al. 2020))

The overall evidence for outcome effects detected in this systematic review were graded as being 1b (moderate quality) for all outcome measures except QoL measures, which were graded as 2b (low quality) evidence. This relatively low quality of evidence for the use of IV Iron as a pre-operative treatment to improve [Hb] and operative outcomes suggests that further studies are required. Chapter 5 describes the methodology and results of one such trial (CAVIAR-UK) which the author contributed to. This has subsequently led to a larger international RCT (ITACS), for which the author is also an investigator, which aims to provide high quality evidence on the effects of IV iron on patient-centred outcomes in cardiac surgery.

Transfusion practices in cardiac surgery

Cardiac surgery generally requires the opening of large vessels and/or cardiac chambers, which pre-disposes patients to surgical bleeding. This propensity for bleeding and the dilutional effect on [Hb] seen with CPB mean that intra-operative anaemia is common. To maintain adequate circulating [Hb] for oxygen delivery, transfusions of red blood cells are commonly used.

Prevalence of transfusion in cardiac surgery

The rate of transfusion varies enormously between countries and between centres. In one analysis of US centres, the transfusion rate in cardiac surgery ranged from 0% to 85% depending on location (Maddux et al. 2009) and 8% to 93% in another.(Bennett-Guerrero et al. 2010) Similar heterogeneity in transfusion practices is seen in the UK with one study showing a range of 25% to 75% between centres. Transfusion practices in Australia appear to be similarly variable with a range between 17% and 79%(Daly et al. 2007) or more recently 22% to 67%.(McQuilten et al. 2014) What is broadly evident, is that overall peri-operative transfusion rates for cardiac surgery are generally high compared to other surgery with approximately one-third of all patients undergoing CABG surgery receiving transfusions (Stover, Siegel, and Parks 1998).

Normal peri-operative [Hb] trends in cardiac surgery

During cardiac surgery, there is a significant dilution of the circulating blood volume due to the CPB priming solution and additional IV fluids, most commonly crystalloid solution (Protsyk et al. 2017) that is infused during the peri-operative period. In addition to this, beginning almost immediately after the initiation of CPB, there is an inflammatory response

which causes an increase in capillary permeability, causing a reduction in circulating blood volume and shift of fluid to the extravascular space. (Cremer et al. 1996)(Hamada et al. 2004) This effect appears to peak at 4-hours post-operatively and can increase the extravascular volume by several litres over the ensuing 24 hours (Tschaikowsky et al. 2000). The combination of a restricted blood volume and early minor bleeding tends (which is almost ubiquitous) can lead to haemodynamic effects that result in patients receiving further crystalloid or colloid resuscitation which causes a dilutional fall in [Hb] in the early post-operative period and further extracellular fluid shift. As the capillary membranes regain integrity, the lost fluid is gradually retuned to the blood volume, sometimes causing the BV to exceed pre-operative levels, which further dilutes the circulating Hb. As such, it has been shown that [Hb] continues to fall until a nadir point, which generally occurs at post-operative day 3 or 4 as the inflammatory process dissipates. Gradually, the now increased blood volume and restoration of capillary integrity results in a significant diuresis, returning blood volume to normal levels and concentrating the circulating Hb. This, with a possible and likely variable contribution by haematopoiesis cause [Hb] to recover approximately 50% of its downward drift by the tenth post-operative day. (George 2012)

These findings were reproduced in an audit of patient undergoing cardiac valve surgery at St Vincent's Hospital (Sydney, Australia) which was undertaken by medical students under the author's supervision as part of a research project with the University of Notre Dame. In this audit of 73 patients, who did not receive an allogenic blood transfusion, there was a relatively predictable reduction in [Hb] from an average of 104g/L immediately post-operatively to 97.6g/L on post-operative day 3, returning to 100.4g/L by day 6. In most cardiac surgical units, the early post-operative period is a common time for patients to receive RBC transfusions as they cross transfusion triggers as [Hb] descends over the first 3 post-operative days. These results suggest that a slightly more restrictive approach may be acceptable during this period and provides some guidance on the timeline of expected [Hb] improvement without intervention.

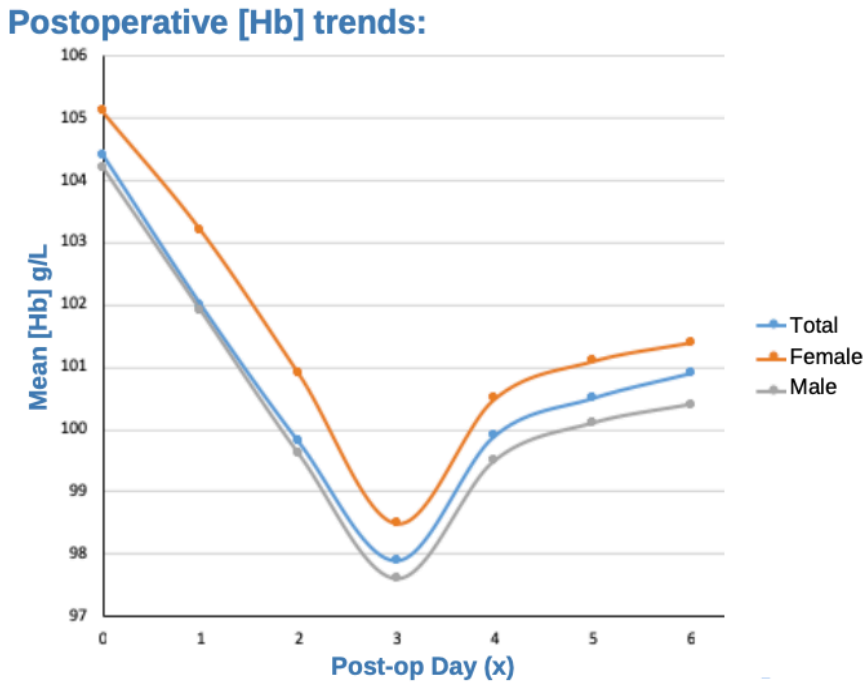

Figure 2.4: Post-operative [Hb] trends in cardiac valve surgery patients who did not receive blood product transfusions (reproduced from poster presentation at St Vincent's Hospital, Sydney)(Shahbaz and Carroll 2019)

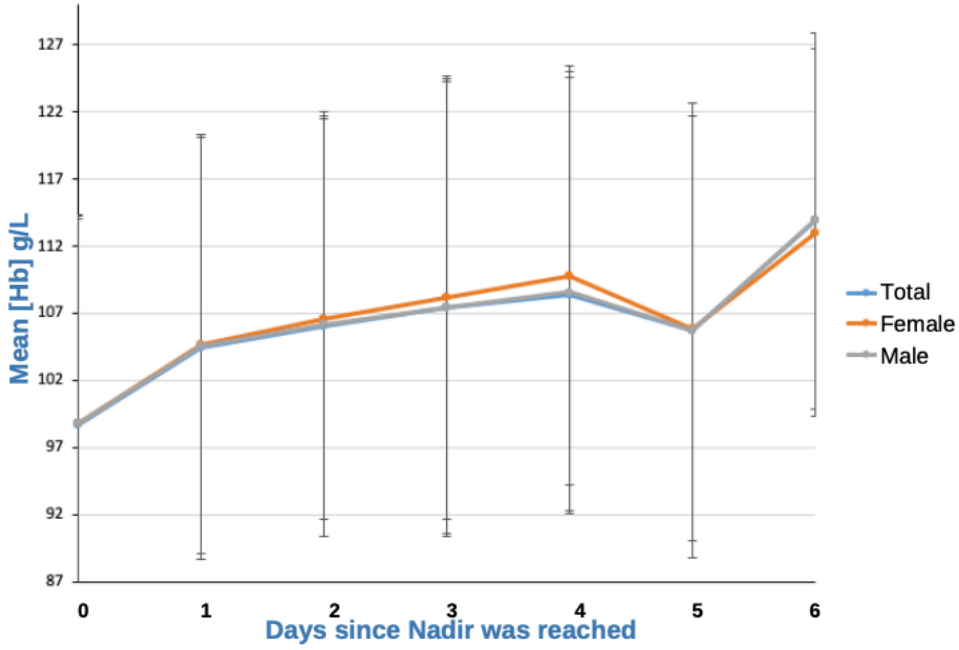

Fig 2. Post-operative [Hb] changes following Nadir in Cardiac Valve surgery patients who have not recieved blood products perioperatively

Figure 2.5: Post-operative [Hb] trends following time of nadir [Hb] in cardiac surgery patients not receiving blood products (reproduced from poster presentation at St Vincent's Hospital, Sydney)(Shahbaz and Carroll 2019)

Transfusion strategies in cardiac surgery

Some of the detrimental effects of anaemia can be rapidly corrected with blood transfusion, and in some acute settings and chronic conditions this therapy may be life-saving(Armstrong 2008). Transfused red blood cells act as an excellent blood volume expander and remain in the intravascular space far more effectively than crystalloid or colloid solutions. The theoretical improvement in oxygen delivery is supported by studies showing that RBC transfusion results in a demonstrable increase in exercise capacity in anaemic patients (Wright et al. 2014; Solheim et al. 2019) and as discussed, transfusions are frequently used in the context of cardiac surgery to this end.

Although it is clear that anaemia is associated with poor outcomes in a cardiac surgery, the treatment of anaemia with allogenic blood products can also have significant detrimental effect on outcomes(Rawn 2007), and it is difficult to establish which effect is greater. (A. Shander et al. 2011) Although the evidence suggests that avoiding transfusion altogether is likely to improve outcomes, sometimes transfusion is thought to be essential to maintain oxygen delivery to tissues, particularly in the presence of organ dysfunction. There is ongoing debate about the [Hb] at which transfusion should occur. Historically, many physicians targeted [Hb] values of over 100g/L, particularly in those with organ dysfunction(Armstrong 2008). There has been a trend towards lower transfusion triggers and many guidelines have shifted recommendations for values around 90g/L to those closer to 70g/L. There is some evidence that a restrictive approach is similarly effective (Hajjar et al. 2010) or non-inferior (Mazer et al. 2017) and this may also apply to long-term outcomes (Mazer et al. 2018). Another study in post-valve-replacement patients showed that non-symptomatic patients had no difference in 1-yr outcomes if they were discharged with a [Hb] of <80g/L or >80g/L, and concluded that these patients should not be transfused to meet arbitrary criteria (Ad et al. 2015).

While evidence supporting restrictive transfusion strategies has been welcomed due to the potential to reduce the significant costs associated with transfusion, it has been recently challenged by the well-known TITRe2 trial, which suggested that a liberal transfusion strategy was not inferior in terms of outcomes or cost, but may slightly improve 90-day mortality. (G. J. Murphy et al. 2015). Restricting transfusion may also cause an increase in the incidence of cardiogenic shock in post-operative patients (Nakamura et al. 2015)

Effects of transfusion on mortality in cardiac surgery

Although transfusion is frequently utilised in cardiac surgery and cardio-pulmonary bypass to maintain oxygen delivery and can thus be organ-sparing or lifesaving in an acute setting, the broader effects of transfusion on mortality appear generally to be detrimental. One US study suggested a 2-fold increase in risk of death in anaemic patients who were transfused compared to those who were not (M. Engoren et al. 2014). Transfusion is associated with an increased risk of in-hospital mortality and also appears to increase the probability of all major complications in a dose-related manner. (Colleen Gorman Koch, Li, Duncan, et al. 2006)

It appears to have an independent effect on longer term survival with one study suggesting a hazard ratio for 3-yr mortality of 1.340 (95% CI: 1.109-1.620) for those transfused intraoperatively.(von Heymann et al. 2016). Another US study suggested a 70% increase in 5-yr mortality for transfused patients, when data was corrected for co-morbidities and other factors(M. C. Engoren et al. 2002). Interestingly, a trial examining the effect of anaemia on outcomes in "off-pump" CABG surgery showed a trend toward higher mortality in the anaemic group (1.6% vs 0.3%), although this was not statistically significant which suggests the process of cardiopulmonary bypass may be a significant factor in anaemia-mortality relationships. (Matsuda et al. 2013)

Effects of transfusion on morbidity in cardiac surgery

Beyond effects on mortality, transfusions of allogenic blood products are associated with significant morbidity on a variety of organ systems.

The immediate risks associated with allogenic blood product transfusions are well described. Transfusion is associated with a variety of potential complications including acute transfusion reactions, transmissible infection(Squires 2011), transfusion-related lung injury (TRALI) (Bernard et al. 1994). In non-surgical cardiac patients presenting with acute coronary syndrome (ACS), there is an association between transfusion and 30-day mortality(Rao et al. 2004). Transfusion may have immunomodulating effects, which may have both immediate and longer-term consequences(H. G. Klein and Weiskopf 1999) and may increase risk of cancer recurrence (Blumberg and Heal 1994)

Allogenic blood products shown to increase rate of post-operative infection in cardiac surgery (Horvath et al. 2013) Transfusion has been linked to post-operative graft occlusion in CABG patients(M. Engoren et al. 2015) and higher rates of post-operative atrial fibrillation (Colleen Gorman Koch, Li, Van Wagoner, et al. 2006; Sood et al. n.d.) and need for inotropic or mechanical support post-operatively(S. D. Surgenor et al. 2006). In a Canadian study of

12,388 patients, erythrocyte transfusion was shown to increase the rate of acute kidney injury in a dose-dependent manner, and this effect was more marked in anaemic patients. (Karkouti et al. 2011). Rates of infections in both the surgical site and other septic complications appear to be increased in those who are transfused(Banbury et al. 2006; P. J. Murphy et al. 1992; G. J. Murphy 2009). Length of both ICU and hospital stays are reliably shown to be longer in transfused patients (Galas et al. 2013). The incidence of TRALI has been reported to be as high as 2.4% in cardiac surgical patients(Vlaar et al. 2011)

Economic impact of transfusion

In addition to the human-cost of blood transfusion in terms of the negative effect on patient outcomes, transfusion comes at a significant financial and societal price. The costs associated with transfusion are very different between countries, although they universally create a significant burden on health services(Amin et al. 2003a).The cost of RBC transfusion is difficult to estimate. On systematic review of data from the USA, Canada and the UK suggests that the estimated cost of a unit of RBCs was US$155-$550 in around 2000 and demonstrated those costs has risen significantly over the studied period. The systemic cost of providing transfusions continues to rise as the processes of collection, testing and processing are refined. For example, between 1997 and 1999, the cost per unit rose by 170% in the UK, while in the USA the cost rose 61% between 2000 and 2001. (Amin et al. 2003b) A 2008 paper from the US suggested that the true cost of a RBC transfusion in the surgical setting was as high as US$3433, of which the cost of the blood itself contributed US$1158. (Aryeh Shander et al. 2008)

It is clear that transfusion is associated with a significant cost and that surgery is a major consumer of global blood resources. Peri-operative services generally seem to account for about a third of all blood product use in many countries (Wallis, Wells, and Chapman 2006) One analysis suggested that CABG surgery alone accounted for 11% of all RBC transfusion resources in the USA in the late 1990s (D. M. Surgenor et al. 1998), and more from that decade suggesting that cardiac surgery was responsible for almost 20% of all blood transfusions nationally.(L. Goodnough, Johnston, and Toy 1991). The most recently published data from Australia suggests cardiothoracic surgery patients received 5.6% of all blood products nationally(Shortt et al. 2009).

Minimising transfusion in cardiac surgery

The effects of transfusion on morbidity, mortality and cost have prompted research into whether by simply reducing transfusion rates, it is possible to improve outcomes.

As populations age, and the demand for blood increases as older demographics consistently require more transfusions (Wells et al. 2002). This effect is compounded by the relative reduction in the proportion of the population that are eligible to donate blood. The combination of supply-demand disparity and increasing costs have stimulated more interest in methods to reduce transfusion costs and minimise associated harm.

Patient Blood Management

The concept of Patient Blood Management (PBM) developed in the late 20th century and became widely accepted in the 2000s(Thomson et al. 2009). This notion is not new, and some attribute its genesis to early attempts to avoid blood product use in Jehovah's Witness groups undergoing cardiac surgery in the 1960s and '70s which was shown to be comparably safe (Ott and Cooley 1977) despite the concept of "bloodless" cardiac surgery being quite radical at the time. Refining of the processes involved has ultimately resulted in studies demonstrating these patients can do better than their matched peers. The appearance of HIV/AIDS in the 1980s and the fear of transfusion-related infections drove a significant push to minimise blood transfusion and this combined with pre-existing factors led to a broad questioning of transfusion practices (James P Isbister 1988)

Modern PBM is a broad, multi-disciplinary approach that focusses on patient-outcomes using a variety of both pre-, intra-, and post-operative techniques aimed at reducing the rate of transfusion overall and assuring that blood products are used appropriately and only when considered beneficial.

The introduction of PBM techniques has been validated in its capacity to reduce cost and improve outcomes (Leahy et al. 2017; Freedman et al. 2008; Brevig et al. 2009) and furthermore the cost of PBM programs has been shown to be less than the financial saving showing it represents value to health systems (DeAnda et al. 2006)

The three "pillars" of PBM (J. P. Isbister 2015) are described as:

1. Maximising the total red cell mass
2. Minimizing blood loss
3. Tolerate anaemia and harness and optimize physiological reserves

each of these pillars involve pre- intra- and post-operative techniques and are summarised in the below diagram reproduced from the Australian Patient Blood Management guidelines:

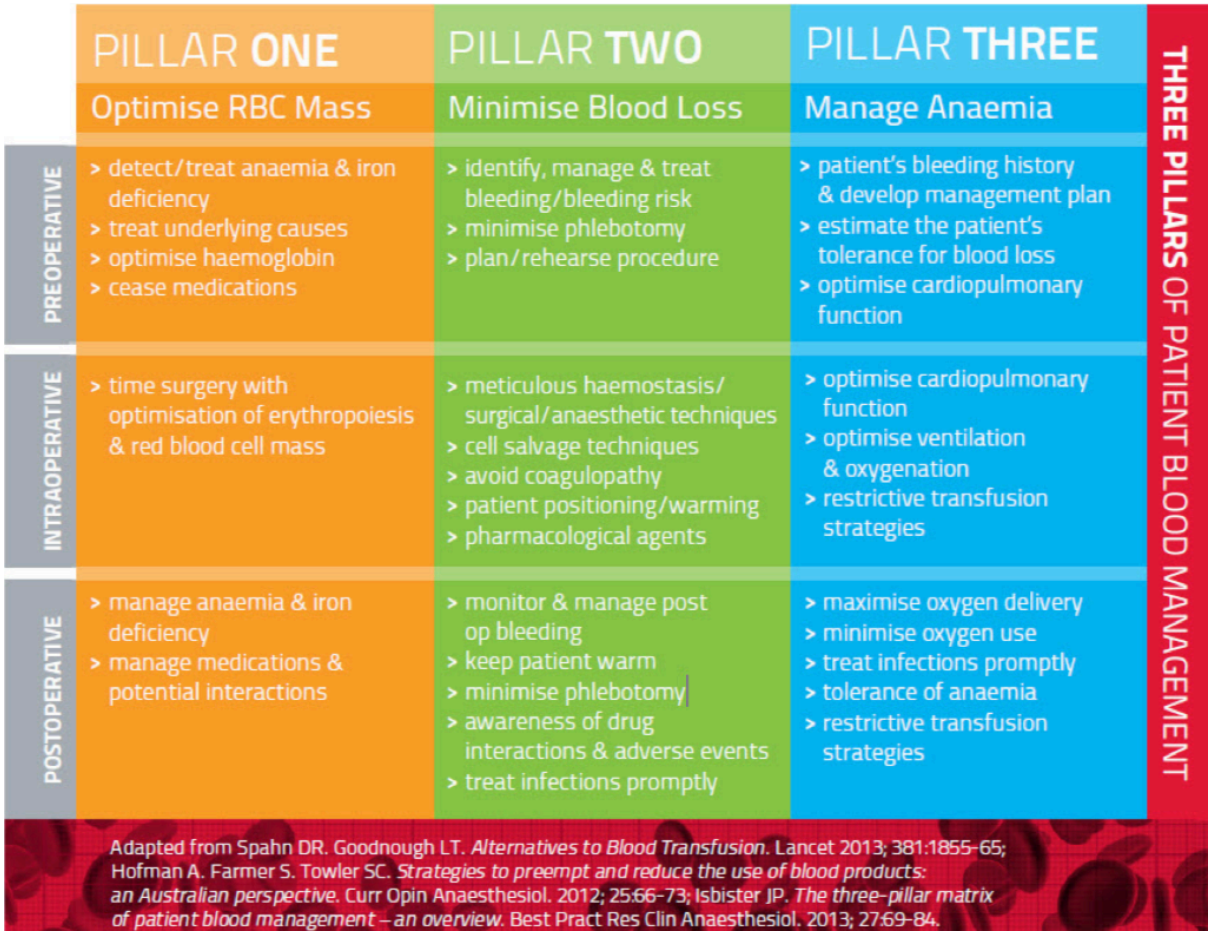

Figure 2.6: The three pillars of PBM. (reproduced from Blood.gov.au)

Pre-operative techniques for optimising haematological parameters are discussed extensively throughout this thesis and many of these have been demonstrated to be highly effective in populations who are, for religious reasons, unable to consent to blood transfusions, such as Jehovah's Witnesses. Interestingly, one scenario in which Jehovah's Witness patients appear to do worse than those that accept transfusions is in major trauma (Varela et al. 2003) which suggests that intra- and post-op improvements may not be adequate to make "bloodless" medical management equal to the standard. This highlights the role of pre-optimisation, one of the key tenets of PBM.

There are a variety of intra-operative techniques that can also be utilised which may reduce transfusion requirements. The most well-known of these are the use of antifibrinolytics, topical haemostatic agents and cell salvage techniques. Various CPB techniques such as retrograde autologous priming of the CPB circuit have also been shown to reduce RBC transfusions.(Ševerdija et al. 2011) The increasing use of bedside coagulation testing using thromboelastographic techniques such as TEG® and ROTEM® to guide blood component administration may also have a beneficial effect on RBC transfusion rates(Rahe-Meyer et al. 2009), although a meta-analysis of the topic concluded that while there appears to be a beneficial effect (RR 0.86), the quality of this evidence remains low. (Wikkelsø A and Afshari 2016).

Targeting PBM strategies

While transfusion is expensive, many of the strategies used in PBM also come at a significant cost, including equipment such as cell salvage, but also the significant resource-drain of setting up PBM programs. Given the cost associated with these PBM strategies, there has been some effort to target resource-use to those who are likely to derive the greatest benefit. As with all medical interventions, strategies to mitigate transfusion risk are best used in a targeted manner. Indiscriminate use of these resource-intensive interventions is not only costly but may not actually improve outcomes and, in some cases, could be harmful. In order to best direct use of PBM resources, potential risk of transfusion needs to be assessed and resources allocated to those in the highest risk categories. With this in mind, various methods have been used to predict those who are likely to require transfusion using prediction models and scoring systems.

Predicting Transfusion requirements using scoring systems

Scoring systems in medicine

The ability to predict outcome such as mortality and, in this example, transfusion requirements using pre-existing variables has led to the development of scoring systems, which combine the predictive power of multiple variables and allow for more accurate prediction of outcomes.

Critical care medicine has utilised scoring systems for decades with early examples such as the Therapeutic Intervention Scoring System(Cullen et al. 1974), the Sepsis Score(Elebute and Stoner 1983) developed in the 1970s and 1980s. The APACHE score(Knaus et al. 1981) was developed in the US, and was the first widely used scoring system for predicting outcome in

critically ill patients and is still in clinical use in the latest iteration, the APACHE IV(Zimmerman et al. 2006). The Simplified Acute Physiology Score (SAPS) was a far simpler system was developed from European data shortly after the original APACHE and is still currently used as SAPS III (Moreno et al. 2005)

Scoring systems in surgery

As with medical scoring systems, those specific to surgical outcomes have evolved over decades, from the widely used, albeit very simplified American Society of Anaesthetists (ASA) physical status score. The score was first described in 1941(Saklad 1941) and although designed as an indicator of pre-operative health for statistical analysis, has developed into a proxy for surgical risk prediction. Although its accuracy at predicting surgical risk, due to its inherently subjective nature, is only moderate (Sankar et al. 2014), it remains widely used, largely thanks to its simplicity. Others such as the POSSUM score (Prytherch et al. 1998) have been adapted to predict surgical risk in various surgical sub-specialities such as vascular and colorectal surgery. Dozens of these scoring systems exist with some more popular examples including the UK-developed SORT score (D. J.N. Wong, Oliver, and Moonesinghe 2017) and now widely used US NSQIP score which applies to breadth of surgical specialties(M. E. Cohen et al. 2009). Additionally, there are composite scores such as the Surgical Risk Score(Sutton et al. 2002), which combine values for 3 other risk scores (CEPOD, BUPA and ASA) to provide a more widely applicable score, albeit somewhat complicated.

In addition to the multitude of scoring systems for overall surgical risk, there are systems that predict organ system specific risk such as the well-known Lee's Revised Cardiac Risk Index (RCRI) to predict peri-operative cardiac morbidity(H. et al. 1999) which joined preceding scores (L. Goldman et al. 1977). Other exist to predict respiratory complications(Canet et al. 2010; Miskovic and Lumb 2017; Scholes et al. 2009), acute renal failure

In cardiac surgery, a multitude of scoring systems for predicting surgical outcomes have also been developed from regional to international levels including the Parsonnet score(Parsonnet, Dean, and Bernstein 1989), Pons score (Pons et al. 1997), Ontario Province Risk (OPR)(Tu, Jaglal, and Naylor 1995), ARCtIC score (Shahin et al. 2016), AusSCORE (Reid et al. 2009a), EuroSCORE (Nashef et al. 1999) and the subsequent and improved EuroSCORE II (Nashef et al. 2012),. Despite the fact that anaemia has been shown to independently predict mortality and many causes of morbidity, very few including those published by MacGovern (Magovern, Sakert, Magovern, et al. 1996), Higgins (Higgins et al. 1992) and the Cleveland

Clinic score (Geissler et al. 2000) include anaemia as an input, although there is increasing evidence that the impact on outcomes might warrant broader inclusion.(Hari Padmanabhan, Siau, et al. 2019) This may reflect the lack of data collected on regional or national databases, for example the Australian and New Zealand Cardiothoracic Surgery (ANZCTS) database did not reliably collect pre-operative [Hb] until 2014. A similar heterogeneity is seen in general surgical scoring systems where some (the POSSUM score, for instance) do include [Hb] as a predictor of morbidity and mortality, while others, including the widely used US-based NSQIP scoring system developed by the American College of Surgeons, do not. Although there may be some association between risk groups, predicting mortality and morbidity is very different from predicting the need for transfusion. This is because there are different variables in effect and various patient-factors have different strength of association with need for transfusion. The ability to predict transfusion requirement has significant value, as it allows measures to be taken to prevent or minimise transfusion. As such, several scoring systems exist to directly predict the requirement for transfusion in surgical patients as a broader group (Rutten and Grobbee 2001), while others have been refined to apply to various surgical specialties including orthopaedics (Guerin et al. 2007)(Salido et al. 2002)(Larocque, Gilbert, and Brien 2003), spinal surgery (Nuttall et al. 2000) head and neck oncology (Weber 1995), or for extracorporeal membrane oxygenation (ECMO) patients (Tauber et al. 2016). The history of such scoring systems was based on the desire to reduce cost and blood-product wastage, and was first explored in detail in the 1970s in the United States via the maximum surgical blood order schedule (MSBOS) (Friedman et al. 1976), which aimed to predict blood product usage for 50 common surgical procedures in order to reduce the outdating of blood products caused by excessive crossmatching. As medicine has increasingly focused on evidence-based improvement in outcomes and reduction of associated costs, these scoring systems have multiplied.

While cardiac surgery is known to frequently be associated with high risk of bleeding, the development of models to identify those at high risk has been relatively recent. By the 1970s, various factors had been described which predisposed to excessive bleeding including type of surgery (Gomes and McGoon 1970) and coagulopathy. (Bachmann et al. 1975) Subsequent factors were identified including aspirin use (S. Goldman et al. 1988) and prolonged CPB time. (Czer 1989) One of the earlier multivariate analyses looking at variables associated with excessive blood loss after cardiac surgery identified further factors including combined or repeat procedures, low heparin dose, female gender, increasing age, hypothermia in ICU (Despotis et al. 1996) A subsequent multivariate analysis looking at variables associated with

re-exploration after CABG included pre-operative heparin use as a factor as well as the requirement for 5- or more distal anastomoses. (Karthik et al. 2004)

The BRiSc score, which was developed at Papworth Hospital (Vuylsteke et al. 2011a) aimed at analysing and organising these variables into a scoring system that could predict excessive post-operative bleeding. This scoring system analysed a large group of patients that had post-operative bleeding in excess of 2mL/kg/hr, required return to theatre for re-exploration, or required FFP, cryoprecipitate or platelets. The cohort of 11592 patients, taken over 8 years from the single institution was divided into 2 groups: 60% percent of these cases were used to develop the scoring system and the remaining 40% were used for the purposes of validation. They identified several variables that were associated with increased risk of post-operative bleeding: type of surgery (with single valve procedures and CABG surgery being less likely to cause bleeding), age, BMI, presence of aortic disease and the urgency of the surgery. The presence of each of these factors was given a value of 1 in the scoring system and various values were apportioned into risk groups (0=low risk, 1-2= medium risk and >3= high risk). While the authors conceded that the applicability of the score was limited by its development from a single-centre database, and that the use of anti-fibrinolytics changed significantly during the study timeline, the score had a strong negative predictive value. The accuracy of the score was challenged by the fact that the positive predictive value was somewhat limited with only 27% of patients in the highest risk group suffering from major bleeding, with this limitation confirmed in the external validation set. The score was later analysed in a university in Norway and found have an similarly high negative predictive value of 98% in the low risk group, but with a PPV of 15% in the high risk and a low AUC of <0.75. (Greiff et al. 2015) This raises further concerns about the generalisability of the score to other settings. Other groups were using the requirement for transfusion, particularly excessive transfusion as an indicator of major bleeding and various and as such various analyses were directed at factors predisposing to this endpoint, rather than at the presence of bleeding itself.

One of the first papers to attempt to predict RBC transfusion as an outcome was a multivariate analysis that identified various factors that increased likelihood of large transfusion (>5 units RBCs), which was designed really as an indicator of excessive bleeding. (Ferraris and Gildengorin 1989) Although other predictive variables were identified, red blood cell volume and bleeding time were identified and used to develop a nomogram which used the ratio of bleeding time: RBC volume to predict likelihood of massive transfusion. A ratio of 0.0071 or greater was associated with a >70% chance of large volume RBS transfusion. While both bleeding time and red cell volume has fallen out of popularity in

more recent times, the predictive value of red cell volume is of particular interest as it is generally a calculated figure (usually derived from BSA and Hct) as it is difficult to measure. Newer methods, though, have been developed for measuring this value with reasonable accuracy and will be discussed in later chapters.

In 2006, the Reducing Bleeding in Cardiac Surgery (RBC) research group from Canada published their clinical prediction rule to identify those at risk of massive transfusion from a group of 10667 patients undergoing cardiac surgery. (Karkouti et al. 2006) They identified 12-variables including many of those discussed but with the addition of some novel indicators including pre-operative shock, platelet count, length of circulatory arrest, nadir haematocrit while on CPB and, interestingly, the presence of a "High-blood-loss-surgeon". The negative predictive value of this score was 95% in the low-risk group and the positive predictive value in the high-risk group was 60%, however its performance was less accurate in the moderate-risk groups and was found to be no more accurate than standard clinical predictors. The score was also internally validated, with 4016 of the total group which limited it applicability to other institutions. Furthermore, using the presence of a "High-blood-loss-surgeon" as part of a scoring system is fraught with challenges, especially when attempting to apply such a predictive score to a broader context.

As evidence developed that transfusion itself was independently associated with risk, a new breed of scoring systems emerged which shifted the focus from major bleeding and massive transfusion to the requirement for any RBC transfusion. Although the need for transfusion is common in cardiac surgery, it is not universally required and there are several factors that allow for prediction of this requirement. As discussed earlier, most cardiac surgical procedures are undertaken using cardiopulmonary bypass (CPB) support, which generally involves dilution of the circulating blood volume with a crystalloid and/or colloid priming solution. This reduces the circulating [Hb] in a predictable manner depending on the volume and composition of priming solution and the circulating blood volume. In addition to surgical factors, there are many patient factors which can be identified which contribute to risk of blood loss. For instance, in most cases, the volume of CPB priming solution is fixed (usually c.1.5L). As a result, the relative dilution will be greater in those with a smaller circulating volume, or lower total haemoglobin mass. As transfusion trigger values are generally based on [Hb] only, it therefore means that those with smaller blood volumes are more likely to receive a blood transfusion, therefore a low body surface area (BSA), which is frequently associated with a low blood volume, is a reliable predictor of transfusion requirement. Females tend to have lower body surface areas, and therefore are often found to have higher transfusion rates.

Similarly, transfusion rates tend to increase with age. In addition to baseline characteristics, there are many patient co-morbidities that have been linked to perioperative transfusion risk and by analysing these factors, it is possible to estimate risk of transfusion around the time of surgery. As with other outcome prediction scores, scoring systems have been designed to predict need for any RBC transfusion in cardiac surgery.

The first of these was developed (Magovern, Sakert, Benckart, et al. 1996) at a single US centre using data from 2033 patients and internally validated with data from a further 422 patients. They identified some familiar pre-operative risk factors including emergent, urgent or redo operation, shock or low LVEF, BMI, age, gender, low red cell mass, diabetes, and some novel ones such as low albumin, catheterisation-induced coronary occlusion, and peripheral vascular disease (PVD). They described an AUC of 0.78 in both the test and validation groups, meaning it appeared to be relatively accurate.

Contemporaneously to the development of the bleeding-risk scoring system developed by Karkouti's group and also in Canada; another predictive model, the TRUST score (Alghamdi et al. 2006) was developed. This scoring system was developed using a data set of 11113 consecutive patients at Toronto General Hospital, of whom two thirds (7446) were used for the score development and the remaining third (3667) for the validation group. They looked at factors predicting the need for any allogenic blood transfusion. They identified 8 variables that were ultimately used in the score, which had all previously been used in various scoring systems. The score was then externally validated, with data from 5316 patients at another campus in Toronto. The TRUST score was shown to be reasonably accurate with an AUC of 0.8 described.

The Transfusion Risk and Clinical Knowledge (TRACK) score (M. Ranucci et al. 2009) was a simplified scoring system based on 5 factors that were shown to increase risk of transfusion in 8989 patients from a single institution. They based the score on the 5 variables that had been described in the literature as being most associated with risk of transfusion: pre-operative haematocrit, age, complex surgery, weight, and gender. They showed an AUC of 0.73 on the development set, with a slightly lower value (AUC = 0.71) on an external validation set of 2371 patients from another institution. While this score represented a major advantage in simplicity, and thus an implied improvement in utilisation and factuality, the accuracy was not a significant improvement from previous scores.

In 2015, Goudie and colleagues published a paper describing dual scoring systems that attempted to predict both the requirement for any transfusion, and severe blood loss (defined by large-volume blood transfusion, or LBVT) generated from 39970 patients from databases

in the UK and Italy. (Goudie et al. 2015) They also compared the performance of their scores to the BRiSc, TRACK and TRUST scores. They demonstrated in their dataset that the any-transfusion score showed an AUC of 0.77 although they acknowledged that the calibration was sub-optimal. This still compared favourably to the TRUST and TRACK scores, which both showed AUCs of 0.71 using the same data set, they also tested the scores against a total of 4 cardiac datasets and demonstrated a better AUC by around 5% in each. In their LVBT score, they described an AUC of 0.81 which compared favourably to the BRiSc score, with an AUC of 0.69. While the score showed itself to be accurate and more widely applicable than many that preceded it, it is significantly more complex than the preceding scoring systems and has remained relatively under-utilised. The shortcomings in the existing scoring systems

A summary of the existing scoring systems for predicting transfusion in cardiac surgery is outlined in the table overleaf:

Score	Cohort	Location	Outcome	No of variables	AUC	AUC external	AUC subsequent
Macgovern, 2006	2455	US Single centre	Any transfusion	15	0.78	0.78	
TRUST, 2006	11,113	Canada, single centre	Any transfusion	8	0.8		
TRACK, 2009	8989	Italy, single centre	Any transfusion	5	0.73	0.71	0.77(Leff et al. 2019)
BRiSC, 2011	11592	UK, single centre	Major bleeding	5	N/A	N/A	0.69(Goudie et al. 2015)
Goudie, 2015	39970	Multi-centre, UK & Italy	Any transfusion	24	0.77		
Goudie, 2015	39970	Multi-centre, UK & Italy	Large volume transfusion	24	0.81		

Table 2.2: Comparison of major transfusion risk scores in cardiac surgery

While these scores all retain reasonable accuracy, they vary in simplicity and applicability. The ideal scoring system for this purpose would be accurate, widely applicable, relatively simple (in terms of number of inputs) and practical to use by clinicians.

With this in mind, a new scoring system was developed and validated from a large multicentre UK audit, the process of which is described in the following chapter. In order to increase applicability, we then modified the risk score and re-calibrated it to Australasian data as described in chapter 4.

Chapter 3: Development of a scoring system to predict risk of RBC transfusion in cardiac surgery.

Although scoring systems already exist to predict the need for RBC transfusion in cardiac surgery, analysis of these existing scores highlights several common shortcomings. Firstly, developing a scoring system from a single-centre database has clear effects on its reproducibly, as demonstrated by the poor results in AUC analysis when these scores are applied to external data sets. Secondly, while a complex scoring system may improve accuracy, it creates challenges where certain data points may not be collected in some areas, thus providing challenges in testing the accuracy of the score and its applicability.
With this in mind, our group aimed to design a robust, simple integer-based scoring system with wide applicability using a national database collected for the Association of Cardio-Thoracic Anaesthetists (ACTA) audit. The development of the scoring system occurred largely prior the author's involvement, and statistical analysis was undertaken by Tim Collier (London School of Hygiene and Tropical Medicine). The author led the writing committee for the paper, co-ordinated the submission of the manuscript for publication and was responsible for the development of the subsequent online and app-based integration(Yeates 2018). The following is a description of the study methodology and results which were published in the BJA in 2017 and found in Appendix 1. (A.A. Klein et al. 2017)

Methods

The ACTA audit was a national service audit of NHS cardiac surgery centres, whereby data was collected on all cardiac surgery patients between 1st January 2010 and 31st July 2013. Data were collected from 10 UK centres that perform cardiac surgery and from this dataset, and an additional report was developed and published regarding the effect of anaemia on patient outcomes (A. A. Klein, Collier, Brar, Evans, Hallward, Fletcher, Richards, et al. 2016), as was discussed in chapter 2. Data from another centre during the same period were also collected and this was used as the dataset for the external validation. The score was then compared in its performance against some of the previously published scoring systems, using the same data. The design was approved by the Research and Ethics Committee of the London School of Hygiene and Tropical Medicine, and they concluded that individual patient consent was not required for the study.

The baseline data that were collected included gender, age, weight, height, preoperative [Hb], [creatinine], logistic EuroSCORE(S. A M Nashef et al. 1999), presence of diabetes or hypertension, type of surgery(CABG or single-valve procedures, combination procedures or other procedures) and a history of previous cardiac surgery. Height and weight figures were used to derive BMI and body surface area (BSA). The outcomes that were explored were number of units of blood transfused blood, duration of stay in ICU, duration of hospital stay and mortality. These were selected as they had previously been shown in other studies to be associated with the chosen outcomes. One of the aims of the study was to provide an integer-based scoring system, and as such, the continuous variables (age, [Hb], [creatinine], logistic EuroSCORE, BMI and BSA were assigned integer values based on clinical judgement. The use of EuroSCORE was unfortunate in that its use has now largely been superseded by the subsequent EuroSCORE II(Samer A M Nashef et al. 2012). This was unfortunately not being used at the time and was such unavailable in the collected data. The use of EuroSCORE as a variable was also somewhat controversial, as it is distinct scoring system used to predict mortality rather than transfusion, and further as it creates a score-within-a-score scenario. It was felt that its use was justified by its standard practice use in UK cardiac centres and by its strong association with risk of transfusion as was confirmed in the analysis. This may have implications on international applicability, as it not a scoring system used globally, though the input data are generally included in other scoring systems. Alternatively, the EuroSCORE component of the ACTA-PORT could potentially be substituted for any risk scoring system to predict local transfusion risk.

Each of these baseline variables was examined for univariate association with the outcome of need for any RBC transfusion using logistic regression. A multivariate logistic regression model was constructed using the forward and backward stepwise method and associations with a p value<0.05 were included in the final score. Both forward and backwards analyses resulted in the same model structure. Likelihood ratio tests were used to explore possible interactions from a number of pre-specified factors. It was our hope that the score was generalisable, and as such it was important that the centre from where the data was taken did not greatly affect the score's accuracy. This was examined comparing two logistic regression models where one included the centre as a random effect and the other did not. The overall performance of the model and the estimate odds ratios for each were very similar and as such it was felt that centre could be excluded entirely from the model as a variable without significantly impacting on its accuracy.

For each of the variables, the logistic odds ratio was multiplied by 0.2 and rounded to the nearest whole number to create an integer value. The reference point for each of these variables was set as the lowest possible risk group, so the score would be a simple addition of integer values, the sum of which would form the overall score. For final model, we calculated adjusted odds ratios, 95% confidence intervals and p-values, which were calculated from a likelihood ratio test. We then calculated the area under the receiver operating characteristics (ROC) curve (AUC) for the integer score, as well as the full model in order to test and compare their relative discriminatory capacity. The Hosmer-Lemeshow goodness-of-fit test was then utilised to compare predicted risk generated by the model, to the observed transfusion rate.

For each integer value of the scoring system, the predicted risk of transfusion was calculated, and these results were presented in both table and figure form, and then the 30 integer scores were grouped into 6 risk-categories (with 5-integer values each) and the predicted vs observed risk of transfusion was tabulated for each. The multiple imputation method was used test the score's resilience to missing datapoints.

The external validation, as mentioned, was performed on a dataset from an additional UK centre. For each of these patients, an integer score was calculated and the AUC and Hosmer-Lemeshow goodness-of-fit test used to test the performance of the score. Again, the score results were grouped into the 6 risk-categories and the predicted and observed transfusion rates compared. The same dataset was used as an independent external validation of the TRACK score, using the DeLong method to compare score-performance. This was unfortunately only able to be performed on the TRACK score, as the collected data was not adequate to fulfil the input requirements of the BRiSc, TRUST or Goudie scores.

Results

The baseline characteristics of the 20,036 patients in the derivation dataset are shown below, both in total and divided into those who received transfused and those who did not:

	All n=20,036	Not transfused n=11,041	Transfused n=8,638	p-value
Age; years	67.1 (11.9)	65.2 (12.0)	69.7 (11.3)	<0.001
Sex; men	14,303 (71.4%)	9,093 (79.8%)	5210 (60.3%)	<0.001
Pre-operative Hb; g/L Missing data	132 (17) 3237 (16.2%)	138 (15) 2077 (18.2%)	125 (17) 1160 (13.4%)	<0.001
Body surface area; m2	1.9 (0.2)	2.0 (0.2)	1.9 (0.2)	<0.001
Body mass index; kg/m2	28.4 (5.1)	29.0 (5.0)	27.6 (5.0)	<0.001
EuroSCORE Missing data	4.3 (2.1-8.7 (0.4-98.4)) 393 (2%)	3.2 (1.7-6.6 (0.4-98.4)) 238 (2.1%)	6.0 (3.1-11.7 (0.4-97.9)) 155 (1.8%)	
Creatinine; µmol/L Missing data	88 (71-106 (9-1547)) 2172 (10.8%)	88 (71-97 (9-1547)) 1254 (11%)	88 (71-106 (9-1450)) 918 (10.6%)	<0.001
Diabetes Missing data	3916 (22.0%) 2267 (11.3%)	2114 (20.7%) 1208 (10.6%)	1802 (23.8%) 1059 (12.3%)	<0.001
Hypertension Missing data	13,325 (67.8%) 384 (1.9%)	7,511 (67.2%) 224 (2.0%)	5814 (68.6%) 160 (1.9%)	0.04
Operation type				
CABG or valve*	14,575 (73%)	8,778 (77%)	5,797 (67%)	
Double procedure	2,858 (14%)	1,008 (9%)	1,850 (21%)	
Other Missing data	2,594 (13%) 9 (<0.1%)	1608 (14%) 7 (0.1%)	986 (11%) 2 (<0.1%)	<0.001

Table 3.1: Baseline characteristics in ACTA-PORT dataset. Values are mean (SD), number (proportion or median (IQR (range)).

* Indicates isolated CABG or single valve surgery.

As is indicated 8635 (43%) of the total group received blood transfusions, which was a rather high proportion compared to published global results. The overall average [Hb] was 132g/L

with an overall rate of anaemia of 31% using WHO criteria, although there was no recorded [Hb] in 16% of patients. More of the patients were male (71%) although there were relatively less in the transfused group. All of these variables were shown to be strongly associated (p-values less than 0.001) with the rate of transfusion on univariate analysis other than the documented diagnosis of hypertension, which barely reached statistical significance. As expected, rates of transfusion increased with increasing age, EuroSCORE and creatinine; were higher in females, diabetics and those undergoing combination procedures. Increasing [Hb], BSA and BMI were associated with a reduction in transfusion rates. As described in international literature, there was significant heterogeneity between centres with rates ranging between 31% and 56% and this is shown in Table 2 below. Interestingly, while the differences achieved statistical significance, they are less pronounced than in some for the international literature where transfusions rates have ranged enormously between centres.

Centre	All n=20,036	Not transfused n=11,401	Transfused n=8,638	Transfusion rate
A	2559 (13%)	1268 (11%)	1291 (15%)	50%
B	732 (3%)	425 (4%)	307 (4%)	42%
C	2058 (10%)	1410 (12%)	648 (8%)	31%
D	2371 (12%)	1233 (11%)	1138 (13%)	48%
E	5371 (27%)	3283 (29%)	2088 (24%)	39%
F	500 (3%)	292 (3%)	208 (2%)	42%
G	960 (5%)	423 (4%)	537 (6%)	56%
H	1986 (10%)	1029 (9%)	957 (11%)	49%
I	1099 (6%)	618 (5%)	481 (6%)	44%
J	2400 (12%)	1420 (12.5%)	980 (11%)	41%

Table 3.2: Transfusion results in ACTA-PORT dataset by anonymised centre. The difference in transfusion rates between centres was statistically significant ($p < 0.001$).

The significant variation in transfusion rate provides a challenge to any scoring system that aims to be widely applied. The causes of this variation are many and include complexity of surgery performed, pre-operative health of patients and institutional transfusion culture. It raises the question of whether a scoring system should correct for baseline transfusion rate in order to more accurately predict risk at each location. In the ACTA-PORT it was decided

not to correct for regional variation to maintain broader applicability, although this would naturally impact the specific accuracy of the score at locations on either extreme of practice. When the variables shown in Table 1 were analysed as independent variables, it was shown that a history of hypertension or diabetes were not able to predict the need for transfusion and that BSA was superior to BMI at predicting risk of transfusion. As such these were not included in the final score which was comprised of the remaining 7 variables. For each of these variables, the calculated odds ratio (with 95% confidence interval), including p-value and log odds ratio (with standard error) as well as the calculated integer value for each sub-group are outlined in Table 3.3 overleaf:

Characteristic	Category	Odds ratio (95%)		p-value	Log odds ratio (SE)		Points
Age; years	<70	Ref.					+0
	70+	1.11	(1.01, 1.21)	0.02	0.10	(0.04)	+1
Sex	Male	Ref.					+0
	Female	1.27	(1.15, 1.40)	<0.001	0.24	(0.05)	+1
Haemoglobin	<110	6.36	(5.38, 7.52)		1.85	(0.09)	+9
g/L	110-	4.60	(3.93, 5.38)		1.53	(0.08)	+8
	120-	3.19	(2.79, 3.65)		1.16	(0.07)	+6
	130-	1.93	(1.70, 2.20)		0.66	(0.07)	+3
	140-	1.55	(1.37, 1.77)		0.44	(0.07)	+2
	150+	Ref.		<0.001			+0
Body surface area	<1.7	3.62	(2.97, 4.42)		1.29	(0.10)	+6
m^2	1.7-	2.21	(1.85, 2.64)		0.79	(0.09)	+4
	1.9-	1.56	(1.31, 1.85)		0.44	(0.09)	+2
	2.1-	1.24	(1.04, 1.49)		0.22	(0.09)	+1
	2.3+	Ref.		<0.001			+0
EuroSCORE	<1	Ref.					+0
	1-	1.36	(1.10, 1.70)		0.31	(0.11)	+2
	2-	1.73	(1.39, 2.15)		0.55	(0.11)	+3
	3-	2.16	(1.75, 2.68)		0.77	(0.11)	+4
	9+	2.76	(2.20, 3.46)	<0.001	1.01	(0.12)	+5
Creatinine	<88	Ref.					+0
µmol/L	88-	1.33	(1.23, 1.44)		0.29	(0.04)	+1
	177-	1.93	(1.54, 2.42)	<0.001	0.66	(0.12)	+3
Operation type	CABG/Valve	1.38	(1.22, 1.55)		0.32	(0.06)	+2
	Combination	2.84	(2.46, 3.29)		1.05	(0.07)	+5
	Other	Ref.		<0.001			+0
Intercept		NA			-3.0	(0.15)	

Table 3.3: Multivariable Risk Score outlining corresponding odds ratios, log odds ratios and how ACTA-PORT score was constructed, showing the number of score-points that were attributed to each group.

The strongest predictors of transfusion risk were pre-operative [Hb], BSA and EuroSCORE (in that order), although the remaining variables, with the exception of age (which demonstrated a p-value of 0.02), were also strongly associated with RBC transfusion, with all other p-values being less than 0.001. The area under the ROC curve (AUC) was calculated as 0.76, with a 95% confidence interval of 0.752 to 0.768. The same calculations were performed on the non-integer-based scores, which were calculated using the log-odds ratios and these showed only a slightly higher AUC of 0.762, demonstrating that the gain in simplicity did not significantly compromise the accuracy of the score. The Hosmer-Lemeshow goodness-of-fit test was applied, with a p-value of 0.23 suggesting there was no evidence of a poor fit.

To calculate the ACTA-PORT score for any given patient, the integer value for each value is added together to give a score between 0 and 30, with a higher integer value predicting a higher risk of the patient requiring a transfusion.

For each integer value of the score, a corresponding predicted risk of transfusion was calculated, as shown in table 3.4 overleaf:

Integer risk score	Predicted risk of transfusion	Integer risk score	Predicted risk of transfusion
0	0.0470	15	0.500
1	0.0570	16	0.550
2	0.0690	17	0.599
3	0.0830	18	0.646
4	0.1000	19	0.690
5	0.1190	20	0.731
6	0.1420	21	0.769
7	0.1680	22	0.802
8	0.1980	23	0.832
9	0.2310	24	0.858
10	0.2690	25	0.881
11	0.3100	26	0.900
12	0.3540	27	0.917
13	0.4010	28	0.931
14	0.4500	29	0.943
15	0.5000	30	0.953

Table 3.4: Integer risk score totals and associated predicted risk of transfusion.

It demonstrates that low scores attract a very low risk of transfusion (i.e., a score of 1 gives a risk of transfusion as less than 5%) whereas a patient with a score of 30 would have more than a 95% risk of requiring a transfusion.

The risk of transfusion in the dataset was normally distributed throughout the group and the range of scores ranges from essentially zero to over 90%, with very small proportions of patients appearing at either extreme. The median score was 14, which represented a predicted risk of transfusion of 45%. This was tabulated and can be seen in figure 1 below: The distribution of risk scores in our population; shows that the distribution follows a relatively normal curve and superimposed is a line showing the increasing risk of transfusion associated with higher scores.

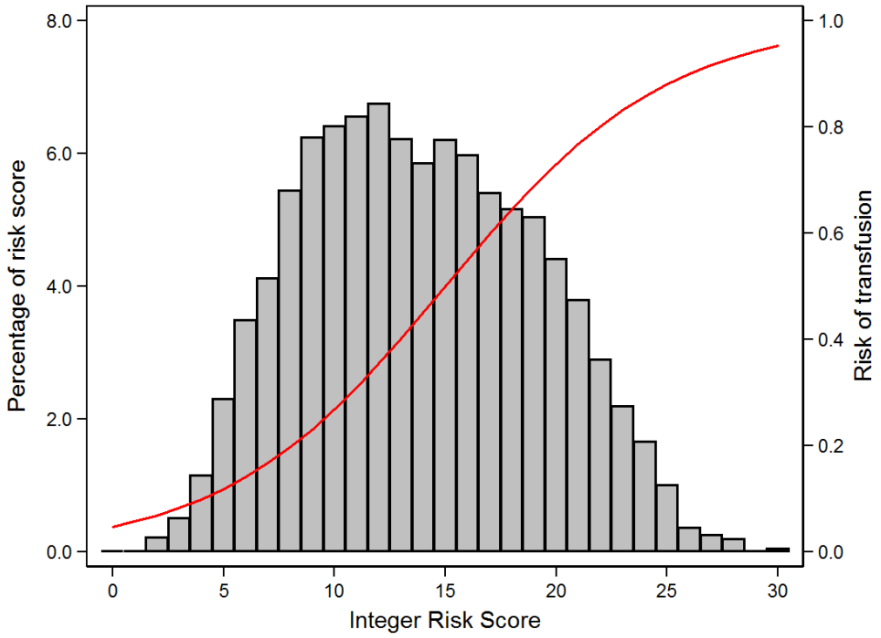

Figure 3.1: Distribution of ACTA-PORT risk scores

For each of the 6 broader risk categories, the predicted risk of transfusion matched quite well with the observed risk, as is demonstrated in figure 2 below:

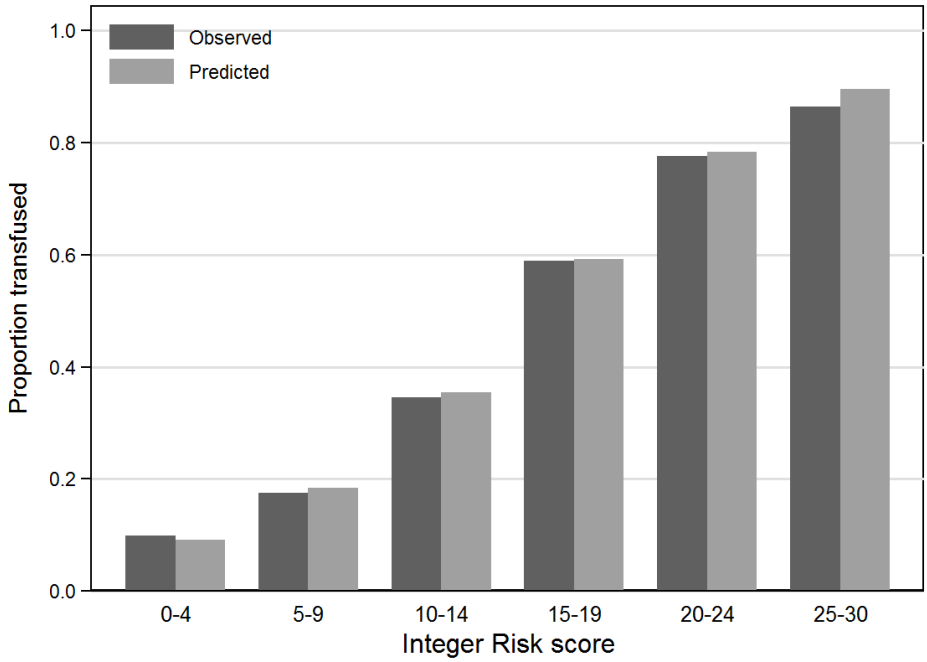

Figure 3.2: Observed vs predicted transfusion rates in the derivation dataset, demonstrates the close correlation between predicted and observed rates of transfusion using our score across the range of scores in our derivation dataset.

As can be seen, in the higher range of the integer score, there is a slight separation of the columns, as the score tends to underestimate the risk of transfusion in those in higher risk categories. Despite the excellent AUC of 0.835 (95% CI 0.810, 0.859) in the external validation, this effect was more pronounced in that group with a significant separation of values at higher risk scores. For example, in the 15-19 category, the predicted risk was 60% although 79% received transfusions and in the highest risk 73% were predicted to receive transfusions and 89% were observed to. Figure 3 below is the same graph in figure 2, using the date from the validation group.

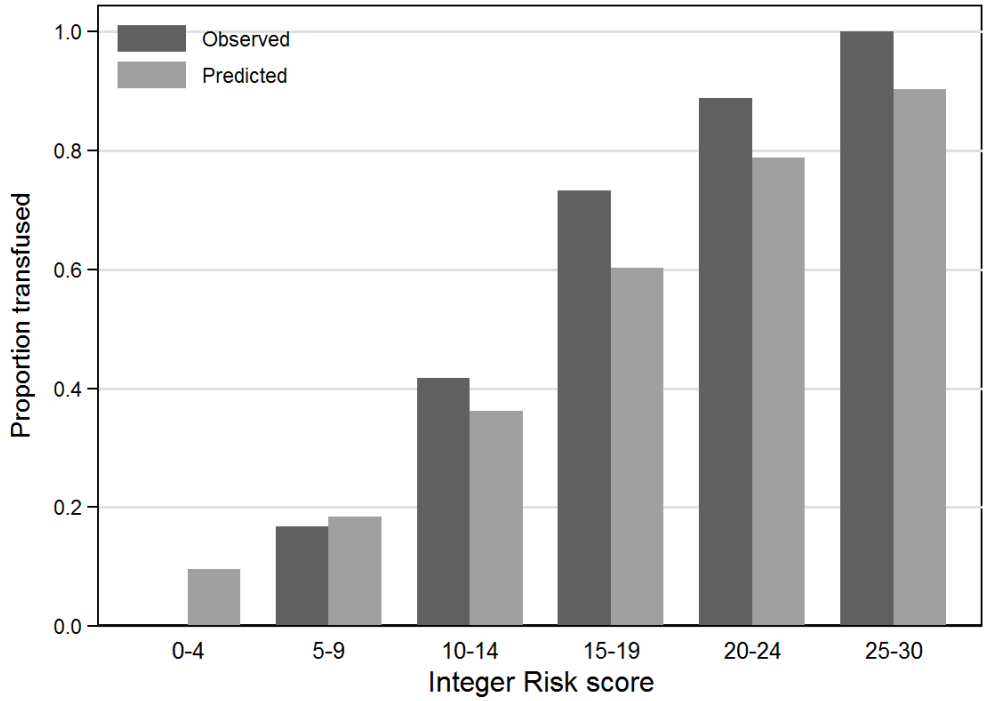

Figure 3.3: Observed and predicted risk of transfusion by group categories of the integer risk score in the external validation dataset.

There are many potential explanations for this discrepancy, including fact that the validation group was a single centre whose transfusion practices may vary from the larger cohort, consistent with the heterogeneity that has been demonstrated globally.
Although the ACTA-PORT score was intended to predict the risk of transfusion as a binary outcome, it was noted (not surprisingly) that an increasing score was associated with an increase in the number of RBC units that were transfused with an increase in median number of units ranging from 0 in the 0-14 range, to 1 in the 15-19 range and in in the 20-14 group

up to 3 in the 25-30 group. Using multiple imputation in the sensitivity analysis did not significantly affect the scoring system.

The predictive values of the score were calculated in the full range of the integer values and the optimum cut point was demonstrated to be 15, at which there was a positive predictive value (PPV) of 69.5% and negative predictive value (NPV) of 70.9%, which are both very respectable. At this value, 70.3% of scores accurately predicted the risk of transfusion.

Discussion

ACTA-PORT is by no means a revolution in transfusion-risk prediction, as many similar scores were in existence at the time of development and publication, but rather represented a significant improvement on existing scores in cardiac surgery. As demonstrated, it is accurate, importantly not only in a small external validation dataset, as many of the other scores, but also in a broader context, as demonstrated by the impressive performance using the Australian ANZCTS database as described in the following chapter. Where its accuracy is comparable to more complex scoring systems, such as the one described by Goudie et al, the ACTA-PORT score retains much of the simplicity achieved by less accurate scores such as the TRACK-score, and thus represents an improvement in functional-value that should result in increased uptake, and therefore practical value.

As was discussed after the publication of the ACTA-PORT score by correspondence in the British Journal of Anaesthesia, the scoring systems in place to predict transfusion are excellent, but despite their accuracy and theoretical usefulness, if uptake remains low, then they are unable to make any meaningful impact on patient care (Bartoszko and Karkouti 2017). The subsequent addition of online and app-based based access using the widely-used Calculate by RxMed© (Yeates 2018) represents a significant improvement in usability and it is hoped that this may translate to an increase in utilisation. The use of the scoring system pre-operatively aims to allow for the outcome-directed use of valuable PBM resources, with the aim of improving patient outcomes in an efficient manner, which is invaluable to an increasingly outcome-focussed health budget. Many pre- and intra-operative PBM strategies come at a significant cost and targeting these to the populations where they are needed most is the most efficient and sustainable of these precious resources. Specifically, those with a low ACTA-PORT (for instance below 15) could perhaps undergo surgery without a costly crossmatch being performed in advance and cell-salvage could be reserved for those with a score above a certain cut-off point. With increasing pressure on global health resources, this could prove to represent a significant improvement in resource allocation.

The performance of the score on a wider global population remains to be seen, but future work may focus on validating the score against other European and North American databases. Since the publication of the ACTA-PORT score, more transfusion-prediction scores have been developed including one developed and validated in India (Madhu Krishna et al. 2019) which was shown to perform better (AUC 0.749) than the TRUST score (AUC 0.72) although the TRACK score's performance was comparable (AUC 0.756). Another group subsequently demonstrated the TRACK score performed relatively well on a single-centre US study, with an AUC of 0.768(Leff et al. 2019).

Perhaps most importantly, while many of the risk factors that increase the ACTA-PORT score are unmodifiable, [Hb] remains as not only one of the strongest predictors of transfusion requirement but also perhaps the most easily modifiable. By improving the [Hb] of patient from 120g/L to 130g/L pre-operatively, the score suggests that reduction in transfusion could be as much as 40%. While this has obvious implications in resource use and associated cost, it could also translate into a significant reduction in per-operative morbidity and mortality.

As with all retrospective studies, there are significant limitations. PBM and transfusion practices have evolved significantly over the last decade and this may have a significant impact on the applicability of the score to current practise. The under-estimation of transfusion risk in higher-risk groups seen in the single-centre external validation dataset further highlights that transfusion practices are heterogenous. While this may represent inadequacy of statistical capacity, it is highly likely that it represents either a higher rate of transfusion than the average, which may be caused by surgeon, perfusionist, anaesthetist or intensivist preference in that centre. This could also represent a higher-complexity group of patients or type of surgery performed at that centre. It was decided that centre should not be included as a variable in the scoring system in order to maintain widespread applicability of the score, and while this discrepancy demonstrates the negative aspect of this approach, it may also represent a method of benchmarking transfusion practices by comparing their observed rates to the predicted rates generated by the national database.

Use of EuroSCORE, rather than EuroSCORE-II.

Finally, the score was developed from a dataset of only UK cardiac surgery patients, limiting broader applicability. In fact, all the scoring systems developed to predict transfusion requirement in cardiac surgery (including ACTA-PORT) have been derived from UK, US, Canadian or European populations and none have yet been developed or validated using Australian or New Zealand data. Despite similarities in clinical practice, risk prediction tools

derived from a UK population do not necessarily retain precision when directly applied to an Australasian population(Yap et al. 2006a; Campbell et al. 2019). Despite this, many northern hemisphere scoring systems are used in this region, without solid evidence of their accuracy. With this is in mind, after the publication of ACTA-PORT, in conjunction with colleagues from Sydney and Melbourne, the author set out to establish the accuracy of the ACTA-PORT score in Australian and New Zealand cardiac surgical patients and then adjust and recalibrate the score to suit locally available data. This process is described in the following chapter.

Chapter 4: Modification & validation of the ACTA-PORT score in cardiac surgical patients in Australia and New Zealand

Given that a scoring system to predict transfusion in cardiac surgery had never been validated in the southern hemisphere, and the excellent availability of high-quality data for this population, the author and a number of interested colleagues, decided to test the reliability of the ACTA-PORT score to the Australian and New Zealand population.

The data for this chapter was obtained from the Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) database, a comprehensive database that aims to continuously record data for all cardiac surgical procedures in the region and currently collects data from 26 public and 32 private hospitals with approximately 15000 procedures each year. At time of writing, it had captured data for a total of 170,103 procedures since its inception, providing an excellent resource for research.

The EuroSCORE is not routinely used in this region, and the ANZSCTS database does not routinely collect all the data-points required to retrospectively calculate a EuroSCORE. This required some modification of the ACTA-PORT score prior to validation. Firstly, a previously described local modification of the EuroSCORE (Yap et al. 2006b) was used as an input and tested. In order to create a more usable system for the given population, the ACTA-PORT score was modified, with the EuroSCORE component being replaced with the equivalent local cardiac surgical risk scoring system, the AusSCORE (Billah et al. 2014). This was then recalibrated and validated using the data to form a new Australasian scoring system, the AntiPORT score. External validation was then performed on a pre-selected cohort and the results showed the new score retained the accuracy of the original score in our population. This concept was developed in conjunction with Tim Coulson (Alfred Hospital & Monash University, Melbourne) who coordinated the statistical analysis and contributed to the paper, and Lachlan Miles (Austin Health & University of Melbourne) who contributed to the paper, which has been submitted for publication. Team members from the ANZSCTS database (Jenni Williams-Spence, Michael Bailey and Chris Reid) contributed to the paper, and Kate Blatchford contributed to the paper and coordinated the submission process. The final copy submitted for publication to *Anaesthesia & Intensive Care* is found in appendix 2.

Methods

We used the ANZSCTS database to identify an appropriate validation cohort. Data from all cardiac surgery procedures performed between 1 September 2016 and 31 December 2018 was used in the analysis. The database currently collects peri-operative data for all patients undergoing cardiac surgery across 26 public and 30 private hospitals in the region. Since mid-2016 data collection has routinely included pre-operative [Hb]. The table overleaf shows the components of the ACTA-PORT score as described in chapter 3. With the exception of EuroSCORE, all other data is collected routinely in the ANZSCTS database.

Characteristic	Category	Odds ratio (95%)		p-value	Log odds ratio (SE)		Points
Age; years	<70	Ref.					+0
	70+	1.11	(1.01, 1.21)	0.02	0.10	(0.04)	+1
Sex	Male	Ref.					+0
	Female	1.27	(1.15, 1.40)	<0.001	0.24	(0.05)	+1
Haemoglobin	<110	6.36	(5.38, 7.52)		1.85	(0.09)	+9
g/L	110-	4.60	(3.93, 5.38)		1.53	(0.08)	+8
	120-	3.19	(2.79, 3.65)		1.16	(0.07)	+6
	130-	1.93	(1.70, 2.20)		0.66	(0.07)	+3
	140-	1.55	(1.37, 1.77)		0.44	(0.07)	+2
	150+	Ref.		<0.001			+0
BSA	<1.7	3.62	(2.97, 4.42)		1.29	(0.10)	+6
m^2	1.7-	2.21	(1.85, 2.64)		0.79	(0.09)	+4
	1.9-	1.56	(1.31, 1.85)		0.44	(0.09)	+2
	2.1-	1.24	(1.04, 1.49)		0.22	(0.09)	+1
	2.3+	Ref.		<0.001			+0
EuroSCORE	<1	Ref.					+0
	1-	1.36	(1.10, 1.70)		0.31	(0.11)	+2
	2-	1.73	(1.39, 2.15)		0.55	(0.11)	+3
	3-	2.16	(1.75, 2.68)		0.77	(0.11)	+4
	9+	2.76	(2.20, 3.46)	<0.001	1.01	(0.12)	+5
Creatinine	<88	Ref.					+0
µmol/L	88-	1.33	(1.23, 1.44)		0.29	(0.04)	+1
	177-	1.93	(1.54, 2.42)	<0.001	0.66	(0.12)	+3
Operation	CABG/Valve	1.38	(1.22, 1.55)		0.32	(0.06)	+2
	Combination	2.84	(2.46, 3.29)		1.05	(0.07)	+5
	Other	Ref.		<0.001			+0
Intercept		NA			-3.00	(0.15)	

Table 4.1: Multivariable Risk Score outlining corresponding odds ratios, log odds ratios and how ACTA-PORT score was constructed, showing the number of score-points that were attributed to each group.

Integer risk score	Predicted risk of transfusion	Integer risk score	Predicted risk of transfusion
0	0.0470	15	0.500
1	0.0570	16	0.550
2	0.0690	17	0.599
3	0.0830	18	0.646
4	0.1000	19	0.690
5	0.1190	20	0.731
6	0.1420	21	0.769
7	0.1680	22	0.802
8	0.1980	23	0.832
9	0.2310	24	0.858
10	0.2690	25	0.881
11	0.3100	26	0.900
12	0.3540	27	0.917
13	0.4010	28	0.931
14	0.4500	29	0.943
15	0.5000	30	0.953

Table 4.2: ACTA-PORT Integer risk score totals and associated predicted risk of transfusion. It demonstrates that low scores attract a very low risk of transfusion (i.e., a score of 1 gives a risk of transfusion as less than 5%) whereas a patient with a score of 30 would have more than a 95% risk of requiring a transfusion.

ACTA-PORT used EuroSCORE as a surrogate for operative mortality. This is not routinely calculated for cardiac surgery patients in Australasia and was thus not immediately available in the data. Some of the EuroSCORE components differed slightly from components collected in the ANZSCTS database, and one (systolic pulmonary artery pressure) was not available at all. We therefore calculated EuroSCORE I (excluding pulmonary artery pressure) based on the closest available variables, as has been previously described by Yap et al. (Yap et al. 2006b). To make future calculations easier using Australian data we subsequently replaced the EuroSCORE mortality prediction ranges in ACTA-PORT with an equivalent Australasian risk prediction score developed from the ANZSCTS database. This score is known as AusSCORE (originally developed for use in CABG surgery only) or 'all procedures

score' (the latter developed subsequently and used for any surgery type)(Reid et al. 2009b)(Billah et al. 2014). In the absence of previously documented techniques of finding equivalent score ranges, we calculated the interquartile range of the AusSCORE in our population for each of the five possible categories of EuroSCORE included in the original ACTA-PORT score. Approximations of these interquartile ranges of AusSCORE replaced the EuroSCORE categories in the new model. These two techniques in turn yielded two scores:

(1) the original ACTA-PORT score using the calculated EuroSCORE (the ACTA-PORT-ES),
(2) the original ACTA-PORT score using the AusSCORE ranges to replace EuroSCORE (the ACTA-PORT-AS). Discrimination and calibration of each of these two scores was then assessed.

Finally, given that it is likely transfusion practices will vary across the two populations, it is also likely that the calibration of the score will be affected. We determined *a priori* that we would recalibrate the score using data derived from the local population. In order to achieve this, the dataset was split randomly into two populations (by hospital) in an approximate 75:25, resulting in a training set and validation set. Logistic regression was carried out in the training set using allogeneic red cell transfusion as the outcome and the ACTA-PORT-AS as the independent variable. Predictions based on this logistic regression were generated for the validation set using the ACTA-PORT-AS integer score. We termed this the 'AntiPORT' recalibration (Antipodean Peri-Operative Risk of blood Transfusion). Discrimination and calibration were then assessed in the validation set.

Baseline characteristics were compared between those patients transfused and those not transfused using Chi-square for categorical data, Student's t-test for normally distributed data and Wilcoxon Rank-Sum for non-normally distributed data. Discrimination of AntiPORT was assessed using the area under the receiver operator characteristic (ROC) curve. Calibration was assessed using calibration plots. Both were assessed using the Brier score. All analyses were carried out by Dr Tim Coulson using Stata version 16.1(StataCorp LLC 2019).

Results

Data from 30,393 patients from 37 hospitals was analysed and the baseline characteristics are displayed in Table 4.3 below:

Columns by RBC transfusion	No RBC	RBC	Total	P-value
n (%)	20358 (67.0)	10030 (33.0)	30388 (100.0)	
Age, median (IQR)	66 (16)	69 (17)	67 (16)	0.00
Sex, n (%)				
Male, n (%)	16247 (79.8)	6427 (64.1)	22674 (74.6)	
Female, n (%)	4111 (20.2)	3603 (35.9)	7714 (25.4)	0.00
Procedure type, n (%)				
0, n (%)	2 (0.0)	0 (0.0)	2 (0.0)	
CABG, n (%)	10991 (54.0)	4367 (43.5)	15358 (50.5)	
Valve surgery, n (%)	4769 (23.4)	1948 (19.4)	6717 (22.1)	
Combined CABG/Valve, n (%)	1445 (7.1)	1384 (13.8)	2829 (9.3)	
Other, n (%)	3151 (15.5)	2331 (23.2)	5482 (18.0)	0.00
Preoperative [Hb], median (IQR)	142 (19)	126 (29)	138 (24)	0.00
Preoperative [Cr], median (IQR)	84 (27)	89 (42)	85 (31)	0.00
Diabetes, n (%)				
No diabetes, n (%)	14605 (71.8)	6680 (66.6)	21285 (70.1)	
Diabetes, n (%)	5731 (28.2)	3343 (33.4)	9074 (29.9)	0.00
Hypertension, n (%)				
No hypertension, n (%)	6064 (29.8)	2689 (26.8)	8753 (28.8)	
Hypertension, n (%)	14272 (70.2)	7332 (73.2)	21604 (71.2)	0.00
NYHA status, n (%)				
1, n (%)	8740 (43.0)	3489 (34.8)	12229 (40.3)	
2, n (%)	7702 (37.9)	3515 (35.1)	11217 (36.9)	
3, n (%)	3306 (16.3)	2217 (22.1)	5523 (18.2)	
4, n (%)	596 (2.9)	803 (8.0)	1399 (4.6)	0.00
Estimated Ejection Fraction, n (%)				
Normal, n (%)	10173 (50.8)	4779 (48.8)	14952 (50.2)	
45-60%, n (%)	6862 (34.3)	2844 (29.1)	9706 (32.6)	
30-45%, n (%)	2368 (11.8)	1453 (14.8)	3821 (12.8)	
<30%, n (%)	604 (3.0)	710 (7.3)	1314 (4.4)	0.00

BMI, median (IQR)	29 (7)	27 (7)	28 (7)	0.00
Urgency, n (%)				
Non-urgent, n (%)	14969 (73.5)	5872 (58.6)	20841 (68.6)	
Urgent, n (%)	4907 (24.1)	3245 (32.4)	8152 (26.8)	
Emergency, n (%)	468 (2.3)	860 (8.6)	1328 (4.4)	
4, n (%)	12 (0.1)	51 (0.5)	63 (0.2)	0.00
Previous cardiac surgery, n (%)				
No, n (%)	19368 (95.2)	8862 (88.4)	28230 (92.9)	
Yes, n (%)	985 (4.8)	1163 (11.6)	2148 (7.1)	0.00
Perfusion time, median (IQR)	91 (49)	112 (75)	97 (57)	0.00
Mortality, n (%)				
Survived, n (%)	20262 (99.5)	9504 (94.8)	29766 (98.0)	
Died, n (%)	96 (0.5)	526 (5.2)	622 (2.0)	0.00

Table 4.3: Baseline characteristics in ANZSCTS cohort used to construct the AntiPORT score

Five patients (0.02%) had missing red cell transfusion data and were excluded, resulting in a final analysis cohort of 30388 patients. Of these, a total of 10,030 (33%) patients were transfused. Age, female sex, NYHA class 4 status, diabetes mellitus, hypertension and elevated creatinine were positively associated with risk of transfusion following univariate analysis. Additionally, patients undergoing combined surgery or emergency surgery were more likely to require transfusion. BMI and pre-operative [Hb] were both negatively associated with transfusion risk.

The range of EuroSCORE used in ACTA-PORT versus the corresponding AusSCORE are shown in Table 4.4 below:

EuroSCORE	AusSCORE	ACTA-PORT score value
<1	-3 to 0	0
1	1 to 3	3
2	1 to 3	3
3-8	4 to 9	4
9+	10+	5

Table 4.4: Assigned EuroSCORE vs. AusSCORE equivalents for the purposes of calculating the ACTA-PORT score

Due to the significant overlap between a EuroSCORE of 1 and 2 these two categories were combined. Assigned ACTA-PORT scores for AusSCORE based on these equivalents are shown in Table 4.4. The resultant integer AntiPORT scoring system is shown in Table 4.5 below:

Characteristic	Category	Points
Age; years	<70	+0
	70+	+1
Sex	Male	+0
	Female	+1
Haemoglobin	<110	+9
g/L	110-	+8
	120-	+6
	130-	+3
	140-	+2
	150+	+0
Body surface area	<1.7	+6
m^2	1.7-	+4
	1.9-	+2
	2.1-	+1
	2.3+	+0
AusSCORE	<1	+0
	1-3	+3
	4-9	+4
	10+	+5
Creatinine	<88	+0
µmol/L	88-	+1
	177-	+3
Type of Operation	CABG/Valve	+2
	Combination	+5
	Other	+0

Table 4.5: The AntiPORT score

Discrimination in the Australian dataset was comparable to the UK dataset with an AU-ROC for ACTA-PORT-ES and red cell transfusion of 0.76 (0.75-0.76, n=30,071), and for ACTA-PORT-AS of 0.76 (0.75-0.76, n=29,487). Brier scores were 0.19 for both. Calibration

was poorer with overprediction of transfusion on calibration plots (ACTA-PORT-AS calibration plot in figure 2).

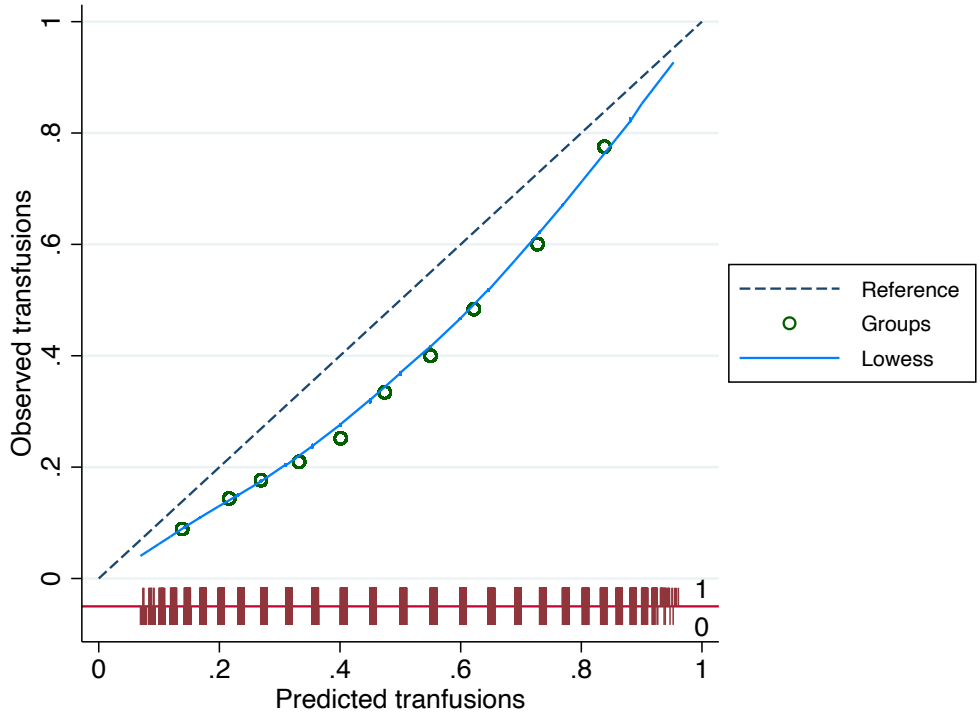

Figure 4.1: Observed vs predicted transfusions demonstrating over-prediction of transfusion requirement requiring re-calibration

Splitting the dataset yielded a training set (n = 22,412, comprising 28 hospitals) and a validation set (n =7981, comprising 9 hospitals). Logistic regression in the training set showed ACTA-PORT-AS was strongly associated with red cell transfusion (OR 1.22, 1.21-1.23, p<0.001, n=21,743). AU-ROC in the validation set was 0.76 (0.75-0.77, n=7744). Brier score was 0.18. Calibration was improved, as shown in Figure 3 overleaf:

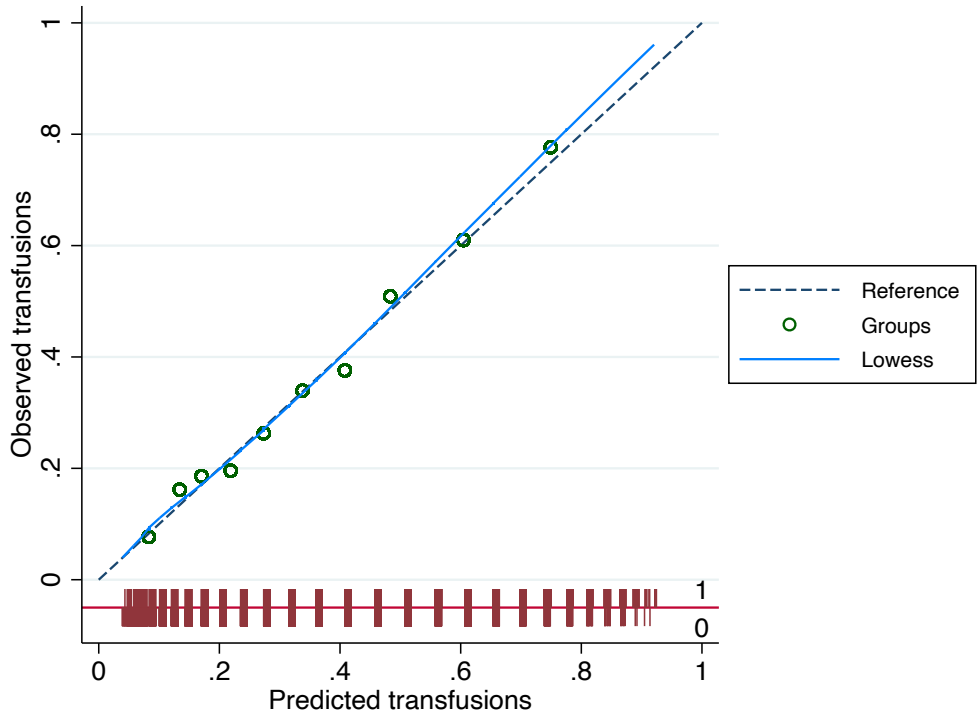

Figure 4.2: Observed vs Predicted transfusion following re-calibration demonstrating much improved agreement

The predicted rate of transfusion based on the AntiPORT recalibration is shown in Table 4.6 overleaf:

AntiPORT score	Predicted risk of transfusion	AntiPORT score	Predicted risk of transfusion
2	0.0393398	16	0.4080969
3	0.0477112	17	0.4575633
4	0.0577569	18	0.5078807
5	0.0697628	19	0.558039
6	0.0840418	20	0.6070406
7	0.1009262	21	0.6539779
8	0.1207557	22	0.6980972
9	0.1438578	23	0.7388374
10	0.1705224	24	0.7758453
11	0.2009692	25	0.808965
12	0.2353102	26	0.8382115
13	0.2735105	27	0.8637347
14	0.3153549	28	0.8857802
15	0.360425	29	0.9046528
16	0.4080969	30	0.9206862

Table 4.6: Predicted risk of transfusion (the AntiPORT recalibration) corresponding to the original ACTA-PORT integer score

Discussion

The AntiPORT score represents a retrospective validation and calibration of the previously developed ACTA-PORT score to an Australasian cohort. As discussed earlier in the chapter, the ANZSCTS database does not routinely collect all of the datapoints required to calculate the EuroSCORE, moreover, the EuroSCORE has been shown to significantly overpredict mortality in Australian cardiac surgical practice(Yap et al. 2005, 2006a). While modifying the inputs for EuroSCORE to available data allowed for good discrimination, it was decided to modify the score to allow input of a local risk scoring system, the AusSCORE. Using either mortality score as an input to the ACTA-PORT score yielded good results in terms of the new score's discriminative capacity but with a significant calibration mismatch. It was considered more likely to have local clinical relevance if a region-specific scoring system was used, particularly as this scoring system is routinely calculated for most cardiac surgical procedures. Re-calibrating the AntiPORT score using the AusSCORE as an input resulted in a scoring system that had good discrimination, was well calibrated and performed well on external validation using ANZSCTS data from a 9-hospital cohort with an AU-ROC of 0.76

The AntiPORT retains the potential uses and benefits of the original ACTA-PORT score on which it was based. The modification and re-calibration of the ACTA-PORT score broadens it applicability and allows clinicians in Australia to use it with stronger confidence in its predictive value. As with the ACTA-PORT, the ability to predict those with higher risk of transfusion allows for better use of expensive and resource-intense PBM strategies, which can have benefits in terms of risk-avoidance but also has potential health-economic benefits. Having any transfusion scoring system can facilitate direct comparison between centres, allowing for benchmarking of transfusion practices. By having a score that can be directly compared to an international standard, this could allow for benchmarking against an international cohort. It also has implication in future trial design by allowing researchers to screen patients using a common 'risk prediction language' , as was demonstrated recently by Wong et al. who validated multiple risk prediction tools as part of an international cohort study (Danny J. N. Wong et al. 2020). Additionally, from the perspective of the bedside clinician, applying the results of clinical trials from other countries to the individual patient is certainly easier if the risk prediction models used in the trial have been validated in the local population first. Consequently, the transformation of ACTA-PORT to 'AntiPORT' could allow common enrolment of patients in Australasia and the UK into trials that attempted to recruit patients at a high risk of receiving allogeneic blood transfusion as part of cardiac surgery, or alternatively, allow a clinician in the UK to apply the results of an Australian trial to their individual practice (once the appropriate recalibration factor was considered). Critically, both ACTA-PORT and AntiPORT use data which is collected routinely for the purposes of clinical audit (including the EuroSCORE in the UK and AusSCORE in Australia), raising the possibility of using these scores as automatically calculated metrics to screen patients for embedded clinical trials.

While there are similarities between UK and antipodean cardiac surgical and transfusion practices, previous studies that have attempted to adapt risk prediction scores developed in the northern hemisphere to Australasian patients have shown that this process is not simple. A recent study by Campbell et al. that attempted to adapt a surgical risk prediction score from the UK to a New Zealand cohort showed that an unadjusted model under-predicted mortality by a factor of five (Campbell et al. 2019). In cardiac surgery, the commonly used EuroSCORE model was found by Yap et al. to significantly over-estimate mortality in a representative cohort of Australian patients drawn from six hospitals (Yap et al. 2006a), necessitating the development of a prediction model that was trialled and validated in a local patient cohort – the AusSCORE (Reid et al. 2009a).

We used a large national database incorporating multiple surgical units. A common criticism of external validation of scoring systems is that the data is taken from the same source as the derivation dataset. In the AntiPORT, the external validation cohort selected randomly by surgical unit, rather than using individual patient data. This suggests the score retains accuracy across surgical units with differing practices. In fact, AntiPORT is calibrated against a much larger group of hospitals (37 centres) than the original ACTA-PORT score (12 centres), which could suggest even wider applicability than its parent score. It is notable that PBM and transfusion practices have evolved significantly over the last decade, impacting on the applicability of the score to current practice. It is likely that future recalibrations will be necessary as patient risk profile and medical practice changes. Nonetheless, the relative recency of the dataset (from late 2016 to the end of 2018) suggests practice is likely to be relevant. One criticism that could be levelled at the methodology is that it is likely that given the global heterogeneity of transfusion practices, and hence risk scoring systems, that it may be more accurate to design a risk score from scratch. While this may be true, it was considered more valuable that the score be able to be directly compared to a comparable international standard, as discussed.

The AntiPORT score is an accurate scoring system to predict peri-operative blood transfusion in patients undergoing cardiac surgery in Australia and New Zealand. The score has a number of potential uses, including the effective allocation of PBM resources, as a quality control initiative as part of a program of comprehensive audit and as a means of achieving consistent appreciation of risk in patients enrolled in international clinical trials of transfusion practice across different countries. As with the ACTA-PORT, the AntiPORT score is a clinically useful transfusion-prediction score that can be utilised easily at the bedside by clinicians using the popular QxMD software package including an online calculator and the popular "Calculate by QxMD" app for mobile devices. The hope is that this may allow the score to have practical value to clinicians in guiding patient care.

A scoring system to predict the need for transfusion is of little value as a purely academic exercise. In order for such scoring systems to have any meaningful clinical relevance, they must allow clinicians to identify those who may benefit most from pre- and intra-operative interventions. As previously discussed, and further highlighted in the development of the ACTA-PORT and AntiPORT scores, the most easily modifiable risk factor for peri-operative transfusion is pre-operative [Hb]. The following chapter describes an attempt to establish the feasibility and effectiveness of optimising [Hb] in the available timeframe prior to cardiac surgery using IV iron to rapidly optimise iron stores.

Chapter 5: Pre-operative IV Iron in Cardiac surgery and effect on [Hb], patient outcomes and the feasibility of provision of this service

Given that pre-operative [Hb] is the most easily modifiable risk factor for peri-operative transfusion identified by the described scoring systems, and that blood transfusion has been shown to negatively impact on surgical morbidity and mortality, it follows that if [Hb] can be optimised prior to surgery, there is potential to improve patient outcomes. As described in chapter 2, the most common cause of pre-operative anaemia is iron-deficiency and there is generally inadequate time prior to surgery to improve iron stores with oral preparations. As IV iron has the capacity to rapidly improve iron stores, if given early enough it seems logical that it should have an impact on haematopoiesis and result in an improvement in [Hb]. If this rise is adequate to prevent or reduce transfusion, a resultant improvement in outcomes should follow. As discussed in chapter 2, IV iron has been shown to improve a number of outcomes in non-surgical patients and appears to improve [Hb] reasonably consistently in anaemic patients, may reduce blood transfusions(Elhenawy et al. 2021) and limited evidence suggests it may improve some outcomes in surgical candidates(Froessler et al. 2016) so it would be hoped that this would apply to cardiac surgery.

There remain hypothetical risks, and significant cost associated with giving IV iron pre-operatively and as such this should not become standard-of-care without high-quality evidence to demonstrate that it does indeed improve not only physiological parameters, but also patient outcomes. A review of the topic, published in 2014(Hogan et al. 2014) identified 16 trials that addressed the link between pre-operative anaemia and cardiac surgical outcomes, and demonstrated a relatively close correlation. At that time, only 4 trials had been published examining the effect of perioperative IV iron in cardiac surgery patients. And concluded that all four demonstrated 2C level evidence of an impact on [Hb] levels. One trial, which combined IV iron sucrose and rhEPO over a 4-week period prior to valve surgery, demonstrated an improvement in transfusion rates, length of hospital stay and mortality(Cladellas et al. 2012). Although the results were impressive, it was a small study, with only 75 receiving the treatment and with multiple potential confounding factors as it was a consecutive cohort analysis. While this limited evidence is promising, it is probably inadequate to recommend a widespread change in practice, is a relatively small subset of cardiac surgery patients and raises a number of practical issues. Specifically, cardiac surgery is

often undertaken in a semi-urgent manner and it is common for the period for time between listing for surgery and the operation to be far shorted than 4 weeks. In addition, diagnosis treatment of cardiac surgical patients with IDA in the limited timeframe before surgery is challenging, for various local and systemic logistical reasons(M. Muñoz et al. 2015; Kotzé et al. 2015). Further, the practical challenges of administering IV iron in 5 doses over 4 weeks are potentially enormous and require significant resources, with many obstacles that may prevent effective treatment. With this in mind, our group of interested consultants from the Association of Cardiothoracic Anaesthetists (ACTA) anaemia audit (Klein, Collier, Brae, Evans, Hallward, Fletcher, Richards, et al. 2016)., designed a multi-centre prospective trial which aimed to identify whether treatment with a single dose of IV iron, up until 10 days pre-operatively, had the capacity to improve [Hb] prior to surgery and looked for any impact of this treatment on transfusion rates and a variety of measures of surgical morbidity and mortality. To increase the practical applicability of the results, our study used a shortened and simplified treatment regime. One of the greatest difficulties in the provision of this treatment is the establishment of diagnostic and therapeutic pathways to allow for this treatment, so the study also served as a feasibility assessment for the establishment of such pathways. The CAVIAR-UK study was designed to assess this feasibility and explore any impact such treatment might have on [Hb] and whether any improvement in [Hb] might be associated with a significant change in transfusion rate or morbidity outcomes. The author's involvement with CAVIAR-UK began subsequent to the design of the trial where he was an investigator at Papworth Hospital and lead investigator of the total-Hb mass substudy. He led the writing committee which involved writing the draft manuscript and co-ordinating the revisions, submission and eventual publication of the paper in the BJA in 2020, a reproduction of which is found in appendix 3 (Andrew A Klein et al. 2020). The published manuscript from the same study, focussing on vascular surgery patients is included in appendix 4, as the author contributed to the manuscript, although it is not discussed in further detail in this thesis.

Methods

The UK Cardiac and Vascular Surgery Interventional Anaemia Response (CAVIAR-UK) study was a stepped observational pilot and feasibility study in 11 UK cardiac surgical centres (from a total of 19 nationally) who had previously expressed interest in establishing pre-operative anaemia treatment pathways after the ACTA audit. UCL was the trial sponsor and funding obtained from the National Institute of Academic Anaesthesia (NIAA) via grants from the Association of Cardiothoracic Anaesthesia and Critical Care (ACTACC), the Vascular Anaesthesia Society of Great Britain and Ireland (VASGBI) and the British Journal of Anaesthesia (BJA). Pharmacosmos A/S, manufacturers of Monofer® provided additional funding for personnel.

The study was approved nationally by the UK Ethics Committee (ref 15/LO/1569, IRAS 188848) and each centre sought local approval to establish treatment pathways via national and local NHS standard procedures. Existing protocols and literature were shared amongst centres as they were developed, and core members of the study group visited each site and provided educational briefings. Centralised meetings were held with investigators on a regular basis where ongoing issues were addressed, and regular communication was maintained between these meetings via newsletters and email communication.

The trial was a prospective observational study of adult patients awaiting elective cardiac surgery (CABG, valve surgery or both). Using the standard WHO definition of anaemia (<120g/L in females and <130g/L in males), subjects were divided into anaemia and non-anaemic groups and the anaemia group was further divided into those that received treatment with IV iron and those that, for any reason, did not. Pregnant or lactating females were secluded from the trial as were renal dialysis patients, prisoners and any subjects lacking mental capacity to properly consent for the trial. The protocol was published in advance of commencement of recruitment (Chau et al. 2017)

The CAVIAR-UK sub-study, of which the author led the cardiac surgery arm, aimed to recruit a further subset of the treatment group to be tested before and after IV iron treatment for total Hb-mass, CPET or 6-minute walk test. The total-Hb mass was to be assessed in the Papworth Hospital respiratory function using the Schmidt & Prommer CO-rebreathing technique (Schmidt and Prommer 2005) described in detail in chapter 6. Consent for the sub-study was sought in suitable candidates and additional exclusion criteria were applied to the sub-study including those with NYHA III or IV symptomatic heart failure, current smokers, or those with severe lung disease.

Recruitment commenced in April 2016 and continued until March 2018 although commencement date was not the same at all centres due to approvals or delays in setting up treatment pathways. Those consenting to inclusion on the trial were tested for [Hb] and full iron studies. Those who qualified for IV iron treatment, by having a [ferritin] <100 and a transferrin saturation (TSAT)<20% greater than 10 days pre-operatively were given a standard dose of either iron isomaltoside 1000 (Monofer®, Pharmacosmos A/S) at a total dose calculated at 20mg/kg or ferric carboxymaltose (Ferrinject®, Vifor Pharma UK), to a maximum of 1000mg, both by infusion over at least 15-30 minutes according to local policy. Treated patients were observed for a total of 45-60 minutes during and after infusion and monitored for significant changes in ECG, Sp02 and NIBP. Any subjects who, for whatever reason were not able to receive IV iron formed the "anaemic non-treated" group, and those who were non-anaemic formed the control group. Those that received IV iron treatment were followed up on the day of surgery for re-testing of [Hb] and Iron studies and change in [Hb] pre- and post-IV iron was the primary outcome measure. Secondary outcome measures for the efficacy part of the trial included mortality, DAH-30 (days-alive-and-at-home, a composite outcome measure of 30-day mortality and length of hospital stay(Myles et al. 2017)), ICU and total hospital length of stay, renal function, peri-operative change in [Hb] and total number of RBC units transfused. Those receiving transfusions of more than 4-units of RBS were excluded as this was considered frequently to be a result of unpredictable surgical misadventure and not represent the intended focus of the investigation.

Statistical analysis was performed by a member of the study-group, Tim Collier of the London School of Hygiene and Tropical Medicine.

Sample size was calculated based on a pre-operative 10g/L increase in [Hb] in the treatment group, which was considered a clinically significant rise. Using a standard deviation of 12g/L from the ACTA audit(A. A. Klein, Collier, Brar, Evans, Hallward, Fletcher, Richards, et al. 2016), with 5% significance, and allowing for 10% loss to follow-up, it was calculated that 62 anaemic patients would provide 80% power to detect a treatment effect. 72 patients would provide 90% power to detect the [Hb] change.

Mean and standard deviation or median and interquartile range were used for continuous variables depending on their distribution. For categorical variables, frequencies (n) and percentages were used. To compare baseline characteristics across the three groups of patients, chi-square tests were used for categorical variables and F-tests (if normally distributed) or Kruskal-Wallis rank test (if not normally distributed) for continuous variables.

A one-sample t-test (including 95% CI and p-value) were used to compare the pre- and post-treatment mean [Hb] for the primary outcome measure, and the pre- and post-operative [Hb] change. Transfusion outcome measures were analysed using multiple logistic regression. Length of stay and DAH-30 were compared using the Wilcoxon rank sum test. Mortality and hospital readmissions were compared between groups using the chi-squared test.

Results

The attempts at establishing clinics and/or treatment pathways for IV iron in anaemic pre-operative cardiac surgery patients were mixed. In seven of the eleven centres, these pathways were successfully established allowing for ongoing involvement in the interventional arm of the study. Of the four that were unable to institute such a service, two were prevented doing so by the failure to gain approval for the use of IV iron by the pharmacy department and a further two were unable to have the business case approved by the local NHS trust. This was not surprising given our experience at Papworth Hospital, where the process was challenging; at one point during the study, the anaemia clinic was de-funded and only with significant pressure from senior clinicians and researchers was it allowed to re-open.

In total, 228 subjects were recruited in the 2-year study period, of which 136 (60%) were anaemic. Of these, 72 (32% of total) were treated with IV iron and 64 (28%) remained un-treated. There was significant variability in each centre's ability to recruit patients with the breakdown shown in the following table:

Centre	Non-anaemic n=92		Anaemic non-treated n=72		Anaemic treated n=64	
Blackpool	5	(5%)	5	(7%)	0	
Cardiff	19	(21%)	4	(6%)	21	(33%)
Castle Hill	13	(14%)	10	(14%)	4	(6%)
Derriford	13	(14%)	2	(3%)	2	(3%)
Essex	3	(3%)	18	(25%)	0	
James Cook	0		2	(3%)	14	(22%)
Kings College	0		1	(1%)	2	(3%)
Liverpool	18	(20%)	12	(17%)	12	(19%)
Manchester RI	0		1	(1%)	0	
Papworth	10	(11%)	5	(7%)	9	(14%)
RI Edinburgh	11	(12%)	12	(17%)	0	

Table 5.1: Distribution of patient groups across centres.

Two-hundred and twenty-eight patients were recruited over 2 years in 11 UK cardiac centres (Supplementary figure 1). The most frequently recorded reasons for failure to recruit patients were: administrative and lack of research staff, no date for surgery, date of surgical procedure outside of study treatment window (within 10 days) and refusal of patients to give consent for the trial (19% of approached patients). Of the anaemic patients, who would ideally receive IV iron pre-treatment, only 47% (64/136) were treated, due to various logistical barriers.

Of the 9 patients eligible for the total Hb-mass sub-study at Papworth, only one gave consent for the additional testing. The reasons given for non-consent included concerns about the testing process and logistical challenges. The one subject who consented for total-Hb mass testing was not able to complete testing due to an error in the process. The sub-study was ultimately abandoned due to failure of recruitment. Further discussion of these challenges and where they led is contained in the following two chapters.

The baseline characteristics of the recruited patients are outlined in the table overleaf:

	Non-anaemic n=92	Anaemic non-treated n=72	Anaemic treated n=64	p-value
Age; years	67.0 (9.7)	69.3 (11.8)	70.2 (10.9)	0.158
Sex; men	66 (72%)	55 (76%)	46 (72%)	0.767
Weight; kg	85.8 (17.9)	83.2 (18.0)	81.6 (17.9)	0.120
Height; cm	170.0 (10.0)	168.9 (10.6)	167.6 (8.9)	0.340
BMI; $kg.m^{-2}$	29.3 (5.3)	29.2 (5.9)	29.0 (5.4)	0.929
EuroSCORE-2	1.3 (0.9-2.7 [0.5-19.8])	1.7(0.9-3.3 [0.6-15.0])	2.2 (1.0-3.4 [0.5-16.9])	0.072
Cardiac function				
Good	66 (73%)	55 (76%)	48 (75%)	
Moderate	24 (26%)	15 (21%)	15 (23%)	
Poor	1 (1%)	2 (3%)	1 (2%)	0.866
NYHA				
1	28 (31%)	23 (32%)	12 (19%)	
2	43 (47%)	26 (37%)	32 (50%)	
3	20 (22%)	17 (24%)	19 (30%)	
4	0	5 (7%)	1 (2%)	0.042
Creatinine; $umol.\mathbf{L}^{-1}$	84 (73-96 [56- 205])	85 (72-113 [47-311])	104 (75-120 [46-192])	0.008
Medical history				
Iron deficiency	4 (4%)	9 (13%)	25 (39%)	<0.001
Anaemia	10 (11%)	21 (29%)	35 (55%)	<0.001
Operation				
CABG	44 (48%)	29 (40%)	24 (38%)	
Single Valve	29 (32%)	33 (46%)	22 (34%)	
CABG +Valve	12 (13%)	4 (6%)	9 (14%)	
Other	7 (8%)	6 (8%)	9 (14%)	0.229

Table 5.2 Baseline characteristics. Values are mean (SD) or number (proportion).

Those anaemic patients who were treated with IV iron were more likely to have a previous diagnosis of anaemia, iron deficiency and chronic kidney disease. The median time from IV iron administration was 33 days with an interquartile range of 15-53 days. A large majority of patients (60 out of 64) were treated with iron isomaltoside, with a mean dose (SD) of 1314 (303) mg and the remaining 4 were given 1000mg of iron carboxymaltose. The overall mean dose (SD) was 1293(303)mg in total.

The pre- and post-operative [Hb] values for all three groups are shown in the following table:

	Non-anaemic (n=92)	Anaemic, not treated (n=72)	Anaemic, treated (n=64)
Mean (SD) [Hb] (g/L)			
Pre-treatment	NA	NA	114.2 (9.3)
Pre-surgery	141.1 (10.4)	116.7 (10.2)	122.7 (13.3)
Postoperative	98.3 (13.6)	93.2 (10.5)	93.7 (11.9)
Mean (95% CI) change in [Hb](g/L)			
Pre/post-treatment	NA	NA	8.4 (5.0, 11.8)
Pre/post-surgery	-42.8 (-45.7 to -39.9)	-23.4 (-26.5 to -20.3)	-29.0 (-32.4 to -25.6)

Table 5.3: Haemoglobin concentration by group and time

The non-anaemic group had a higher baseline [Hb] than both anaemic groups by approximately 25g/L and anaemic group receiving IV iron had 2.5g/L lower starting mean [Hb] than the non-treated group. In the post-operative follow-up [Hb] was similar in all three groups which represented a much greater drop in [Hb] in the non-anaemic group of 42.8g/L.

After IV Iron, there was statistically significant increase in [Hb] when treated patients were re-tested before surgery. The treated anaemic group had a mean [Hb] gain of 8.4g/L and at that stage as a result had a higher pre-operative [Hb] than the untreated anaemic group by 6g/L.

Consistent with international data, there was significant inter-centre variability in transfusion rates from 30% to 65% amongst our study centres. 10% of the total (23 patients, including 9 non anaemic, 5 anaemic non-treated and 9 anaemic-treated) received more than 4-units of RBCs and were excluded from analysis.

When the two anaemic groups were combined and compared to the non-anaemic controls, the results were consistent with previously reported data. The anaemic group had statistically significant increases in transfusion rates (48.8% vs 26.5%, p=0.001 & adjusted OR 3.18, p=0.001), received more units overall (median 1 vs 0, p=0.005) and were more likely to receive 3-4 units (12% vs 8%, p=0.016). They had longer median hospital stays (9 vs 8 days, p=0.014) and the composite measure, DAH-30 was similarly worse (20 vs 21, p=0.033). Mortality, ICU length-of-stay, and re-admissions were not significantly different.

	Non-anaemic (N=92)		Anaemic (N=136)		p-value
Number (%) transfused	22	(26.5)	59	(48.8)	0.001
Adj OR (95% CI)			3.18	(1.60-6.31)	0.001
Units transfused; **n** (%)					
1-2	15	(16%)	43	(32%)	
3-4	7	(8%)	16	(12%)	0.016
Median (IQR)	0	(0-1)	1	(0-2)	0.005
Died, **n** (%)	3	(3.3)	5	(3.7)	0.867
Readmissions; **n** (%)	15	(16.3)	16	(11.8)	0.327
ITU length of stay-days					
Median (IQR)	2	(1-4)	2	(1-5)	0.571
Hospital stay; days					
Median (IQR)	8	(6-11)	9	(7-14.5)	0.014
DAOH-30; days Median (IQR)	21	(17-23)	20	(14-22)	0.033

Table 5.4: Study outcomes, anaemic versus non-anaemic patients, excluding 23 patients who were transfused > 4 units red cells. Adj OR: odds ratio for blood transfusion adjusted for sex, BMI, diabetes, and operation type.

Amongst the two anaemic groups there were minimal differences in outcomes between those treated with IV iron and those who were not. These are outlined in the table overleaf:

	Anaemic non-treated n=72		Anaemic treated n=64		p-value
Number (%) transfused	28	(42%)	31	(56%)	0.127
Adj OR (95% CI)			1.33	(0.52-3.40)	0.553
Units transfused; **n** (%)					
1-2	18	(25.4)	25	(39.1)	
3-4	10	(14.1)	6	(9.4)	0.107
Median (IQR)	0	(0-2)	1	(0-2)	0.082
Died; **n** (%)	3	(4%)	2	(3%)	0.747
Readmissions; **n** (%)	5	(7%)	11	(17%)	0.064
ITU length of stay; days					
Median (IQR)	2	(1-4)	3	(1-5)	0.158
Hospital stay; days					
Median (IQR)	9	(7-14)	10.5	(7-15)	0.492
DAOH-30; days Median (IQR)	21	(14-22)	19	(15-23)	0.768

Table 5.5: Study outcomes, anaemic non-treated versus anaemic treated after exclusion of 14 patients transfused > 4 units of blood. Adj OR, odds ratio adjusted for baseline haemoglobin, sex, BMI, diabetes, iron tablets, hypertension, and operation type.

No significant outcome differences were able to be demonstrated between the treated and non-treated anaemic patients. Interestingly, there was a non-significant trend toward higher transfusion rates in the treated group.

Discussion

In terms of feasibility, the results of CAVIAR demonstrated that in most centres, it was possible to establish effective anaemia treatment pathways before cardiac surgery, although results suggested that a significant number of these patients were not treated with intravenous iron despite being identified as having iron-deficiency anaemia. Results were consistent with those published from Frankfurt, where IV iron was only given to small fraction of patients(Meybohm et al. 2017). There were several barriers to the effectiveness of these treatment pathways at an organisational and systemic level. Although there has been a significant increase in the research focus on peri-operative treatment of anaemia over the last decade, actual progress in implementing treatment pathways has been slow (A Shander et al. 2012). This highlights the complexity of setting up and running multi-disciplinary treatment pathways and the need for co-operation from all levels of organisational hierarchy and multiple specialty groups. CAVIAR was designed to treat patients at least 10 day pre-operatively as it appears that 7-9 days is required for IV iron to have it peak effect on ferritin levels(Blunden et al. 1981) and it appears that [Hb] increase begins to plateau between 5-14 days.(Bhandal and Russell 2006)

In terms of efficacy, the results suggest that in those given IV iron, there is a significant improvement in [Hb], even in the relatively short timeframe that is often available. This is consistent with findings of a broad meta-analysis in the effects of IV iron (Clevenger et al. 2016) in non-dialysis patients and another trial specific to cardiac surgery (Litton, Xiao, and Ho 2013), but has not been replicated in at least one other RCT(H Padmanabhan et al. 2017). This is the largest trial to date in cardiac surgery, that demonstrates that IV iron can effectively treat anaemia in cardiac surgery patients pre-operatively.

It is important to note, however, that an observational trial such as CAVIAR is not ideal to detect a treatment effect and this finding will hopefully be confirmed in randomised control trials specific to cardiac surgery.

As with previous studies, including the ACTA audit(A. A. Klein, Collier, Brar, Evans, Hallward, Fletcher, Richards, et al. 2016), our data showed that anaemic patients have higher rates of transfusion and worse outcomes than those that are non-anaemic. The significantly larger fall that was observed in [Hb] in the non-anaemic group was interesting and may represent the increased use of RBC transfusion in the anaemic group or the more rigorous use of PBM strategies, such as use of intra-operative blood conservation techniques, or a combination of these factors.

While improving [Hb] is likely to have value, demonstrating an effect on patient-centred outcomes is essential before a widespread uptake of any medical intervention should occur. Limited evidence exists that IV Iron can reduce transfusion both broadly (Litton, Xiao, and Ho 2013), and in the context of cardiac surgery (Johansson, Rasmussen, and Thomsen 2015)as well as possible effects on other outcomes such as mortality and length of admission(Cladellas et al. 2012). Despite the relative weakness of this evidence, it has already been translated into expert consensus(M. Muñoz et al. 2017) and practice guidelines.("National Institute for Health and Care Excellence. Blood Transfusion. NICE Guideline (NG24)." 2017)

Since the publication of this trial, more evidence has emerged to support the hypothesis that IV iron pre-treatment can reduce transfusion rates in other types of surgery, supported by a recent meta-analysis in major abdominal surgery (Elhenawy et al. 2021), although results of this kind are not ubiquitous with the very recent PREVENTT trial (Richards et al. 2020) not detecting a treatment effect in a similar population. Another more recent trial where patients were given IV Iron and EPO immediately before cardiac surgery showed a reduction in transfusion rates across the admission (Spahn et al. 2019) without any other demonstrable patient benefit. Our results were not able to demonstrate any beneficial effect on patient-centred outcomes, although it was not designed to have adequate power to detect these.

As is often the case with observational research, the baseline characteristics of the study groups varied significantly. In this study population, anaemic patients who received intravenous iron had a significantly higher rate of pre-existing renal impairment. Renal impairment has a major influence on transfusion requirement as demonstrated in many predictive scores for transfusion risk in cardiac surgery. For example, if we were to calculate the risk of transfusion in a patient at intermediate risk of transfusion prior to cardiac using the ACTA-PORT score,(A.A. Klein et al. 2017) the presence of a pre-operative creatinine > 177 µmol/L would shift the predicted transfusion rate from 45% up to 60%. The higher rates of previously diagnosed anaemia (55% vs 30%), previous iron deficiency (39% vs 13%), and symptomatic angina (63% vs 39%) in our treatment group, compared with the non-treated anaemic patients, may have contributed to the outcome results.

Observational studies are also far more likely to suffer from effects of bias and have confounding factors present. Although no major sources of bias were identified, this cannot be excluded from the analysis of CAVIAR.

The complex nature of cardiac surgery and multifactorial causes for coagulopathy with need for significant use of blood resources(Manuel Muñoz et al. 2015) may override the positive effects of intravenous iron in the preoperative setting.

As discussed, ongoing research using larger cohorts powered to detect patient-centred outcome differences are required to establish whether IV Iron can meaningfully improve morbidity, mortality, and quality of life after cardiac surgery. Members of the CAVIAR study group and other designed such a trial, that is currently underway and for which the author is a principal investigator. The Intravenous Iron for Treatment of Anaemia before Cardiac Surgery (ITACS) is a blinded RCT that is underway (NCT02632760) which aims to recruit 1000 patients who are then randomised to receive either IV iron or placebo before cardiac surgery with the primary outcome measure of 90-DAH (days alive and out of hospital in the first 90 post-operative days). Secondary outcomes include [Hb] change, ICU stay, hospital stay, anaphylaxis, infection, disability-free survival, 90-day survival, blood product usage, quality of life and cost-effectiveness. At the time of writing, ITACS had recruited 619 patients from 29 sites in 9 countries but have faced significant delays in recruitment that were unexpected. Given the results of audits of anaemia rates in cardiac surgical patients, which suggested that somewhere approaching one-third of patients could be anaemic, the challenges in recruiting suitable patients for anaemia studies were somewhat surprising. As a result, some further exploration of anaemia rates and potential effects of recent prescribing patterns was undertaken. The following chapter contains a description of this process and the results obtained.

Chapter 6: Knowledge from failure & Epidemiology of changing anaemia rates & IV Iron prescription

The described challenges of recruiting anaemic patients for the ITACS study and the failure to recruit patients for the CAVIAR sub-study and ensuing PREFIX trial in Sydney raised a number of questions. Given that the rates of anaemia seen in large audits such as the ACTA audit(A. Klein et al. 2016) suggest that many cardiac surgery patients are anaemic, it was assumed that identifying and recruiting patients for these trials would be relatively straightforward and rapid. Anecdotal evidence from other ITACS study-centres also suggested that recruitment was significantly slower than anticipated and as such it was decided to investigate further. There are a number of challenges to recruitment for these trials:

1. Number of anaemic patients
2. Failure to identify suitable participants
3. Adequate time to surgery to allow recruitment (usually greater than 7 days)
4. Willingness to participate in study
5. Exclusion of patients treated with IV iron prior to pre-admission appointment

Addressing point 3 is challenging as many surgical patients will not be sent to pre-admission until they have a date for surgery and then will eventually be given a date within the 7-10 pre-operative day window, thus precluding them from recruitment. Fixing this requires major systemic changes, which are underway but significant improvement is still required. Willingness to participate has not been identified as a major issue and is also a difficult point to address. The 3 remaining points were considered and thought worth exploring further. One hypothesis was that the incidence of anaemia may be dropping due to increased awareness of PBM strategies and a potential increase in optimisation of iron stores pre-operatively with supplementation via oral or IV routes. This had been noted locally in certain populations such as women of childbearing age(Shand et al. 2020) but not investigated specifically in the context of cardiac surgery.

Broadly speaking, the global anaemia burden appears to be improving in the last few decades. A large worldwide study showed the global incidence of 32.9% in 2010 had reduced significantly from 40.2% in 1990 (N. J. Kassebaum et al. 2019) and this finding paralleled an estimate in another analysis that suggested that global anaemia prevalence is dropping at 0.2-0.3% per year.(Mason et al. 2013)

There is some evidence that increases in understanding of concepts of absolute and functional iron deficiency have translated into increases in testing for ferritin and TSAT as seen in the figure below taken from a French paper examining testing utilisation in those receiving iron treatments. (Cacoub, Nicolas, and Peoc'h 2020) This suggests a greater understanding of the concepts involved in iron deficiency and may be associated with better treatment strategies across the range of medical providers.

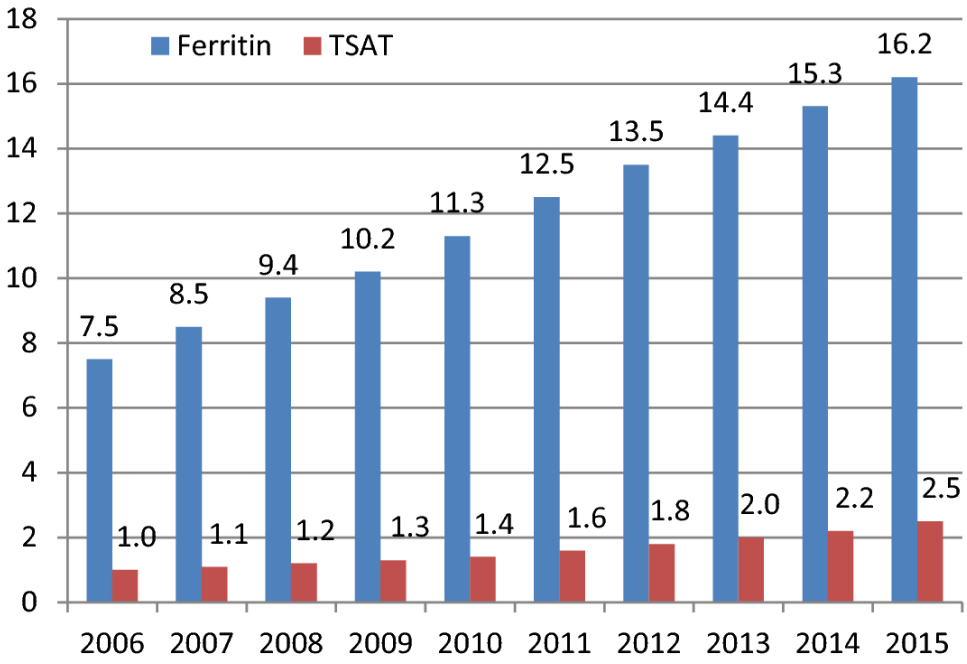

Figure 6.1: Annual frequency (%) of the biological assessments of iron deficiency (transferrin saturation index [TSAT] and/or serum ferritin)—from French healthcare coverage databases (EGB) 2006–2015. Reproduced from (Cacoub, Nicolas, and Peoc'h 2020)

As discussed in chapter 2, there are many interventions directed at reducing global anaemia rates, ranging from fortification of staple foods with iron, oral iron supplementation and the addition of further dietary supplements to enhance iron absorption. Appropriate treatment of contributing infections can also influence anaemia rates, although this is not likely significant in our study population with the possible exception of *h.pylori* infections.

Since the release of the better tolerated iron-carbohydrate complexes and the gradual increase in evidence of their efficacy, they are being prescribed much more frequently. This combined with their increasing presence in national and local guidelines and the inevitable marketing and publicity associated with new pharmacological interventions has seen a significant increase in their clinical use. While single hospital use is relatively easy to track via

local pharmacy services, IV iron is now often given in an outpatient setting and is increasingly being used in primary care. This makes monitoring its use at a regional or national level more challenging. Much like the UK, Australia is fortunate to have a centralised national service for obtaining and distributing subsidised pharmaceuticals, the national Pharmaceutical Benefits Scheme (PBS) who publish data annually on various categories of medication and their usage and cost. Very little has been published about trends in iron prescription since the introduction of newer preparations, but one analysis of IV iron prescription in Australian women showed that there has been a rapid increase in its use in that demographic over the last decade. So much so, that in 2017, IV iron was prescribed to 1 in 50 women of child-bearing age, five times greater than the proportion in 2013(Shand et al. 2020)

In order to better understand the trends in IV iron use, the author sought to explore available national data since the introduction of the newer IV preparations to examine how rapidly their use has increased amongst the general population. The hypothesis is that increased IV iron use could be contributing to a reduction in rates of anaemia in patients presenting for cardiac surgery. The increased use of IV iron in outpatient or primary care settings may also contribute to patients being unrecruitable to IV iron studies as they have already received the treatment prior to pre-admission.

In addition to Australian prescriber data, the author sought similar data from the UK and the US to see if any global pattern was detectable. The NHS presents annualised costing for all prescriptions in England called the Prescription Cost Analysis. Unfortunately, this report includes data from only community and primary health prescriptions and excludes data from secondary health. Centralised hospital data is not routinely collected by the NHS. The US Medicare and Medicaid provide insurance to approximately 35% of the US population including subsidised prescriptions. The costs of these prescriptions are published annually by medication type. These resources were examined to seek prescribing trends in the US and UK in order to establish whether local trends were consistent with a broader pattern.

The effects of an increase in iron prescription at a population level is difficult to measure without large-scale high-quality research, however it is possible to assess overall patterns in our target-group thanks to various local and national databases. With this in mind, the author undertook an audit to ascertain if a downward trend in anaemia rates was observable in our local (St Vincent's Hospital) and national (ANZSCTS) databases during the time of the rapid rise in IV iron prescription. A more detailed examination of the most recent anaemic patients who were not recruited was undertaken to ascertain if any were missed who were suitable. It should be noted that these trends are purely an observed association, are full of potential bias

and may not reflect causality. They are, however, of significant interest and may represent a significant improvement in the diagnosis and treatment of iron deficiency, with or without anaemia.

Methods

Data for each subcategory was obtained as detailed below and analysed using Microsoft Excel (version 16.35 for Mac, Microsoft 2020) or SPSS software (version 27 for Mac, IBM 2020) for statistical significance where appropriate.

Australian National Iron prescription trends

Data were obtained from the Australian national Pharmaceutical Benefits Scheme (PBS) via their published financial-year reports from 2013/14 to 2018/19. Data on total annual (by financial year) subsidised PBS prescriptions for IV iron including cost to government, cost to patients and average total cost per dose were retrieved from the data and prepared into a table and then represented graphically by year. Prior to 2013/14, no data were available on IV iron prescriptions, so the data is limited to this range. Data is only provided for total IV iron expenditure and not divided into preparation or brand

UK national prescribing trends – primary care

Data were obtained from official NHS data released under the prescriber cost analysis (PCA). Total expenditure data were analysed, and annual totals compiled for BNF category 0901012, which is the sub-category for IV iron preparations in the "Anaemias and some other blood disorders" category. The annualised data were tabulate and presented in graphical form.

US Medicare and Medicaid prescription trends

Data were obtained from the Centers for Medicare & Medicaid Services (CMS) who publish annual reports on total prescription costs comprising of costs to Medicare/Medicaid, deductible, and co-insurance, representing the full value of the product. Medicaid data is presented separately to Medicare data, which is further divided into parts depending on type of service. Part B consists of medications administered through doctors' offices or outpatient settings. Group D represents medications taken by patients at home and thus did not contain data on IV iron preparations. Therefore, data from Medicaid and group B Medicare for the calendar years 2014-2018 were combined and examined by IV Iron type (brand) and

presented in tabular and graphical form. Total spending on each of the IV Iron preparations (listed by brand and generic name) were included for each year and presented in both tabular and graphical form.

Local anaemia trends and outcomes

The St Vincent's Hospital (Sydney, Australia) department of cardiothoracic surgery collects perioperative data on all patients undergoing cardiac surgery including pre-operative [Hb] and this presented an opportunity to undertake a local audit of anaemia rates and recent trends. Prior to September 2015, the database did not reliably collect preoperative [Hb], so that was selected as a start point for data analysis and 4 years of data were analysed in total ending in August 2019. This audit was approved by the St Vincent's Hospital research office with project number 2018/ETH00738. Patients undergoing urgent or emergency surgery were excluded as it was assumed that these patients were not allowed the usual time for their various treating practitioners to undertake the pre-operative assessment and management that was of interest.

National anaemia trends and outcomes

Data were obtained from the ANZSCTS database on patients presenting for cardiac surgery between September 2016 and December 2018, inclusive. Prior to this, data for pre-operative [Hb] was inconsistently recorded on the database and thus was not available for analysis. The baseline characteristics for the entire dataset for the AntiPORT cohort were analysed and are presented in tabular form. Mean and standard deviation or median and interquartile range were used for continuous variables depending on their distribution. For categorical variables, frequencies (n) and percentages were used. To compare baseline characteristics across the three groups of patients, chi-square tests were used for categorical variables and F-tests (if normally distributed) or Kruskal-Wallis rank test (if not normally distributed) for continuous variables. Data for emergency and urgent cases was then excluded as these patients would not generally be appropriate for trial recruitment due to the inadequate timeframe to observe a treatment effect. The remaining data were analysed for annualised anaemia rate and presented in tabular form.

Results

Australian Iron prescription trends

As the table below demonstrates, the number of annual prescriptions for IV Iron increased enormously from 104 prescriptions in 2014 to 248,205 prescriptions in 2018, which was associated with a corresponding increase in annual government cost from $26,277 to $68,160,786, representing a 259,293% increase. In the 2018/19 financial year, data were not published on the total expenditure on iron, but ferric carboxymaltose, which is just one of the 3 available IV iron preparations in Australia, was prescribed 289,042 times at a total cost of $86,518,572, making it the 29th on the list of the highest cost drugs to the PBS. This data was excluded from the table as it likely underestimates the total IV iron use and can thus not be compared accurately to the preceding data.

Year	PBS subsidised prescriptions	Government cost	Patient Contribution	Total Cost	Average Price
2013-14	106	$26,277	$2,787	$29,064	$274.19
2014-15	67,435	$18,740,502	$1,479,961	$20,220,463	$299.85
2015-16	123,597	$33,858,891	$2,718,655	$36,577,546	$295.94
2016/17	182,188	$49,834,425	$4,156,865	$53,991,290	$296.35
2017/18	248,205	$68,160,786	$5,962,430	$74,123,217	$298.64

Table 6.1: Financial year costings of IV iron prescriptions 2014-2019 (data from PBS)

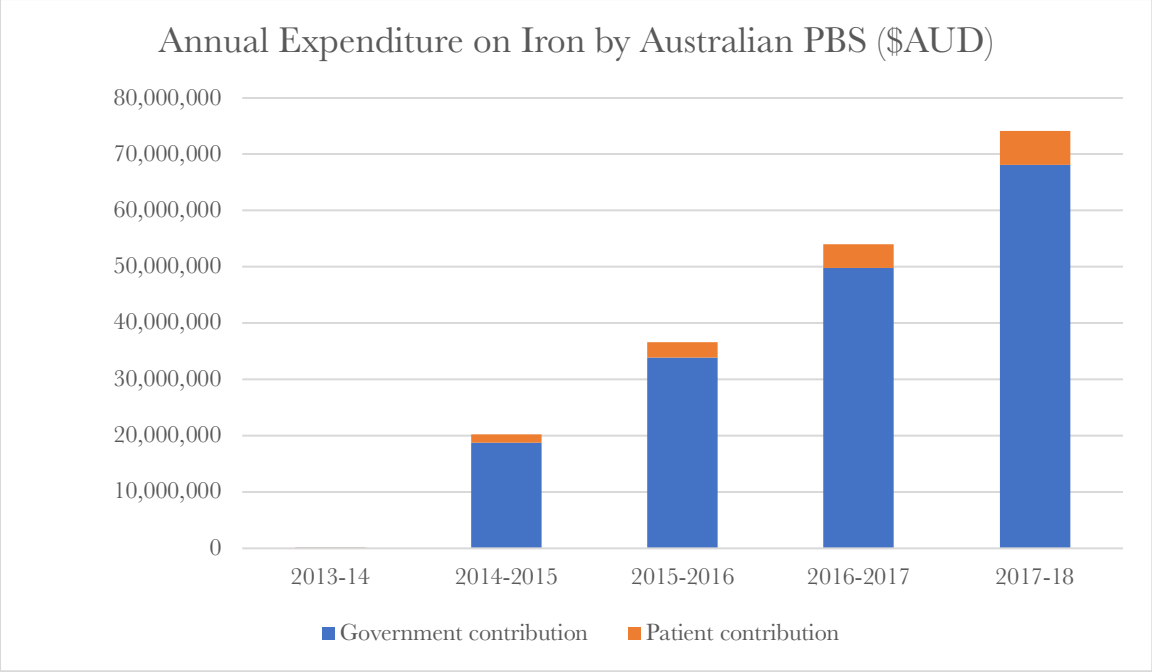

Figure 6.2: Total expenditure (with government and patient contribution) for all PBS subsidised IV iron prescriptions 2013/14-2017/18. Generated from published PBS data.

US Medicare and Medicaid IV Iron spending 2014-18:

Data from the US Medicare and Medicaid programs showed a large increase in total IV Iron prescription costs from US$112 million to US$416 million. The rise was not uniform however, with a rapid rise in some preparations such as ferric carboxymaltose, which increased from US$13 million in 2014 to US$173 million in 2018, and a commensurate decrease in older preparations such as iron dextran, which dropped from US$14 million to US$6 million over the same period.

Brand Name (Generic)	Funding source	Total Spend 2104	Total Spend 2105	Total Spend 2016	Total Spend 2017	Total Spend 2018
Dexferrum (Iron Dextran)	Medicaid	$188,058	$46,690	$12,016	$5,190	$547
	Medicare	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Feraheme (Ferumoxytol)	Medicaid[1]	$3,474,356	$4,456,058	$6,340,899	$7,976,301	$9,687,203
	Medicaid[2]	$39,705,438	$42,280,018	$46,892,275	$49,615,147	$61,123,991
	Medicare	$1,129,795	$931,663	$1,123,488	$1,057,760	$888,939
	Total	$44,974,118	$50,478,977	$59,823,308	$62,799,523	$75,892,162
Ferrex 150 (Iron Polysaccharide)	Medicaid	$326,103	$99,190	$17,836	$22,228	$51,583
	Medicare	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Iferex 150 (Iron Polysaccharide)	Medicaid	$20,751	$70,320	$129,127	$132,899	$137,484
	Medicare	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Infed (Iron Dextran)	Medicaid	$2,271,383	$2,943,397	$3,249,843	$3,798,311	$1,655,318
	Medicare	$11,667,411	$11,294,558	$9,646,182	$10,268,635	$4,016,261
	Total	$13,938,794	$14,237,955	$12,896,025	$14,066,947	$5,671,579
Injectafer (Ferric Carboxymaltose)	Medicaid	$902,635	$5,363,788	$15,213,046	$24,359,967	$32,430,067
	Medicare	$12,318,625	$50,495,429	$85,641,974	$114,114,931	$140,612,426
	Total	$13,221,260	$55,859,217	$100,855,020	$138,474,898	$173,042,493
Poly-Iron (Iron Polysaccharide)	Medicaid	$71,450	$84,822	$4,548	$9,643	$7,156
	Medicare	Not Reported	Not Reported	Not Reported	Not Reported	Not Reported
Venofer (Iron Sucrose)	Medicaid	$29,486,489	$45,399,346	$54,469,754	$82,221,970	$150,260,652
	Medicare	$6,944,836	$5,332,426	$5,073,750	$4,171,311	$4,254,354
	Total	$36,431,325	$50,731,772	$59,543,504	$86,393,281	$154,515,006
Ferrlecit (Ferric Gluconate/Sucrose)	Medicaid	$2,727,413	$2,072,804	$2,013,080	$3,068,418	$5,903,099
	Medicare	$423,237	$322,129	$682,543	$592,708	$545,997
	Total	$3,150,650	$2,394,933	$2,695,623	$3,661,126	$6,449,096
Total Spending		$112,322,510	$174,003,875	$235,977,007	$305,565,734	$415,767,105

Table 6.2: Annual total cost of IV iron preparations from US Medicare/Medicaid data displayed by drug and year.

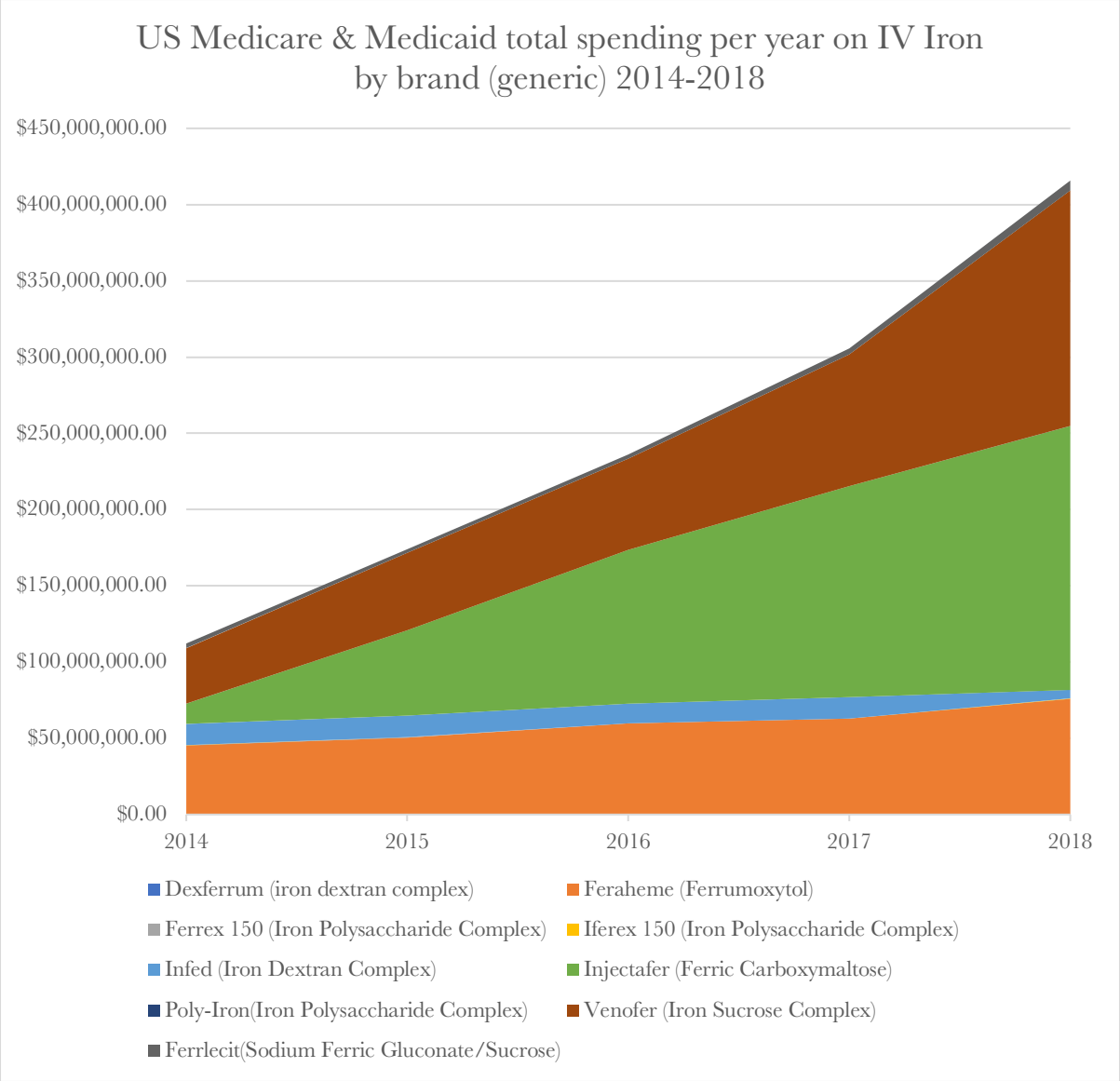

Figure 6.3: Medicare/Medicaid spending on IV iron by year divided into different IV iron preparations

UK IV Iron prescription in primary care

The results from the PCA show the annual prescription rates for IV Iron preparations in primary care has approximately halved between 2014 and 2019. This represented in graphical form in the chart overleaf:

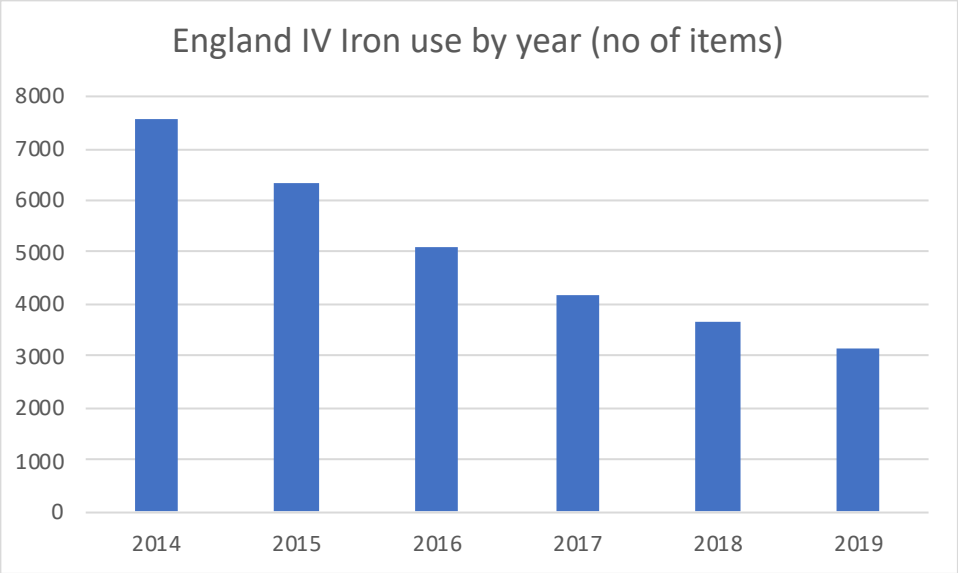

Figure 6.4: IV Iron use in primary care in England

Of important note, this data does not include IV Iron prescriptions outside of primary care, where the majority of IV Iron is given in the UK setting.

Local anaemia trends and outcomes, St Vincent's Hospital, Sydney

The data show that during the same period, over which iron prescriptions have risen exponentially, there has been a corresponding drop in the rate of anaemia in those presenting for elective cardiac surgery and a corresponding rise in average [Hb]

	2015-16	2016-17	2017-18	2018-19
Number of cases	150	170	131	91
Anaemic patients	41	35	25	14
Anaemic patients (%)	27%	21%	19%	15%
Average Hb (g/L)	137	138	139	142

Table 6.3: Annual anaemia rates for patients presenting for elective cardiothoracic surgery at St Vincent's Hospital Sydney

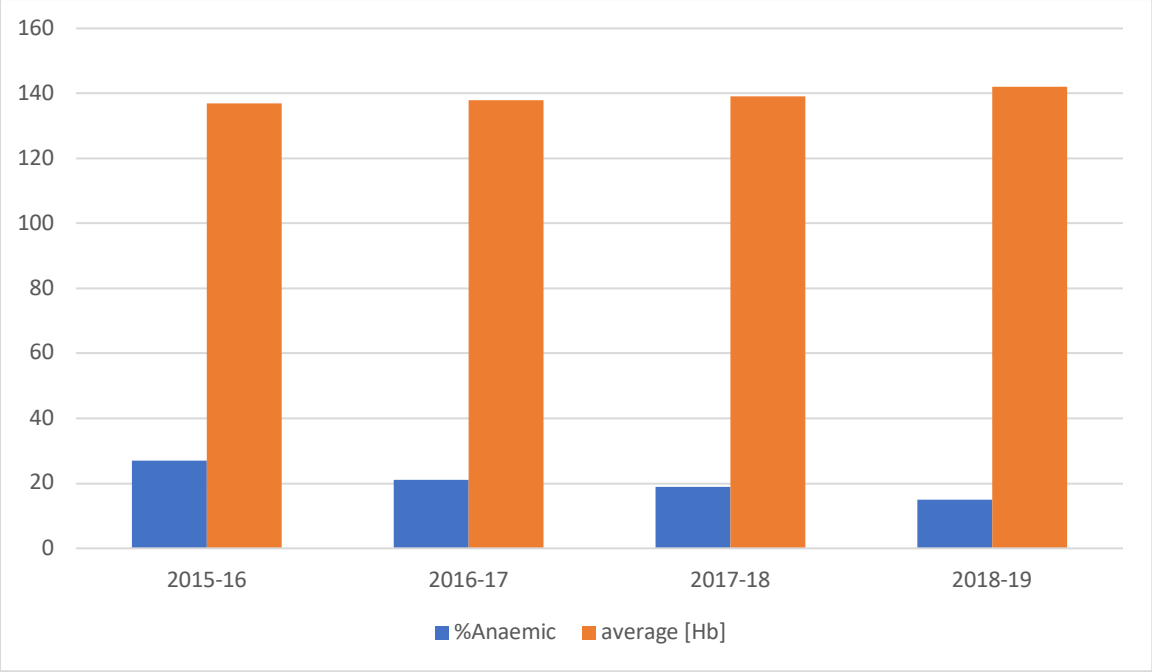

Figure 6.5: Annual proportion of anaemic cardiac surgery patients and average [Hb] from the St Vincent's Hospital cardiac surgical database.

Detailed examination of the data showed that in the last year of the audit, of the 14 anaemic patients, all had iron status checked pre-operatively. Of these, only 1 patient had iron deficiency using the standard definition of a [ferritin] <30. In the context of cardiac surgery, where a standard blood loss is expected, the criteria for pre-operative IV iron is often broadened to include those patients with a [ferritin] < 100 and a TSAT <20%, using these criteria, only 4 of the 14 patients identified would qualify for a pre-operative iron infusion and of these only one was seen in the pre-admission clinic more than 1 week prior to the planned surgery date. The one patient who would have been eligible for recruitment for any study was a male with a [Hb] of 127g/L, a [ferritin] of 95 and a TSAT of 17% and presumably as all the results were close to the normal range, was not identified by the attending specialist as having iron-deficiency anaemia, despite technically qualifying for IV iron replacement.

National anaemia trends and outcomes

The baseline characteristics of the patients in the data obtained from the ANZSCTS database is displayed in the table overleaf. It is divided into those who received red blood cell transfusions and those who did not. Of these patients, data for patients having urgent (6168 patients) and emergency (818 patients) were excluded from the subsequent analysis.

	No Transfusion	Transfusion	Total	p-value
n (%)	15878 (69.8)	6860 (30.2)	22738 (100)	
Age, median (IQR)	66 (16)	70 (15)	67 (16)	0.00
Sex:				
-Male, n (%)	12624 (79.5)	4405(64.2)	17029 (74.9)	0.00
-Female, n (%)	3254 (20.54)	2455 (35.8)	5709 (25.1)	
Procedure Type:				
-CABG, n (%)	8669 (54.6)	3081(44.9)	11750 (51.7)	
-Valve surgery, n (%)	3682 (23.2)	1344 (19.6)	5026 (22.1)	
-Combined, n (%)	1139 (7.2)	969 (14.1)	2108 (9.3)	
-Other, n (%)	2388 (15.0)	1466 (21.4)	3854 (16.9)	
Pre-op [Hb], median (IQR)	142 (19)	127 (27)	138 (23)	
Pre-op [Cr], median (IQR)	84 (26)	87 (38)	85 (29)	
BMI, median (IQR)	29 (7)	27 (7)	28 (7)	
Urgency:				
-Non-urgent, n (%)	11528 (72.6)	4224 (61.6)	15752 (69.3)	
-Urgent, n (%)	4012 (25.3)	2156 (31.4)	6168 (27.1)	
-Emergency, n (%)	338 (2.1)	480 (7.0)	818 (3.6)	
Hypertension:				
-no, n (%)	4739 (29.8)	1750 (25.5)	6489 (28.5)	
-yes, n (%)	11139 (70.2)	5110 (74.5)	16249 (71.5)	0.00
Perfusion time, median (IQR)	89 (52)	108 (75)	94 (58)	0.00
Diabetes:				
-No, n (%)	11393 (71.8)	4622 (67.4)	16015 (70.4)	
-Yes, n (%)	4485 (28.2)	2238 (32.6)	6723 (29.6)	0.00

Table 6.4: Baseline characteristics of patients in the ANZSCTS dataset undergoing cardiac surgery between September 2016 and December 2018 (inclusive)

The anaemia rates for these patients, divided into groups by year, to allow comparison to the St Vincent's Hospital data are displayed in table 6.5 below.

	2016	2016-17	2017-18
Number of operations	1643	6748	7248
Anaemic patients	405	1503	1585
Anaemic patients (%)	24.65%	22.27%	21.87%

Table 6.5: Annual anaemia rates for patients presenting for elective cardiothoracic surgery in Australia & New Zealand (September 2016-December 2018) from ANZSCTS database.

Discussion

IV Iron prescription data

The results of these audits show that there has been a dramatic increase in the numbers of prescriptions of IV iron between 2014 and 2018 in the USA and Australia. While the overall increase in IV iron has been large in both regions, the US data suggest that the growth is mostly due to increases in the newer IV iron-carbohydrate preparations, such as Iron carboxymaltose (Injectafer® in the USA, Ferrinject® in Australia) and iron sucrose (Venofer®). Iron isomaltoside, which has been popular in Europe has only recently been approved by the FDA as Monoferric® and such does not feature in this data. Older preparations such as Iron-dextran appear to be falling.

The pattern of prescription is also interesting, with primary care appearing to account for the bulk of prescriptions. The same paper showed that between 2013 and 2017, 43% of all IV iron prescriptions were written by GPs. This does not appear to be replicated in the limited UK data, which negative rates of change in primary care IV iron prescription, although given the suspiciously low numbers, possibly reflects differing data collection techniques within the NHS and without complete secondary care data should not be over-interpreted. Also of note, the different licencing requirements for the administration of IV iron in the UK means that many primary care facilities do not have the available resources to administer IV iron preparations. Given the increasing presence of IV iron in pre-operative guidelines and treatment pathways, and anecdotal evidence of its increased use, it is likely that the data from secondary care prescriptions would show significant growth over the same period.

Unfortunately, as discussed earlier, these data were not available as they are not routinely published by the NHS. The private company responsible for collection of secondary-care prescription data unfortunately did not respond to requests for this data.

Local anaemia rates (St Vincent's Hospital, Sydney)

The local figures obtained from the St Vincent's Hospital database suggest a significant downward trend in anaemia rates for those presenting for cardiac surgery although the numbers are relatively small and not necessarily representative of the wider population.
A more detailed examination of those potentially recruitable for IV iron trials found that almost all anaemic patients were iron replete at time of pre-admission, suggesting that the cause of anaemia was not iron-deficiency, or that iron-stores had been successfully replenished. Data on treatment modality was not available. It is important to note that the numbers in this cohort are small and observed trends may be misleading and/or not representative of the wider population.

Australian national anaemia rates (ANZSCTS database)

The national data from the ANZSCTS database demonstrated that this trend may also be present at a broader level although the observed trend is less dramatic than the results from St Vincent's Hospital Sydney. This may reflect coincidence, or over-interpretation of trends in the St Vincent's data or differences in primary and secondary care in the St Vincent's cohort compared to the broader population. Results from other countries were unfortunately not able to be obtained for the purposes of this audit, although it would be valuable to understand if this local trend is representative of a broader change. Hopefully, widespread awareness of these results will encourage audit and publication of similar data from other regions.

Implications of findings

The findings show that along with the increasing prescription of intravenous iron during the study period, there has been a contemporaneous decrease in anaemia rates in our centre and, to a lesser-extend nationally in our target population. This could represent a degree of effectiveness of PBM strategies and the increased awareness of the importance of treating pre-operative anaemia, but similarly could reflect any number of societal and socio-economic trends that we have not examined such as reduction in the burden of associated diseases or broader changes in dietary patterns or oral supplementation.
Iron studies, although now routinely collected prior to cardiac surgery in most centres in Australia are not recorded in the local or national database, and their inclusion would provide enormous benefit to researchers.

The discrepancy between changes at our centre compared to the national trend are important to consider. SVH is a quaternary referral centre, in a catchment area with high mean socio-economic indicators, although does receive many non-local referrals from surrounding and distant regions. The access to primary care in the local area is very good and this may have an impact on the rate of early diagnosis and treatment of anaemia.

The observed trend in anaemia rates fits with previously recorded global decreases in anaemia rates, although the rate of change appears to be significantly greater than previously described.

The rapid increase in expenditure on IV iron has a significant implication on health economics and especially given the evidence for the benefit over oral iron is limited to certain scenarios. Even if the trend of reduction in anaemia rates was wholly due to the increased use of IV Iron, it is difficult to clearly demonstrate that this would have any benefit over oral replacement therapy, and whether it represents a significant enough clinical benefit to justify the enormous cost. If the increase in cost of subsidised IV iron to the health system continues, it is likely to come under significant health economic scrutiny. With the current lack of strong evidence for effectiveness, it may become difficult to justify this cost, and thus there is a strong need for continued research to demonstrate that this treatment can have positive effects on patient outcomes.

While the data on the increase in IV iron prescription rates and costs is freely available, there has been minimal interpretation of this data in the literature, with only a handful of papers describing this trend. Previous published data have suggested that global anaemia rates are improving, although there is no recent data to demonstrate this effect in the cardiac surgery population. To our knowledge, this represents the first analysis of trends in anaemia in cardiac surgery patients. While the more dramatic trend noted in the St Vincent's data is from a single centre, and thus of limited applicability, the ANZSCTS data suggests that there is a more modest, but still consistent fall in rates of pre-operative anaemia.

This audit was purely observational and retrospective and therefore is full of potential confounders and bias and result should be interpreted with a significant degree of caution. The observed trends are temporally associated, and biologically plausible, but in no way can causality be confirmed.

To firmly establish a downward trend in anaemia rates, longer term data would be more useful, particularly at the national level. Another UK-wide audit of the cardiac surgical population, as a follow-up to the ACTA audit (A. A. Klein, Collier, Brar, Evans, Hallward,

Fletcher, and Richards 2016) would be of great value in ascertaining if a similar trend exists elsewhere.

Data between iron prescriptions and anaemia rates do not perfectly align due to availability of PBS data.

The Australian PBS data contains information on subsidised prescriptions only, and thus underestimates the total national usage as hospital inpatient, and private prescriptions are not included in the analysis.

In conclusion, the observed changes in anaemia rates in those undergoing cardiac surgery may represent a treatment effect from the rapid rise in the prescription of IV iron preparations. This has implications for future studies. It may make recruiting anaemic patients more difficult, which may delay the necessary evidence that IV iron can have any meaningful impact on clinical outcomes, as we have found with our studies. It may also mean that recruitment populations are skewed over the recruitment timeframe, given the relatively rapid rate-of-change and the expected longer recruitment periods with reduced available patients.

This chapter has explored anaemia in cardiac surgery as defined by [Hb]. In chapter 2, the limitations of [Hb] as a measure, in the context of the pathophysiology of anaemia were discussed, as were the advantages of measurement of total-Hb mass. This chapter, as with the preceding chapter have attempted to link iron treatment with improvements in [Hb]. One of the main advantages of total Hb-mass measurement is that it appears to be more sensitive to smaller changes due to interventions than [Hb]. This has significant implications to future studies into the biological efficacy of anaemia interventions, as significant treatment effects could be detected using smaller sample populations. Widespread uptake of total Hb-mass testing is currently impractical due to the difficulties in establishing such a service. The following chapter describes the evolution of testing techniques of measuring total Hb-mass and their strengths and limitations. It then goes on to describe the development of a novel testing technique, which attempts to address some of these limitations and make the testing of this important biomarker simpler and more accessible.

Chapter 7: A method of estimating total Hb-mass in the hospital setting by modification of a commonly used respiratory function test.

As discussed in chapter 2, the first pillar of PBM is "Maximising the total red cell mass" and as stated in this paper by one of the world's leading proponents of PBM, "while [Hb] does not correlate particularly well with aerobic capacity", it is the "only feasible surrogate measurement" because "measurement of a patient's total red cell mass is not practical"(J. P. Isbister 2015)

The established methods for measuring total red cells of total haemoglobin are all variations on the principal of indicator dilution and have been through various iterations over the decades. The generally accepted gold standard techniques of measuring total Hb mass is radioisotope scanning using RBCs radiolabelled with Chromium-51("Standard Techniques for the Measurement of Red-Cell and Plasma Volume*" 1973) and subsequent research has shown that it can also be accurately calculated from plasma volume measured using Iodine-125 or 131(Fairbanks et al. 1996) . While accurate, these tests are expensive, require exposure to radiation and are now performed in very few centres. Other methods have been described to simplify the technique such as haemodilution methods (Hahn 1987) which relies on the dilution of circulating Hb with known volumes of crystalloid or colloid solutions, and using the measurable change in [Hb] to calculate the initial blood volume. This method, though undoubtedly practical in a hospital setting is prone to significant error (M. B. Wolf 2017).

Carbon monoxide binds tightly to haemoglobin forming the measurable molecule carboxyhaemoglobin and this makes it suitable as an alternative dilution technique. It was first described as early as 1900 (Haldane and Smith 1900) and has been revised in various forms since. Burge & Skinner described a method in 1985 using a 10-minute rebreathing time with 50mL of CO in oxygen (Burge and Skinner 1995)

The direct CO rebreathing method, been shown to correlate well with the 125I-albumin method and also radiolabelled Hb techniques. (Thomsen et al. 1991)

Since the first description of the technique, it has undergone a number of modifications and been applied using various methodologies. In 2005, a new iteration of the CO-rebreathing technique was described, which shortened the rebreathing time and simplified the technique (Schmidt and Prommer 2005) resulting in the widespread uptake of the technique in sports medicine.

The Schmidt & Prommer, or optimised CO Rebreathing (oCOR) technique is a shortened 2-minute CO-rebreathing technique using a known dose of CO in oxygen. It utilises a proprietary glass spirometer manufactured by BloodTec, which is show in the diagram below. It is fitted with a 3L standard anaesthetic bag, flushed and pre-filled with 100% oxygen. Between the main spirometer and mouthpiece is a small chamber, through which the participant breathes, which is filled with soda lime, in order to absorb exhaled CO2. The participant's nose is obstructed using a standard nose-clip. A 15-minute period of rest is suggested to stabilise blood volumes and a baseline capillary blood gas is taken to establish [COHb] and end tidal [CO] is measured using a CO detector. Upon commencement of the test, the subject exhales fully and then begins breathing through the spirometer. A calculated volume of pure CO is introduced into the spirometer via a 100mL syringe which is attached via an injection port early in the first inspiration to enhance early absorption. The mixture is rebreathed for 2-minutes after which they are asked to fully exhale into the spirometer and the contents of the reservoir are tested for volume and [CO]. The participant's end-tidal [CO] is rechecked after 4 minutes and repeat blood gas analyses are performed at 6- and 8-minutes, or a single sample at 7-minutes. Results are entered into password-protected software provided by BloodTec and a value for total Hb is calculated. The custom spirometer is shown in the diagram overleaf:

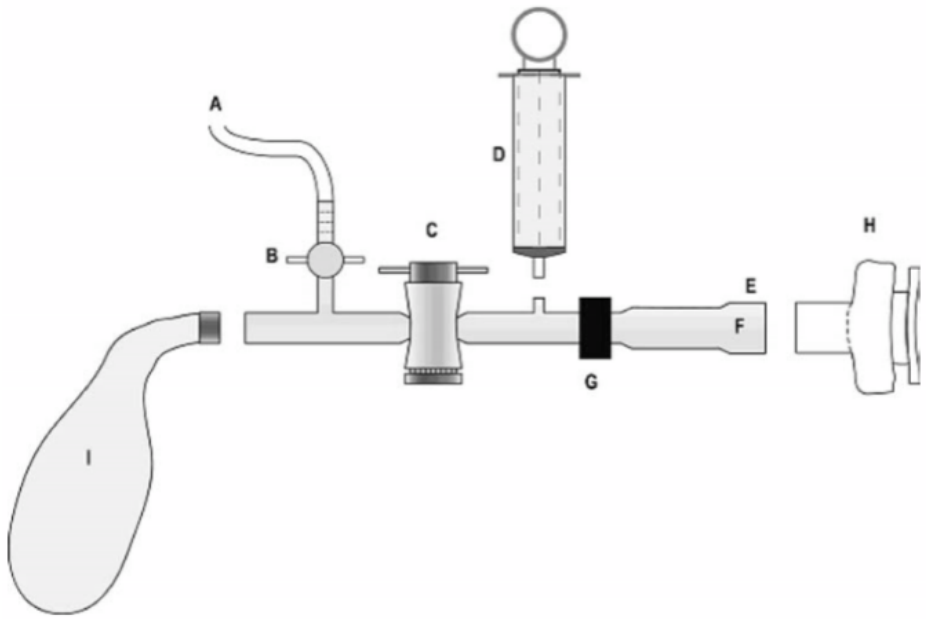

Figure 7.1. Custom made spirometer developed for the optimised carbon monoxide rebreathing (oCOR) method by Schmidt & Prommer. (A) oxygen (O2) tube, (B) O2 valve, (C) valve of the O2 reservoir, (D) prefilled CO syringe (100 ml capacity), (E) adapter to enable mouth-piece connection, (F) netting bag of soda lime (CO2 scrubber), (G) sleeve, (H) mouthpiece, (I) 3 litre anaesthetic bag (containing pure medical grade O2). Image reproduced from the original paper(Schmidt and Prommer 2005) Patent numbers: US20050075552 A1; DE10222750C1.

The Schmidt & Prommer oCOR technique has been used to demonstrate the effect of altitude training on total-Hb mass(Gore et al. 2013) and postulated to be an effective means of detecting blood doping in elite sports (PROMMER et al. 2008)

The safety of the method is not firmly established in hospital patients, as the development and much of the use has been in sports medicine, where the bulk of trial-participants are athletes. There are, however, some studies which suggest it is safe in the context of coronary artery disease (Karlsen et al. 2016), and those with liver disease and heart failure (James M. Otto et al. 2017)

While the technique itself is relatively straightforward, setting up such a testing method in an existing lab is costly and time-consuming. The equipment, which includes the custom-made glass spirometer is costly (c.£4000), fragile and difficult to clean and maintain. To satisfy the training requirement of the developers of the technique, the author was required to travel to the physiology laboratory at the Institut für Sportwissenschaft, University of Bayreuth (Bavaria, Germany) to be trained in its use and the formula for calculating the total Hb mass

is retained by the developers with all analysis occurring via their software package. Once trained, and the specialist equipment obtained it was then necessary to arrange for 99.97% purity Carbon Monoxide to be available for the laboratory in which the testing is to occur. There are very few industrial or scientific uses for such a gas and as it is colourless, odourless, and extremely lethal, it is challenging to acquire in a hospital-lab setting. Ordering a small cylinder of research-grade CO for the respiratory lab in Papworth (Cambridgeshire, UK) involved approvals from the research office, head of pharmacy, head of physiology and loading-dock staff and further education and signage for those using the outdoor gas cylinder storage area to ensure the gas was stored safely. Furthermore, to ensure such a gas was being used for the correct purposes, we were required to undergo an audit from the gas supplier (BOC) and acquire an unusual regulator for the small cylinder (eventually obtained for approximately £200 which included delivery, dangerous gas fee, pharmacy processing fee and then an ongoing monthly cylinder rental fee). The process of arranging this cylinder took approximately 11 weeks. When equipping the lab at St Vincent's Hospital (Sydney, Australia) the first quote obtained by the author for CO was AUD$1090(c.£570), though the gas was of inadequate purity (99.5%). To obtain the appropriately pure gas mixture, the next quote involved importing a cylinder from Europe to be shipped in an individual shipping container at a cost of over AUD$14,000 (c.£7300) with a 12-week lead-in time. Eventually a more suitable arrangement was found via another gas supplier, although the quoted purity of the gas was at worst 99.9%, which could potentially lead to further, albeit minor inaccuracies. Once obtained, it was necessary to store the gas in an outdoor storage site, which necessitated a 3-story commute to fill syringes with the gas for each testing procedure and further signage and training for others using the storage facility. With these barriers in place, it seemed highly unlikely that the establishment of such testing technique would easily become widespread in hospital laboratories, without significant disturbance to standard lab routine or major investment in appropriate in-lab storage facilities for pure CO.

The challenges involved, while surmountable, led to some consideration of methods with which the process might be adapted or modified to be more easily established in an existing hospital respiratory laboratory.

As part of routine respiratory function tests (RFTs), most respiratory function labs would include a measure of the diffusing capacity for carbon monoxide (DLCO, also referred to as transfer factor for carbon monoxide or TLCO). It is a technique that was first described by Marie Krogh in 1915 (Krogh 1915), whereby a known volume of CO is inhaled and uptake by the subject measured. As CO is so rapidly and avidly bound to Hb, forming stable

carboxyhaemoglobin (COHb), its uptake & binding to circulating Hb is limited only by the availability of CO and the diffusion capacity of the barrier across which it must diffuse, in this case the alveolar membrane. When there is damage to the alveolar membrane, reduction in available alveolar surface area, or reduction in pulmonary capillary blood volume, the diffusion capacity of CO is reduced. DLCO thus becomes a good measure of the overall gas-exchanging capacity of the lung and is still the primary reliably used measure of this in the laboratory setting.

While Krogh described using CO "prepared from formic acid and sulphuric acid and washed with 20% caustic potash and potassium permanganate", modern respiratory labs use a readily available mixture of CO (usually 0.3%) with the addition of a tracer gas (usually methane 0.3% or helium 0.3%) in order to calculate alveolar volume. In a standardised DLCO test, the subject exhales fully to residual volume (RV) then inhales the gas mixture rapidly to total lung capacity (TLC), holds at TLC for approximately 10 seconds and then exhales again to RV (MacIntyre et al. 2005). A sample of the exhaled alveolar gas is then analysed for dilution of tracer gas and uptake of CO across the alveolar membrane.

During the breath-holding phase of the test, the inhaled CO is diluted into the alveolar gas and is transferred into the pulmonary capillaries at an exponential rate. The log of the change in concentration per minute is calculated as a rate constant (kCO). The total accessible alveolar volume is calculated using the aforementioned tracer gas and by measuring its concentration in the inhaled and exhaled samples and the relevant volumes. If V_1 is the inhaled volume and F_1 is the concentration of tracer in the inhaled sample, and V_2 is the exhaled volume and F_2 is the concentration of tracer in the exhaled sample, then $F_1V_1 = F_2V_2$ and therefore: $V_2 = V_1F_1/F_2$ or $VA = V_I \times (F_I \text{ tracer}/F_A \text{ tracer})$

(where F_A tracer is the alveolar (exhaled) fraction of tracer gas, F_I tracer is the inspired fraction of tracer gas and V_I is the volume of inspired gas)

VA multiplied by kCO gives the rate at which CO is taken up (V_{CO}) in mL/min as demonstrated in the formula: $VA \times kCO = V_{CO}$

DLCO is dependent on the driving atmospheric pressure, therefore both sides are divided by the difference between barometric pressure and water vapour pressure, or P_B-P_{H2O} giving the formula $(VA \times kCO)/(P_B\text{-}P_{H2O}) = V_{CO}/P_B\text{-}P_{H2O}$ = DLCO mL/min per mmHg.

KCO, or the carbon monoxide transfer coefficient, which represents the efficiency per lung unit can be calculated by dividing DLCO by VA, as $KCO = kCO/(P_B\text{-}P_{H2O})$, and thus many labs report this as DLCO/VA. This usage has been disputed as it incorrectly implies that the measure is independent of lung volume, which has been demonstrated to be false.

In order to demonstrate a reproducible result, the process is repeated (with at least 4 minutes between breaths) until 2 acceptable test results are within 2mL/min/mmHg of each other. A maximum of 5 breaths can be used as DLCO begins to decrease significantly as the %COHb rises and after 5 breaths it can be reduced by as much as 3-3.5% (Graham et al. 2017) as the back-pressure from increased COHb increases due to the reduced concentration gradient. Given that DLCO testing involves the inhalation and exhalation of a measurable amount of CO, the addition of pre- and post-test measures of COHb% should provide similar information as the inputs for Schmidt & Prommer's oCOR technique. A method to measure total Hb mass using existing equipment without need for significant further training, rather only a slight modification of standard testing technique would be potentially useful and far easier to implement as a standard technique. With this in mind, the following describes an alternative method for calculating the total Hb mass using the standard equipment and software used in a DLCO test with slight modifications, the Modified DLCO method for measuring total haemoglobin, henceforth "MoDLCO". This project was approved by the St Vincent's Hospital (Sydney) Research Office (PID15112). This was designed as a proof-of-concept study to test the validity and reliability of the new method in estimating total Hb-mass.

Using a more dilute form of CO to test Δ%COHb is theoretically no different from using pure CO, especially given that using the Schmidt & Prommer technique, the pure CO is diluted into a 3L circuit, giving a concentration of 1.5-2%, depending on the dose used. In fact in the time in which the following method was developed and tested, a new technique using a rebreathed mixture of 0.15% CO was described and demonstrated to be accurate (Falz and Busse 2018). Difficulties begin to arise in detecting smaller differences due to the limitations of the measurement devices. The blood gas analyser that is used (Radiometer ABL90 Flex, Radiometer Medical ApS, Brønshøj, Denmark) provide a value of %COHb to 1 decimal place only and according to the user information, has a CV of 3.4-5.2% in the 5-10%COHb range. To minimise differences between the MoDLCO method and the Schmidt & Prommer method, we aimed to have a similar average of both inhaled CO volume and similar Δ%COHb. Approximating the amount of pure CO to be inhaled, using a 0.3% CO mixture was relatively simple. Assuming a vital capacity of 3-5L, in inspiration of 0.3% CO would equate to 12-20mLs of CO per breath. Schmidt & Prommer's technique suggests a dose of 0.8mL/kg for untrained males and 0.6mL/kg for untrained females. This would equate to 56mL for an average male of 70kg and 36ml for a 60kg female. The standardised DLCO test requires a maximum of 5 breaths, which should equate to 60-100mLs of total

inspired CO, with a higher expiratory loss due to it being a single breath technique, so it was inferred that a full 5-breath DLCO test would provide an approximation of Schmidt & Prommer's CO dose. Furthermore, the dose correction for weight and gender required in the Schmidt & Prommer technique is effectively compensated for by the corresponding increase in tidal volume seen in males and in those with higher ideal body weight.

Calculating the expected rise in %COHb in DLCO testing was somewhat more challenging. While estimating the rise is relatively straightforward, there was only one recent study in healthy volunteers that estimated the %COHb rise in those who performed a 5-second DLCO breaths to be 0.44% per breath and 0.64% rise in those that undertook 10-second DLCO breaths(Zavorsky 2013). Another older paper suggested that each standardised DLCO breath resulted in a COHB rise of approximately 0.7%(Frey et al. 1987), although this was again in healthy non-smokers.

Given the lack of clarity regarding the expected rise in COHb in hospital patients and given that the patient population undergoing testing for tHb-mass in a hospital setting may have multiple cardio-respiratory co-morbidities, it was thought wise to explore the issue further by undertaking an audit in local DLCO testing, to determine what the anticipated COHb rise would be in a usual hospital patient undergoing testing in the respiratory function laboratory. This was undertaken in order to provide information in the development of the new testing technique (MoDLCO) and also to ensure the magnitude of the expected rise would not provide any potential risk to subjects with cardiovascular co-morbidities.

With this in mind, the ACADEMY audit, a simple observational study was designed and undertaken by the author, with the help of respiratory laboratory staff. This project was approved by the St Vincent's Hospital Research Office (project identifier: D/2016/26323) and was a prospective audit of 181 patients undergoing DLCO testing at the St Vincent's Hospital respiratory function laboratory. Pre- and post-procedure %COHb was measured via earlobe sample and a per-breath mean rise in %COHb calculated.

Number of DLCO breaths:	2	3	4	5	6
Number of patients	113	51	14	2	1
Mean %COHb rise	0.861	1.278	1.414	2.15	2.1
Mean %COHb rise per DLCO breath	0.43	0.43	0.35	0.43	0.35
Overall mean %COHb per DLCO breath					0.423%

Table 7.1: Results of %COHb rise in the ACADEMY audit

Based on this audit it was assumed that the expected COHb rise in a 5 breath DLCO test would be in the order of 2.1%

With this information contributing to the development of the MoDLCO method, a trial was designed to test the accuracy and reproducibility of results gained from this novel technique. This was reported based on the structure recommended by the STARD checklist for reporting diagnostic accuracy studies.(J. F. Cohen et al. 2016)

Methods

The trial was a prospective crossover study to examine the test-retest accuracy of the new total Hb-mass (MoDLCO) technique and then compare its performance to the oCOR technique, which was considered to be the most established method of measuring total Hb-mass in recent years.

Eligibility criteria were adults without significant cardio-respiratory comorbidities and volunteers were advertised for via email and poster.

10 healthy adult volunteers were recruited, and after written consent they were testing twice using the MoDLCO technique and once using the oCOR technique. Tests were undertaken with a maximum of one test per day, to allow COHb levels to fall to baseline. Tests were non-consecutive but not randomised and undertaken in a convenience series over a period of 47-days due to laboratory and subject availability. All tests were undertaken with the assistance of a physiologist in the Respiratory Physiology Laboratory, St Vincent's Hospital, Sydney, Australia.

oCOR method

Testing was undertaken in accordance with the methods and equipment described in Schmidt & Prommer's original paper (Schmidt and Prommer 2005) and consistent with the training undertaken by this author at their institution (Institut für Sportwissenschaft, University of Bayreuth, Germany).

Ambient temperature and barometric pressure were recorded for each test. Subjects were seated quietly for approximately 10 minutes to allow for plasma volume stabilisation during which time a cream was applied to an earlobe to vasodilate and therefore "capillarise" samples. A small 1.75mm earlobe incision was made with a Tenderlett™ finger incision device (Accriva diagnostics, San Diego, CA, USA) baseline sample was taken in a 45uL heparinised (70IU/mL) blood gas collection tube (safeCLINITUBEs, Radiometer, Copenhagen, Denmark) and then analysed using the laboratory gas analyser, a Radiometer ABL90 Flex (Radiometer, Copenhagen) for pre-test %COHb. A baseline exhaled CO in PPM was recorded using a handheld CO gas analyser (Dräger Pac® 7000, Drägerwerk AG & Co., Lübeck, Germany) as used in the oCOR technique. Subjects exhaled slowly into the analyser using the Dräger calibration attachment and a 10cm length of disposable oxygen tubing. After a further period of quiet breathing, subjects were asked to exhale fully to residual volume (RV) and a nose clip applied. A 100mL syringe was flushed 3 times with pure CO (99.9% purity, Coregas Pty Ltd, Villawood, NSW, Australia) and then filled to the 60mL level before being left to equilibrate to room temperature and pressure, sealed with a 3-way tap after which it was emptied to the 50mL level which was used as the standard dose for each subject. The measurement was checked by the attending physiologist.

They were then connected to the proprietary spirometer manufactured by the authors of the technique (Spico-CO Respirations-Applikator, Blood Tec, Bayreuth, Germany) with the addition of a low volume standard anaesthetic circuit HME filter (DAR™, Covidien LLC, Mansfield, MA, USA) to decrease any risk of cross-contamination. A 3L anaesthetic bag was pre-filled with 100% oxygen and attached to the spirometer. The soda-lime cannister was filled with approximately 30mL of standard anaesthetic soda lime (Drägersorb® 800+, Drägerwerk AG & Co. KGaA, Lübeck, Germany)

They were asked to inhale fully as the 3-way tap was opened and the CO injected into the circuit and the spirometer spigot opened to allow early inhalation of the CO, then asked to breath hold for greater than 10 seconds enhancing early absorption. After this, they continued to rebreathe the oxygen/CO mixture for a total of 2 minutes during which the CO-analyser was used to check for leaks in the mouthpiece seal, nose-clip, or spirometer. At

the end of the 2-minutes, they were asked to exhale fully (to RV) into the spirometer and the spigot closed at the end of this process. The remaining gas in the circuit was then analysed for remaining CO in PPM. At 4-minutes post-test, end tidal CO was again measured and further capillary samples taken at 6- and 8-minutes post-test and blood-gas analysis performed. Measurements were entered into the proprietary software and a figure for total-Hb mass obtained.

This method was chosen as the reference standard for several reasons. Firstly, it has been demonstrated to have accuracy in a variety of settings, it is safe and of minimal discomfort to the participant and was readily available to use at short notice to increase convenience to the participant. While an official gold-standard for measuring total Hb mass does not exist, an attempt was made to compare the MoDLCO test to the historical gold-standard test for measuring Red Blood Cell Volume using radiolabelled red cells. Unfortunately, this was not available in any local centre at the time of the study. Carbon-monoxide rebreathing is considered to be the best method of estimating total Hb-mass currently(Siebenmann et al. 2017)

MoDLCO method

In the new technique, testing was undertaken in the same laboratory and tests were carried out in no set order and on days where the laboratory and subjects were available. The technique was designed to be as consistent with the oCOR process as possible, to minimise discrepancies, whilst using currently available standard respiratory laboratory equipment where possible.

Ambient temperature and barometric pressure were recorded for each test. Subjects were seated quietly for approximately 10 minutes to allow for plasma volume stabilisation during which time a cream was applied to an earlobe to vasodilate and therefore "capillarise" samples. A baseline sample was taken in a 45uL heparinised (70IU/mL) blood gas collection tube (safeCLINITUBEs, Radiometer, Copenhagen, Denmark) and then analysed using the laboratory gas analyser (Radiometer ABL90 Flex, Radiometer, Copenhagen) for pre-test %COHb. A baseline exhaled CO in PPM was recorded using a CO gas analyser (Dräger Pac 7000, Drägerwerk AG & Co. KGaA, Lübeck, Germany) with subjects being asked to exhale slowly and fully into the analyser using a calibration attachment and a short length of disposable oxygen tubing.

The lung function testing equipment that is used for DLCO testing in our laboratory is the Vmax™ Encore PFT & CPET system (VIASYS Healthcare Inc, Conshohoken, Pennsylvania, USA)

Subjects were then seated in the lung function console and allowed a further short period of quiet breathing before a nose-clip was applied. 5- standard DLCO breaths were undertaken in accordance with normal laboratory DLCO testing protocol. We used the maximal breath hold time of 15s to enable maximal absorption of CO into the pulmonary circulation. Sequential 15-second single-breath DLCO tests were undertaken at 2-minute intervals. At the end of each test, the subject was asked to take an additional VC breath of room air and hold that breath for as long as possible to minimise unmeasured loss of inhaled CO. After this VC breath, end-tidal CO was measured using the portable CO-analyser to ascertain if a significant amount of CO was exhaled on the second exhalation after the DLCO test. The results of these second breath-exhalations were tabulated per breath and in total mL CO loss. The standard plateau measurement window was used to calculate expired [CO] on the attached software.

A further capillary sample was taken at between 5- and 8-minutes after the final breath and blood-gas analysis performed in a similar manner to the initial test.

To calculate total Hb mass, the formula was based on that used in all standard CO-rebreathing methods, namely:

$$tHb\ mass = K \times MCO \times 100 \times (\Delta COHb\% \times 1.39)^{-1}$$

To estimate dead-space, there are several described methods, all of which have been questioned and none are generally considered superior. It has been demonstrated that anatomic dead space increases with age and has a reasonable correlation with age. Hart et al (Hart, Orzalesi, and Cook 1963). Therefore, in the calculation we used the formula that is used in our laboratory routinely:

$$Anatomic\ dead\ space = (weight \times 2.2) + age$$

Equipment dead space was calculated by measuring the volume of the mouthpiece/filter attached to the VIASYS spirometer. It was measured by filling the mouthpiece with water and detecting the weight gain in grams, which was then translated to mL. In this experiment, the SureGard™ (Bird Healthcare, Sydney, Australia) mouthpiece was used with a measured volume of 110mL.

After the inhalation of each breath of CO is taken, there is a progressive loss of CO from the blood to both myoglobin and exhalation. The most recently published estimation of this rate of loss was published by Prommer & Schmidt in 2007 (Prommer and Schmidt 2007) where

they showed that 0.32 ± 0.12% min^{-1} is lost to ventilation and 0.32 ± 0.18% min^{-1} is lost to myoglobin giving a total of 0.64% loss of CO per minute. This would mean that after each minute, 99.36% of the inhaled dose should remain bound to Hb and be measurable as COHb. To correct for this loss by the time of the final blood gas analysis, a correction factor was added to the calculation such that absorbed dose (at time of final sample) was calculated as:

Absorbed CO dose = (inhaled CO − dead space loss − exhaled CO) x $0.9936^{\text{time(mins)}}$

Where:

Inhaled CO dose (mL) = IVC(L) x 3

Dead space loss (mL) = calculated anatomic & equipment dead space(mL) x 0.003

Exhaled CO (mL) = IVC (L) x FE CO x 10

Time(mins) = time from breath to final blood gas sample

Adding the 5 absorbed CO doses gave a total absorbed CO in mLs.

The total-Hb Mass (MoDLCO) was then calculated using the following formula:

Total Hb mass (MoDLCO) = K x total absorbed CO x 100 x $(\Delta COHb\% \times 1.39)^{-1}$

Where:

K = barometric pressure/760 x 273/temp(K)

ΔCOHb (%) = post-test [COHb] − pre-test [COHb]

The results were tabulated and calculated using Microsoft Excel for Mac (version 16.35, Microsoft 2020), and then analysed using SPSS (version 27, IBM)

Descriptive statistics were performed on baseline characteristics including mean values and standard deviations for age, height, and weight.

For the testing techniques, mean and standard deviation were calculated for each test. Paired students t-tests were performed for MoDLCO test-1 and test-2 and then for oCOR and MoDLCO-average to determine if there was a statistically significant difference between these tests and Pearson's correlation coefficients were calculated and presented in tabular and graphical form.

Typical error was calculated for MoDLCO test 1 vs 2 and MoDCLO-average vs oCOR. A Bland-Altman plot was constructed plotting error of MoDLCO test 1, 2 and average against

the oCOR result. The limits of agreement (as described by Bland and Altman) were calculated as 1.96 x the mean difference between tests and were calculated for MoDLCO test 1 vs 2, and MoDLCO average vs oCOR and plotted on the Bland-Altman plot.

Results

The baseline characteristics of the subjects are shown below:

Subject:	Age	Gender	Height	Weight	Smoker	Comorbidities
1	40	Male	170	82	No	No
2	35	Male	177	72	No	No
3	46	Male	178	80	No	No
4	34	Male	187	90	No	No
5	29	Male	180	80	No	No
6	39	Female	165	89	No	No
7	35	Male	175	74	No	No
8	38	Male	196	87	No	No
9	30	Male	187	92	No	No
10	40	Male	183	81	No	No
Mean (SD)	36.6(5.1)		179.8 (9.0)	82.7 (6.7)		

Table 7.2: Baseline characteristics

The mean ΔCOHb in the new testing technique was 3.4(SD=0.43)

Calculated total-Hb mass results for the 3 tests are shown by subject in the table overleaf:

Subject	Total Hb - oCOR	Total Hb - MoDLCO (test-1)	Total Hb - MoDLCO (test-2)	Total Hb - MoDLCO (average)
1	842.2	876.7	810.9	843.8
2	1064.8	948.8	1091.1	1020.0
3	1002.5	1035.3	994.1	1014.7
4	964.0	979.4	992.6	986.0
5	946.2	1045.9	953.9	999.9
6	642.2	620.3	616.7	618.5
7	718.9	771.7	733.2	752.4
8	1238.5	1153.8	1162.1	1158.0
9	1040.6	1110.5	987.0	1048.7
10	1098.5	1133.2	1117.3	1125.3
Mean (SD)	955.8 (179.2)	967.6 (170.3)	945.9 (174.9)	956.7 (168.8)

Table7.3: Calculated total-Hb mass for each of the tests (2x MoDLCO and 1x oCOR)

The table below shows the results of statistical analysis when MoDLCO test 1 and test 2, then MoDLCO average and oCOR were tested as paired samples.

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	MoDLCO - test 1	967.5600	10	170.28667	53.84937
	MoDLCO - test2	945.8900	10	174.89670	55.30719
Pair 2	MoDLCO average	956.7300	10	168.77711	53.37201
	oCOR	955.8400	10	179.21633	56.67318

Table 7.4: results of paired statistics.

The means of the 2 MoDLCO tests were different by 21.7g Hb, which was quite high although the overall calibration of the MoDLCO results were excellent with a difference in means of only 0.9g Hb compared to the oCOR result.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Pair 1	MoDLCO - test 1 - MoDLCO - test2	21.67000	72.48592	22.92206	-30.18331	73.52331	.945	9	.369
Pair 2	MoDLCO average - oCOR	.89000	40.09191	12.67817	-27.79002	29.57002	.070	9	.946

Table 7.5: Descriptive statistics for MoDLCO

Paired sample t-tests showed that there was a non-significant difference between MoDLCO tests 1 and 2, and between the average MODLCO result and the result obtained from oCOR testing.

Calculating the typical error (TE) showed that when comparing MoDLCO test 1 vs test 2, the typical error was 51g (95 % confidence interval 37.4-84.3). When compared to the mean result, this represents a typical error of 5.3% in this series.

Paired Samples Correlations

		N	Correlation	Sig.
Pair 1	MoDLCO - test 1 & MoDLCO - test2	10	.912	.000
Pair 2	MoDLCO average & oCOR	10	.975	.000

Table 7.6: Correlation between MoDLCO tests and between MoDLCO (average) and oCOR

Calculating the Pearson's coefficient demonstrated that there was good correlation (r=0.912) between MoDLCO tests 1 &2 and an excellent correlation between the average of the MoDLCO results and the oCOR result (r =0.975). The results were charted in a scatter plot, and then a separate scatter plot for test 1 and test 2 were generated, which demonstrate that MoDLCO test 2 was more reliable than test 1. As is shown on the graph, there was also an improvement in the correlation from MoDLCO test 1 (r^2=0.86) to test 2 (r^2=0.96) vs oCOR.

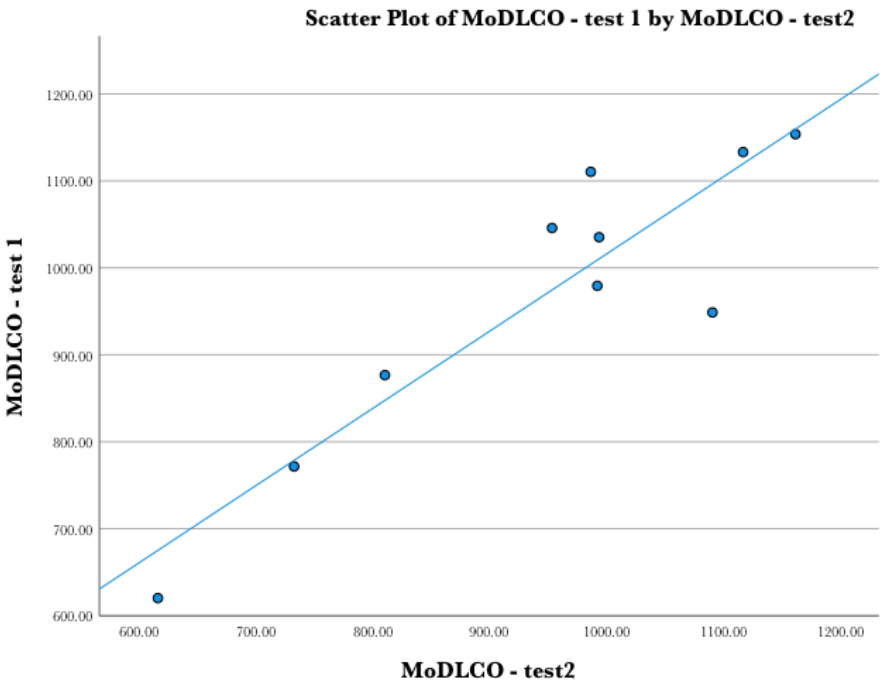

Figure 7.2: Scatter plot of MoDLCO test-1 vs test-2

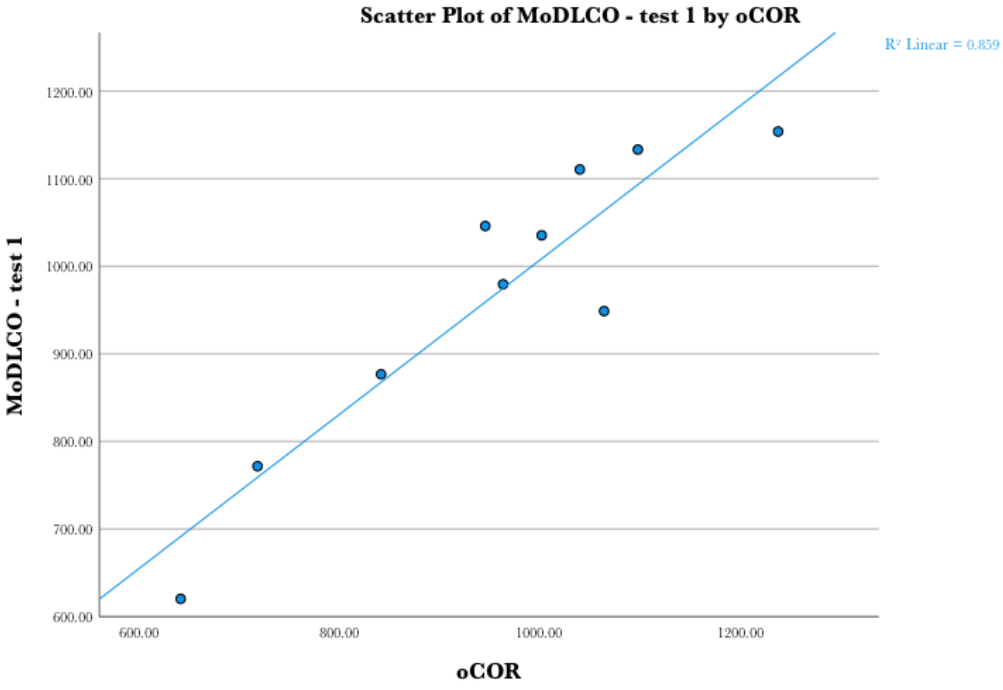

Figure 7.3: Scatter plot of MoDLCO test-1 vs oCOR

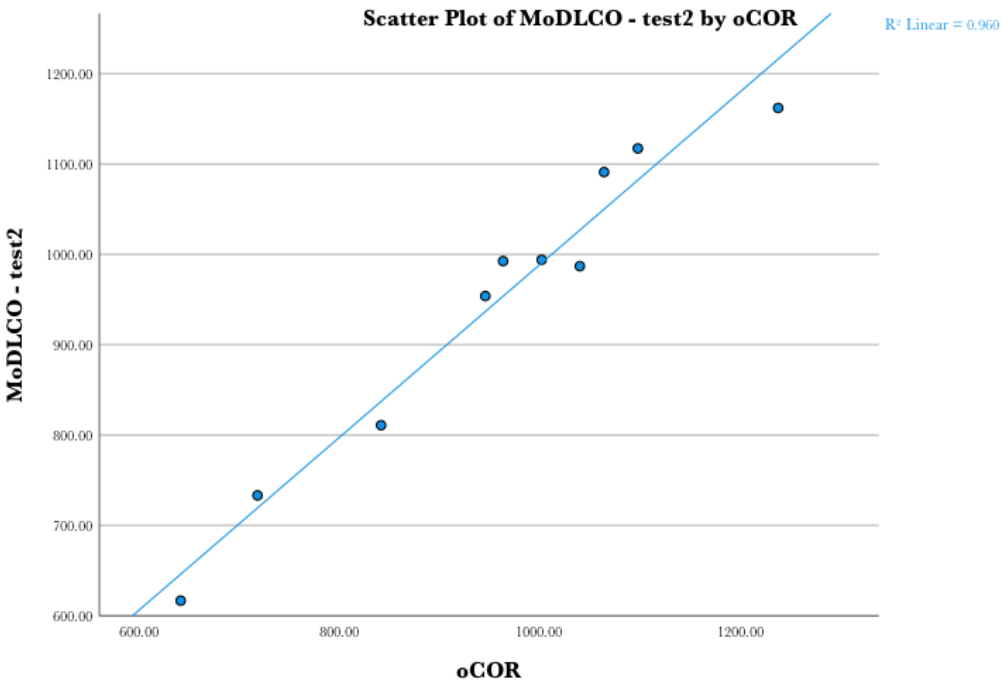

Figure 7.4: Scatter plot of MoDLCO test-2 vs oCOR

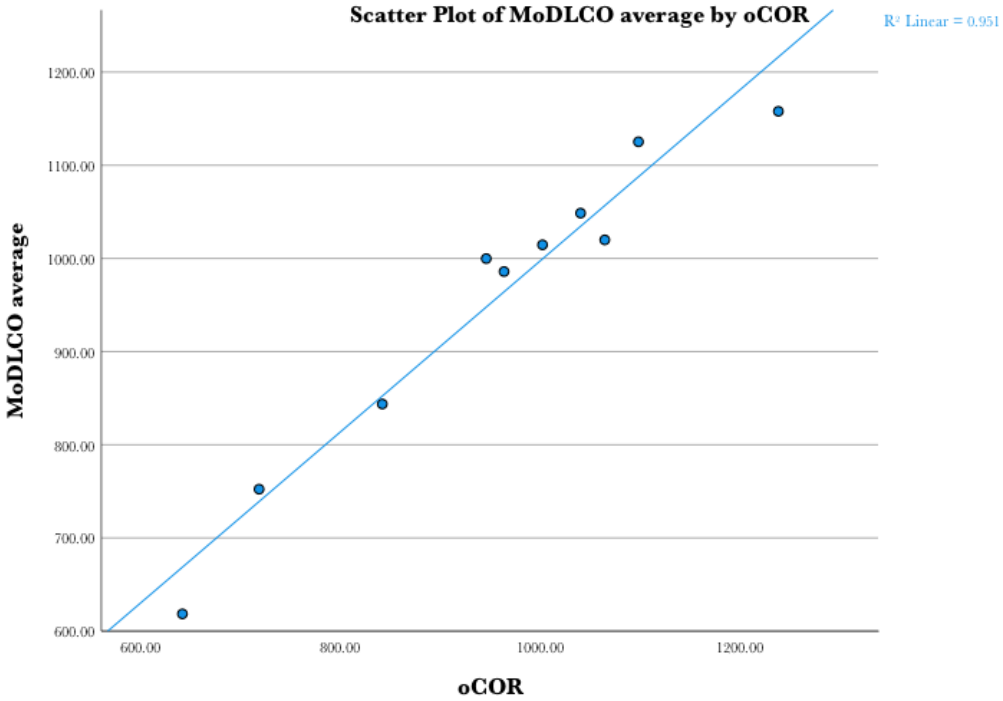

Figure 7.5: Scatter plot of MoDLCO average vs oCOR

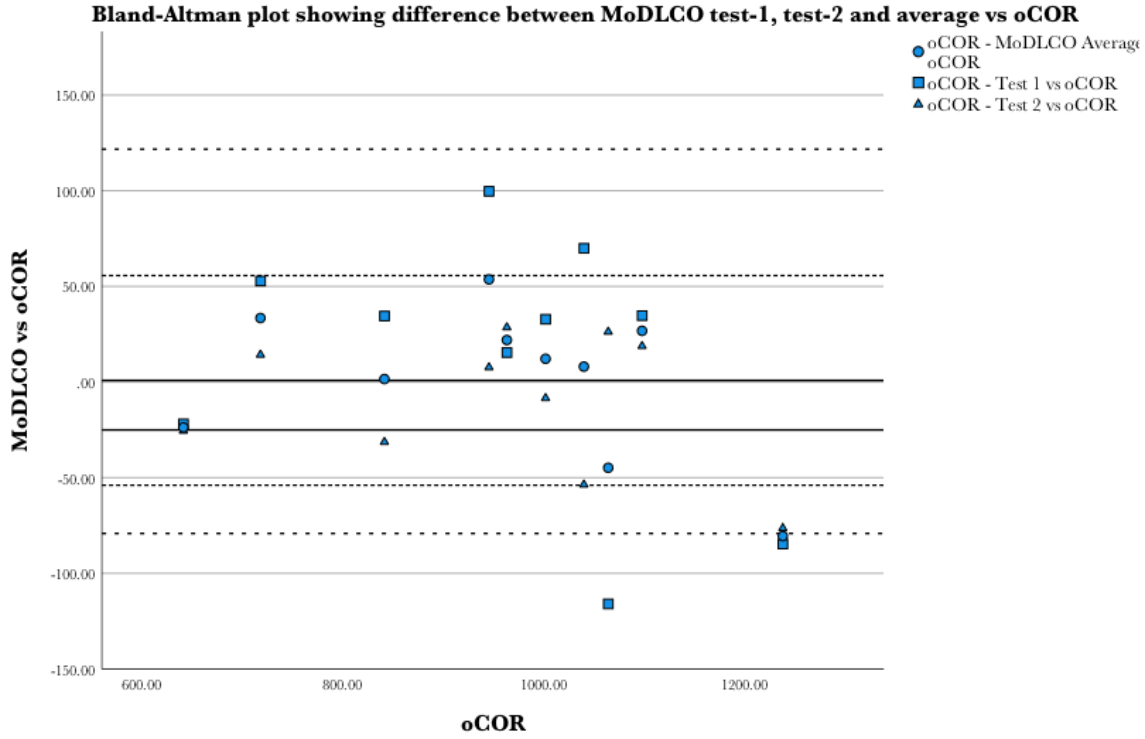

Figure 7.6: Bland Altman Plot showing differences between MoDLCO test 1, 2 and average vs oCOR. The middle solid line represents the mean difference in measured total Hb-mass between techniques (0.89g). Dotted lines represent the limits of agreement (LOA) of MoDLCO test-1 & test-2 (- - - -) and MoDLCO average & oCOR (-----)

Discussion

The results of the first assessment of the MoDLCO testing technique show that it is possible to provide a reasonable estimation of total Hb-Mass and plasma volume with only minor modification of routine lung function tests. The novel test has significant advantages in ease-of-establishment. It demonstrates that it may be possible to utilise standard respiratory lab equipment and gases to measure total-Hb mass without the need for extra equipment, laborious training, or difficult to acquire and dangerous to store pure Carbon Monoxide. This has various implications. Firstly, it makes the routine measure of total Hb-mass, which has been demonstrated to have advantages over [Hb] as an indicator of anaemic pathophysiology, practical. In addition, it allows simple estimation of plasma volume without the need for radioisotope scans. With further development, this could easily become a routine part of lung function testing or a stand-alone test in any hospital respiratory function lab.

No significant set-up cost, no additional equipment and not much additional training given that it is a slight modification of a routine test, with less accuracy than other CO-rebreathing techniques described in the literature.

The typical error rate of 51g for the new technique was less than ideal, and not indicative that this new technique would be a replacement for the oCOR technique, although it can be seen when comparing the scatterplots of test-1 vs test-2 that the accuracy improved between the two, suggesting that further improvements in accuracy may be achievable with repeated practising of the technique.

The correlation between the average of the MoDLCO tests was excellent with a Pearson's coefficient of 0.98 and good calibration with the means of the MoDLCO scores and the oCOR scores differing by only 0.9g, which suggests that with some improvement of the technique to improve reliability, it could be of value.

Longer time to test, although this may have some beneficial effects as a recent paper has suggested that perhaps 10 minutes is required to achieve complete CO-Hb equilibration, even in healthy volunteers.(Garvican et al. 2010)

There are a number of potential sources of error in the new technique that could be improved with bespoke software changes. For example, the exhaled [CO] is calculated in the Vmax™ Encore PFT & CPET system based on a small section of the exhaled gas analysis, selected by the operating physiologist, based on where the [CO] appears to plateau. If the software allowed for the full expired gas curve to be sampled, an AUC could be calculated thus allowing for accurate measurement of the total amount of CO exhaled in each breath, which should significantly improve measurement accuracy.

Other potential sources for error exist. The blood gas analyser used gives a measurement to only 1 decimal place. Given the importance of ΔCOHb% in the calculation of total Hb-mass, the accuracy of this measure is paramount to the accuracy of the overall test, and an error of 0.1% percent can change the overall result significantly. As discussed, the blood gas analyser available to us was the Radiometer Flex 90. Despite claiming overall excellent accuracy in measurement of most parameters, the quoted CV% of 3.4-5.2% is still significant enough to impact on accuracy of MoDLCO. This was touched on by a recent paper, which seemed to conform that this particular analyser has questionable accuracy in the measurement of MetHb and COHb (García-Payá et al. 2013). It is possible that by using a different analyser, specifically one that has greater accuracy in %COHb measurement, that the MoDLCO typical error could be improved. Similarly, duplicating each pre- and post-test measurement of COHb% may have

helped to reduce error, and though this was not routinely done in this study, it would be recommended in future studies.

The CO that was used for the oCOR technique was from a local supplier who were unable to confirm with certainty that it was of the minimum purity specified by Schmidt and Prommer (99.97%) which could theoretically lead to a slight reduction in the accuracy of the oCOR reference measurement. This effect should be minimal, however. Additionally, for simplicity a standard dose of 50mL of CO was given to each subject, which was in some cases lower than the dose specified in the original paper, although was in the quoted range of 0.5-1mL/kg in all cases. The accuracy of the oCOR test in our laboratory was not tested. On reflection, it would have been worthwhile to test the reliability of the oCOR technique in our hands, as it is possible the practical accuracy with our relative lack of experience and different equipment would not equal the described accuracy of the original technique. A direct comparison of test-retest results for each technique would have provided a fairer comparison of techniques.

With software modifications to standard PFT or CPET testing techniques, it should be relatively straightforward to add total-Hb mass testing to routine respiratory function testing techniques using the addition of only a pre- and post-test blood gas analysis for COHb%. At present, blood sampling is the only reliable method of measuring COHb%. Although non-invasive oximetry sensors exist, they are currently not accurate enough to detect the relatively small differences that are achieved in these tests, rather they are designed to detect the large rises seen in CO toxicity. If accuracy of these monitors was improved at lower ranges, it would theoretically be possible to estimate total Hb-mass entirely non-invasively, although PV estimation would require a blood sample to calculate from this. Further studies are required to assess whether small doses of CO would provide adequate COHb% change to estimate tHb-mass, in which case it is possible that it could be estimated using as little as one breath of the standard 0.3% CO mixture. It is feasible that if such techniques were developed, they could be used on ventilated patients in ICU, where estimation of plasma volume could be exceedingly valuable.

In conclusion, with further development, the MoDLCO technique could provide a means for any hospital respiratory function lab to measure total-Hb mass using standard lab equipment and gases, and with minimal additional training or the additional challenges of sourcing and safely storing pure Carbon Monoxide.

Chapter 8: General discussion, conclusions, and future research implications

As this thesis describes, there has been increased interest in the effect of iron-deficiency and anaemia on outcomes in cardiac surgery. While transfusion of blood products still has an important role in maintaining blood-oxygen carriage in this context, the increasing evidence of the negative effects on morbidity combined with the high cost have led to strategies to minimise or prevent transfusion. This thesis describes a number of linked projects which aim to help identify those at higher risk of peri-operative transfusion and evaluates the feasibility and effectiveness of intravenous iron therapy in those identified as being most-likely to benefit from this treatment. The scoring systems described in chapters 3 and 4 are useful tools to accurately predict those who are likely to require blood transfusions at the time of surgery and are shown to be accurate in the UK and Australian/New Zealand populations. While the CAVIAR trial did show that pre-operative IV Iron can have a beneficial effect on [Hb], it was not able to detect an improvement in either transfusion rates or outcomes. Finally, while other studies have shown that characterising total Hb-mass may have some advantages over the traditional [Hb] as a marker of anaemic physiology, current methods for testing this are difficult to establish. The new method described in chapter 7 represents an interesting early step in allowing total-Hb mass to be tested for with a slight modification of a commonly used test in hospitals worldwide. While accuracy remains inferior to the gold standard test, it has significant advantages in simplicity and could be set-up by any respiratory lab with lung-function testing capability. With further improvement in accuracy, it has the potential to bring total-Hb measurement into a more prominent role in the characterisation of anaemia in hospital patients, rather than just in athletes. It could quite easily be incorporated into standard respiratory function tests, which could increase exposure of clinicians to this measurement and give it more clinical relevance. The ability to use this test to easily calculate plasma volume increases its potential applicability to a broad range of medical conditions and settings, where estimation of fluid compartment volumes could be of enormous value. Further modification of this technique could feasibly allow for it to be performed as an add-on into ICU ventilators allowing rapid estimation of plasma volume, information which could be very valuable in guiding therapy in the critically ill.

The described studies make some contribution to the increasing body of evidence surrounding anaemia and iron replacement in cardiac surgery, but also provide direction to

future research. The ACTA-PORT and AntiPORT scores could be validated in other regions to provide greater applicability and a global risk-stratification language that could be used to provide a basis for future research into high-risk patients. As the CAVIAR study demonstrated, IV Iron has the capacity to improve [Hb] in pre-operative patients and this provides a strong suggestion that future, adequately powered research can show that it can improve transfusion- and surgical-outcomes. One such trial, ITACS, is well underway with involvement of many of the author's collaborators from the CAVIAR trial. Importantly, as these trials are being undertaken across multiple international sites, as is the case with ITACS, they provide broader applicability and may foster global interest in future research. Chapter 6 provides some early evidence that the use of IV Iron is rapidly increasing in advance of strong evidence that it is effective. It also suggests that anaemia rates that have been slowly decreasing globally for some time may now be reducing more rapidly. Whether there is any causality in this relationship is difficult to show, but further exploration of this trend may provide some large-scale evidence that IV Iron has an important role in the treatment of anaemia. Further improvement in diagnosis and treatment pathways for pre-operative anaemia and iron-deficiency could make a significant impact on patient outcomes. Chapter 2 discusses the concept of non-anaemic iron-deficiency (NAID) and this area is also being increasingly targeted by research including some significant interest in the targeting of this group in cardiac surgery with IV iron.

Future areas of research by the author will include continuing to explore the use of Hb-mass testing techniques to determine effectiveness of IV iron on improving Hb-mass prior to surgery as is underway with the ongoing PREFIX trial. Further development of the MoDLCO method may allow for this to be used as a testing modality in such research. Further exploration of the ongoing trends in IV iron prescription and anaemia rates in cardiac surgical patients will also continue.

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Appendix 1: The ACTA PORT score published manuscript (*British Journal of Anaesthesia, 2017*)

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Cardiovascular

The ACTA PORT-score for predicting perioperative risk of blood transfusion for adult cardiac surgery

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Abstract

Background. A simple and accurate scoring system to predict risk of transfusion for patients undergoing cardiac surgery is lacking.
We conducted a retrospective analysis of data collected from the ACTA National Audit. For the derivation dataset, we included data from 20 036 patients, which we then externally validated using a further group of 1047 patients.
Methods. We identified independent risk factors associated with transfusion by performing univariate analysis, followed by logistic regression. We then simplified the score to an integer-based system and tested it using the area under the receiver operator characteristic (AUC) statistic with a Hosmer-Lemeshow goodness-of-fit test. Finally, the scoring system was applied to the external validation dataset and the same statistical methods applied to test the accuracy of the ACTA-PORT score.
Results. Several factors were independently associated with risk of transfusion, including age, sex, body surface area, logistic EuroSCORE, preoperative haemoglobin and creatinine, and type of surgery. In our primary dataset, the score accurately predicted risk of perioperative transfusion in cardiac surgery patients with an AUC of 0.76. The external validation confirmed accuracy of the scoring method with an AUC of 0.84 and good agreement across all scores, with a minor tendency to under-estimate transfusion risk in very high-risk patients.
Conclusions. The ACTA-PORT score is a reliable, validated tool for predicting risk of transfusion for patients undergoing cardiac surgery. This and other scores can be used in research studies for risk adjustment when assessing outcomes, and might also be incorporated into a Patient Blood Management programme.

Key words: anaesthesia; cardiovascular; risk prediction; transfusion

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Editor's key points

- The authors performed a retrospective analysis of data from 20 036 patients undergoing cardiac surgery in the UK to derive and validate a simple scoring system for risk of transfusion.
- The ACTA PORT-score accurately predicted risk of perioperative blood transfusion in cardiac surgery patients.
- This tool will be useful in risk assessment and preoperative optimization approaches.

Cardiac surgery is associated with comparatively high rates of blood product transfusion. Blood products are a limited resource and are both expensive and resource-intensive; cardiac surgery consumes a significant proportion of global blood resources. There is conflicting evidence supporting the relative merits of restrictive[1] or liberal[2] transfusion triggers, but there is a significant body of evidence that *any* perioperative transfusion is associated with higher risk of mortality in both the short-[3] and long-term.[4]

Several factors have been shown to be independently associated with transfusion in cardiac surgery patients. These include age,[5] gender, preoperative haemoglobin concentration (Hb), elevated plasma creatinine[3] and low body weight.[3] Various attempts have been made to synthesise these predisposing factors into a predictive scoring system,[6–8] but as yet none have become widely established.

Patient blood management (PBM) is an increasingly important concept in perioperative medicine. As with any risk-reduction strategy, the first step is to predict individual risk, followed by targeted strategies to mitigate this risk.[9] This allows for appropriate and focussed use of PBM strategies, which can be expensive or result in harmful side-effects, and should therefore be reserved for those at higher risk of transfusion. Scoring systems to predict general mortality and morbidity are widely used in cardiac surgery and critical care, such as the EuroSCORE[10] and the recently published ARCTIC score[11] and Surgical Outcome Risk Tool (SORT).[12] A similar scoring system to guide effective perioperative PBM could have a major impact on resource allocation and potentially on perioperative morbidity. We therefore decided to design the Association of Cardiothoracic Anaesthetists (ACTA) perioperative risk of blood transfusion score – the ACTA-PORT score, using a large national database collected by members of ACTA.

Methods

This study comprises a national service audit of National Health Service (NHS) cardiac surgery centres that collected relevant patient data as part of routine institutional practice. The Research and Ethics Committee of the London School of Hygiene and Tropical Medicine approved the study, and individual patient consent was not required. Between 1st January 2010 and 31st July 2013, data were collected from 10 cardiac surgery centres in the UK during the first ACTA national audit; an analysis of the effect of anaemia has already been published.[11] After the analysis was complete, a further centre provided data from the same study period – this was analysed as the external validation dataset.

Baseline data collected included age, gender, preoperative haemoglobin (Hb) creatinine, weight, height, logistic EuroSCORE, diabetes, hypertension, type of surgery proposed and previous cardiac surgery. BMI and body surface area (BSA) were derived from weight and height. These variables were chosen because we a priori expected them to be associated with outcome. Outcomes recorded included number of units of blood transfused, duration of ICU and hospital stay, and death.

As our goal was to produce a simple-to-use integer risk score, the continuous variables age, preoperative haemoglobin, creatinine, logistic EuroSCORE, BMI and BSA were all categorised using clinical judgement where available or otherwise following graphical inspections and taking into account the distribution of the outcome. We used logistic EuroSCORE as EuroSCORE-2 was not in routine use in the NHS during the study period. Although EuroSCORE was designed to calculate the risk of mortality (as opposed to transfusion), we included it as a separate variable to aid in the calculation of risk of transfusion. Operation type was grouped into three categories: isolated coronary artery bypass graft (CABG) or valve surgery, combination surgeries (CABG and valve, or valve and valve), and other (including operations on the aorta).

The univariate association between each of the baseline variables and the outcome of blood transfusion was assessed using logistic regression. Forward and backward stepwise model building approaches were used in developing a final multivariable logistic regression model using a threshold for inclusion or exclusion of $P<0.05$. Both approaches yielded identical final models. A restricted set of pre-specified potential interactions were investigated using likelihood ratio tests.

As our goal was to produce a risk score that is generalisable beyond the centres involved in this audit, centre was not included as a fixed effect in the model. We compared multivariable logistic regression models omitting centre completely and multivariable mixed effects logistic regression models including centre as a random effect. These two approaches produced almost identical results in terms of the estimate odds ratios and the overall model performance, and we therefore decided to proceed with the former.

Adjusted odds ratios and 95% confidence intervals for the final multivariable model are presented along with *P*-values from a likelihood ratio test. For each variable in the model, we set the lowest risk category as the reference group so that the risk score would only involve the addition of points. The logistic odds ratio for each category was converted into an integer by dividing by 0.2 and rounding to nearest the nearest whole number. The total integer risk score for each patient was then calculated by summing the points associated with their combination of baseline risk factors.

The discriminatory performance of the risk score before and after simplification was assessed using the area under the receiver operating characteristic curve (AUC) statistic. The goodness of fit of the models, (i.e. how closely predicted risk matched observed risk), was assessed using the Hosmer-Lemeshow goodness-of-fit test.

The predicted risk of transfusion associated with each value of the total integer risk score was calculated and presented in a table and figure. We grouped the risk score into six equally spaced categories (0-4, 5-9, 10-14, 15-19, 20-24 and 25-30) and plotted the observed *vs* predicted proportion of patients transfused in each category.

We assessed the sensitivity of our results to the influence of missing data using multiple imputation. Multiple imputation with chained equations was used to generate 20 completed datasets. The selected model was then fitted to each of the 20 completed datasets and the estimated coefficients were combined according to Rubin's rules.

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An external validation of the integer risk score was carried out using data from a further cardiac surgical centre. The integer risk score was calculated for each patient in the external dataset and the performance of the risk score was assessed using the AUC and the Hosmer-Lemeshow goodness-of-fit test. We grouped the risk score for the validation patients into the same categories as described above for the derivation data and plotted the observed *vs* predicted risk of transfusion. We also used our validation dataset to compute the TRACK score and compared our model with TRACK using the DeLong method. We were unable to calculate any other published risk scores as we did not collect the required variables.

The analysis was carried out using Stata 14 (StataCorp, College Station, TX, USA).

Patient involvement

Patients/service users/lay people were not involved in the design of this study.

Results

We analysed data from 20 036 patients, whose baseline characteristics are shown in Table 1. A total of 8635 (43%) patients were transfused.

Table 1 shows the baseline characteristics of the patients overall and by the outcome of blood transfusion. The mean age of patients in this audit was 67 yr [range 18, 111], and 71% were male. Mean preoperative haemoglobin was 132 g L^{-1}; 31% of patients were anaemic (<130/<120 g L^{-1} for males/females, respectively). Haemoglobin was not available for 16% of patients. Of the 20 036 patients 8635 (43%) received a blood transfusion perioperatively.

With the exception of a known history of hypertension, all baseline variables were strongly associated with risk of blood transfusion (all $P<0.001$) in the univariate analysis. Age, EuroSCORE, female gender, diabetes mellitus and elevated creatinine were positively associated with risk of transfusion. Haemoglobin, BMI and BSA were negatively associated with risk of transfusion. Patients undergoing combined surgery were more likely to be transfused. There were marked differences in transfusion rates among the 10 centres, which ranged from 31% to 56% (Table 2).

Table 3 shows the adjusted odds ratios, 95% CIs and *P*-values for the 7 variables included in the final multivariable risk score. During the model building process, it was found that BSA was a stronger predictor of transfusion than BMI. Neither history of hypertension nor diabetes mellitus were found to be independently associated with risk of transfusion. Table 2 also shows the log-odds ratio, their standard errors and the integer points associated with each category. Other than age ($P=0.02$), all variables in the multivariable risk score were strongly associated with the outcome ($P<0.001$). No statistically significant interactions were found. The strongest predictor of transfusion was baseline Hb, followed by BSA and EuroSCORE. The AUC for the integer risk score model was 0.760 (95% CI 0.752, 0.768), and the Hosmer-Lemeshow goodness-of-fit test provided no evidence of a poor fit ($P=0.23$). The AUC from the non-integer risk model (i.e. using the log-odds ratios) was 0.762 indicating that little predictive power had been lost through the simplification process.

The risk score for any patient is simply calculated by adding the points associated with their baseline characteristics. For example, a 65 yr old (+0 points) male (+0 points), with baseline Hb of 135 g L^{-1} (+3 points), BSA of 2.0 (+2 points), logistic EuroScore of 1.5 (+2 points), creatinine of 1.5 (+1 point) and undergoing CABG surgery (+2 points) would have a total risk score of 10 points. A 75 yr old (+1 point) female (+1 point), with baseline Hb of 125 g L^{-1} (+6 points), BSA of 1.8 (+4 points), logistic EuroScore of 2 (+3 points), creatinine of 2.5 (+3 points) and undergoing valve surgery (+2 points) would have a total risk score of 20 points.

Table 4 and Fig. 1 show the predicted risk of transfusion associated with each value of the risk score. Figure 1 shows the distribution of risk score among patients in the audit. The risk

Table 1 Baseline characteristics. Values are mean (SD), number (proportion or median (IQR (range)). *Indicates isolated CABG or single valve surgery

	All $n=20\,036$	Not transfused $n=11\,041$	Transfused $n=8638$	P-value
Age; yr	67.1 (11.9)	65.2 (12.0)	69.7 (11.3)	<0.001
Sex; men	14 303 (71.4%)	9093 (79.8%)	5210 (60.3%)	<0.001
Preoperative Hb; g L^{-1}	132 (17)	138 (15)	125 (17)	<0.001
Missing data	3237 (16.2%)	2077 (18.2%)	1160 (13.4%)	
Body surface area; m^2	1.9 (0.2)	2.0 (0.2)	1.9 (0.2)	<0.001
BMI; kg m^2	28.4 (5.1)	29.0 (5.0)	27.6 (5.0)	<0.001
EuroSCORE	4.3 (2.1–8.7 (0.4–98.4))	3.2 (1.7–6.6 (0.4–98.4))	6.0 (3.1–11.7 (0.4–97.9))	
Missing data	393 (2%)	238 (2.1%)	155 (1.8%)	
Creatinine; μmol L^{-1}	88 (71–106 (9–1547))	88 (71–97 (9–1547))	88 (71–106 (9–1450))	<0.001
Missing data	2172 (10.8%)	1254 (11%)	918 (10.6%)	
Diabetes mellitus	3916 (22.0%)	2114 (20.7%)	1802 (23.8%)	<0.001
Missing data	2267 (11.3%)	1208 (10.6%)	1059 (12.3%)	
Hypertension	13 325 (67.8%)	7511 (67.2%)	5814 (68.6%)	0.04
Missing data	384 (1.9%)	224 (2.0%)	160 (1.9%)	
Operation type				
CABG or valve*	14 575 (73%)	8778 (77%)	5797 (67%)	
Double procedure	2858 (14%)	1008 (9%)	1850 (21%)	
Other	2594 (13%)	1608 (14%)	986 (11%)	<0.001
Missing data	9 (<0.1%)	7 (0.1%)	2 (<0.1%)	

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Table 2 De-identified centres. The difference in transfusion rates between centres was statistically significant ($P<0.001$)

Centre	All $n=20\,036$	Not transfused $n=11\,401$	Transfused $n=8638$	Transfusion rate
A	2559 (13%)	1268 (11%)	1291 (15%)	50%
B	732 (3%)	425 (4%)	307 (4%)	42%
C	2058 (10%)	1410 (12%)	648 (8%)	31%
D	2371 (12%)	1233 (11%)	1138 (13%)	48%
E	5371 (27%)	3283 (29%)	2088 (24%)	39%
F	500 (3%)	292 (3%)	208 (2%)	42%
G	960 (5%)	423 (4%)	537 (6%)	56%
H	1986 (10%)	1029 (9%)	957 (11%)	49%
I	1099 (6%)	618 (5%)	481 (6%)	44%
J	2400 (12%)	1420 (12.5%)	980 (11%)	41%

Table 3 Multivariable Risk Score outlining corresponding odds ratios, log odds ratios and how ACTA-PORT score was constructed, showing the number of score-points that were attributed to each group

Characteristic	Category	Odds ratio (95%)	P-value	Log odds ratio (SE)	Points
Age; yr	<70	Ref.			+0
	70+	1.11 (1.01, 1.21)	0.02	0.10 (0.04)	+1
Sex	Male	Ref.			+0
	Female	1.27 (1.15, 1.40)	<0.001	0.24 (0.05)	+1
Haemoglobin	<110	6.36 (5.38, 7.52)		1.85 (0.09)	+9
$g\,L^{-1}$	110-	4.60 (3.93, 5.38)		1.53 (0.08)	+8
	120-	3.19 (2.79, 3.65)		1.16 (0.07)	+6
	130-	1.93 (1.70, 2.20)		0.66 (0.07)	+3
	140-	1.55 (1.37, 1.77)		0.44 (0.07)	+2
	150+	Ref.	<0.001		+0
Body surface area	<1.7	3.62 (2.97, 4.42)		1.29 (0.10)	+6
m^2	1.7-	2.21 (1.85, 2.64)		0.79 (0.09)	+4
	1.9-	1.56 (1.31, 1.85)		0.44 (0.09)	+2
	2.1-	1.24 (1.04, 1.49)		0.22 (0.09)	+1
	2.3+	Ref.	<0.001		+0
EuroSCORE	<1	Ref.			+0
	1-	1.36 (1.10, 1.70)		0.31 (0.11)	+2
	2-	1.73 (1.39, 2.15)		0.55 (0.11)	+3
	3-	2.16 (1.75, 2.68)		0.77 (0.11)	+4
	9+	2.76 (2.20, 3.46)	<0.001	1.01 (0.12)	+5
Creatinine	<88	Ref.			+0
$\mu mol\,L^{-1}$	88-	1.33 (1.23, 1.44)		0.29 (0.04)	+1
	177-	1.93 (1.54, 2.42)	<0.001	0.66 (0.12)	+3
Type of Operation	CABG/Valve	1.38 (1.22, 1.55)		0.32 (0.06)	+2
	Combination	2.84 (2.46, 3.29)		1.05 (0.07)	+5
	Other	Ref.	<0.001		+0
Intercept		NA		−3.00	(0.15)

score can in theory take values ranging from 0 to 30, with a higher score associated with a higher risk. For example, the risk of transfusion for a patient with a risk score of 10 is estimated to be 27% compared with an estimated risk of transfusion of 73% for a patient with a risk score of 20. Figure 1 shows that the risk score is fairly normally distributed in this sample of patients with very few patients having a risk score below 5 (1.9%) or above 24 (1.8%). The median risk score was 14, for which the estimated risk of transfusion was 45%.

Figure 2 shows the observed *vs* predicted risk of transfusion across categories of the risk score. The score performs well in stratifying the transfusion. Among patients with a score below 10, less than 20% were transfused compared with close to 80% of patients with a score of 20 or above, a four-fold higher risk. There is good agreement between the predicted and observed probability of transfusion.

The AUC for the risk score in the external validation dataset was 0.835 (95% CI 0.810, 0.859). However, the score tends to underestimate risk of transfusion, particularly at higher levels of the score, (e.g. 73% and 89% observed *vs* 60% and 79% predicted in the 15–19 and 20–24 categories, respectively).

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The score was not designed to predict number of units of blood transfused. However, increasing ACTA-PORT score was associated with increased number of units of blood transfused perioperatively: risk score 0-14, median units of blood transfused 0; score 15-19, median 1 unit; score 20-24, median 2 units; and score 25-30, median 3 units.

Table 4 Integer risk score totals and associated predicted risk of transfusion. Low scores have a very low risk of transfusion (i.e. a score of 1 gives a risk of transfusion<5%), whereas a high score of 30 has >95% risk of requiring a transfusion

Integer risk score	Predicted risk of transfusion	Integer risk score	Predicted risk of transfusion
0	0.0470	15	0.500
1	0.0570	16	0.550
2	0.0690	17	0.599
3	0.0830	18	0.646
4	0.1000	19	0.690
5	0.1190	20	0.731
6	0.1420	21	0.769
7	0.1680	22	0.802
8	0.1980	23	0.832
9	0.2310	24	0.858
10	0.2690	25	0.881
11	0.3100	26	0.900
12	0.3540	27	0.917
13	0.4010	28	0.931
14	0.4500	29	0.943
15	0.5000	30	0.953

The results from the sensitivity analysis using multiple imputation did not make substantial changes to the risk score. We also calculated the performance of the ACTA-PORT score at various integer risk score cut-points; the optimum cut-point was 15, with a positive predictive value of 70% and a negative predictive value of 71%, with 70% of values correctly predicted.

Discussion

We developed a simple, integer-based scoring system that accurately predicted the likelihood of transfusion. We also externally validated this, demonstrating its applicability in a real-world scenario.

The concept of a scoring system designed to predict the risk of bleeding or transfusion during cardiac surgery is not new. One of the first efforts in this area was from Papworth Hospital.[6] This system aimed to measure blood loss exceeding 2mL kg^{-1} hr^{-1}, requirement for fresh frozen plasma, platelets or cryoprecipitate, or return to theatre after arrival in the ICU. Whilst the negative predictive value of this score was high, only 27% of patients who the score placed in the highest risk category subsequently demonstrated major bleeding. This low positive predictive value was confirmed by a subsequent external validation.[7]

Whilst the Papworth Bleeding Risk Score sought to identify those patients at risk of excessive blood loss in the ICU after cardiac surgery, subsequent scoring systems have sought to predict the risk of transfusion. A relatively recent example of this is the Transfusion Risk and Clinical Knowledge (TRACK) score.[8] TRACK aimed to create a simple, easily applied system, based on five predictors of transfusion risk, assigning each variable a proportional risk score based on the clinical condition of the patient. This scoring system was subsequently validated against

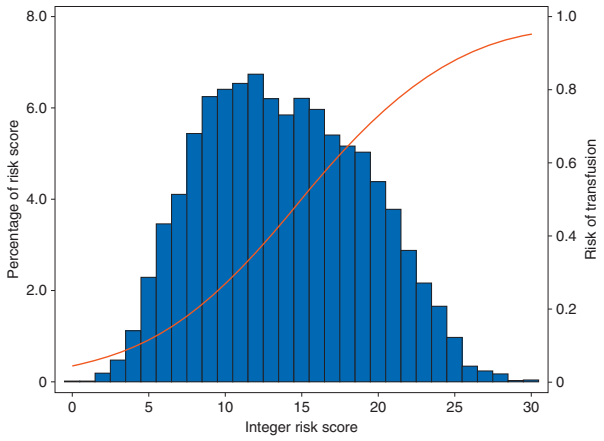

Fig 1 Distribution of risk scores in the patient population. The distribution follows a relatively normal curve. Superimposed is a line showing the increasing risk of transfusion associated with higher scores.

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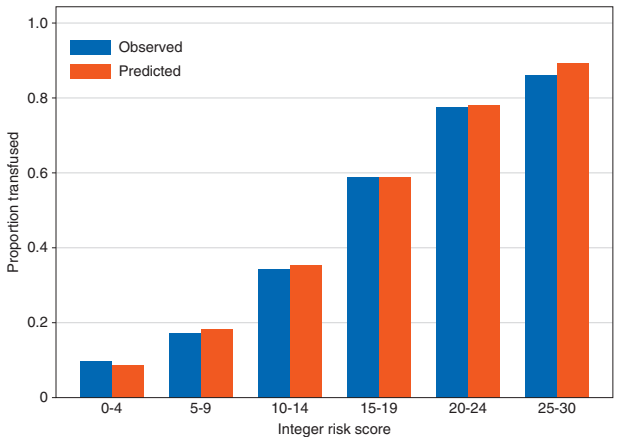

Fig 2 Observed *vs* predicted transfusion rates in the derivation dataset. There is a close correlation between predicted and observed rates of transfusion across the range of scores in the derivation dataset.

an external cohort, and proved to be superior to three earlier systems[13–15] with an AUC of 0.70. Like the ACTA-PORT score, TRACK aimed to improve the utility of the scoring system for clinical practice as a result of its relative simplicity, whist at the same time remaining sensitive and specific for predicting transfusion risk. We used our validation dataset to also compute the TRACK score; the AUC of TRACK was 0.781 (ACTA-PORT vs. TRACK $P<0.001$ using the DeLong method for comparing risk scores). We therefore conclude that the ACTA-PORT score performs significantly better than TRACK.

Recently, Goudie and colleagues[16] published two risk prediction models: one for any red cell transfusion, and another for a requirement for massive transfusion. This is considerably more complex than the simpler TRACK and ACTA-PORT prediction models. When the Goudie model was published, it represented an advance on many existing scoring systems,[10,15,17] with an AUC of 0.77 for any red blood cell transfusion. We were unable to calculate the risk of transfusion from our dataset using the Goudie method as we did not collect all the necessary data.

The use of risk scores for transfusion such as ACTA-PORT might allow clinicians to quantify this risk before surgery, thereby potentially allowing modification of important risk factors during preoperative optimisation. Transfusion has been shown to be associated with increased 30-day mortality,[18] morbidity related to ischaemia,[19] infection,[20–23] renal impairment,[24] post-CABG graft occlusion[25] and acute lung injury.[26] With respect to longer term outcomes, Engoren and colleagues found that blood transfusion during cardiac surgery was associated with a doubling of the risk of death at five yr.[27] Yet this clinical intervention, with appropriate preoperative warning and preparation, can potentially be avoided.

In our study, the only realistically modifiable risk factor associated with requirement for blood transfusion was Hb. Patients with an Hb $< 130\,g\,L^{-1}$ accounted for nearly 50% of all transfusions, despite making up only one-third of the total cohort. Using the risk profile of those patients included in the ACTA-PORT cohort, a PBM program able to increase haemoglobin from 120 to $130\,g\,L^{-1}$ would theoretically decrease risk of transfusion during the perioperative period by 40%, with an implied reduction in perioperative morbidity and mortality.

Similar to previous studies that have used the retrospective analysis of large databases to generate a risk score, our study suffers from some limitations. First, the preoperative management of patients presenting for cardiac surgery at the centres involved in the study was not standardised. The possibility that patients at certain centres were exposed to different PBM strategies therefore cannot be excluded, and could potentially confound any subsequent analysis. Such strategies might include differences in the cessation of anti-platelet therapies and use of cell salvage, and transfusion preferences of individual surgeons and centres. All centres administered tranexamic acid routinely, but at different doses depending on institutional preference.

Secondly, despite demonstrating overall reliability in predicting risk of transfusion, the score does slightly underestimate transfusion risk in the higher risk categories. Patients with risk score >20 had a roughly 10% higher observed rate of transfusion relative to predicted risk. This might reflect the nature of the validation cohort, being from a single centre, as opposed to the multi-centre model derivation dataset. Consequently, the transfusion practices in the specific centre might not accurately reflect general transfusion practice. This could be as a result of regional variation in anaemia incidence as described,[17] or a higher incidence of complex cardiac surgery at this specific

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centre. The decision by the authors to not specifically correct for regional variation was made in order to retain generalisation, enabling the scoring system to be used at centres outside those that participated in the initial cohort. Consequently, if a centre has policies or surgeons that make transfusions more likely (compared with the average in the audit), the score will underestimate risk, as evidenced by the results of the validation cohort. Whilst ACTA-PORT will be useful to stratify risk of transfusion in any patient presenting for cardiac surgery, the system will need to be recalibrated if centres outside of the control cohort wish to use it to predict absolute risk. We plan to design a simple App/online calculator to calculate the ACTA-PORT score when planning surgery or discussing risk with patients. In addition, we were only able to compare the ACTA-PORT score with the TRACK score, and were unable to compute other risk scores because of lack of appropriate data.

Finally, the score makes use of the EuroSCORE[10] as an overall marker of patient mortality. This might limit the applicability of the scoring system beyond health systems that routinely collect this information, particularly centres in China[28] and Australia.[29] Furthermore, risk prediction models are subject to constant revision,[30] potentially further limiting the applicability of derived models that make use of them.

In summary, using a large, multicentre cohort of patients collected from multiple cardiac centres, we derived a robust, simple and accurate system for predicting risk of transfusion for patients undergoing cardiac surgery. Future research will ideally include independent validation against a further external cohort, comparing ACTA-PORT with other bleeding/transfusion risk scores. This and other scores could be used in research studies for risk adjustment of patients when assessing the outcome of an intervention, and could also be incorporated into Patient Blood Management programmes.

Authors' contributions

Study design/planning: A.A.K., T.R.
Study conduct: A.A.K., N.F., C.E.
Data analysis: T.C.
Writing paper: T.C., J.Y., L.F.M.
Revising paper: all authors

Declaration of interest

All authors have completed the ICMJE uniform disclosure form at http://www.icmje.org/coi_disclosure.pdf and declare: A.A.K. has received funding for research/education and/or honoraria and travel support from Pharmacosmos, Vifor Pharma, CSL Behring and Fisher and Paykel; C.E. has received honoraria and travel support from Pharmacosmos; T.R. has received funding for research/education and/or honoraria and travel support from Pharmacosmos and Vifor Pharma. S.F. is the President of the Association of Cardiothoracic Anaesthesia and Critical Care (ACTACC). The authors received no support from any organisation for the submitted work; and no financial relationships with any organisations that might have an interest in the submitted work in the previous three yr; and no other relationships or activities that could appear to have influenced the submitted work.

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Appendix 1

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Appendix 2: AntiPORT paper (submitted to *Anaesthesia & Intensive Care, 2021)*

AntiPORT: adaptation of a transfusion prediction score to an Australian cardiac surgery population

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Summary:

Introduction

Risk scoring systems exist to predict perioperative blood transfusion risk in cardiac surgery. None have been validated in the Australian or New Zealand population. The ACTA-PORT score was developed in the UK for this purpose. In this study we validate and recalibrate the ACTA-PORT score in a large national database.

Methods

We used data from the Australian and New Zealand Society of Cardiac and Thoracic Surgeons Database between September 2016 and December 2018. ACTA-PORT score was calculated using an equivalent of EuroSCORE. Discrimination and calibration was assessed using area under the receiver operating curve (AU-ROC), Brier scores and calibration plots. ACTA-PORT was then recalibrated in a development set using logistic regression and the outcome of transfusion to develop new predicted transfusion rates – termed AntiPORT. Accuracy of these new predictions was assessed as for ACTA-PORT.

Results

30,388 patients were included in the study at 37 centres. The rate of red blood cell transfusion was 33%. Discrimination of ACTA-PORT was good (AU-ROC =0.76), but calibration was poor with overprediction of transfusion. The re-calibrated AntiPORT showed significantly improved calibration in both development sets and validation sets without compromising discrimination.

Discussion

The AntiPORT is the first red cell transfusion risk scoring system for cardiac surgery patients to be validated using Australian data. It is accurate and simple to calculate. AntiPORT may help facilitate benchmarking and future research in the area of patient blood management (PBM) as well as providing a useful tool to help clinicians target PBM strategies.

Introduction

Peri-operative anaemia and the requirement for allogeneic red blood cell (RBC) transfusion are independently associated with increased morbidity and mortality following cardiac surgery (Hung, Besser, Sharples, Nair & Klein, 2011;Klein, Collier, Brar, Hallward, Fletcher, & Richards, 2016). Both nationally and internationally, surgery accounts for around a third of national blood product usage (Wallis, Wells, and Chapman 2006). Cardiothoracic surgery patients received 5.6% of all blood products in Australia (Shortt et al. 2009). While transfusion costs vary internationally, the burden on health service providers is universally significant (Amin et al. 2003a).

Various strategies to reduce overall blood product use by both pre-operative optimisation of haemoglobin and blood conservation strategies have been developed and are grouped together under the term 'Patient Blood Management' (PBM). PBM programs incur a significant economic burden, and in order to best allocate resources a number of scoring systems have been developed to identify those at increased risk of peri-operative transfusion (Vuylsteke et al. 2011a; Goudie et al. 2015; Alghamdi et al. 2006; M. Ranucci et al. 2009). Those at higher risk of transfusion can be subsequently targeted for pre-operative optimisation or more aggressive peri-operative blood conservation strategies. A recently published example is the UK generated ACTA-PORT score (Klein et al., 2017), which is a relatively simple, integer-based scoring system that proved accurate in a UK context.

To date, all relevant RBC transfusion risks scores (including ACTA-PORT) have been derived from UK, US, Canadian or European populations. None have yet been developed or validated using data from Australia or New Zealand. One paper described a score to predict

requirement for platelet transfusion in cardiac surgery using Australian and New Zealand data (Flint et al. 2020). Despite similarities in clinical practice, risk prediction tools derived from a UK population do not necessarily retain precision when directly applied to an Antipodean population (Yap et al. 2006a; Campbell et al. 2019). As noted by Reilly et al., "risk prediction tools are developed and initially validated in a specific patient population, in a specific health-care setting, at a specific point in time" (Reilly et al. 2020). This precludes direct translation of risk prediction models between countries and highlights the importance of validating such models with local data before applying them.

In this study we determine the accuracy of the original ACTA-PORT score in a cohort of Australian cardiac surgical patients. We subsequently develop and validate a recalibrated version of this score (henceforth the AntiPORT score) in our population.

Methods

We used the Australian and New Zealand Society of Cardiac and Thoracic Surgeons (ANZSCTS) Database and included data from all cardiac surgery procedures performed between 1 September 2016 and 31 December 2018. The Database currently collects peri-operative data for all patients undergoing cardiac surgery across 26 public and 30 private hospitals in the region. Since mid-2016 data collection has routinely included pre-operative haemoglobin [Hb]. The components and calculation of the ACTA-PORT score(Klein, Collier, Brar, Hallward, Fletcher et al., 2017) are included in the table below. With the exception of EuroSCORE, all other data is collected routinely by the ANZSCTS Database.

Characteristic	Category	Points
Age; years	**<70**	+0
	70+	+1
Sex	**Male**	+0
	Female	+1
Haemoglobin	**<110**	+9
g/L	**110-**	+8
	120-	+6
	130-	+3
	140-	+2
	150+	+0
Body surface area	**<1.7**	+6
m^2	**1.7-**	+4
	1.9-	+2
	2.1-	+1
	2.3+	+0
EuroSCORE	**<1**	+0
	1-	+2
	2-	+3
	3-	+4
	9+	+5
Preoperative Creatinine	**<88**	+0
µmol/L	**88-**	+1
	177-	+3
Type of Operation	**CABG/Valve**	+2
	Combination	+5
	Other	+0

Table 1: Multivariable Risk Score showing how ACTA-PORT score is calculated, showing the number of score-points that were attributed to each group.

ACTA-PORT used EuroSCORE as a surrogate for operative mortality. This was not available in our dataset. Some of the EuroSCORE components differed slightly from components collected in the ANZSCTS Database, and one (systolic pulmonary artery pressure) was not available at all. We therefore calculated EuroSCORE I (excluding pulmonary artery pressure) based on the closest available variables, as has been previously described by Yap et al. (Yap et

al. 2006b). Correlation between EuroSCORE and "All-procedures" AusSCORE was assessed. To make future calculations easier using Australian data we subsequently replaced the EuroSCORE mortality prediction ranges in ACTA-PORT with an equivalent Australasian risk prediction score developed from the ANZSCTS Database. This score was originally developed for CABG procedure only as AusSCORE (Reid et al. 2009b) and subsequently developed to include all cardiac surgical procedures, known as the 'all procedures' model (Billah et al. 2010). This "all procedures" version was used in our analysis, which is referred to as AusSCORE-AP. In the absence of previously documented techniques of finding equivalent score ranges, we calculated the interquartile range of the AusSCORE-AP in our population for each of the five possible categories of EuroSCORE included in the original ACTA-PORT score. Approximations of these interquartile ranges of AusSCORE-AP replaced the EuroSCORE categories in the new model. These two techniques in turn yielded two scores:

(1) the original ACTA-PORT score using the calculated EuroSCORE (the ACTA-PORT-ES),

(2) the original ACTA-PORT score using the AusSCORE-AP ranges to replace EuroSCORE (the ACTA-PORT-AS).

Discrimination and calibration of each of these two scores was then assessed.

Finally, given that it is likely transfusion practices will vary across the two populations, it is also likely that the calibration of the score will be affected. We determined *a priori* that we would recalibrate the ACTA-PORT score using data derived from the local population. In order to do this, the dataset was split randomly into two populations (by hospital) in an approximate 75:25, resulting in a training set and validation set. Logistic regression was carried out in the training set using allogeneic red cell transfusion as the outcome and the ACTA-PORT-AS as the independent variable. Predictions based on this logistic regression were generated for the

validation set using the ACTA-PORT-AS integer score. We termed this the 'AntiPORT' recalibration (Antipodean Peri-Operative Risk of blood Transfusion). Discrimination and calibration were then assessed in the validation set.

Baseline characteristics were compared between those patients transfused and those not transfused using Chi-square for categorical data, Student's t-test for normally distributed data and Wilcoxon Rank-Sum for non-normally distributed data. Correlation was assessed using Pearson's pairwise correlation. Discrimination of AntiPORT was assessed using the area under the receiver operator characteristic (ROC) curve. Calibration was assessed using calibration plots. Both were assessed using the Brier score. All analyses were carried out using Stata version 16.1 (StataCorp LLC 2019).

Results

We analysed data from 30,393 patients from 37 hospitals, whose baseline characteristics are displayed in Table 2.

Columns by Red blood cell transfusion	No RBC	RBC	Total	P-value
n (%)	20358 (67.0)	10030 (33.0)	30388 (100)	
Age, median (iqr)	66 (57-73)	69 (60-76)	67 (58-74)	<0.001
Sex, n (%)				
Male	16247 (79.8)	6427 (64.1)	22674 (74.6)	
Female	4111 (20.2)	3603 (35.9)	7714 (25.4)	<0.001
Procedure type, n (%)				
CABG	10991 (54.0)	4367 (43.5)	15358 (50.5)	
Valve surgery	4769 (23.4)	1948 (19.4)	6717 (22.1)	
Combined CABG/Valve	1445 (7.1)	1384 (13.8)	2829 (9.3)	
Other, n (%)	3153 (15.5)	2331 (23.2)	5482 (18.0)	<0.001
Preoperative haemoglobin, median (iqr)	142 (19)	126 (29)	138 (24)	<0.001
Preoperative creatinine, median (iqr)	84 (72-99)	89 (73-115)	85 (72-103)	<0.001
Diabetes, n (%)				
No diabetes	14605 (71.8)	6680 (66.6)	21285 (70.1)	
Diabetes	5731 (28.2)	3343 (33.4)	9074 (29.9)	<0.001
Hypertension, n (%)				
No hypertension	6064 (29.8)	2689 (26.8)	8753 (28.8)	
Hypertension	14272 (70.2)	7332 (73.2)	21604 (71.2)	<0.001
NYHA status, n (%)				
1	8740 (43.0)	3489 (34.8)	12229 (40.3)	
2	7702 (37.9)	3515 (35.1)	11217 (36.9)	
3	3306 (16.3)	2217 (22.1)	5523 (18.2)	
4	596 (2.9)	803 (8.0)	1399 (4.6)	<0.001
Estimated Ejection Fraction, n (%)				
Normal	10173 (50.8)	4779 (48.8)	14952 (50.2)	
45-60%	6862 (34.3)	2844 (29.1)	9706 (32.6)	
30-45%	2368 (11.8)	1453 (14.8)	3821 (12.8)	
<30%	604 (3.0)	710 (7.3)	1314 (4.4)	<0.001
BMI, median (iqr)	29 (25-32)	27 (24-31)	28 (25-32)	<0.001
Urgency, n (%)				
Non-urgent	14969 (73.5)	5872 (58.6)	20841 (68.6)	
Urgent	4907 (24.1)	3245 (32.4)	8152 (26.8)	
Emergency	468 (2.3)	860 (8.6)	1328 (4.4)	
Salvage	12 (0.1)	51 (0.5)	63 (0.2)	<0.001
Previous cardiac surgery, n (%)				
No	19368 (95.2)	8862 (88.4)	28230 (92.9)	
Yes	985 (4.8)	1163 (11.6)	2148 (7.1)	<0.001
Perfusion time, median (iqr)	91 (70-119)	112 (82-157)	97 (73-130)	<0.001
Mortality, n (%)				
Survived	20262 (99.5)	9504 (94.8)	29766 (98.0)	
Died	96 (0.5)	526 (5.2)	622 (2.0)	<0.001

Table 2 Baseline characteristics of the dataset used in AntiPORT derivation and validation

CABG: Coronary Artery Bypass Grafting
NYHA: New York Heart Association
BMI: Body Mass Index

Five patients (0.02%) had missing red cell transfusion data and were excluded, resulting in a final analysis cohort of 30388 patients. Of these, a total of 10,030 (33%) patients were transfused. Age, female sex, NYHA class 4 status, diabetes mellitus, hypertension and elevated creatinine were positively associated with risk of transfusion following univariate analysis. Additionally, patients undergoing combined surgery or emergency surgery were more likely to require transfusion. BMI and pre-operative [Hb] were both negatively associated with transfusion risk.

EuroSCORE and AusSCORE-AP were strongly correlated ($r=0.83$, $p<0.001$). The range of EuroSCORE used in ACTA-PORT versus the corresponding AusSCORE-AP are shown in Figure 1.

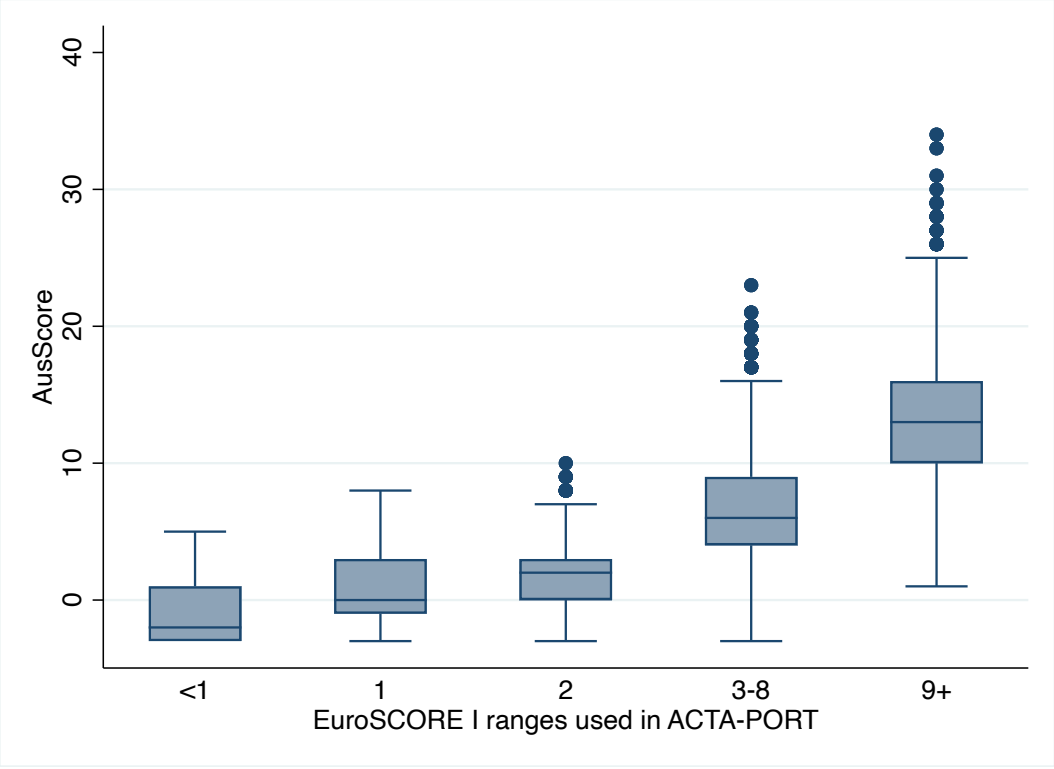

Figure 1 Box plot of AusSCORE plotted against EuroSCORE categories from ACTA-PORT.

Due to the significant overlap between a EuroSCORE of 1 and 2 these two categories were combined. Assigned ACTA-PORT scores for AusSCORE-AP based on these equivalents are shown in Table 3.

EuroSCORE	AusSCORE	ACTA-PORT score value
<1	-3 to 0	0
1	1 to 3	3
2	1 to 3	3
3-8	4 to 9	4
9+	10+	5

Table 3: Assigned EuroSCORE vs. AusSCORE equivalents for the purposes of calculating the ACTA-PORT score.

The resultant integer AntiPORT scoring system is shown in Table 4.

Characteristic	Category	Points
Age; years	<70	+0
	70+	+1
Sex	Male	+0
	Female	+1
Haemoglobin	<110	+9
g/L	110-	+8
	120-	+6
	130-	+3
	140-	+2
	150+	+0
Body surface area	<1.7	+6
m^2	1.7-	+4
	1.9-	+2
	2.1-	+1
	2.3+	+0
AusSCORE	<1	+0
	1-3	+3
	4-9	+4
	10+	+5
Creatinine	<88	+0
µmol/L	88-	+1
	177-	+3
Type of Operation	CABG/Valve	+2
	Combination	+5
	Other	+0

Table 4: The AntiPORT score with AusSCORE values substituted for EuroSCORE

Discrimination in the Australian population was similar to the UK population with an AU-ROC for ACTA-PORT-ES and red cell transfusion of 0.76 (0.75-0.76, n=30,071), and for ACTA-PORT-AS of 0.76 (0.75-0.76, n=29,487). Brier scores were 0.19 for both. Calibration was poorer with overprediction of transfusion on calibration plots (ACTA-PORT-AS calibration plot in figure 2).

Splitting the dataset yielded a training set (n = 22,407, comprising 28 hospitals) and a validation set (n =7981, comprising 9 hospitals). Logistic regression in the training set showed ACTA-PORT-AS was strongly associated with red cell transfusion (OR 1.22, 1.21-1.23, p<0.001,

n=21,743). AU-ROC in the validation set was 0.76 (0.75-0.77, n=7744). Brier score was 0.18. Calibration was improved, as seen in Figure 3 below.

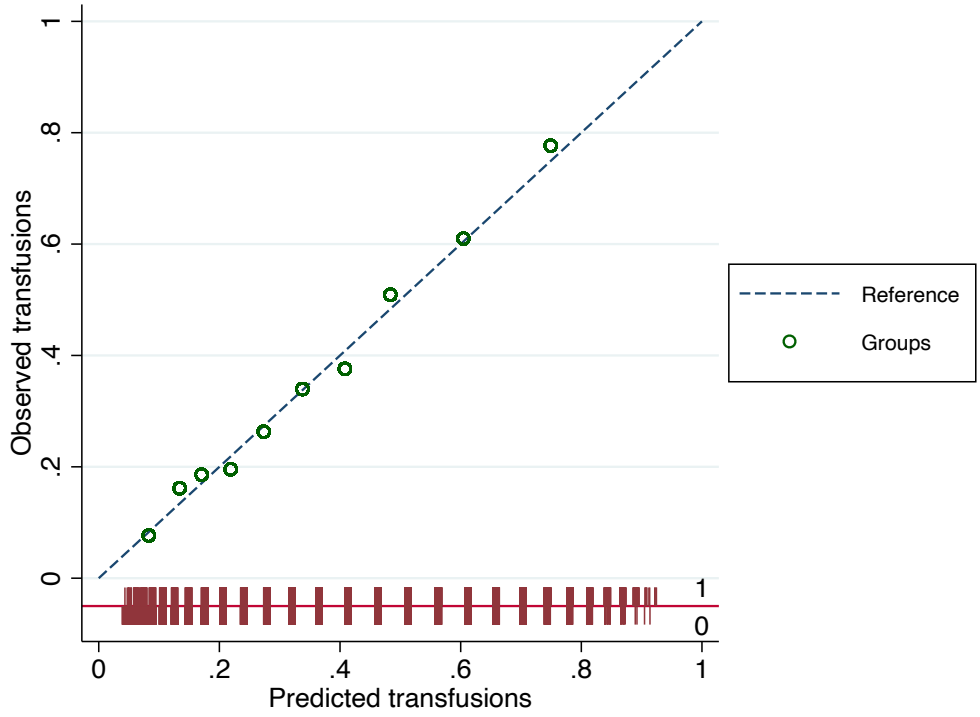

Figure 3: Calibration plot showing predicted vs observed transfusions for recalibrated ACTA-PORT-AS: the 'Anti-PORT'

The predicted rate of transfusion based on the AntiPORT recalibration is shown in Table 5, with corresponding predicted rate of transfusion from the original ACTA-PORT score as reference.

AntiPORT/ ACTA-PORT score	Predicted risk of transfusion (ACTA-PORT)	Predicted risk of transfusion (AntiPORT)
0	4.7%	**2.7%**
1	5.7%	**3.2%**
2	6.9%	**3.9%**
3	8.3%	**4.8%**
4	10.0%	**5.8%**
5	11.9%	**7.0%**
6	14.2%	**8.4%**
7	16.8%	**10.1%**
8	19.8%	**12.1%**
9	23.1%	**14.4%**
10	26.9%	**17.1%**
11	31.0%	**20.1%**
12	35.4%	**23.5%**
13	40.1%	**27.4%**
14	45.0%	**31.5%**
15	50.0%	**36.0%**
16	55.0%	**40.8%**
17	59.9%	**45.8%**
18	64.6%	**50.8%**
19	69.0%	**55.8%**
20	73.1%	**60.7%**
21	76.9%	**65.4%**
22	80.2%	**69.8%**
23	83.2%	**73.9%**
24	85.8%	**77.6%**
25	88.1%	**80.9%**
26	90.0%	**83.8%**
27	91.7%	**86.4%**
28	93.1%	**88.6%**
29	94.3%	**90.5%**
30	95.3%	**92.1%**

Table 5: Predicted risk of transfusion for **AntiPORT** recalibration, with corresponding ACTA-PORT predicted risk as comparison.

Discussion

We performed a retrospective validation study of the ACTA-PORT score for patients undergoing cardiac surgery in Australia. We demonstrated that discrimination between UK and Australian populations for the original ACTA-PORT score was similar when either derived EuroSCORE or AusSCORE-AP was used, but calibration was inaccurate, with over-prediction of transfusion based on observed rates. Recalibrating the ACTA-PORT score to the local population resulted in a score that was both discriminative and well calibrated.

The ability to accurately predict transfusion risk allows for better-directed, and more cost-effective use of PBM strategies. Patients with a low score could proceed to surgery without further optimisation, reserving more intensive methods for those at higher risk. Many strategies to optimise anaemia require delays to be effective, and attempts to avoid such delays may necessitate expensive combination treatments such as IV iron, vitamin B12, folate, and EPO (Spahn et al. 2019). In addition to guiding the graduated use of PBM, and potentially improving resource allocation, this score could be used for benchmarking. Studies of transfusion in cardiac surgery regularly show that practices vary enormously between centres(Andrew A Klein et al. 2020; McQuilten et al. 2014). It is possible that practices at units with lower risk-adjusted transfusion rates could be investigated and emulated at other hospitals. Finally, there are significant implications to future PBM research. When designing an international clinical trial, the capacity to screen and assess using a common 'risk prediction language' can have major impacts on feasibility, as was demonstrated recently by Wong et al. who validated multiple risk prediction tools as part of an international cohort study (Danny J. N. Wong et al. 2020). Additionally, from the perspective of the bedside clinician, applying the results of clinical trials from other countries to the individual patient is both easier, and more reliable if the risk prediction models used in the trial have been validated in the local population first.

Consequently, the transformation of ACTA-PORT to 'AntiPORT' could allow common enrolment of patients in Australasia and the UK into trials that attempted to recruit patients at a high risk of receiving allogeneic blood transfusion as part of cardiac surgery, or alternatively, allow a clinician in the UK to apply the results of an Australian trial to their individual practice, once the appropriate recalibration factor was considered. Critically, both ACTA-PORT and AntiPORT use data which is collected routinely for the purposes of clinical audit (including the EuroSCORE in the UK and AusSCORE-AP in Australia), raising the possibility of using these scores as automatically calculated metrics to screen patients for embedded clinical trials.

While there are similarities between UK and antipodean cardiac surgical and transfusion practices, previous studies that have attempted to adapt risk prediction scores developed in the northern hemisphere to Australasian patients have shown that this process is not simple. A recent study by Campbell et al. that attempted to adapt a surgical risk prediction score from the UK to a New Zealand cohort showed that an unadjusted model under-predicted mortality by a factor of five (Campbell et al. 2019). In cardiac surgery, the commonly used EuroSCORE model was found by Yap et al. to significantly over-predict mortality in a representative cohort of Australian patients drawn from six hospitals (Yap et al. 2006a), necessitating the development of a prediction model that was trialled and validated in a local patient cohort – the AusSCORE (Reid et al. 2009a).

Multiple scoring systems have been developed to predict RBC transfusion risk in cardiac surgery with varying degrees of accuracy, simplicity and generalisability. The best-known of these are the BRiSc (Vuylsteke et al. 2011b), TRACK (M. Ranucci et al. 2009), TRUST (Alghamdi et al. 2006) and Goudie scores (Goudie et al. 2015). All of these scores have significant merits, but also weaknesses, most driven by the inverse relationship between

simplicity and accuracy. The ACTA-PORT score was chosen for our study as we feel it bests strikes the balance between simplicity, accuracy and practicality for use by clinicians. As mentioned previously in the literature, these scores have little value to patients unless they have a positive impact on clinical management and patient outcomes, and to do this they must, above all, be usable (Bartoszko and Karkouti 2017)

Strengths and limitations of this study should be recognised. We used a large national database incorporating multiple surgical units. The split into development and validation cohorts was carried out by surgical unit (randomly), rather than using individual patients. This suggests the score is valid across surgical units with differing practices. It is notable that PBM and transfusion practices have evolved significantly over the last decade, impacting on the applicability of the score to current practice. It is likely that future recalibrations will be necessary as patient risk profile and medical practice changes. Nonetheless, the relative recency of the dataset (from late 2016 to the end of 2018) suggests practice is likely to be relevant. It is possible that a more discriminative risk prediction tool could have been generated in this population by bespoke design, rather than recalibration of an existing tool. However, for reasons described previously it was felt that the ability to apply a common risk score internationally was more important.

To conclude, we have developed a local variation of a UK-based transfusion risk prediction score. The AntiPORT score is an accurate scoring system to predict peri-operative blood transfusion in patients undergoing cardiac surgery in Australia and New Zealand. The score has a number of potential uses, including the effective allocation of PBM resources, as a quality control initiative as part of a program of comprehensive audit and as a means of achieving consistent appreciation of risk in patients enrolled in international clinical trials of transfusion practice across different countries. Finally, AntiPORT score is a clinically useful transfusion-

prediction score that can be utilised easily at the bedside by clinicians using the popular QxMD software package including an online calculator and the popular "Calculate by QxMD" app for mobile devices. In addition to its academic value, we hope that this might be become a practical and useful tool for peri-operative physicians in Australia and New Zealand when caring for patients in preparation for cardiac surgery.

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Appendix 3: The CAVIAR study published manuscript (*British Journal of Anaesthesia, 2020*)

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Preoperative intravenous iron before cardiac surgery: a prospective multicentre feasibility study

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Abstract

Background: Preoperative anaemia affects one third of patients undergoing cardiac surgery and is associated with increased mortality and morbidity. Although it is recommended that perioperative teams should identify and treat patients with preoperative anaemia before surgery, introducing new treatment protocols can be challenging in surgical pathways. The aim of this study was to assess the feasibility and effectiveness of introducing a preoperative intravenous iron service as a national initiative in cardiac surgery.

Methods: We performed a multicentre, stepped, observational study using the UK Association of Cardiothoracic Anaesthesia and Critical Care Research Network. The primary feasibility outcome was the ability to set up an anaemia and intravenous iron clinic at each site. The primary efficacy outcome was change in haemoglobin (Hb) concentration between intervention and operation. Secondary outcomes included blood transfusion and hospital stay. Patients with anaemia were compared with non-anaemic patients and with those who received intravenous iron as part of their routine treatment protocol.

Results: Seven out of 11 NHS hospitals successfully set up iron clinics over 2 yr, and 228 patients were recruited into this study. Patients with anaemia who received intravenous iron were at higher surgical risk, were more likely to have a known previous history of iron deficiency or anaemia, had a higher rate of chronic kidney disease, and were slightly more anaemic than the non-treated group. Intravenous iron was administered a median (inter-quartile range, IQR [range]) of 33 (15–53 [4–303]) days before surgery. Preoperative intravenous iron increased [Hb] from baseline to pre-surgery; mean (95% confidence interval) change was +8.4 (5.0–11.8) g L^{-1} ($P<0.001$). Overall, anaemic compared with non-anaemic patients were more likely to be transfused (49% [59/136] *vs* 27% (22/92), $P=0.001$) and stayed longer in hospital (median days [IQR], 9 [7–15] *vs* 8 [6–11]; $P=0.014$). The number of days alive and at home was lower in the anaemic group (median days [IQR], 20 [14–22] *vs* 21 [17–23]; $P=0.033$).

Conclusion: The development of an intravenous iron pathway is feasible but appears limited to selected high-risk cardiac patients in routine NHS practise. Although intravenous iron increased [Hb], there is a need for an appropriately powered clinical trial to assess the clinical effect of intravenous iron on patient-centred outcomes.

Keywords: anaemia; cardiac surgery; haemoglobin; intravenous iron; iron deficiency; outcome; preoperative; transfusion

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Editor's key points

- The introduction of preoperative treatment protocols for anaemia remains difficult.
- This study investigated the feasibility and effectiveness of introducing a preoperative intravenous iron service as a national initiative in cardiac surgery.
- Preoperative treatment of anaemia with intravenous iron resulted in an increase in haemoglobin concentration before cardiac surgery.
- Owing to the small sample size, no effect of preoperative intravenous iron on transfusion rates or patient outcome could be demonstrated.
- A preoperative intravenous iron pathway is feasible, but an appropriately powered clinical trial is required to assess its effect on patient-centred outcomes.

In a large UK-wide study conducted by the Association of Cardiothoracic Anaesthesia and Critical Care (ACTACC) in 2016, anaemia was found to be common before cardiac surgery and independently associated with worse outcomes, including length of stay in ICU and hospital, and mortality. Patients with anaemia had an increased risk of death after cardiac surgery.[1] Anaemia in the general population is common, with a global prevalence in excess of 30%.[2] This prevalence increases with age, affecting half the geriatric population across the UK.[3] An expanding body of evidence continually links preoperative anaemia to increased surgical mortality, higher rates of transfusion, length of hospital stay, and surgical complications.[4–6] This is particularly relevant in cardiac surgery where comorbidities are common, blood loss is greater, and transfusion requirements are higher.[7–9]

Patient blood management (PBM) is a patient-centred, multidisciplinary approach to reducing blood transfusion and consists of a raft of preventative and reactive measures that highlight best practice and quality in blood transfusion. Introduction of PBM is associated with improved outcomes and reduction in cost.[10] The first pillar of PBM has focused on early detection and correction of anaemia. However, these recommendations for the timely diagnosis of anaemia, with appropriate and early treatment, present significant healthcare organisational challenges in an often busy setting before operation.[11,12]

The most common cause of anaemia is iron deficiency, whether absolute or functional; folate and vitamin B_{12} deficiency are less frequent.[13] Treating iron deficiency anaemia effectively in the time available before most cardiac procedures is not possible with oral iron. Intravenous (i.v.) iron is an ideal alternative given newer preparations that can be administered rapidly and with less side-effects and a good safety profile.[14] The use of i.v. iron is more effective in increasing haemoglobin (Hb) than oral iron[15] and has been observed to reduce transfusion in many surgical specialties.[16]

In 2016, the National Institute for Health and Care Excellence (NICE) reviewed transfusion practice and recommended the timely identification of anaemia before surgery and to consider alternatives to transfusion.[17] However, sequential audits on PBM practices across NHS organisations show management of preoperative anaemia is frequently inadequate.[18,19] Similarly, the implementation of PBM in Europe is limited, and considerable variations exist in the assessment and treatment of preoperative anaemia.[20]

Previously, with the ACTA, we developed a network of enthusiastic and knowledgeable consultants interested to develop PBM in cardiac surgery.[1] We wished to assess the feasibility of the introduction of a preoperative i.v. iron pathway into routine clinical practice, in line with NICE guidance. The aim of the Cardiac and Vascular Surgery Interventional Anaemia Response (CAVIAR) study was to assess the introduction and efficacy of a preoperative i.v. iron pathway to treat anaemia in patients before cardiac surgery.

Methods

Design

The UK CAVIAR study was a multicentre, stepped, observational pilot and feasibility study in patients undergoing cardiac and vascular surgery, the protocol for which has already been published.[21] From a network developed with ACTACC, we invited 11 UK cardiac surgical centres (from a total of 19 in the UK) that had expressed an interest in setting up anaemia treatment clinics to take part in this study. Educational support was provided by a core team of experts in PBM, i.v. iron, and cardiac surgery. National UK Ethics Committee approval (ref 15/LO/1569, IRAS 188848) was granted, and each centre obtained local approval to set up their anaemia pathway following UK NHS procedures, which included proformas, business plans, and formulary application approval. Site visits were performed to every unit with advice and educational briefings; literature and protocols for anaemia management were shared between centres once developed. Three annual meetings were held to exchange feedback and support, and regular newsletters were sent to all those involved.

The study was designed as a stepped, prospective, observational platform comprising three groups of patients awaiting cardiac surgery: non-anaemic patients (control); anaemic patients of any cause who were not treated with i.v. iron for any reason; and anaemic patients treated with i.v. iron before surgery. The trial design was published in advance.[21]

Anaemia was defined according to the WHO definition (Hb <130 g L^{-1} in men and <120 g L^{-1} in women). Inclusion criteria were any patient older than 18 yr undergoing elective cardiac surgery (coronary bypass, valve surgery, or both). Exclusion criteria included: pregnant or lactating women; patients on renal dialysis; prisoners and patients who lacked the capacity or were unwilling to consent to the study.

Recruitment

Centres recruited patients consecutively, between April 2016 until March 2018, but over different periods depending on the centre's progress in setting up preoperative anaemia services and availability of study teams. All patients provided full written informed consent and were recruited as controls (non-anaemic) or those with anaemia. Those with anaemia and able to receive i.v. iron were recruited if they fulfilled local criteria for diagnosis and treatment of iron deficiency anaemia (generally ferritin <100 and transferrin saturation [TSAT] <20%) and could attend preoperative clinic at least 10 days

before surgery. Those who were anaemic and qualified for i.v. iron treatment, but did not receive i.v. iron, for any reason, formed the anaemic non-treated group. This was largely for logistical or geographical reasons. After consent was obtained, and collection of baseline data and blood samples, patients were treated with a single dose i.v. iron infusion. This comprised either iron isomaltoside 1000 (Monofer®; Pharmacosmos A/S, Holbaek, Denmark) at a total dose of calculated at 20 mg kg^{-1} or ferric carboxymaltose (Ferinject®; Vifor Pharma UK, Surrey, UK), to a maximum of 1000 mg, both by infusion over at least 15–30 minutes according to local policy. Patients were observed during and for 30 minutes after infusion (noninvasive blood pressure, ECG, and oxygen saturations). Anaemic patients who had received i.v. iron were reassessed on the day of surgery, and further laboratory data and blood samples collected.

Outcomes

The primary feasibility outcome was success in setting up an anaemia clinic in the NHS, and the primary efficacy outcome was the ability to increase Hb concentration between treatment and immediately before surgery with i.v. iron. Secondary outcomes were: blood transfusion (proportion of patients transfused, and number of units transfused, excluding patients who received a large transfusion defined as four or more units of red cells); ICU and hospital length of stay; renal function; change in Hb from before treatment to after surgery and mortality. Patients were followed up for 30 days before operation and asked about re-admission to hospital so that days-alive-and-at-home (DAH-30)[22] could be calculated. DAH-30 is a composite measure that combines hospital length of stay and mortality, although we included all three outcomes measures for reference.

Analysis

For descriptive statistics, mean and standard deviation (SD) or median and inter-quartile range (IQR) and range were used for continuous variables as appropriate. Frequencies and percentages were used for categorical variables. Baseline characteristics were compared across the three groups of patients using χ^2 tests for categorical variables and *F*-tests (if normally distributed) or Kruskal–Wallis rank test (if not normally distributed) for continuous variables. For the primary efficacy outcome, a one-sample *t*-test was used to estimate the mean change in Hb along with its 95% confidence interval (95% CI) and *P* value. Multiple logistic regression models adjusted for important baseline predictors were used for the secondary outcome blood transfusion. The Wilcoxon rank sum test was used to compare length of stay (excluding patients who had died) and for days alive and out of hospital. One-sample *t*-tests were used to estimate the mean pre–post-surgery change in Hb and its 95% CI in each group of patients. The proportion of deaths and hospital readmissions were compared using a χ^2 test.

Sample size was calculated based on change in Hb from baseline to pre-surgery in patients who received i.v. iron. Assuming the SD for Hb would be 12 g L^{-1} based on national audit data,[1] we calculated that 72 patients would provide 90% power at a 5% significance level and 62 would provide 80% power (allowing for up to 10% loss to follow-up) to demonstrate a difference in the change from baseline in Hb of 10 g L^{-1}. The report was prepared according to the Strengthening the Reporting of OBservational studies in Epidaemiology (STROBE) framework.

Results

Feasibility

Seven of the 11 (64%) NHS hospitals successfully set up anaemia pathways or clinics as part of their preoperative cardiac surgical service over the study period. Two centres had difficulties getting approval for i.v. iron onto their pharmacy formulary, and two had the business plans refused by the hospital Trust or commissioners (funders).

A total of 228 patients were recruited over 2 yr in 11 UK cardiac centres (Supplementary Fig. S1). The most common reasons for failure to recruit patients were: administrative and lack of research staff, no date for surgery, date of surgical procedure outside of study treatment window (within 10 days), and 19% of patients approached to take part refused to give consent.

Of the 228 patients recruited before cardiac surgery, 92 (40%) patients were not anaemic, 72 (32%) were anaemic but not treated, and 64 (28%) were anaemic and received i.v. iron before operation. Of the anaemic patients who would ideally receive i.v. iron pre-treatment, only 47% (64/136) were treated, owing to various logistical barriers. Individual TSAT/ferritin results for all participants have not been included as the data were not complete at the time of analysis.

Patients treated with i.v. iron were more likely to have a history of anaemia and iron deficiency, and have chronic kidney disease (Table 1). Those treated were slightly more anaemic than the patients in the non-treated group, with mean (95% CI) difference in [Hb] −2.5 (Table 2).

I.V. iron was administered at a median (IQR [range]) of 33 (15–53 [4–303]) days before surgery. The mean (SD) dose of i.v. iron was 1293 (303) mg with 60 patients receiving iron isomaltoside at a mean dose of 1314 (303) mg and four receiving 1000 mg of iron carboxymaltose. No adverse event was reported.

Efficacy

Intravenous iron was efficacious and increased the average Hb in anaemic patients before surgery compared with those without treatment; the mean (95% CI) change in Hb in patients treated with i.v. iron was +8.4 (5.0–11.8) g L^{-1} between treatment and surgery ($P<0.001$) (Table 2). Overall, transfusion rates varied from 30% to 65% across the study centres. Twenty-three (10%) patients received a large blood transfusion with more than four units of red cells and were excluded (nine from the non-anaemic group, five from the non-treated anaemic group, and nine from the treated anaemic group). Non-anaemic patients were less likely to be transfused than anaemic patients, 22/92 (27%) *vs* 59/136 (42%), adjusted odds ratio (OR)=2.53 (1.38–4.63, $P=0.003$; Table 3). Non-anaemic patients were also transfused fewer units of red cells and had a shorter stay in hospital, and days alive at home (DAH) was higher (Table 3). There was no difference in transfusion rate, quantity of blood transfused, or other outcomes between untreated anaemic patients and anaemic patients treated with i.v. iron (Table 4). After surgery, Hb was similar in all three groups. The largest drop in Hb was in the non-anaemic group (42.8 [−45.7 to −39.9] g L^{-1}, $P<0.001$; Table 2).

Table 1 Baseline characteristics. CABG, coronary artery bypass graft; EuroSCORE II, European System for Cardiac Operative Risk Evaluation II; NYHA, New York Heart Association; SD, standard deviation.

	Non-anaemic (*n*=92)	Anaemic non-treated (*n*=72)	Anaemic treated (*n*=64)	*P* Value
Age, yr, mean (SD)	67.0 (9.7)	69.3 (11.8)	70.2 (10.9)	0.158
Sex; men, *n* (%)	66 (72)	55 (76)	46 (72)	0.767
Weight, kg, mean (SD)	85.8 (17.9)	83.2 (18.0)	81.6 (17.9)	0.120
Height, cm, mean (SD)	170.0 (10.0)	168.9 (10.6)	167.6 (8.9)	0.340
BMI, kg m^{-2}, mean (SD)	29.3 (5.3)	29.2 (5.9)	29.0 (5.4)	0.929
EuroSCORE II	1.3 (0.9–2.7 [0.5–19.8])	1.7 (0.9–3.3 [0.6–15.0])	2.2 (1.0–3.4 [0.5–16.9])	0.072
Cardiac function, *n* (%)				
Good	66 (73)	55 (76)	48 (75)	
Moderate	24 (26)	15 (21)	15 (23)	
Poor	1 (1)	2 (3)	1 (2)	0.866
NYHA, *n* (%)				
1	28 (31)	23 (32)	12 (19)	
2	43 (47)	26 (37)	32 (50)	
3	20 (22)	17 (24)	19 (30)	
4	0	5 (7)	1 (2)	0.042
Creatinine, μmol L^{-1}	84 (73–96 [56–205])	85 (72–113 [47–311])	104 (75–120 [46–192])	0.008
Medical history, *n* (%)				
Iron deficiency	4 (4)	9 (13)	25 (39)	<0.001
Anaemia	10 (11)	21 (29)	35 (55)	<0.001
Operation, *n* (%)				
CABG	44 (48)	29 (40)	24	(38)
Single valve	29 (32)	33 (46)	22	(34)
CABG + valve	12 (13)	4 (6)	9	(14)
Other	7 (8)	6 (8)	9	(14)

Table 2 Haemoglobin concentration by group and time. CI, confidence interval; Hb, haemoglobin; NA, not available; SD, standard deviation.

	Non-anaemic (*n*=92)	Anaemic not treated (*n*=72)	Anaemic treated (*n*=64)
Mean (SD) Hb, g L^{-1}			
Pre-treatment	NA	NA	114.2 (9.3)
Pre-surgery	141.1 (10.4)	116.7 (10.2)	122.7 (13.3)
Postoperative	98.3 (13.6)	93.2 (10.5)	93.7 (11.9)
Mean (95% CI) change in Hb, g L^{-1}			
Pre/post treatment	NA	NA	8.4 (5.0, 11.8)
Pre/post surgery	−42.8 (−45.7 to −39.9)	−23.4 (−26.5 to −20.3)	−29.0 (−32.4 to −25.6)

Table 3 Study outcomes, anaemic *vs* non-anaemic patients, excluding 23 patients who were transfused >4 units red cells. Adj OR, odds ratio for blood transfusion adjusted for sex, BMI, diabetes mellitus, and operation type; CI, confidence interval; DAOH, days alive and out of hospital; IQR, inter-quartile range.

	Non-anaemic (*n*=92)	Anaemic (*n*=136)	*P* Value
Transfused, *n* (%)	22 (26.5)	59 (48.8)	0.001
Adj OR (95% CI)		3.18 (1.60–6.31)	0.001
Units transfused, *n* (%)			
1–2	15 (16)	43 (32)	
3–4	7 (8)	16 (12)	0.016
Median (IQR)	0 (0–1)	1 (0–2)	0.005
Died, *n* (%)	3 (3.3)	5 (3.7)	0.867
Readmissions, *n* (%)	15 (16.3)	16 (11.8)	0.327
ITU length of stay, days, median (IQR)	2 (1–4)	2 (1–5)	0.571
Hospital stay, days, median (IQR)	8 (6–11)	9 (7–14.5)	0.014
DAOH-30, days, median (IQR)	21 (17–23)	20 (14–22)	0.033

Table 4 Study outcomes, anaemic non-treated *vs* anaemic treated after exclusion of 14 patients transfused >4 units of blood. Adj OR, odds ratio adjusted for baseline haemoglobin, sex, BMI, diabetes mellitus, iron tablets, hypertension, and operation type; CI, confidence interval; DAOH, days alive and out of hospital; IQR, inter-quartile range; ITU, intensive treatment unit.

	Anaemic non-treated (*n*=72)	Anaemic treated (*n*=64)	*P* Value
Transfused, *n* (%)	28 (42)	31 (56)	0.127
Adj OR (95% CI)		1.33 (0.52–3.40)	0.553
Units transfused, *n* (%)			
1–2	18 (25.4)	25 (39.1)	
3–4	10 (14.1)	6 (9.4)	0.107
Median (IQR)	0 (0–2)	1 (0–2)	0.082
Died, *n* (%)	3 (4)	2 (3)	0.747
Readmissions, *n* (%)	5 (7)	11 (17)	0.064
ITU length of stay, days, median (IQR)	2 (1–4)	3 (1–5)	0.158
Hospital stay, days, median (IQR)	9 (7–14)	10.5 (7–15)	0.492
DAOH-30, days, median (IQR)	21 (14–22)	19 (15–23)	0.768

Table 5 Distribution of patient groups across centres. Data are presented as *n* (%).

Centre	Non-anaemic (*n*=92)	Anaemic non-treated (*n*=72)	Anaemic treated (*n*=64)
Blackpool	5 (5)	5 (7)	0
Cardiff	19 (21)	4 (6)	21 (33)
Castle Hill	13 (14)	10 (14)	4 (6)
Derriford	13 (14)	2 (3)	2 (3)
Essex	3 (3)	18 (25)	0
James Cook	0	2 (3)	14 (22)
Kings College	0	1 (1)	2 (3)
Liverpool	18 (20)	12 (17)	12 (19)
Manchester RI	0	1 (1)	0
Papworth	10 (11)	5 (7)	9 (14)
RI Edinburgh	11 (12)	12 (17)	0

Discussion

Although feasible, it has proven difficult to detect, diagnose, and treat anaemia in cardiac surgical patients within the time frame before surgery. Only a minority of potential patients undergoing cardiac surgery in the UK were entered into a pathway involving i.v. iron in a timely manner before operation (Table 5). Hurdles for care were institutional (set up) and local (pathways). Implementation of anaemia identification and management in line with NICE guidance appears to have changed little in the past decade.[20] Our study to develop and i.v. iron service before operation was in keeping with the Frankfurt PBM programme results, where only 57 of 1830 patients scheduled for surgery received i.v. iron.[23] In order to set up anaemia detection and treatment pathways, organisational change is required, and this must be multifactorial and cross boundaries. Expertise and buy-in are essential from surgery, anaesthesia, haematology, pharmacy, nursing, and finance departments.

Although as a non-randomised observational study it is difficult to draw firm conclusions regarding efficacy, treating anaemic patients with i.v. iron before cardiac surgery appears to be efficacious, with a statistically and clinically significant increase in Hb concentration, which is consistent with a meta-analysis on the effect of i.v. iron.[15] Our work also supports the findings of the ACTA audit data that anaemic patients are more likely to be transfused and have worse outcomes than non-anaemic patients.[1] Although we did not show any effect of treating anaemia with i.v. iron on transfusion rate or other patient outcomes, this study was not powered to demonstrate such a difference and not dissimilar to results seen in a small pilot RCT.[24]

As is often the case with observational research, the baseline characteristics of the study groups varied significantly. In this study population, anaemic patients who received i.v. iron had a significantly higher rate of pre-existing renal impairment. Renal impairment has a major influence on transfusion requirement as demonstrated in many predictive scores for transfusion risk in cardiac surgery. For example, if we were to calculate the risk of transfusion in a patient at intermediate risk of transfusion before cardiac surgery using the ACTA-PORT (perioperative risk of blood transfusion) score,[25] the presence of a preoperative creatinine >177 μmol L^{-1} would shift the predicted transfusion rate from 45% up to 60%. The higher rates of previously diagnosed anaemia (55% *vs* 30%), previous iron deficiency (39% *vs* 13%), and symptomatic angina (63% *vs* 39%) in our treatment group, compared with the non-treated anaemic patients, may have contributed to the outcome results.

The complex nature of cardiac surgery and multifactorial causes for coagulopathy with need for significant use of blood

resources[26] may override the positive effects of i.v. iron in the preoperative setting. Successful treatment of anaemia before cardiac surgery is possible, but there is currently no strong evidence it can improve cardiac surgical outcomes. In a more general context, it appears that i.v. iron has the ability to significantly improve Hb concentration and reduce risk of transfusion.[27] This has been translated into expert consensus[28] and practice guidelines.[17] Trials examining preoperative cardiac surgical patients have been encouraging, although not definitive. One large retrospective cohort trial showed an improvement in mortality, transfusion rate, renal failure, and admission length,[29] and a smaller RCT suggested that an increase in [Hb] and a reduction in transfusion rates is possible.[30] However, another small RCT failed to demonstrate that i.v. iron can improve [Hb],[31] making the evidence rather inconclusive and inadequate to firmly validate the widespread use of i.v. iron in this population. To date, this study is the largest of its kind that demonstrates i.v. iron is effective in treating anaemic cardiac surgical patients, within the time constraints of the preoperative period. CAVIAR-UK was not designed to be the definitive study of the effect of i.v. iron on improving clinical outcomes in this patient group, but rather designed to provide information to guide design and implementation of a subsequent randomised controlled trial, which is now underway.

These results suggest that further research is both possible and necessary to demonstrate that by effectively treating anaemia before operation, we may be able to reverse or improve some of the associated adverse outcomes. The study also demonstrates that, in the UK centres studied, there were significant deficits in infrastructure and process to allow timely diagnosis and treatment to achieve a meaningful clinical result in an appropriate time frame. CAVIAR was designed to treat patients at least 10 days before surgery as it appears that 7–9 days is required for i.v. iron to have its peak effect on ferritin levels,[32] and it appears that [Hb] increase begins to plateau between 5 and 14 days.[33] A recent RCT showed that giving i.v. iron and erythropoietin (EPO) immediately before surgery showed a reduction in transfusion, but no demonstrable patient benefit.[34] Greater access to anaemia treatment centres, such as multidisciplinary preoperative anaemia clinics, should facilitate prompt and effective treatment for those patients identified at risk.

By the nature of its design, this study had significant limitations. As an observational study, the likelihood of significant differences in baseline data is high, and the presence of bias and confounders is common. There were significant differences in baseline characteristics between the groups that could plausibly lead to changes in the treatment effect and measured outcomes. The study was designed to detect an increase in Hb concentration and therefore underpowered to detect outcome changes.

Authors' contributions

Grant submission: AK, CE, SA, TR
Trial design: AK, CE, SA, TR
Investigation: AK, JY
Manuscript preparation and revision: AK, CE, JY, SA, TR
Trial management: MC
Data collection and preparation: MC
Data analysis: TC

Declaration of interest

AK or his institution has received educational grant funding, honoraria or travel expenses from Pharmacosmos, Vifor Pharma, Massimo, Hemonetics, Hemosonics, and Fisher and Paykel. MC's salary is supported by Pharmacosmos. CE has undertaken consulting work for Pharmacosmos. SA has received research funding and honoraria from Pharmacosmos. TR reports grants, personal fees, and non-financial support from Pharmacosmos; grants, personal fees, and non-financial support from Vifor Pharma; grants, personal fees, and non-financial support from Acelity; grants, personal fees, and non-financial support from Stroke Association; grants from Mason Medical Research Foundation; grants from UCH league of Friends; and grants and non-financial support from Libresse/Bodyform.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bja.2019.11.023.

CAVIAR investigators by site. Appendix 1:

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Papworth Hospital	Andrew Klein(PI) and James Yeates
Cardiff (University Hospital of Wales)	Caroline Evans
Liverpool Hospital	Seema Agarwal
Freeman Hospital	Michael Clarke
Royal Infirmary of Edinburgh	Peter Alston
Castle Hill Hospital	Ajith Vijayan
Manchester Royal Infirmary	Akbar Vohra
Essex Cardiothoracic Centre	Anirudda Pai
Brighton & Sussex University Hospitals	Anita Sugavanam
Guy's & St. Thomas Hospital	Jugdeep Dhesi
Royal Oldham Hospital	Damian Kelleher
Royal Cornwall Hospital	Harvey Chant
Royal Blackpool Hospital	Palanikumar Saravanan
Royal Bournemouth Hospital	Richard Green
University Hospitals of Leicester	Matthew Bown
Derriford Hospital	Mark Bennett
King's College Hospital	Gudrun Kunst
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Appendix 4: The CAVIAR study (vascular surgery) published manuscript (*British Journal of Surgery, 2020*)

Rapid research communication

Preoperative anaemia management in patients undergoing vascular surgery

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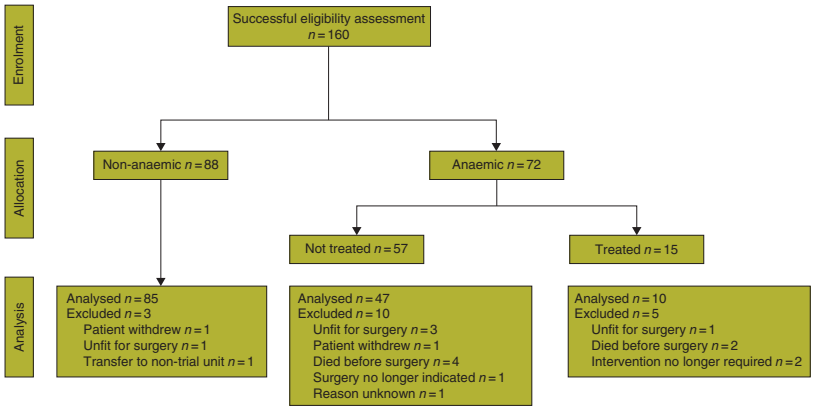
Introduction

Preoperative anaemia is associated with increased morbidity and mortality rates[1–3]. Judicious blood transfusion practice has been encouraged, with traditional top-up use questioned by RCT evidence supporting a restrictive practice, prompting a dilemma in perioperative care to balance the risk of preoperative anaemia with the risk of blood transfusion[4,5]. The use of intravenous iron (IVI) therapy is an alternative intervention for patients who are anaemic before operation. Historical reservation regarding IVI and the risk of anaphylaxis has been mitigated by use of contemporary non-dextran iron preparations that enable the infusion of large doses of IVI in minutes. In 2015, National Institute for Health and Care Excellence (NICE) guidelines[6] recommended the timely identification of anaemia before surgery and, in the 2019/2020

Fig. 1 Study flow chart



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Commissioning for Quality and Innovation (CQUIN) guidance[7], the use of IVI was supported to correct preoperative anaemia and reduce postoperative blood transfusion.

The CAVIAR (UK Cardiac and Vascular Surgery Interventional Anaemia Response) study[8] was undertaken to assess these recommendations. The aim of the study was to explore the feasibility and efficacy of introducing preoperative anaemia investigation and treatment pathways into routine National Health Service (NHS) practice in high-risk surgical patients[9].

Methods

The CAVIAR study is a multicentre, stepped observational study of patients undergoing cardiac or vascular surgery[9]; cardiac data have been presented separately[10]. The report was prepared according to the STROBE framework[11].

Between April 2016 and March 2018, patients undergoing vascular surgery at ten UK centres were recruited. National UK Ethics Committee approval was obtained (15/LO/1569, IRAS 188848). Patients aged 18 years or over, undergoing open or endovascular surgery, were included. Exclusion criteria were: pregnancy, renal dialysis, and lack of capacity or unwillingness to consent to the trial.

Three groups of patients were compared: non-anaemic, those with anaemia and anaemic patients treated with IVI before surgery. In the latter group, patients with anaemia underwent IVI infusion according to local criteria (ferritin level below 100 μg/l and/or transferrin saturation less than 20 per cent) at least 10 days before surgery. Anaemia was

Table 1 Baseline variables

	Not anaemic (n = 85)	Anaemia not treated (n = 47)	*P* (not anaemic *versus* anaemia not treated)†	Anaemia treated (n = 10)	*P* (not anaemic *versus* anaemia treated)†
Age (years)*	74·0(8·5)	74·4(9·0)	0·658‡	71·8(7·1)	0·427‡
Sex ratio (M : F)	67 : 18	40 : 7	0·380	7 : 3	0·527
Weight (kg)*	78·7(15·8)	74·8(16·9)	0·153‡	84·9(18·5)	0·249‡
Height (cm)*	170·5(8·6)	170·7(9·0)	0·726‡	172·1(7·8)	0·615‡
BMI (kg/m^2)*	27·0(4·4)	25·5(4·8)	0·047‡	28·5(5·5)	0·415‡
Smoking status					
Current smoker	20 (24)	19 (40)	0·042	3 (30)	0·653
Ex-smoker/non-smoker	65 (76)	28 (60)		7 (70)	
Creatinine (μmol/l)*	91·0(30·2)	103·1(43·4)	0·169‡	104·3(47·9)	0·607‡
eGFR (ml per min per 1·73 m^2)	67·6(18·1)	62·4(21·8)	0·161‡	49·7(13·2)	0·021‡
ASA grade			0·013		0·082
I	7 (8)	0 (0)		0 (0)	
II	20 (24)	8 (17)		2 (20)	
III	43 (51)	30 (64)		6 (60)	
IV	1 (1)	3 (6)		2 (20)	
Missing	14 (16)	6 (13)		0 (0)	
Previous myocardial infarction	14 (16)	8 (17)	0·935	2 (20)	0·779
Hypertension	56 (66)	34 (72)	0·447	7 (70)	0·795
Ischaemic heart disease	6 (7)	6 (13)	0·265	2 (20)	0·162
History of anaemia	6 (7)	10 (21)	0·017	4 (40)	0·001
Liver disease	0 (0)	1 (2)	0·179	0 (0)	0·645
Bleeding tendency	2 (2)	6 (13)	0·017	0 (0)	0·626
Iron deficiency	5 (6)	10 (21)	0·008	5 (50)	< 0·001
Chronic obstructive pulmonary disease	22 (26)	15 (32)	0·462	3 (30)	0·781
Stroke	18 (21)	8 (17)	0·567	1 (10)	0·696
Diabetes	25 (29)	13 (28)	0·832	3 (30)	0·969
Atrial fibrillation	10 (12)	9 (19)	0·249	3 (30)	0·114
Peripheral artery disease	21 (25)	24 (51)	0·002	4 (40)	0·301
Aspirin	53 (62)	24 (51)	0·569	5 (50)	0·451
Clopidogrel	14 (16)	17 (36)	0·011	4 (40)	0·074
Statin	67 (79)	35 (74)	0·569	9 (90)	0·406
Preoperative anticoagulation	5 (6)	5 (11)	0·325	2 (20)	0·108

Values in parentheses are percentages unless indicated otherwise; *values are mean (s.d.). †χ^2 test or Fisher's exact test, except. ‡Kruksal-Wallis test.

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defined according to WHO criteria, by a haemoglobin (Hb) level below 130 g/l in men and under 120 g/l in women.

Outcomes

Primary outcomes were the feasibility and efficacy of the IVI pathway. Secondary outcomes included blood transfusion (proportion of patients transfused; number of units transfused), duration of hospital stay and change in Hb from before treatment to after surgery.

Sample size

Based on national audit data, inclusion of 72 patients would provide 90 per cent power (62 patients for 80 per cent power) at the 5 per cent significance level to demonstrate a difference in the change from baseline in Hb level of mean(s.d.) 10(12) g/l[1], allowing for up to 10 per cent loss to follow-up.

Results

Some 160 patients were recruited between May 2016 and February 2018, from ten UK vascular centres. It proved feasible to set up a preoperative IVI clinic in four of the ten hospitals. Six centres had business plans refused by the hospital Trust or commissioning body. The four successful centres established pathways only after approval for IVI on to their hospital formulary. Of the 160 patients recruited, 72 (45·0 per cent) had anaemia, of whom only 15 (21 per cent) received IVI; after exclusions, 142 patients were included in the final analysis (*Fig. 1*).

Anaemic (non-treated) patients were overall at higher surgical risk (*Table 1*). Anaemic patients who were treated with IVI had a significantly lower baseline estimated glomerular filtration rate (eGFR) than non-anaemic patients (mean(s.d.) 49·7(13·2) *versus* 67·6(18·1) ml per min per 1·73 m^2; $P = 0·021$). IVI was administered to the treated group a median of 32 (i.q.r. 19–69, range 12–122) days before surgery, with a median dose of 1000 (i.q.r. 675–1000) mg. IVI resulted in a mean increase in in Hb level of 5·7 (94 per cent c.i. 4·5 to 6·9) g/l before surgery.

Secondary endpoints

Patients with preoperative anaemia (treated and untreated combined) had a higher rate of blood transfusion than those without anaemia (21·3 *versus* 4·7 per cent; $P = 0·003$) with no difference in quantity transfused (median (i.q.r.) 3 (1–5) *versus* 2 (1·5–3) units; $P = 0·661$), and a longer hospital stay (5·5 (3–17) *versus* 4 (3–7) days; $P = 0·029$). IVI did not influence the duration of hospital stay; patients whose anaemia was treated had the longest hospital stay overall (median (i.q.r.) 10·5 (7–16) days) ($P = 0·009$).

The postoperative Hb level was lower in those with preoperative (untreated) anaemia than in those without anaemia before surgery (97·8 *versus* 138·6 g/l; $P < 0·001$), but comparable to that among patients who received preoperative IVI ($P = 0·171$). There was no difference in mortality, duration of intensive care stay or readmissions between the groups.

Discussion

The CAVIAR observational study, which involved ten UK vascular units, demonstrated that it was difficult to set up a dedicated identification and treatment pathway for preoperative anaemia. It does not appear to be feasible to meet NHS England commissioning targets. The use of IVI did increase Hb levels, but the data were too limited to allow comment on its effect in reducing blood transfusion in vascular surgical patients.

Limitations of this study include an apparent selection bias for inclusion in the trial. Over 7000 major vascular procedures are performed annually in the UK, and the patients receiving IVI therapy were at higher surgical risk. With small numbers recruited, it was apparent that only the sickest received intervention; preoperative IVI was administered to only 15 patients, with ten successfully followed through to surgery, approximately 6 per cent of the total recruited.

The efficacy of IVI was lower than expected, with only a small increase in Hb level compared to before surgery (mean 5·7 (95 per cent c.i. 4·5 to 6·9) g/l), but this was comparable to the increase of 8·4 (5·0 to 11·8) g/l in cardiac surgical patients[10]. In a meta-analysis[12] of 65 studies including 9004 patients, IVI resulted in higher Hb levels than in controls (mean difference 1·04 (95 per cent c.i. 0·52 to 1·57) g/l), but there was considerable heterogeneity ($I^2 = 93$ per cent), with a point estimate of the mean difference in Hb levels ranging from −7 to 30 g/l. The assumption is that iron deficiency would be the predominant cause of anaemia[13], whether absolute or functional, which would respond to IVI therapy. The small rise in Hb among the patients whose anaemia was treated may have been skewed by the disproportionately greater baseline eGFR in this group, this limiting the treatment effect anticipated by an isolated iron defiency anaemia.

Preoperative anaemia management is not a new concept and is one aspect highlighted by NHS Blood and Transplant in a series of programmes for better blood transfusion[14,15]. Despite this, and recent CQUIN IVI recommendations influenced by NICE guidance, CAVIAR revealed a resistance owing to local business plans, perhaps emphasizing the constraints of NHS preoperative pathways.

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Patient outcomes from treatment with IVI in existing RCTs of the use of preoperative IVI were not conclusive. In patients with anaemia or iron deficiency before cardiac surgery, a combined package of interventions with IVI, including erythropoietin, vitamin B12 and folic acid, resulted in a reduced mean volume of packed red cells transfused per patient, with no difference in the number of patients transfused between groups and no impact on postoperative complications or duration of hospital stay[16].

The CAVIAR trial has highlighted not only the need for well constructed clinical trials, with patient outcomes as the endpoint, but also the problem of recruitment to clinical trials in this setting[12]. It is not possible to implement change without good clinical trial data and high-quality evidence. Conversely, this also brings into question the validity of CQUIN guidelines where the evidence is based on association and clinical pathways are challenging.

Collaborators

UK Cardiac and Vascular Surgery Interventional Anaemia Response (CAVIAR) study team members: G. Kunst (King's College Hospital, London); A. Mellor (James Cook University Hospital, Middlesbrough); A. Sugavanam (Royal Sussex County Hospital, Brighton); J. Dhesi (St. Thomas Hospital, London); M. Clarke (Freeman Hospital, Newcastle upon Tyne); R. Green (Royal Bournemouth and Christchurch Hospital, Bournemouth); H. Chant (Royal Cornwall Hospital, Truro); D. Kelleher (Royal Oldham Hospital, Oldham); D. Martin (Royal Free Hospital, London); M. Bown (Leicester General Hospital, Leicester); A. Vohra (Manchester Royal Infirmary, Manchester).

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Disclosure: The authors declare no conflict of interest.

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