Investigation of Patient Blood Management in Colorectal Surgery

by

Barrie D Keeler

BSc MBBS PGDipLATHE FRCS *(Gen Surg)*

Thesis submitted to the University of Nottingham for the degree of Doctor of Philosophy

OCTOBER 2015

Declaration:

Except where acknowledged, I declare that this Thesis is the result of my own work which has been mainly undertaken during my period of registration for this degree at The University of Nottingham.

Barrie D Keeler.

Table of Contents:

List of Tables:

List of Figures:

Abstract:

Introduction:

Perioperative allogeneic red blood transfusions (ARBT) are associated with impaired short and long term outcomes. Consequently, perioperative ARBT should be avoided, yet preoperative anaemia increases this need. The study aimed to compare the efficacy of preoperative intravenous (IVI) and oral iron (OI) in reducing ARBT use in anaemic patients undergoing colorectal cancer (CRC) surgery.

Methods:

116 anaemic patients with non-metastatic CRC adenocarcinoma were recruited preoperatively and randomised to receive either OI (ferrous sulphate) or IVI (ferric carboxymaltose). Perioperative changes in Haemoglobin (HB) and ARBT were recorded across groups. Parametric data was compared with 2 tailed T-test and non-parametric paired data with Wilcoxon Rank test, and Mann-Whitney U test. Nominal data was compared with 2-tailed Chi squared test.

Results:

There was no difference in demographic data between groups. HB levels at recruitment were comparable (OI 10.4g/dL 95%CI 10.1-10.7; IVI 10.2g/dL 95%CI 9.8-10.5, *P*=0.24), as was median treatment duration (OI 21 days IQR 15-33; IVI 21 days IQR 15-34, *P*=0.75). However, HB levels were higher on the day of Surgery in IVI (11.9g/dL 95%CI 11.5-12.3 vs OI 11g/dL 95%CI 10.6-11.4, *P*<0.01). Median preoperative HB change in patients not transfused preoperatively was higher in IVI (1.5g/dL IQR 0.9-2.6 vs OI 0.5g/dL IQR-0.1-1.3, *P*<0.01). There were fewer anaemic patients at surgery in the IVI group after treatment (75% vs 90%, *P*<0.05). OI patients received a mean 0.63u (95%CI 0.26-1) from recruitment to day 28 postoperatively vs mean 0.47u (95%CI 0.1-0.84) for IVI. Neither number of patients transfused (*P*=0.33) nor mean units transfused (*P*=0.54) differed over this period. When patients with heavy intraoperative losses (>1.5L) were excluded in subgroup analysis, a significant difference in mean units of blood transfused was seen up to 7 days post operatively (*n*= 108; OI 0.6u 95%CI 0.23-0.96; IVI 0.16u 95%CI 0.01-0.3, *P*< 0.05) and significantly less IVI patients were transfused (10% vs 25%, *P*<0.05)

Conclusions:

In patients undergoing CRC surgery, IVI appears more efficacious than OI at treating preoperative anaemia. It does not appear to minimise overall ARBT requirement, but may reduce ARBT use in the immediate perioperative period when the implications of ARBT are probably at their greatest.

Publications arising from this thesis:

The following peer reviewed publications (to date) are based on work documented within Chapters of this thesis:

Chapter 1:

Is there a role for intravenous iron therapy in patients undergoing colorectal cancer resection?

Keeler, BD; Krell, J; Acheson, AG; Brookes, M J;Stebbing, J. & Frampton, AE.

Expert Reviews in Anticancer Therapy (2012) 12**,** 1407-12.

Chapter 3:

The feasibility and clinical efficacy of intravenous iron administration for preoperative anaemia in patients with colorectal cancer.

Keeler, BD; Simpson, JA; Ng, S;Tselepis, C; Iqbal, T; Brookes, MJ; & Acheson, AG.

Colorectal Disease (2014) 16, 794-800.

Chapter 5:

An observational study investigating the effect of platelet function on outcome after colorectal surgery.

Keeler, BD; Simpson, JA; Fox, SC; Stavrou, CL; Briggs, RA; Patel, P; Heptinstall, S & Acheson AG.

International Journal of Surgery (2015) 17:28-33.

Acknowledgements:

The work documented within this thesis has resulted from collaboration and support from several individuals to whom I am extremely grateful. In particular I would like to thank:

- My lead Supervisor and mentor, Austin Acheson, without whose guidance, time and support both in and out of Research, this Thesis would never have come to fruition.
- My Supervisor Dr Matthew Brookes for his patience and guidance which have been invaluable over the past 4 years.
- Chapter 2 – To Christiana Stavrou, Sophia Beeby, Intan Hanisah and Umar Butt, the BMedSci students that I co-supervised who assisted in data collection.
- Chapter 3 – Alastair Simpson for his friendship and tireless efforts and hard work in setting up the Pilot trial and collecting a significant amount of the data (with Shu-Li Ng). Many thanks for allowing me to complete the study and utilise the results for publication and in this Thesis. Thanks also to Tariq Iqbal and Chris Tselepis for their advice regarding hepcidin.
- Chapter 4 – All other members of the IVICA steering committee: Austin Acheson, Matthew Brookes, Alastair Simpson

and Shu Li Ng for their help and support setting up the trial and with securing funding for the study. Acknowledgment is made of this funding from NIHR (RfPB) and hence any views expressed are those of the author and not necessarily those of the NHS, the NIHR or the Department of Health.

- Chapter 4– The PIs at each other site: Matthew Brookes, Robert Longman, Nader Francis, Danilo Miskovic, Jon Lund and Thomas Pinkney, and all research teams at each Centre who assisted in the efficient running of the trial. Without this input the trial would not have been successful, hence I am extremely grateful.
- Chapter 4 –Oliver Ng, Jon White, Karen Robinson, Melanie Lingaya and Yirga (Nadine) Falcone for training me and also assisting in analysis of the biomarker serum samples.
- Chapter 5 – The BMedSci students I co-supervised, Rebecca Briggs, Christiana Stavrou and Priya Patel for their efforts with data collection as part of their BMedSci projects. I would also like to thank Prof Heptinstall and Dr Sue Fox for their guidance on data analysis.
- Chapter 6 – All the Delphi and PPIE participants for their time and experience which formed the basis of this chapter.
- Dr Andrea Venn and Mehluli Ndlovu for statistical advice.
- Parveen Sharma and Ahmed El-Sharkawy for their friendship and support in acting as a soundboard for my research plans.

Most importantly, I am thankful to my wife Anna for her strength in raising our children in my extended absence, for her patience, and for her unending support during this whole time. Completion of this Thesis is as much a testament to this as my own efforts.

And finally to Alex and Chloe for giving me the inspiration and motivation to persevere.

For the memory of my Mum and for my Dad.

Chapter 1:

Patient Blood Management and Anaemia: Background, treatment and extent of clinical implications.

1.1 Introduction to Patient Blood Management:

Patient blood management (PBM) is a relatively new concept developed to improve patient care and outcome. The global principal is that of:

"*The timely application of evidence-based medical and surgical concepts designed to maintain hemoglobin concentration, optimize hemostasis and minimize blood loss in an effort to improve patient outcome.*"

Society for the Advancement of blood management (SABM, 2013)

The basis for the implementation of PBM relates to the deleterious effects related to the use of blood products, in particular allogeneic red blood cell transfusion (ARBT). In surgical patients these effects can be considered as either patient specific, or linked to increases in inherent risks encountered in the perioperative period, or pose consideration for the wider community.

1.1.1 Patient Specific considerations:

The transfusion of blood and blood components are associated with

several known risks which can broadly be considered as either infective, metabolic, biochemical, immunological and "unknown" (Goodnough and Shander, 2007).

Infective risks:

The infective complications are related to the microbial pathogens that may infect the recipient of the transfusion if present within the blood administered. These are widely recognised including viral, bacterial and protozoal organisms, and consequently many are routinely screened for. Despite this, the risk of infection for diseases such as HIV does still persist due to the "window" between infection and seroconversion of the donor (Maxwell and Wilson, 2013). Bacterial contamination resulting in severe sepsis in the recipient is quoted in the region of 1 per 0.5 million transfusions (Blajchman, 2001).

Potential infections that are not routinely screened for include hepatitis A, malaria, Parvovirus B19 and new variant Creutzfeldt-Jakob disease (CJD) (Vamvakas and Blajchman, 2009). The later example demonstrates the "unknown" risks of ARBT use, as prior to the emergence of the disease, it could not be conceived that ARBT administration could be linked with CJD transmission. This

poses the question as to the spectrum and scope of conditions which will be linked with ARBT use in the future. Already, potential links have been made between ARBT administration and increased risks of malignancies such as non-Hodgkin's lymphoma (Castillo et al., 2010), and also as possible "transmission" route for Alzheimer's disease (de Calignon et al., 2012) highlighting further that the risks of ARBT use may still be identified.

Metabolic and biochemical complications:

Biochemical and metabolic complications are most problematic in massive transfusion (Donaldson et al., 1992). ARBT are stored at 4^0C, hence may lower body temperature with implications upon other physiological processes such as clotting. The storage procedure involves addition of citrate, which can bind to calcium and potentially cause hypocalcaemia (Maxwell and Wilson, 2013). Furthermore, ARBT are also high in potassium and also citric and lactic acid (Maxwell and Wilson, 2013), hence may affect potassium levels and acid-base balance after repeated ARBT administration.

Immunological implications of blood transfusion:

There are several forms of immunological "reactions" which are caused by host antibody response to cellular or humoral material present within the administered product (McEvoy and Shander, 2013) (Dean L, 2005). These range from the potentially fatal ABO incompatibility (Vamvakas and Blajchman, 2009) and transfusion associated lung injury (Toy et al., 2005) to the more benign febrile reactions (Yazer et al., 2004). Despite rigorous implementation of safety measures to avoid ABO incompatibility, this remains the second leading cause of ARBT associated death in the US (Vamvakas and Blajchman, 2009) highlighting that this remains a significant risk of ARBT.

1.1.2 Considerations key to Surgery:

ARBT administration is associated with important immunomodulatory effects that are of relevance to all patients. These are particularly important in those undergoing surgery, especially those with malignancy.

Even during "normal" circumstances, the perioperative period poses unique challenges to the immune system, which has important consequences on the anti-tumour activity of the immune

system (Cata et al., 2013). The inflammatory response induced by surgery impairs cytotoxic immune function (Ben-Eliyahu, 2002). Furthermore volatile anaesthetic agents used to induce/maintain anaesthesia, and opioid analgesics have both been demonstrated to also reduce Natural-Killer cell and cytotoxic lymphocyte activity (Mao et al., 2013, Exadaktylos et al., 2006). This perioperative insult is exacerbated by the immediate post-operative stress response which sees a shift in T-Helper cell activity to further reduce cytotoxic function (Cata et al., 2013). This is particularly relevant during CRC surgery, whereby tumour manipulation has been associated with the detection of tumour genetic material within the circulation (Yamaguchi et al., 2000) . The inference is that during routine operative circumstances, patients are exposed to increased circulating malignant cell-loads, coupled with an immune response which is impaired at opposing this, thereby increasing the risk of metastasis and recurrence. Such a theory is believed to be of prognostic significance in relation to CRC recurrence (Bosch et al., 2003).

The perioperative use of ARBT has been independently linked with solid-tumour recurrence including ovarian cancer (De Oliveira et al., 2012) and CRC (Acheson et al., 2012).

The postulated mechanisms by which adverse outcomes are attributed to ARBT relate to immunomodulation, i.e. further changes in the immune response which reduces anti-tumour activity and also impairs the ability to combat infection. In a review by Cata *et al. (Cata et al., 2013)*, three main mechanisms were proposed by which this occurs irrespective of whether leucoreduced ARBT are administered:

1. Cytokines present in transfused blood impair T-Helper cell function and also induce further cytokine release such as IL-6, IL-10 and TNF-α which exacerbate this impairment.
2. Pro-inflammatory lysophosphatidylcholines contained within transfused blood acts to trigger further endogenous cytokine release.
3. Eicosanoids accumulate with the transfused blood and mediate an inflammatory response in the recipient.

These mechanisms are supported by observational data which described a positive correlation between the number of units of ARBT administered with complication rates following major gastrointestinal surgery (Bernard et al., 2009). In this review of over 125,000 patients, intraoperative transfusion of 1 unit or ARBT was associated with increases in infective complications and

mortality which increased incrementally with the number of units transfused. It seems logical to assume that the more units of ARBT administered, the greater the amount of immune mediators will be co-administered, causing greater immunosuppression. However, this study must remain interpreted with caution, as although the authors report matching for multiple variables, preoperative anaemia and intraoperative blood loss are not clearly stated as being matched for.

It could therefore be argued that with worsening anaemia or blood loss, increased complication rates would have been expected, and that the ARBT was merely a confounder. This issue highlights a real problem with interpretation of the impact of ARBT on outcome, due to the difficulty in distinguishing the deleterious effects of perioperative ARBT use from the anaemia which necessitated the ARBT (Isbister et al., 2011).

1.1.3 Implications for the wider community:

Blood products are a finite resource, and although the use of ARBT within the UK is on a downward trend (Tinegate et al., 2013), the current use within the United Kingdom is unsustainable (JPAC, 2014). To further compound the scarcity of availability, the

acquisition, screening, storage and safe administration of a blood transfusion is extremely costly and thus the estimated cost of a blood transfusion is in the region of US$ 700-1200 (Shander et al., 2010).

1.2 Components of Patient Blood Management:

PBM is thus intended to ensure that the risks of ARBT for the patient are minimised, and that resources are used appropriately by utilising all safe means to avoid unnecessary transfusions.

PBM is comprised of 3 core "pillars" which must be implemented in the three phases of surgery, i.e. preoperatively, intraoperatively and postoperatively. These pillars include paying consideration to increasing red cell mass, preservation of existing levels and optimisation of patient response to anaemia (Shander et al., 2012).

Current UK National Blood Transfusion Committee guidelines (NBTC, 2014) have outlined the key aspects of PBM in surgical patients which are summarised in Table 1.

In light of these recommendations, coupled with a personal interest in the colorectal surgical aspects of this concept, this Thesis will focus on investigating future clinical practices in the first of these components relating to the preoperative management of anaemia and haemostasis.

1. Preoperative Management of Anaemia and Haemostasis:
Timely identification and correction of anaemia before elective surgery.
Develop and implement protocols for the management of anticoagulants and antiplatelet drugs.
Avoid transfusion for managing anaemia if alternatives are available e.g. oral and intravenous iron.
2. Intraoperative Management:
Use intraoperative cell salvage if appropriate.
Use pharmacologic agents to reduce blood loss.
Maintain physiologic homeostasis.
Use controlled hypotension whenever indicated.
Position patients minimising central venous pressure and capillary oozing.
Minimise surgical blood loss through use of new technologies.
3. Postoperative Management:
Use postoperative blood salvage where indicated.
Consider alternatives to transfusion for postoperative management.
Consider the effects of intraoperative fluid.

Table 1 - National Blood Transfusion Committee guidelines on the specific aspects of Surgical Patient Blood Management (2014). (NBTC, 2014)

1.3 Definition of anaemia:

Haemoglobin is protein composed of 4 polypeptide subunits, each bound to a single haem molecule. It is essential for the transport and delivery of oxygen to tissues (Lehmann and Carrell, 1969). In general terms anaemia is defined as a total reduction in erythrocyte number, or reduced amount of circulating haemoglobin (HB), or as a decreased circulating red blood cell mass (Perkins, 2006). The net result is a pathophysiological state where erythrocyte oxygen carrying capacity is insufficient to meet physiological demands (Varat et al., 1972, WHO, 2011) and hence can also be defined by this functional state (Spivak, 1994).

More specifically, anaemia is defined by HB values below threshold levels which are themselves affected by variables including age, gender, ethnicity, smoking and altitude, but are generally recognised to correspond to levels below 12 g/dL for non-pregnant females, and 13 g/dL for adult males (see Table 2) (WHO, 2011).

1.3.1 Anaemia in malignancy:

Anaemia is frequently associated with malignancy (Ludwig et al., 2004) and depending on definition employed and location of tumour, can be identified in 30-90% of cancer patients (Knight et

al., 2004). On specific review of Gastrointestinal (GI) malignancy, in the European Cancer Anaemia Survey (ECAS) reviewing over 2,400 patients diagnosed with colorectal malignancy, 38.9% of patients were found to have HB values of less than 12 g/dL (Ludwig et al., 2004). Using such data within the previously discussed parameters of the WHO definition of anaemia, it would indicate a conservative estimate for the prevalence of anaemia at diagnosis of colorectal malignancy in the region of 40%. Within ECAS, the majority of anaemic colorectal cancer patients were diagnosed with either "mild" or "moderate" anaemia (see Table 2 below), with less than 1% of patients having HB values of less than 8 g/dL.

Sex	**Severity of Anaemia (g/dL)**		
	Mild	Moderate	Severe
Male > 15 yrs	11.0-12.9	8.0-10.9	<8.0
Non-Pregnant Female > 15 yrs	11.0-11.9	8.0-10.9	<8.0

Table 2 - The WHO definition of anaemia severity.

Adapted from WHO 2011. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. World Health Organistaion.

As anticipated, it appears that the location of tumour is important in the prevalence of anaemia, with right sided tumours associated with anaemia in approximately 47% of cases, and lower rates with left colonic (34%) and rectal (19%) malignancy (Dunne et al., 2002a). The reason for this relationship has been attributed to delays in presentation of right sided tumours due to late onset of symptoms, with the net result of right sided tumours being more advanced at presentation. The relationship is perhaps more complex than such a link, as the advancing tumour stage and increasing rates of anaemia remains an area of debate. Whilst Raftery et al and Vanek et al reported no difference in rates of anaemia at presentation of all Dukes' stage disease (Raftery and Samson, 1980, Vanek et al., 1986), other groups have identified a relationship; with a 40% prevalence of anaemia demonstrated in patients with Duke's Stage A disease and 76% in Duke's Stage "D" (Cappell and Goldberg, 1992). It should be noted that in latter study the threshold for diagnosis of anaemia was high (14.1 g/dL and 12.1 g/dL for males and females respectively), and is thus perhaps an overestimate given current WHO criteria, but still demonstrates a trend for decreasing patient HB levels with increasing stage.

Observational studies reviewing the relationship between colorectal tumour size and anaemia may provide more evidence for this debate. Although larger tumours are not always associated with increased tumour stage, it would be logical to assume that such a relationship exists. In a retrospective review of over 350 patients with colorectal cancer, increasing tumour size was associated with anaemia (Sadahiro et al., 1998). Interestingly, however, the presence of lymph node metastasis was deemed statistically non-significant, which again throws doubt upon the relationship between tumour stage and anaemia, given the role of nodal involvement within pathological staging.

Consequently, the reason for increased incidence of anaemia at presentation of colorectal cancer may be multifactorial with size and stage playing part of the role and location of the tumour also being of relevance. Such an observation is supported by work by Macrae and St.John, who noted a significant difference in the amount of blood lost from tumours of different colonic location. Mean blood losses were found to be 9.3 ml/day from tumours of the caecum, 1.5 ml/day for transverse and descending colon, 1.9 ml/day for sigmoid colon, and 1.8 ml/day for rectal cancers (Macrae and Stjohn, 1982). Importantly, in this study, tumour stage was unrelated to amount of blood lost.

1.3.2 Iron deficiency:

Anaemia secondary to colorectal adenocarcinoma is predominantly iron deficient in origin (Ho et al., 2008). The chronic loss of blood from the tumour results in a gradual depletion of iron stores, such that when anaemia develops, the patient has been iron deficient for a significant period of time (Sadahiro et al., 1998). As a consequence it has been identified that about 6% of patients referred to secondary care for investigation of iron deficiency anaemia will have CRC (Raje et al., 2007) and 60% of patients diagnosed with CRC are iron deficient (Beale et al., 2005).

Iron deficiency can also develop during malignancy as a consequence of malnutrition (Alleyne et al., 2008). The systemic manifestations of malignancy, such as anorexia, together with more CRC related symptoms of abdominal pain and change in bowel habit, can result in a reduced oral intake (Inui, 1999), and thus inadequate ingestion of iron. Inadequate intake can thus further exacerbate the increased losses from the primary tumour.

1.3.3 Bone Marrow suppression and inflammation:

Bone marrow infiltration from malignant cells can result in a suppression of haemopoiesis (Rogers et al., 2012). As bone

metastases develop, they can replace and impair progenitor cells reducing erythropoietic function (Mercadante 2000). Although bone metastases are uncommon in CRC, it is recognised that some cytokines and inflammatory mediators (e.g. TNF-a, IL-1) released from the primary tumour may have a pathological effect upon erythrocyte production, resulting in an anaemia of chronic disease (Means Jr, 2004).

Anaemia of chronic disease occurs as a consequence of three steps attributable to the hypoproliferative state induced by inflammatory mediators (Faquin et al., 1992). Firstly red cell life span may be reduced (Means Jr, 2004) which is exaggerated by an inability of erythropoiesis to be upregulated by relatively supressed levels of erythropoietin (EPO) (Henry, 1992) and a blunted response to the hormone itself (Baer et al., 1990).

The final step, results from an impairment of iron metabolism (Dallalio et al., 2006). Solid tumours, including CRC, can release IL-6 (Li et al., 2009), and this results in reduced intestinal absorption of iron and also increased enteric iron loss (Jongen-Lavrencic et al., 1996). IL-6 has been shown to upregulate the production of hepcidin (Dallalio et al., 2006, Nemeth et al., 2004) which has also been heavily implicated in the anaemia of chronic

disease and hence anaemia associated with malignancy (Means Jr, 2004).

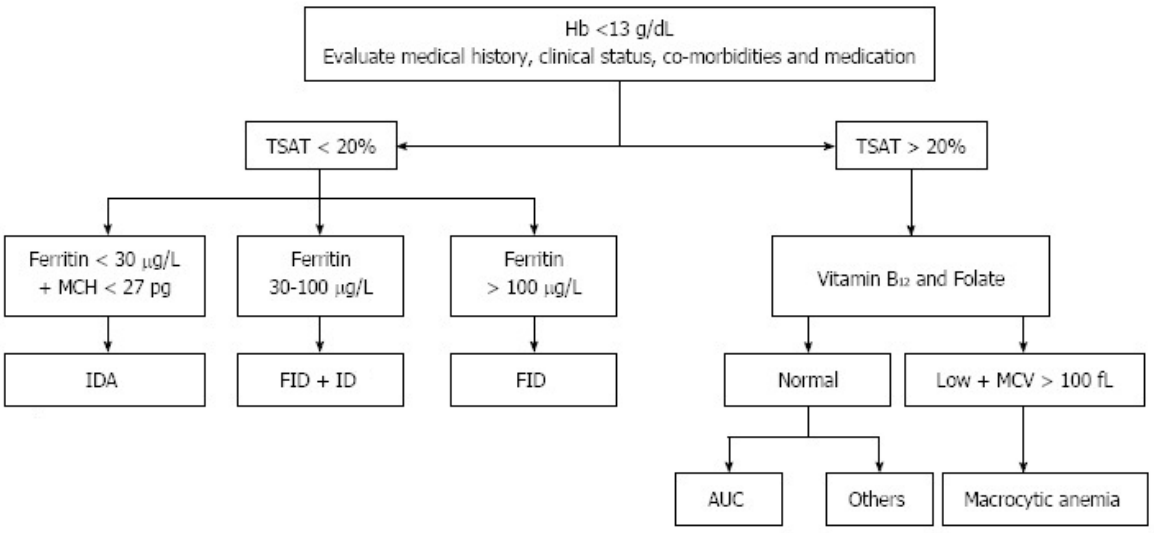
Hepcidin is a key regulatory peptide in normal iron metabolism (Finberg, 2013, Zhang and Rovin, 2013). Hepcidin reduces the export of iron from enterocytes and macrophages by causing internalisation of the ferroportin channel (Knutson et al., 2005). This results in reduced mobilisation of iron into plasma (Ganz, 2011) and thus reduced circulating levels. In hereditary haemochromatosis, where the ferroportin channel is abnormal, the hepcidin inactivation of ferroportin is lost resulting in excessively increased movement of iron into plasma (Ganz, 2011). Similarly, serum hepcidin levels are shown to positively correlate with serum ferritin levels (Dallalio et al., 2003), i.e. as iron levels increase, in normal situations, hepcidin down-regulates further iron absorption and mobilisation.

CRC tumours are thought to induce hepcidin production, both indirectly via IL-6 as discussed previously, and also directly as hepcidin mRNA was found to be expressed in over one third CRC tumours (Ward et al., 2008a) and that the serum hepcidin levels are proportional to CRC stage (Roberts et al., 2008). Furthermore, as hepcidin is an acute-phase protein, it is released in periods of

systemic inflammation. Malignancy may induce such a state, as indeed can the trauma of surgery, further raising the levels of the hormone. As a consequence, CRC may impair iron handling resulting in a relative iron deficiency as a result of the release of inflammatory mediators such as hepcidin, which also suppress the normal bone marrow response to anaemia.

1.3.4 The net effect of iron deficiency and inflammation:

Malignancy associated Iron Deficiency Anaemia (IDA) is a multifactorial process, hence in CRC occurs as a combination of Absolute Iron Deficiency (reduced intake, reduced absorption or increased loss) and Functional Iron Deficiency, where iron mobilisation is insufficient to meet demand often as a consequence of inflammation (Ludwig et al., 2013). A diagnostic algorithm (Munoz et al., 2014) has been proposed to aid in diagnosis of the mechanism of anaemia which encompasses these processes and is illustrated in Figure 1.



Where HB = Haemoglobin, TSAT= Transferrin Saturation, MCH=Mean Cell Haematocrit, MCV=Mean Cell Volume, IDA=Iron deficiency anaemia, FID=Functional iron deficiency, AUC=Anaemia of unknown cause.

Adapted from Munoz, M., Gomez-Ramirez, S., Martin-Montanez, E. & Auerbach, M. 2014. Perioperative anemia management in colorectal cancer patients: a pragmatic approach. *World J Gastroenterol,* 20, 1972-85

Figure 1 – A proposed algorithm to aid in the diagnosis of the cause of anaemia.

1.3.5 The impact of chemotherapy on anaemia:

Current National Institute for Health and Care Excellence (NICE) guidelines for the management of colonic adenocarcinoma advocate surgical resection in suitable patients with adjuvant chemotherapy for selected patients with high risk of micrometastatic disease or recurrence (NICE, 2011). These same guidelines recommend that locally advanced rectal carcinoma should undergo a period of neoadjuvant therapy in the form of infusional 5- Fluorouracil (5-FU) or oral capcitebine and long course radiotherapy.

Capcitebine is a pro-drug of 5-FU, and although there is a slight variation in their side effect profiles (Saif et al., 2008), both are recognised as causes of anaemia. Rates of anaemia have been reported in the region of 10% in patients undergoing capcitebine treatment as a radiosensitising agent for rectal neoadjuvant treatment (Velenik et al., 2006) and 33% of patients being administered 5-FU in the same context (Habr-Gama et al., 1998).

The mechanism of action of these agents induces a relative folate deficiency which causes impaired DNA synthesis. As a result, the anaemia that these drugs cause is predominantly megaloblastic (Grem, 2000, Ulrich et al., 2002), and distinguishable from IDA by an elevated Mean Cell Volume (MCV) (Figure 1).

1.4 Clinical consequences of perioperative anaemia:

Preoperative anaemia is an independent clinical risk factor for adverse outcome (Fearon et al., 2013). These adverse outcomes can be considered in relation to their time point in treatment.

1.4.1 Preoperative implications:

Quality of Life:

Anaemia is recognised to cause a variety of symptoms including fatigue, lethargy and shortness of breath (Campbell, 1996). In the context of malignancy, it has been demonstrated that reducing HB levels are associated with reducing Quality of Life (QOL) scores and hence it has been postulated that reversing this anaemia will improve cancer related QOL (Lind et al., 2002).

More specifically, anaemia has been associated with fatigue (Cella, 1998), reduced functional capacity (Chaves, 2008, Merchant and Roy, 2012), depression (Maccio and Madeddu, 2012) and increased risk of falls in the elderly (Dharmarajan et al., 2006). Anaemia clearly has a multifactorial impact of on patients' quality of life. As maximisation of quality of life of is a key treatment goal for any patient diagnosed with malignancy, it is argued that appropriate

management of anaemia should therefore form part of this treatment (Jamil et al., 2009).

Efficacy of Neoadjuvant Therapy:

As previously discussed, current NICE recommendations for the management of locally advanced rectal adenocarcinoma includes the selective use of neoadjuvant chemoradiotherapy. In an animal model to assess the impact of anaemia on tumour radiosensitivity, Thews *et al.* compared tumour response to radiotherapy in anaemic rats which were either untreated or treated with EPO (Thews et al., 1998). Although there was no evidence of increased tumour growth following administration of EPO (as demonstrated by a comparative group not receiving radiotherapy), it was found that reversing the anaemia resulted in an enhanced response to radiotherapy treatment.

Such a relationship between anaemia and tumour responsiveness has been noted in relation to chemotherapy. In a similar study to that described for radiotherapy, treating anaemia in rats with EPO was demonstrated to increase the efficacy of cyclophosphamide cytotoxicity (Thews et al., 2001).

There is significant speculation regarding the mechanism by which anaemia causes an impaired response to chemoradiotherapy (CRT). Although some early work indicated that anaemia (and tumour hypoxia) had no effect upon tumour growth rates (Thews et al., 1998), this has been contradicted by subsequent investigations. It is now postulated that local tissue hypoxia resulting from anaemia may cause reduced metabolic and mitotic activity, and hence reduces the length of time the cells are replicating, i.e. when the tumour is most sensitive to therapy (McCormack et al., 1990, Thews et al., 2001). As it is recognised that tumours lack the normal regulatory mechanisms to regulate tissue hypoxia, such as altering local blood flow, the metabolic activity remains vulnerable to anaemia, hence reduces in these conditions causing a direct effect on chemoradiosensitivity (Vaupel et al., 2001).

Other reviews have advocated that local tissue hypoxia caused by anaemia also causes a decreased sensitivity to CRT by two indirect mechanisms (Vaupel et al., 2001). Firstly, anaemia has been proposed to induce chemoradioresistance, by upregulating genes responsive to tissue hypoxia which increase both tumour progression and impair the mechanism of action of chemo and radiotherapy (Vaupel and Mayer, 2005). Secondly, oxygen is

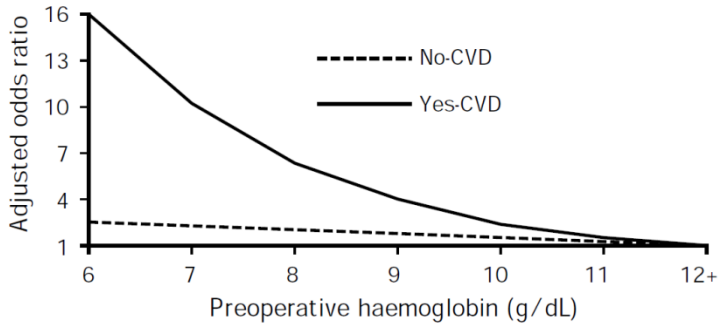
thought to increase the efficacy of radiotherapy, by causing free radical damage to DNA causing increased damage to the rapidly dividing cells of the malignancy (Vaupel et al., 2001). If anaemia causes hypoxia with the tumour, this would result is less oxygen available for such free radical damage to occur.

As a consequence, of these findings, it appears that the efficacy of neoadjuvant therapy in rectal adenocarcinoma is reduced in the context of anaemia (Walter et al., 2013). This could have more far reaching implications for the management of colonic neoplasia if the current FOXTROT trial (Reibetanz and Germer, 2013a) identifies a further role for neoadjuvant treatment for locally advanced colonic malignancy.

1.4.2 Perioperative implications:

Mortality

In studies reviewing perioperative mortality in patients refusing ARBT on religious grounds, a relationship has been demonstrated to exist between decreasing preoperative HB levels and increasing perioperative mortality (Carson et al., 1996). This increased risk is more pronounced in patients with co-existent cardiovascular disease (CVD), as is evident in Figure 2.



Where CVD= Cardiovascular disease

Taken from: Carson, J. L., Duff, A., Poses, R. M., Berlin, J. A., Spence, R. K., Trout, R., Noveck, H. & Strom, B. L. 1996. Effect of anaemia and cardiovascular disease on surgical mortality and morbidity. The Lancet, 348, 1055-1060.

Figure 2 - Perioperative mortality adjusted odds ratio for mortality in relation to preoperative Haemoglobin.

The increased risk is evident in both groups with HB levels below 12 g/dL, but far more evident at levels below 10 g/dL.

Such a relationship is always potentially vulnerable to confounding variables – patients with anaemia frequently have other comorbidities, such as renal and cardiac failure, which in turn increases their perioperative risk. In an attempt to address this, Beattie *et al.* reviewed over 9,000 consecutive patients undergoing non-cardiac surgery, and performed analysis matching for such confounders (Beattie et al., 2009). The results obtained demonstrated similar findings to those illustrated in Figure 2; it was noted that with reducing preoperative HB levels, an increased probability of mortality was observed. It was also of particular

interest that this increased mortality risk increased most greatly at HB levels consistent with the gender specific WHO definitions of anaemia. This is consistent with subsequent studies which indicated that 30-day mortality risk was associated with severity of preoperative anaemia as defined by the WHO (Musallam et al., 2011).

Morbidity and length of stay:

In light of this relationship, it is logical to suggest that rates of post-operative complications are higher in patients with preoperative anaemia, and that this also impacts upon length of stay (LOS). When assessing a composite morbidity outcome, comprised of new onset renal impairment, cerebrovascular accidents and myocardial events in CRC patients, the degree of preoperative anaemia appeared to correlate with the risk of this outcome independent of associated comorbidity (Leichtle et al., 2011). This composite outcome reflects the multisystem effect that preoperative anaemia appears to have, which is confirmed by further data which has implicated preoperative anaemia with postoperative septic, respiratory and thrombotic complications (Beattie et al., 2009).

1.4.3 Mechanisms linking anaemia and complications:

Despite the growing interest in the association of preoperative anaemia and adverse perioperative outcomes, there is little investigation into the reasons why such an association exists. The mechanisms behind this increased risk have been postulated to be both direct and indirect. Indirectly, anaemic patients are at risk of complications as a result of the use of ARBT. Perioperative ARBT have been demonstrated to impair cellular immunity in CRC patients (Ydy et al., 2007) rendering them more susceptible to infective complications (Dunne et al., 2002b, Waymack et al., 1993), and are even implicated in increased risk of anastomotic dehiscence (Tadros et al., 1992). The immunomodulatory basis for these changes has been discussed in section 1.1.2.

Consequently, the direct causality between anaemia and adverse outcome is most likely to be secondary to the physiological effects of reduced haemoglobin, and in particular the effect that such a reduction has on oxygen delivery.

Oxygen delivery is calculated by:

Delivery of Oxygen = Cardiac output x Arterial Oxygen Content

Where Arterial Oxygen Content =

$$(k_1 \times HB \times Sa_{02}) + (k_2 \times Pa_{02})$$

Where:
k_1= Hüfner's constant
HB= Haemoglobin
Sa_{02}= Arterial Oxygen Saturation
k_2= Solubility of oxygen constant
Pa_{02}= Partial Pressure of arterial blood)

Adapted from McLellan and Walsh, Oxygen delivery and haemoglobin, Continuing Education in Anaesthesia, Critical Care & Pain, 4;4 2004

Table 3 - Calculation of oxygen delivery.

From this equation it can be seen that by reducing HB levels, the arterial oxygen content and hence oxygen delivery will be reduced. The net effect is that when breathing room air, an anaemic patient (HB 7.5 g/dL) will have 50% of the oxygen delivery of a non-anaemic patient (HB 15 g/dL) (McLellan and Walsh, 2004). This anaemia associated reduction in end-organ oxygen delivery may result in the increased complications evident within practice (Johannes et al., 2007, Greenburg, 1996).

It is important to note, that due to physiological reserve, this does not mean that such reductions will induce a critical tissue hypoxia. Cardiac output increases to meet demand, pre-capillary oxygen loss is reduced and during chronic anaemia 2,3-DPG levels increase to improve oxygen release from HB all of which improve oxygen delivery (McLellan and Walsh, 2004).

Attempts have been made to establish a "minimum" threshold of HB below which oxygen delivery is insufficient, and hence has led to the notion of transfusion thresholds. This has predominantly been undertaken in critically ill patients, and although indicates that HB levels below 7g/dL represent this threshold (Hebert et al., 1999), it is important to recognise that this proposal relates to the appropriate point to utilise ARBT, balancing absolute HB levels with outcome and not oxygen delivery. From this study, it may be assumed that in a critically unwell patient, transfusion below a trigger of 7g/dL may be more advantageous than transfusion at a trigger of less than 10g/dL, but this does not mean that tissue oxygenation is unaffected between these levels. This merely means that the benefit of improvement of tissue perfusion between these levels by administration of ARBT does not outweigh the negative risks of ARBT use. Furthermore, the reliability of ARBT at

increasing tissue oxygenation in surgical patients is not well established (Napolitano and Corwin, 2004, Vincent et al., 2007)

Consequently we must continue to assume that a HB closer to normal levels is optimal for patients, and speculate that the improvement in oxygen delivery achieved by increasing HB levels continues to increase until a level when blood viscosity then reduces flow. If low HB levels are disadvantageous, and so too is the use of ARBT to correct this in surgical patients, then other modalities need investigation in order to achieve this.

Blood Transfusions

As described, preoperative anaemia in patients undergoing surgery for CRC is associated with the increased perioperative use of ARBT (Dunne et al., 2002b). This is of particular relevance, not only due to the multiple risks of ARBT (discussed in section 1.1) but also because the use of ARBT further increases the risks of complications.

In a recent metanalysis reviewing the perioperative use of ARBT in patients undergoing CRC resectional surgery, it was noted that this was associated with adverse outcomes in terms of of all-cause

mortality, cancer related mortality, combined recurrence–metastasis–death, need for surgical re-intervention, postoperative infections, and length of hospital stay (Acheson et al., 2012). Such findings are consistent with a Cochrane Review investigating ARBT use in CRC surgery, which illustrated the same association between cancer recurrence and ARBT use (Amato and Pescatori, 2006).

1.4.4 Postoperative consequences:

Recurrence:

Aside from the increased risks of cancer recurrence discussed above, anaemia is also directly linked with adverse outcomes from malignant disease. As discussed, anaemia induces hypoxia within tumours, resulting in cellular changes at a genetic level. These alterations are thought to increase the potential for local invasion, local cellular spread, and spread of tumour cell metastases to regional lymph nodes and distant sites (Hockel and Vaupel, 2001). Similarly it is recognised that tumour angiogenesis is a risk factor for post-operative recurrence of colonic malignancy (Frank et al., 1995), and since hypoxia has been shown to induce such activity in CRC (Vaupel and Mayer, 2005), indicates another mechanism for poor long-term prognosis.

Clearly, if this is the case, then a cycle may occur whereby advanced tumours cause anaemia, which results in local hypoxia causing cellular changes, which then causes upstaging of the cancer and a further propagation of the anaemia. As the stage of the CRC at the time of surgical resection is associated with long-term prognosis (NCIN, 2009) and duration of disease free survival (Sakamoto et al., 2004), it follows that anaemia may adversely affect long term outcome independent to the effects of ARBT.

1.5 Treatment of anaemia:

Treatment of anaemia is therefore essential and vital to PBM. Several options are available for the management of anaemia associated with CRC. These are geared at either providing the substrate required for erythropoiesis by iron supplementation; or by stimulating the production or red blood cells with erythropoietin; or by replacing the red cell deficit from an exogenous source, i.e. an allogeneic red blood cell transfusion (ARBT).

1.5.1 Iron supplementation:

Oral Iron:

Iron is most readily absorbed from the gut as haem, which is ingested at either as haemoglobin or myoglobin predominantly from meat (Fuqua et al., 2012). Most non-haem iron is ingested in the ferric form (Fe^{3+}) which is poorly absorbed due to insolubility (McKie et al., 2001). The presence of gastric acid, however, lowers the pH of the stomach, stabilising ingested Ferrous iron (Fe^{2+}), thereby making this form more bioavailable (Fuqua et al., 2012).

The site of most iron absorption is in the proximal small bowel, mainly from the gastro-duodenal junction (Andrews and Schmidt,

2007), duodenum and proximal jejunum (Collins and Anderson, 2012). Non-haem iron is then absorbed into enterocytes by one of two pathways; either via Divalent Metal Transporter 1 (DMT1)(Fleming et al., 1997, Fleming et al., 1998) or by a mucin-integrin-mobilferrin pathway (Conrad and Umbreit, 1993). DMT1 is probably the most important of these pathways, as murine studies have shown the development of significant iron deficiency anaemia in animals deficient in the gene for this transporter (Gunshin et al., 2005).

Once within enterocytes, the iron is then either stored or released in response to iron levels in the body (Roy and Enns, 2000). If not stored within the enterocyte, it is released into the circulation via the Ferroportin channels (Fuqua et al., 2012). At this point, the majority binds to transferrin, and lesser amounts remain free or bind to albumin. As the circulating complex of iron and transferrin encounter transferrin receptors in the bone marrow or target tissue, receptor mediated endocytosis occurs delivering the iron to the tissue (Andrews and Schmidt, 2007).

Oral iron supplementation is perhaps the easiest and cheapest method of administration of iron. Various preparations exist, but most are composed of iron in its ferrous (Fe^{2+}) state. As a

consequence of this, bioavailability of these tablets is highly variable, and absorption is increased with co-administration of ascorbic acid (Teucher et al., 2004, Cook and Reddy, 2001) and reduced with concomitant proton pump inhibitor use (Ito and Jensen, 2010, Golubov et al., 1991).

Oral iron is also associated with many side effects including constipation, diarrhoea (Aronstam and Aston, 1982), abdominal pain and nausea (Rybo and Solvell, 1971, Zimmermann and Hurrell, 2007). This dose related intolerance is noted in up to 20% of patients and affects adherence to prescribed treatment regimens (Bonnar et al., 1969). Simultaneous ingestion with food may improve this intolerance (Macdougall, 1999), but also reduces the already variable absorption rates by as much as two thirds (Brownlie et al., 2002). As a consequence, the benefits of the drug must be of significant magnitude to justify this.

The efficacy of the use of oral iron has been examined in the context of colorectal cancer (CRC) resectional surgery. Lidder et al (Lidder et al., 2007), recruited 49 patients who were planned to undergo elective resection for CRC and randomly assigned each patient to receive either oral ferrous sulphate 200mg three times

daily or “standard” clinical care, i.e. as governed by the clinical team overseeing the patient’s care.

The Investigators reported that patient groups were comparable in demographics, planned operation, and initial mean haemoglobin (HB) and ferritin levels.

Following a mean treatment period of 14 days, both groups experienced a decrease in HB levels. However, this decrease was only statistically significant in the non-treatment group, where a mean decrease of 0.6 g/dL was evident compared to 0.3 g/dL in the treatment arm.

A modest rise in median ferritin was also noted in the treatment arm, increasing from 40mg/l to 73 mg/L, whilst it remained unchanged in the non-treatment group.

The primary endpoint was differences in perioperative blood transfusions between the groups, and indeed 13 patients in the non-treatment group were transfused a total of 47 units of packed red cells. This compared to 6 transfused patients in the iron group who received a total of 15 units.

The authors surmised that oral iron supplementation resulted in a reduced preoperative fall in patient HB, and favourable increases in ferritin levels, which reduced the need for blood transfusions. It is however, important to note several observations regarding this study. Firstly, although not statistically different, the mean initial HB of the treatment group was 1 g/dL higher than the non-treatment arm. Similarly, included patients did not need to be anaemic, and there were 14 anaemic patients at recruitment in the non-treatment group, compared to only 6 patients within the oral iron group. Such data could imply that non-treatment group were more likely to require a blood transfusion on the basis of inclusion HB, and that the lack of statistical difference was merely reflective of small sample numbers.

Secondly, the authors acknowledged the individuality that each clinician has in their rationale for blood transfusion. The transfusion protocol published by the authors included reference to transfusion of blood at HB levels between 8 and 10 g/dL when the patient had:

"..*ischaemic heart disease*, *obstructive lung disease* or at the *Consultant's discretion…"*

The Consultants' decision would undoubtedly have been influenced by comorbidities, including those mentioned in the protocol, hence it would have been relevant for the authors to outline any differences in this key variable between the group. Clearly, within the scope of a pilot study, such in depth reporting is difficult, but considering each patient underwent an operation, then description of patient ASA (American Society of Anaesthesiologist) Grade would have been a simple method of indicating whether one arm had patients with significantly more comorbidity.

On further review of the amount of blood transfused (see Table 4), it was evident that the non-iron group received 47 units of ARBT, with a range of 11 units. It is thus possible that statistical significance was achieved by inclusion of one patient that required an 11 unit transfusion. Given the large volume transfusion, it is probable that this was a consequence of a significant intraoperative event, and unrelated to HB preoperatively. Had the patient been randomised to the treatment group, the 11 unit transfusion would still invariably have been required, and may have heavily influenced the study outcome.

	Group	Number Patients transfused	Total units transfused	Median units transfused (range)	P-value [95% CI]
All Patients	Iron (n=23)	6 (26%)	15	0 (0-4)	0.031 [0.13-2.59]
	No-Iron (n=22)	13 (59%)	47	2 (0-11)	
Anaemic Patients	Iron (n=6)	3 (50%)	6	1 (0-2)	*Non-Significant*
	No-Iron (n=14)	10 (71%)	39	2.5 (0-11)	

Table 4 - Transfusion data from Lidder *et al.*, 2007.

Finally, and perhaps most pertinent of all, relates to subgroup analysis of the efficacy of the iron treatment in relation to anaemia. The authors described a significant difference in the number of blood transfusions administered between the study arms which was only evident when all patients were included (see Table 4 above). When anaemic patients were analysed separately, this statistical significance was lost. Consequently, this could imply that oral ferrous sulphate (OFS) supplementation is not sufficiently efficacious to reduce blood transfusion requirements in anaemic patients undergoing colorectal cancer resection. It is possible that OFS is only mildly potent which is adequate for patients with minimal iron deficiency who are yet to develop anaemia. Invariably there is delay between diagnosis of CRC and a planned date of surgery. Quinn *et al.* investigated the role *of* OFS in this

period in the preoperative optimisation of patients with CRC (Quinn et al., 2010). They enrolled all patients undergoing elective CRC resection regardless of initial HB levels, and administered a standard dose of OFS until the day of surgery, with the aim of reviewing an impact on the incidence of preoperative anaemia and perioperative transfusion.

This group noted a significant mean rise in HB of 1.1 g/dL over a median treatment period of 39 days. Subgroup analysis demonstrated that this increase was larger in those patients who were anaemic (1.7g/dL) compared to those that were non-anaemic (0.5g/dL).

In total, 20 patients were transfused (10 intraoperatively, 10 post-operatively), a total of 71 units. This represented a 54% reduction in units transfused compared to a historical control. Consequently, the authors concluded that OFS was efficacious in increasing preoperative HB levels of CRC patients, and that this increase translated to a reduction in blood transfusion requirements *intra-* and postoperatively.

It is important to note that this was an open label, cohort study with comparison to a historical control. No details describing the

control group were reported, hence it is not possible to ascertain the homogeneity between the groups.

Also, patients who required a preoperative blood transfusion were excluded which reduces the clinical validity of the study. These patients were potentially more iron deficient hence had a differing response to OFS treatment than the rest of the group.

Furthermore, these patients were probably those with the highest volume of tumour induced blood loss and consequently at highest risk of requiring a perioperative transfusion. It is therefore possible that had these patients been included a higher number of blood transfusions would have been subsequently administered. Extrapolation of the findings of the study to *all* patients with CRC should thus be performed with caution.

The findings of these two studies contradict each other with regard to the effect on HB of OFS. This may be reflective of the different length of treatment with OFS, or due to the study design. Although both studies were prospective, both require subgroup analysis to review the efficacy of oral iron in *anaemic* patients undergoing surgery for CRC. As yet, there are no studies which prospectively examine this group of patients, but retrospective data does exist.

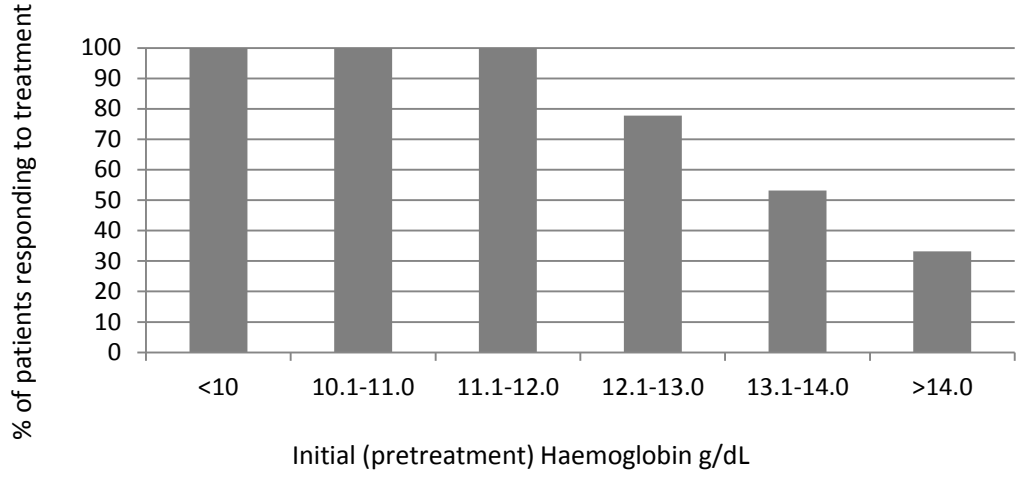
Okuyama *et al.* retrospectively reviewed 6 consecutive years of CRC patients undergoing resectional surgery (except low anterior [LAR] and abdominoperineal resections [APR]) (Okuyama et al., 2005). They identified 116 anaemic patients over this period, of which 32 patients had received preoperative oral iron (iron citrate 100md OD) for 2 weeks. On comparison of the groups it was noted that despite similar initial HB values, the treated group rose by a mean 2 g/dL, whilst the untreated group increased by only 0.9 g/dL. The net clinical result was that more patients required intraoperative blood transfusions in the untreated group compared to those receiving oral iron supplementation (27.4% vs 9.4%). The authors also noted a correlation between low HB on the day of surgery and intraoperative blood transfusion requirement.

It is interesting to note that this study excluded patients undergoing operations with the highest associated blood losses (LAR and APR), perhaps on the assumption that patients undergoing such procedures, the operative loss would be more influential towards subsequent transfusion than starting HB.

It is also interesting to note that the authors defined anaemia as a Hb below 10 g/dL. This corresponds to (low) "moderate" anaemia by the WHO definition (see Table 2). It is possible that worsening

anaemia in patients with CRC may be a crude estimate of worsening iron deficiency, and hence be a predictor of a potentially greater response. Although far less accurate than other biomarkers (which will be discussed in more detail in section 4.5), there is evidence to support that response to oral iron supplementation may be predicted by pre-treatment HB.

Figure 3 represents data taken from a study by Friere (Freire, 1989). In this double-blind trial, pregnant females were randomised to either placebo or OFS, and it is evident that the lower the HB at initiation of treatment, the more likely a patient was to respond to therapy.



Data taken from: W B Freire. Hemoglobin as a predictor of response to iron therapy and its use in screening and prevalence estimates. Am J Clin Nutr *December 1989; 50(6) 1442-1449*

Figure 3 - A graph illustrating response to oral ferrous sulphate in relation to pre-treatment haemoglobin.

A response was defined as any increase in HB, but what is not documented in this study, and remains less clear, is whether the magnitude of response is related to initial pre-treatment HB. This has not been addressed by studies with oral iron in patients with CRC, but has been reviewed in the paediatric population and found to be related, i.e. a greater response to treatment is seen in patients with lower HB (Gera et al., 2007).

These relationships may account for why the study by Okuyama noted such good responses to oral iron in CRC. With inclusion HB levels less than 10 g/dL, it could be argued that these patients were more likely to respond to treatment, and to a greater degree than less anaemic patients. As previously discussed, the majority of anaemic CRC patients are in the "mild" anaemia category, hence the study by Okuyama could be argued to have exaggerated the efficacy of oral iron in the broader clinical context of CRC.

These studies discussed have highlighted a potential role for oral iron in the preoperative optimisation of CRC patients, but have are yet conclusively address whether this is in anaemic patients, and if so, in what severity of anaemia. As a result, it remains challenging to base PBM upon this.

Intravenous Iron:

Intravenous iron was historically associated with serious side effects including anaphylaxis which resulted in limited clinical use (Auerbach and Ballard, 2010). However, over recent years, extensive drug development has been performed resulting in a much improved safety profile (Auerbach and Ballard, 2010).

As discussed, oral iron has been criticised for the associated gastrointestinal side effects causing reduced patient adherence, variable enteric absorption, and poor efficacy in certain pathophysiological states. Intravenous iron (IVI), was thus intended to overcome some of these, allowing larger doses of iron to be administered parenterally.

Several of these IVI formulations have been reviewed in CRC patients with mixed results. Edwards et al. randomised patients awaiting surgery for CRC to either Iron Sucrose (IS) or placebo, and noted no significant change in HB levels or transfusion rates between then groups (Edwards et al., 2009). Initial HB was not an inclusion criterion despite the aim to review the drug in anaemic CRC patients- indeed only 9 anaemic patients were recruited to each arm, hence the median recruitment HB in the treatment arm was 13.4 g/dL.

Furthermore, debate exists around the diagnosis of iron deficiency in the subjects. The authors could argue that IVI was indeed indicated given the median ferritin of the patients in the IS group was 70.8 ng/L, and the corresponding ferritin and TSat levels in the control arm were 100.8 ng/L and 19.9% respectively. These values would conform to the Consensus statement on preoperative use of IVI (Beris et al., 2008) (see Table 5).

Serum ferritin level <100 ng/ml
Ferritin 100–300 ng/ml and transferrin saturation <20%
Undergoing surgical procedures with an expected blood loss >1500 ml
Undergoing surgical procedures with an expected HB drop of 3–5 g d/dL

Adapted from: Beris, P., Munoz, M., Garcia-Erce, J. A., Thomas, D., Maniatis, A. & Van Der Linded, P. 2008. Perioperative anaemia management: consensus statement on the role of intravenous iron. Br J Anaesth, 100, 599-604.

Table 5 - Summary of Perioperative anaemia management: consensus statement on the role of intravenous iron in non-anaemic patients.

However, these guidelines are perhaps inaccurate in the context of CRC. Current British Society of Gastroenterology guidelines recommend a ferritin level of less than 50 ng/L as definition of iron deficiency in the context of coexisting disease (Goddard AF et al., 2005), in light of the fact that that ferritin is an acute-phase

protein and elevated in inflammatory states such as malignancy (Cook, 2005). As a consequence it is thus debatable whether the patients were indeed iron deficient in Edwards' study (Ranganathan and Pramesh, 2010, Simpson et al., 2010b). It could thus be argued, that if the patients were generally neither anaemic nor iron deficient, then the potential benefit of IVI was minimal.

The next consideration from this trial by Edwards et al. relates to the dosing of the IS administered. There are currently four IV iron preparations used within the NHS – Iron dextran (Cosmofer®), Iron Sucrose (Venofer®), Iron III isomaltoside 1000 (Monofer®) and ferric carboxymaltose (Ferinject®). With the exception of Ferinject®, they all require dosing based upon the Ganzoni equation of cumulative iron deficit (CID) (see Table 6).

Cumulative Iron Deficit =

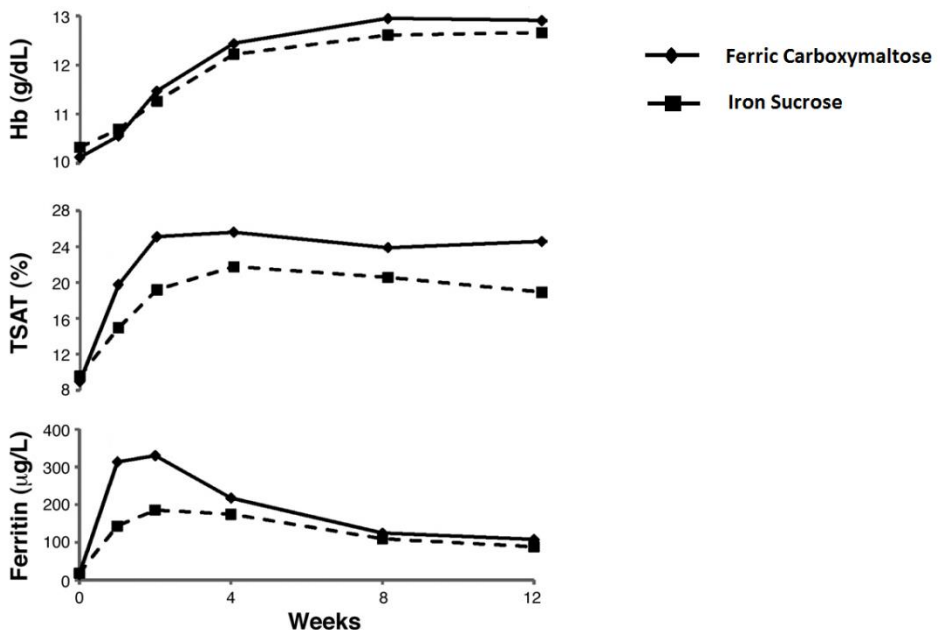
$[Wt_{(kg)} \times (\text{Target HB} - \text{Current HB}_{[g/dL]}) \times 2.4] + \text{Iron Store}^{*}_{(mg)}$

*Iron store = 500mg if body weight > 35kg 15 mg/kg if body weight < 35kg

Table 6 - Calculation of the Cumulative Iron Deficit by Ganzoni Formula.
Where: Wt= Patient body weight, and HB = Haemoglobin level.

As a consequence the fixed dosing regimen that was employed in this study is incorrect. Using the data reported in the study, a 75kg patient with the median HB (13.4 g/dL) would in fact require 698mg of IV iron. The trial design had allowed a maximum of 600mg iron to be administered, so it is reasonable to assume that most patients would have been undertreated.

Finally, although the median treatment period of 17 days was often appropriate in the context of operative waiting times, and thus makes the findings clinically transferrable, it may have been insufficient to allow a treatment response. Review of data from the FERGIcor study (Evstatiev et al., 2011) which compared ferric carboxymaltose (FCM) with iron sucrose (IS) treatment in IBD associated anaemia, a response should be evident in this time-frame. Although the mechanism of anaemia in IBD differs from that of cancer, it is evident from Figure 4 that around treatment day 17, a significant treatment effect should have been evident in all parameters recorded in the study, but maximal HB response occurs much later.



Taken from Evstatiev, R., Marteau, P., Iqbal, T., Khalif, I. L., Stein, J., Bokemeyer, B., Chopey, I. V., Gutzwiller, F. S., Riopel, L. & Gasche, C. 2011. FERGIcor, a Randomized Controlled Trial on Ferric Carboxymaltose for Iron Deficiency Anemia in Inflammatory Bowel Disease. Gastroenterology, 141, 846-853.e2

Figure 4 - Changes in haematinic markers in relation to duration of treatment in patients receiving Ferric Carboxymaltose or Iron Sucrose for the treatment of Inflammatory Bowel Disease associated anaemia.

In summary, there must be limitations to any judgements made from this study on the role of IVI in the preoperative treatment of anaemia in CRC patients. Such caution is particular advisory given the variation in results obtained from the only other published series using preoperative IVI in this patient group. In a small, un-controlled, non-randomised study, iron dextran (ID) was administered to patients awaiting CRC resectional surgery, who had IDA defined as a HB of less than 13.0g/dL in males and less than 11.5g/dL in females, in combination with a serum ferritin level

<20ng/ml (Simpson et al., 2010c). Of the 10 patients treated, a mean HB increase of 1.1 g/dL was noted over a mean treatment period of 27 days. The mean dose of iron administered was 1093 mg, hence had selected IDA patients and administered maximal therapy, both in dose and duration, with good treatment response.

In conclusion, it is evident that there is minimal data which reviews the role of iron supplementation, either oral or IV, in the context of CRC and that there are inherent limitations to the interpretation of the published results. Despite this, a potential role for intravenous iron in the management of anaemia have been identified in several clinical contexts including IBD (Kulnigg and Gasche, 2006, Evstatiev et al., 2011), heart failure (Anker et al., 2009, Okonko et al., 2008), renal failure (Li and Wang, 2008) and post-partum anaemia (Breymann et al., 2008, Giannoulis et al., 2009). This indicates a real need for further clinical trials to review these drugs in anaemic CRC patients (see Chapter 3 and 4).

1.5.2 Erythropoietin:

Aside from iron supplementation there has been debate about the role of erythropoietin (EPO) in the management of CRC related anaemia. EPO is glycoprotein hormone produced and release by

the kidney and is the main regulator of erythropoiesis (Lopez et al., 2009). It is available in three synthetic forms for clinical use: epoetin-α, epoetin-β, and darbepoetin-α (Aapro, 2009), and is now widely used and of proven benefit in the treatment of renal disease associated anaemia (Cody et al., 2005), often in combination with iron supplementation (Macdougall et al., 1996).

EPO is also used in the treatment of malignancy associated anaemia. When reviewing the clinical benefit of the drug in the treatment of anaemia of patients with any form of malignancy, it has been adjudged to improve quality of life and reduce blood transfusion requirement if administered at HB levels of less than 10 g/dL (Seidenfeld et al., 2001). It is therefore unsurprising that the ECAS reported that the most frequent treatment of cancer related anaemia involved EPO (Ludwig et al., 2004), although a large number of those treated had haematological malignancy. Only 1/3 of CRC patients had their anaemia treated in any form.

A reluctance to administer EPO to CRC patients is not ill-founded. Animal models have indicated EPO administration stimulates CRC recurrence (Pascual et al.), whilst human cell line studies have indicated it induces recurrence, chemo-resistance and upregulates tumour activity in head and neck cancers (Abhold et al., 2011).

The clinical efficacy of EPO in CRC is also questionable. In one small RCT, although EPO was found to increase reticulocyte count after a short course of treatment perioperatively, this did not correlate with reduced transfusion requirements (Heiss et al., 1996). Of the 20 patients treated, one was diagnosed with a deep vein thrombosis following initiation of treatment and highlights a real limitation of the treatment in CRC patients undergoing surgery. EPO therapy is recognised to be linked with thrombotic events (Lopez et al., 2009), and indeed the risk of serious cardiovascular events increases when Hb levels rise at a rate greater than 1g/dL per fortnight (Locatelli et al., 2009). Hypertension is also associated in up to 24% of patients receiving EPO (Vaziri, 1999). As malignancy, and prolonged abdominal/pelvic surgery are both risk factors for venous thromboembolism (Arcelus et al., 2012), clearly administration of a further risk factor is less than an ideal therapy, especially with the increased cardiovascular risk.

1.5.3 Allogeneic Red Blood Cell Transfusions:

The final treatment option available for the treatment of anaemia is allogeneic red blood cell transfusion (ARBT). The decision to administer ARBT is a risk benefit analysis based upon the

symptoms and comorbidity of the patient and potential for complications with and without ARBT. As CRC patients are predominantly iron deficient, ARBT is not a targeted therapy, and more of a global treatment for *all* anaemias irrespective of aetiology. Similarly, as established previously, the majority of anaemic CRC patients have mild anaemia (see Table 2) hence have HB concentrations in excess of 10 g/dL. When considered in line with the current UK blood transfusion and tissue transplantation guidelines (UKBT, 2014) summarised in Table 7, it implies that only a small percentage of patients should be considered for ARBT, and that other modalities should be employed instead.

- Transfusion should be considered if HB below 80 g/L.
- If the HB is below 70 g/L transfusion is usually indicated
- The decision to transfuse should be based on the clinical condition of the patient (higher thresholds may be appropriate in individual cases).
- Many clinicians recommend using a higher threshold in patients with acute coronary syndromes, but the evidence for this is limited.
- Patients who are not actively bleeding should be transfused with a single unit of red cells and then reassessed before further blood is given.

Available online at: http://www.transfusionguidelines.org.uk/transfusion-handbook/7-effective-transfusion-in-surgery-and-critical-care/7-1-transfusion-in-surgery

Table 7 - Current UK Blood Transfusion and Tissue Transplantation Guidelines on Blood Transfusion in Surgery.

1.6 Potential timing for treatment of anaemia:

PBM advocates treatment of anaemia in all phases of surgery. It is however, necessary to explore the optimal time point at which to initiate treatment to avoid the need for ARBT and also minimise the risks of anaemia.

1.6.1 Early postoperative period:

It could be argued that if perioperative anaemia is managed postoperatively, the treatment is being administered too late (Keeler et al., 2012). Although conceptually, postoperative iron supplementation may have potential for reducing the need for ARBT, not only will the patient have been exposed to the risks associated with the anaemia, but the iron supplementation may be ineffective. This has been demonstrated in CRC patients, whereby post-operative IVI failed to reduce the incidence of blood transfusions (Titos-Arcos et al., 2012). This can be accounted for by the associated inflammatory response that is generated by surgery. As discussed previously, hepcidin is an acute phase protein, and is a key inhibitor of iron absorption in the small intestine and iron release from macrophages (Nemeth et al., 2003a). Clearly, since abdominal surgery elevates the level of hepcidin (Park et al., 2012) and other inflammatory mediators,

then it may adversely affect iron metabolism, and render iron supplementation less effective. Postoperative management should ideally be targeted at minimising the effects of anaemia, for example ensuring tissue oxygenation with supplementary oxygen (Strachan and Noble, 2001) and correcting anaemia as appropriate with ARBT within the profile of PBM.

1.6.2 Intraoperatively:

Intraoperative treatment of anaemia may involve ARBT in extreme cases, but is predominantly geared towards preservation of existing red cell mass. This can be achieved by either pharmacological or non-pharmacological methods. Tranexamic acid is the mainstay of pharmacological treatment, aiming at stabilising clot and reducing blood loss. This has been of proven efficacy and thus widely used in orthopaedic (Husted et al., 2003) and urological procedures (Kumar et al., 2012). Although it has been of proven use in some abdominal procedures (Wu et al., 2006), it has not been shown of benefit in procedures for CRC and hence is not widely used. This importance of platelet function further highlights the need for clear delineation of guidelines for the perioperative management of antiplatelet therapy as previously outlined in Table 1 as a cornerstone of PBM (this will be covered in Chapter 5).

As a result, the majority of intraoperative measures to reduce blood loss in CRC surgery have been focussed on non-pharmacological measures. These are either surgical or anaesthetic related. Anaesthetic related measures may be as simple as avoidance of hypertension and permission of mild hypotension (Sollevi, 1988) or the use of epidural or spinal analgesia (Kida et al., 1999). Similarly it is now recognised that active patient warming prevents coagulopathy and hence reduces blood loss and transfusion requirements (Bock et al., 1998). Consequently these measures already feature as part of PBM (Table 1).

Other more complex techniques such as acute normovoalemic haemodilution have been utilised within colorectal surgery with mixed popularity. Although some early studies have identified that the practice is associated with reduced ARBT (Nikolov et al., 1990) the system is rarely employed in the UK. This is partly due to the demonstration of the same adverse immunological outcomes when homologous transfusions were given as described for ARBT (Busch et al., 1993).

Surgical techniques to minimise blood loss have been more readily employed. Although cell-salvage devices are contraindicated in the context of malignancy and contamination, as may be encountered

in colorectal surgery, minimally invasive surgery is becoming of increased popularity. With the development of increased familiarity and expertise, laparoscopic surgery has shifted from being comparative or inferior to open surgery in terms of blood loss and transfusion requirement (Guillou et al., 2005, Maxwell-Armstrong et al., 2000) to being generally regarded as superior in this respect in both colonic and rectal cancer surgery (Aly, 2009).

All of the measures discussed are useful in terms of conservation of blood and red cell mass, but will obviously fail to improve a patients' anaemia. They are thus important adjunctive measures in the management of the anaemic patient undergoing surgery for CRC, but will fail to reduce the risks previously discussed.

1.6.3 Preoperative period:

Excluding the use of ARBT, it appears that postoperative treatment of IDA is largely ineffective, and intraoperative measures are mainly aimed at avoidance of exacerbating anaemia rather than treatment of it. As a consequence, more emphasis is thus placed upon the preoperative treatment of the condition. This appears to be the most logical point for treatment – the patient is "optimised" prior to undergoing surgery. This is arguably the point at which

iron supplementation is most effective, and given the limited value of other treatment modalities, lends further justification to preoperative iron use. Furthermore, given the period of inevitable logistical delay between diagnosis, staging and planning of treatment that occurs prior to surgery, this period can be utilised to manage the anaemia with iron supplementation, and indeed, is associated with reduced blood transfusion perioperatively (Espallardo et al., 2011).

1.7 Conclusions and General Aims of Thesis:

PBM is a new concept and as a result, some of the guidance remains vague. As discussed, this Thesis will focus on aspects of PBM relevant to colorectal surgery.

Several of the measures advised by PBM (Table 1) are already employed within colorectal surgical practice, but these predominantly focus on the intraoperative components such as minimially invasive surgery, and the use of electronic surgical devices to minimise intraoperative blood loss. Furthermore, much of the intraoperative techniques advised by PBM are within the realms of anaesthetic practice and are currently under investigation by this medical field.

A key bulk of the surgical onus in implementation of PBM is therefore within the preoperative phase of the model, yet there is currently a scarcity of good evidence to guide best practice. Consequently this Thesis aims to undertake several investigations in order to address these issues with respect to the Preoperative factors proposed in the NBTC guidelines summarised in Table 1 (NBTC, 2014), i.e. the increase and maintenance of red cell mass in the preoperative phase of surgery.

As has been demonstrated, anaemia is a common problem encountered by patients with colorectal pathology. Early identification and management of this is an essential component of PBM, and furthermore, if not adequately treated, can predispose patients to increased perioperative morbidity and mortality irrespective of ARBT use.

Chapter 2 will look to understand whether current practice attempts to identify and treat anaemia early, and to establish the extent of the problem in current practice since the introduction of screening. Furthermore, this Chapter will seek to investigate the influence of absolute HB levels on the risk of transfusion requirements, in order to determine the magnitude of treatment response that will be potentially required to influence clinical outcome.

Chapter 3 will then build upon this by discussing the results of a pilot clinical trial to investigate the efficacy of IV iron the preoperative treatment of IDA in colorectal cancer patients. Current PBM guidance advises that oral iron should be used in preoperative IDA and IV iron in functional iron deficiency (NBTC, 2014). Consequently Chapter 3 investigates if intravenous iron does provide a feasible alternative to oral iron in this context.

The clinical trial documented in Chapter 4 further develops the concepts raised in the previous Chapter. Building on the results of the Pilot study, Chapter 4 discusses the subsequent larger clinical trial which compares the efficacy of oral and IV iron in the preoperative management of CRC related anaemia. This chapter is divided into 3 parts, with the first section comparing efficacy in terms of ARBT use and changes in blood results, whilst the second section compares efficacy in terms of complications and quality of life scores. The final section looks to identify if any biomarkers exist which may predict response to iron therapies in order to potentially select patients for each treatment.

As previously discussed in section 1.6.2, PBM guidelines remain vague with respect to preoperative antiplatelet therapy. Although this stipulates protocols should be in place to ensure the correct management of antiplatelet drugs in the preoperative period, such protocols are not clearly outlined and widespread debate persists. Chapter 5 documents a clinical trial which aims to provide more evidence for delineation of such a protocol.

The final Chapter consolidates the experiences and learning from designing, setting-up and undertaking the Clinical Trials documented within this Thesis. Consequently, Chapter 6

extrapolates the work undertaken into future areas of research by documenting the planning and design of a large multi-centre Clinical Trial which investigates further questions relating to PBM.

Chapter 2:

Timely identification and correction of anaemia before elective surgery: anaemia, treatment and the use of allogeneic blood transfusion in colorectal cancer surgery.

Abstract:

Background:

Preoperative identification and treatment of anaemia is advocated as part of Patient Blood management due to the association of adverse outcome with the perioperative use of blood transfusion. This study aimed to establish the rate of anaemia identification, treatment and implications of this preoperative anaemia on ARBT use.

Methods

All patients who underwent elective surgery for colorectal cancer over 18 months at a single Centre were reviewed. Electronic databases and patient casenotes were reviewed to yield required data.

Results:

Complete data was available on 201 patients. Sixty-seven percent (n=135) had haemoglobin tested at presentation. There was an inverse correlation between tumour size and initial haemoglobin (P<0.01, R_s=-0.3). Initial haemoglobin levels were significantly lower in patients with right colonic tumours (P<0.01). Patients who were anaemic preoperatively received a mean 0.91 units (95%CI

0-0.7) per patient which was significantly higher than non-anaemic patients (0.3 units [95%CI 0-1.3], *P*<0.01). For every 1g/dl preoperative haemoglobin increase, the likelihood of transfusion was reduced by approximately 40% (OR 0.57 [95%CI 0.458-0.708], *P*<0.01). Laparoscopic surgery was associated with fewer anaemic patients transfused (*P*<0.01).

Conclusion:

Haemoglobin levels should be routinely checked at diagnosis of colorectal cancer, particularly those with large or right sided lesions. Early identification of anaemia allows initiation of treatment which may reduce transfusion risk even with modest haemoglobin rises. The correct treatment of this anaemia needs to be established.

2.1 Introduction:

The associations between preoperative anaemia and adverse outcomes following colorectal surgery (Leichtle et al., 2011) were discussed in the previous chapter. Increased requirement of allogeneic red blood-cell transfusion (ARBT) was highlighted as one particular adverse outcome which is also thought to increase complications in both the short and long term (Acheson et al., 2012).

Consequently, preoperative anaemia is both directly and indirectly associated with impaired patient outcome and thus treatment of this forms a key part of patient blood management (PBM).

Advances in CRC management such as the Bowel Cancer Screening Programme (BCSP) could reduce the prevalence of anaemia at diagnosis by identifying malignancy at an earlier stage (McClements et al., 2012). Similarly, laparoscopic surgery (LS) may reduce the overall need for ARBT by minimising blood loss (COLOR, 2005). The continued uptake of LS may be accelerated by screening since this mode of access is well suited to less locally advanced tumours.

Current practice is therefore changing which may impact on the risks outlined in historical data. A contemporary review is therefore required to evaluate this which will be a reflection of current and future practice.

Furthermore, PBM advises:

"…timely identification and correction of preoperative anaemia…"
(NBTC, 2014)

Several key elements need to be known in order to improve this process. This review of current practice in a laparoscopic colorectal National training centre which is part of the BCSP is ideally suited to highlight such issues and thus aimed to:

1. Identify the prevalence of anaemia at presentation of CRC.
2. To delineate the natural changes in haemoglobin levels (HB) across the surgical treatment course.
3. Outline current practices in treatment of anaemia in this patient group and assess their efficacy.
4. Investigate the details of ARBT utilisation.
5. Correlate the impact that LS rates have on ARBT use.
6. To ascertain a regional "baseline" of current outcomes in relation to these variables, including length of stay and complication rates.

The results of this review would thus help to ascertain the current magnitude of this issue, and whether the principles of PBM were being adhered to. The findings were intended to guide the design of a future Clinical Trial.

2.2 Methods:

Patient Population:

Patients who underwent elective surgery for resection of a primary colonic or rectal tumour between 1st January 2011 and 31st May 2012 were identified from the local National Bowel Cancer Audit Programme (NBOCAP) registry.

Two-hundred and twenty seven patients were considered for analysis. Sixteen were excluded for incomplete records, 6 for having undergone emergency surgery, and a further 4 for having had benign disease. Two-hundred and one patients were thus included in analysis.

Data recorded:

Data was retrieved from patient casenotes and hospital electronic records and was reviewed at several time points. Complications were recorded as documented within these records, with infective complications further defined by a complication with a proven documented focus of infection. Infection was defined using recognised criteria (Bone et al., 1992), e.g. the pathological:

"…microbial phenomenon characterised by an inflammatory response to the presence of microorganisms or the invasion of normally sterile host tissues by those organisms…"

ACCP/SCCM consensus statement 1992

All complications were graded using the validated Clavien-Dindo scoring system (Dindo et al., 2004)

The first out-patients appointment (OPA) which prompted investigation resulting in the registered operation was defined as the presentation OPA. Blood test values taken at that appointment or on referral were used as the *"diagnosis"* value. The WHO definition of anaemia (Males, <13g/dL; Females, <12g/dL) was applied to all haemoglobin (HB) levels (WHO, 2011).

The second time point evaluated was the preadmission clinic (PAC) appointment when the patient was assessed for surgery. This occurred within the 7 - 14 days preceding surgery. Blood test values acquired at this visit were used clinically to reflect day of surgery values, and were regarded similarly in this review.

"Initial" HB levels were defined as the earliest available HB level, i.e. the "diagnosis" value when tested, and the PAC result when this was not available.

Tumour details were recorded as documented in the final histopathology report. The site of the tumour was classified as either "Right" (from caecum to distal transverse colon) or "Left" (from splenic flexure to anorectum). Tumour stage was noted per modified Dukes' (Whittaker and Goligher, 1976) and TNM classifications (Poston et al., 2011) . Tumour size was recorded as the maximum tumour diameter in millimetres.

Details were obtained from the operation note, including the American Society of Anesthesiology grade (ASA), operative approach and description including documented blood loss. Blood transfusions including date and volume of administration were delineated from electronic transfusion logs and inpatient charts and recorded from OPA until postoperative discharge. The transfusion policy employed by the clinical teams included a "trigger" of 7g/dL in healthy individuals, or a target of greater than 9g/dL in those with significant cardiovascular or respiratory disease, in line with local policy.

Study Approvals:

Ethical approval was not sought for this review, but data collection was registered with the Clinical Audit and Evaluation office at Nottingham University Hospitals NHS Trust, audit reference 13-027C.

Statistical Analyses:

Statistical significance was defined as $P<0.05$. Non-parametric data was compared using Wilcoxon signed rank test for paired data, Mann-U Whitney for independent variables, and Kruskal-Wallis test when group numbers exceeded two. Categorical data was evaluated using Chi-squared test. Continuous non-parametric data was evaluated with Spearman's rank test to assess correlation. Binary logistic regression was used to investigate the effect of HB levels on transfusion status whilst accounting for confounders. Statistical analyses were performed using SPSS® version 21 (SPSS, Chicago, Illinois, USA).

2.3 Results:

General Demographics

Demographic data for the entire cohort and specific subgroups analysed are illustrated in Table 8. At diagnosis, 135 patients had HB tested, with a median value of 13.65 g/dl (IQR 11.88-14.9) for males and 12.60 g/dl (IQR 10.95-13.2) for females. At this point, 51% of patients were anaemic. Thirty-one patients received oral iron (OI) and a further 3 received intravenous iron.

Haemoglobin Changes in untreated patients:

The median time from OPA to Surgery was 50 days (IQR 26-94). The change in HB levels over this period was significant for patients with results available from both time-points ($P<0.05$). The median fall for males was 0.20 g/dl [IQR-0.9 to 0.25] and 0.15 g/dl [IQR -1 to 0.4] for females.

Haemoglobin changes with oral iron:

In those prescribed OI who did not receive ARBT in this period, the median HB was 13.10 g/dL [IQR 11.55-14.4] at diagnosis with a non-significant median rise of 0.10 g/dl [IQR-0.3 to 1.1] ($P=0.107$). This was significantly higher than the corresponding overall change in untreated anaemic patients ($P<0.05$, untreated change -0.20 g/dl, IQR-0.3-1.5). Median treatment duration was 56 days (IQR 37-126).

	Group		*P* value
	Entire cohort		
Gender (M:F)	201 (109:92)		-
Age years (IQR)	68.3 (61-77.3)		-
ASA (95%CI)	2.1 (1.99 -2.21)		-
	Anaemic at Diagnosis - Untreated†	**Anaemic at Diagnosis - Treated oral iron†**	
Gender (M:F)	43 (23:20)	27(12:15)	0.624
Age years (IQR)	73 (63-79.8)	75 (68-82.8)	0.244
ASA (95%CI)	2.13(1.87-2.38)	2.35 (2.07-2.63)	0.202
Laparoscopic : Open	25:18	17:10	0.238
MCV fl (IQR)	83 (76.8-90)	80 (74.5-87)	0.24
	Anaemic at Surgery	**Non-Anaemic at Surgery**	
Gender (M:F)	87 (41:46)	114 (68:46)	0.09
Age years (IQR)*	76 (67.5-81)	67 (59 – 73)	<0.01
ASA (95%CI)*	2.26 (2.09-2.43)	2.01 (1.87-2.15)	<0.05
Laparoscopic : Open	39:48	46:68	0.566
MCV fl (IQR)*	83.5 (76.5-90)	91 (86-93)	<0.01
	Laparoscopic Surgery	**Open Surgery**	
Gender (M:F)	84 (49:35)	117 (60:57)	0.39
Age years (IQR)	69 (62-78)	70.5 (61-76.3)	0.88
ASA (95%CI)	2.1 (1.93-2.27)	2.1 (1.96-2.25)	0.996
Anaemic at surgery(A:NA)	38:46	49:68	0.667
Converted procedures (converted:completed)	12:72	-	-
Tumour Size mm (IQR)	40 (30-50)	37.5 (25-51.25)	0.447
Tumour site (Right:Left)*	36:48	33:84	<0.05
T stage (95%CI)	2.82 (2.63-3)	2.89 (2.72-3.06)	0.687

Table 8 - Demographic details within groups.

NB† denotes exclusion of patients who did not have blood results at both diagnosis and surgery; IQR=Interquartile range; MCV= Mean Corpuscular Volume; NA=Not anaemic at surgery; A=Anaemic at surgery; -=Not applicable; *statistically significant

Factors influencing Haemoglobin: Tumour Stage, Size, Site:

There was no association between initial HB levels and Dukes' Stage or TNM Stage of disease (P=0.09), However, increasing T-stage was associated with decreasing initial HB levels (P<0.05) and there was a significant inverse correlation between tumour size and initial HB levels (R_S=-0.3, P<0.01, see Figure 5). Consequently, tumour size (P<0.01) and T-stage (P<0.05) were higher in those patients who were anaemic on initial HB.

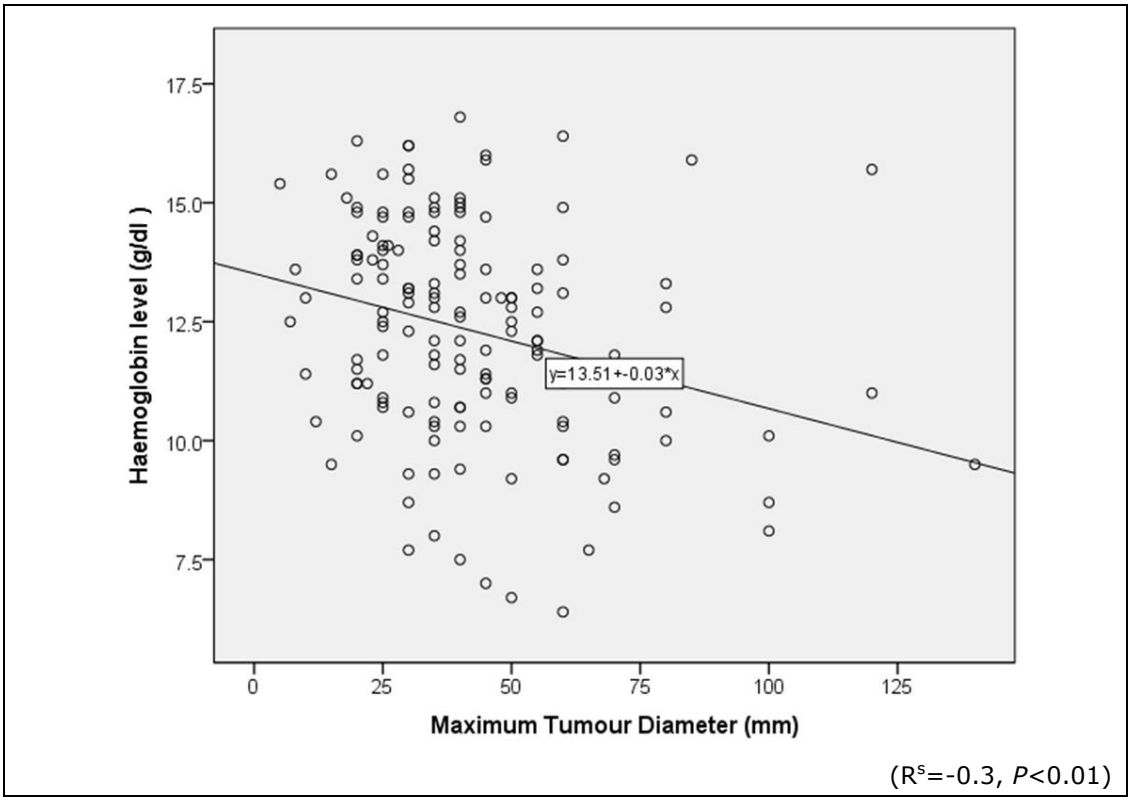


Figure 5 - Scatter-graph illustrating the inverse correlation of tumour size and initial haemoglobin levels.

Initial HB levels were significantly lower in patients with tumours located in the right colon ($P<0.01$) which corresponded to a significant difference in the prevalence of gender specific anaemia of 67% vs 36% for right and left respectively ($P<0.01$).

T-stage ($P<0.05$) and tumour size ($P<0.01$) were significantly higher in right sided tumours. There was no association between Dukes' stage ($P=0.762$) or TNM stage with location ($P=0.77$). No association was found between transfusion rates and tumour location ($P=0.343$).

ARBT use in relation to Haemoglobin levels at Surgery:

Fifty-six percent of patients ($n=114$) were not anaemic at surgery. Of these, 8% ($n=9$) received ARBT from surgical admission until discharge receiving a total of 32 units. This equated to a mean of 0.3 units [95%CI 0-1.3] per patient in this group.

In the 87 anaemic patients at surgery, 30% ($n=26$) had not had HB measured at OPA. Of all those anaemic at surgery, 32% ($n=28$) received ARBT from admission to discharge. Seventy-eight units were administered to this group, a mean 0.91 units [95%CI 0-0.7] per patient. The transfusion rate was significantly higher in the

anaemic group (P<0.01) as was the mean transfusion volume (P<0.01). Figure 6 illustrates the point in surgical treatment when ARBT were administered for each group, and the number of patients transfused.

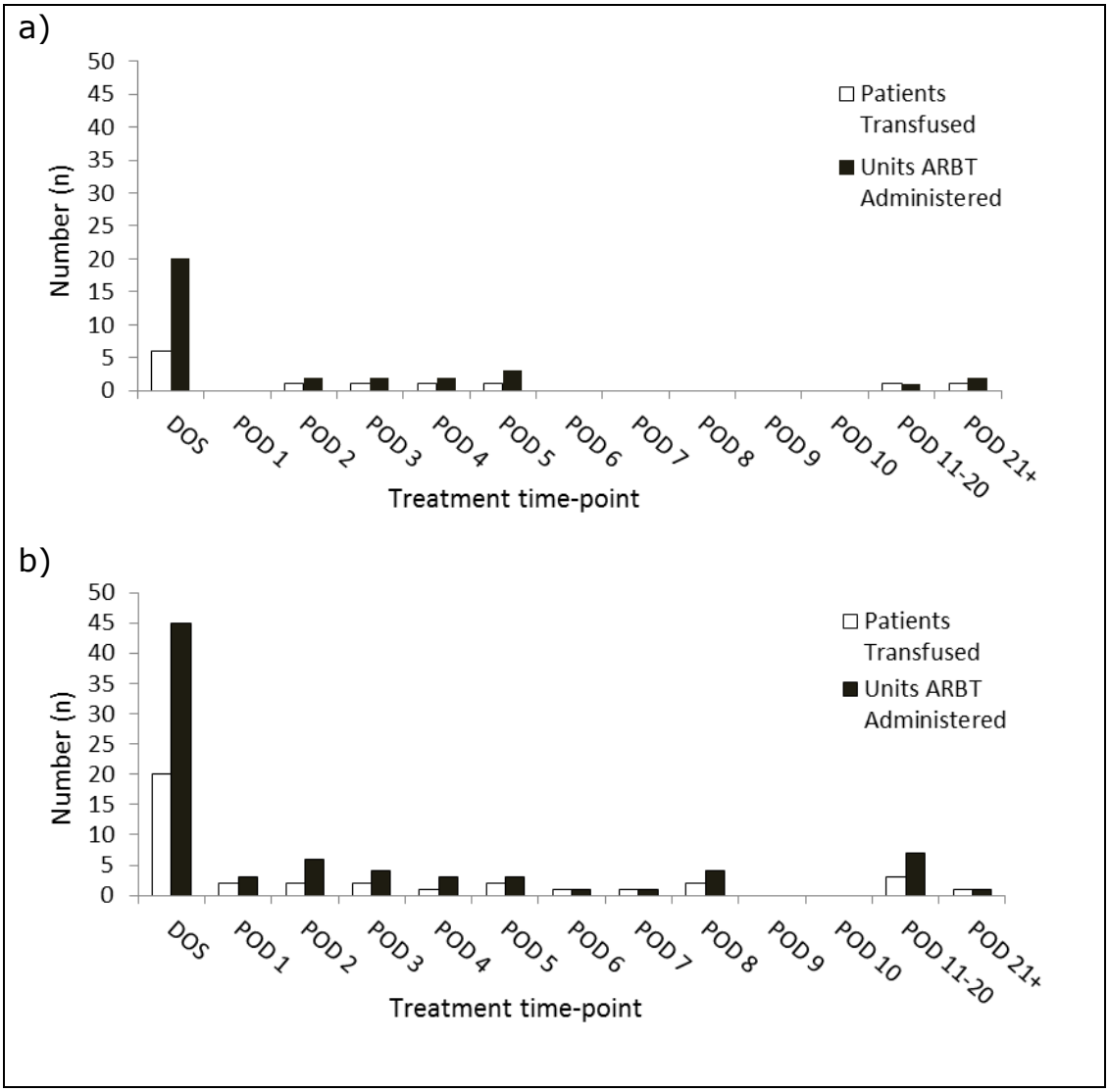


Figure 6 – Bar graphs illustrating the treatment time point at which patients received Allogeneic Red Blood cell Transfusion (ARBT) and the number or ARBT units administered for non-anaemic (Figure 6a) & anaemic patients (Figure 6b) Figure 6.

Where: DOS= day of surgery, POD = Postoperative day.

Regression analysis demonstrated that with every 1 g/dL increase in HB, the likelihood of transfusion was reduced in the order of 40% (OR 0.57 [95%CI 0.458-0.708], *P*<0.01). The magnitude of this was not materially altered when accounting for confounders including ASA, mode of operative access, age and gender (adjusted OR 0.58 [95%CI 0.444-0.754], *P*<0.01).

Table 9 illustrates the odds ratio (OR) and risk ratio (RR) of transfusion in relation to HB levels at surgery, whilst Figure 7 demonstrates the increasing percentage of patients who received ARBT in relation to decreasing HB levels at Surgery.

Haemoglobin level (g/dl)	**Odds ratio (95%CI)**	**Risk ratio (95% CI)**	***P* value**
>13	Standard		<0.01*
12.1-13	1.219 (0.29 – 5.17)	1.2 (0.32-4.53)	
11.1-12	3.25 (0.9 - 11.74)	2.23 (1.05-4.75)	
10-11	5.474 (1.7 - 17.67)	4 (1.52-10.53)	
<10	19.5 (6.16 - 61-78)	8.4 (3.65-19.36)	

Table 9 - Odds and risk ratio variation in comparison to baseline risk with haemoglobin levels above 13 g/dl.

Denotes statistical significance for overall significance of association and also linear trend.

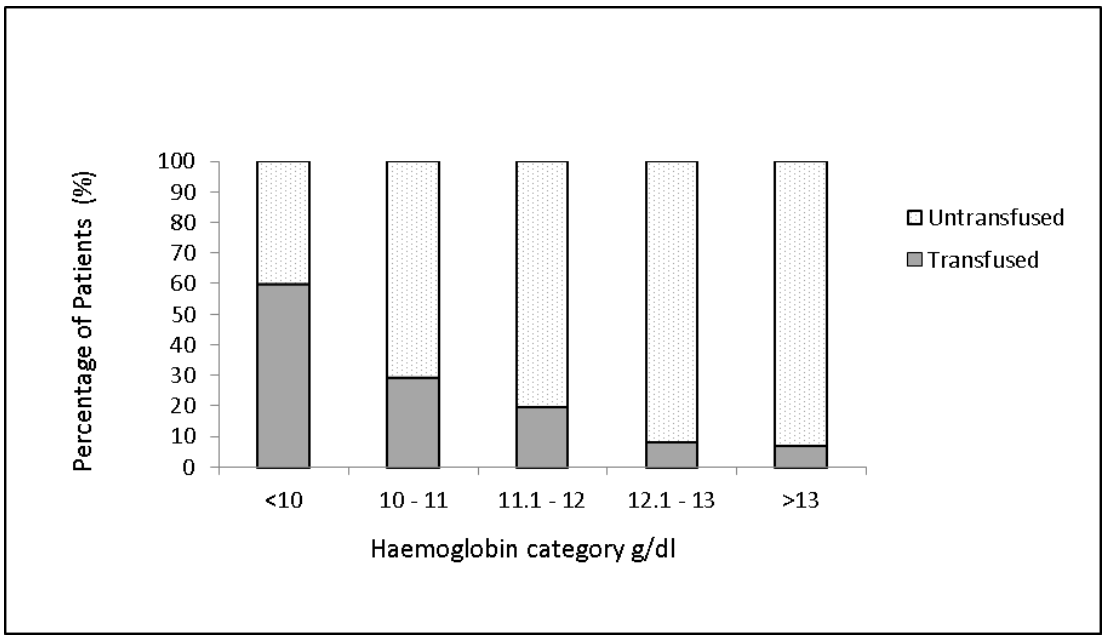


Figure 7 - A stacked bar graph illustrating the change in proportion of patients receiving an allogeneic red blood cell transfusion in relation to the haemoglobin level at surgery.

Preoperative ARBT use in anaemic patients

Twenty-seven patients were anaemic at OPA with available blood results from OPA and Surgery and received OI over this period. There were 43 untreated anaemic patients with corresponding values, one of which was excluded from this analysis due to a diagnosis of myeloma causing relapsing and remitting anaemia.

Of the untreated patients, 21% (*n*=9) received a total of 23 units ARBT between Diagnosis and the day before surgery. This compared to 11% (*n*=3) of the treated patients, who received 9 units between OPA and the day prior to surgery. Neither the mean

units transfused (*P*=0.41) nor transfusion rate (*P*=0.35) differed between groups.

Peri-and postoperative transfusion use in anaemic patients:

Forty percent of untreated anaemic patients (*n*=17) received ARBT from the start of Surgery until discharge, at a mean of 1.1 units [95%CI 0.59-1.6] per patient in the group. This equated to an overall transfusion rate from OPA to discharge of 47%, and a mean 1.67 units (95%CI 0.99-2.3) per patient.

In comparison, 37% of anaemic patients treated with OI (*n*=10) received ARBT from the start of Surgery until discharge, a mean of 1.04 units [95%CI 0.18-1.89] per patient in the group. The overall transfusion rate for this group was 37%, with a mean transfusion rate of 1.4 units [95%CI 0.39-2.3]), which was not different from those untreated (*P*=0.6).

Of note, 58% (n=7) of the patients transfused blood preoperatively required further ARBT on either the day of surgery or postoperatively.

Results of untreated patients with more severe anaemia*:

There were 28 patients (Males, *n*=15; Female, *n*=13) who were anaemic to 1g/dL* below the WHO definition of anaemia at OPA and did not receive IS in the preoperative period. The median HB at Diagnosis for this subgroup of patients was 10.3 g/dL [IQR 9.55–10.85] and at Surgery was 10.0 g/dL [IQR 8.85–10.55] for those not transfused in this period. This was a significant decrease ($P<0.05$).

Five patients (18%) received a total of 12 units ARBT preoperatively. From the morning of Surgery until discharge 11 patients (39%) received 33 units ARBT.

In total, 14 individual patients received at total of 45 units of blood from OPA to discharge following surgery. This equated to a transfusion rate of 46% and a mean of 1.6 units per patient [95%CI 0.2576-1.01].

Footnote:
This category was reviewed independently as a control group for discussion in Chapter 4.

The median LOS was 6 days [IQR 3-11] with 17 patients experiencing 43 complications (61%), with 8 patients experiencing infective complications (29%). Two patients died within 30 postoperative days (POD) (7%).

Length of stay, complications and preoperative HB levels:

The median LOS for all patients was 6 days [IQR 3 -13]. There was no difference between LOS for those that were anaemic and those who were not (*P*=0.52).

Across the entire cohort, 130 patients experienced a postoperative complications (65%). There was no significant difference in HB levels at surgery in relation to the occurrence of postoperative complications (*P*=0.996). There was also no significant difference between the severity (*P*=0.58) or number of complications (P=0.6) and the existence of anaemia at surgery. Although the 30 day mortality rate was higher in the anaemic group (3.5%, 3/87; versus non-anaemic 0.8%, 1/114) this was statistically non-significant (*P*=0.3)

The infective complication rate was 12% (14/114) for non-anaemic patients, compared to 8% (7/87) (*P*=0.36) in the anaemic group.

In those treated with iron preoperatively, this rate was 33% (11/34) which was significantly higher than those not receiving iron supplementation (9/167, $P<0.01$).

Operative access and ARBT use:

No difference was noted in patient HB levels between those who underwent laparoscopic or open procedures ($P=0.643$). Despite this, LS was associated with fewer patients transfused (laparoscopic 9/84; open 28/117, $P<0.01$).

Three patients within the non-anaemic group had documented significant intraoperative losses of 900ml, 1000ml and 7500ml respectively. Two of these patients received ARBT, accounting for 65% of ARBT units administered to this group on the DOS. This compared to 2 events in the anaemic group, with losses of 700ml and 1200ml recorded. These 2 patients received 7% of ARBT units administered to this subgroup on the DOS. All of these patients underwent open surgery.

2.4 Discussion:

This study aimed to investigate key aspects of anaemia, treatment and ARBT use in patients undergoing colorectal cancer surgery. The study was undertaken at a centre with established laparoscopic practice and CRC screening which started 3 years before the study period. The findings are therefore relevant to modern practice. Given the close matching to previous data regarding patient demographics (Brenner et al., 2007) and tumour details (McCallion et al., 2001) the findings should be transferable to the wider population.

Identification of anaemia:

The first key observation was the low proportion of HB levels measured at presentation. Only 2/3 of patients had an HB level measured at presentation. It is most likely that these patients were selected based on symptomatology of anaemia, which may account for the higher prevalence of anaemia (51%) than previous series (Ludwig et al., 2004).

As part of PBM, a suspected diagnosis of CRC should prompt clinicians to actively exclude anaemia. As large tumours, advanced T-stage and right sided lesions were associated with anaemia, this

highlights a particular need to measure HB levels in these high risk patients.

Haemoglobin changes preoperatively:

It would appear that HB levels do continue to fall in the preoperative period, which highlights a further need for early identification and treatment of anaemia. The magnitude of this decline, though small, was potentially underestimated in this study. In an attempt to review the natural history of HB changes preoperatively, patients who received ARBT were excluded from that analysis. However, it is highly likely that patients who require preoperative ARBT for anaemia would probably have higher inherent tumour blood loss, or a longer history of haemorrhage with associated greater depletions in iron stores. As a result, such an exclusion would remove the subset of patients who may have demonstrated larger HB decreases if left untreated.

Anaemia and Blood transfusion:

The PBM principal of early identification of anaemia (Shander et al., 2012) appears essential given the apparent relationship between HB levels at Surgery and ARBT requirement. Anaemia at the point of surgery was found to be associated with an increased ARBT rate

and increased number of units required. As severity of anaemia increased, so too did the proportion of patients who required ARBT.

This is further exemplified within Table 9, which demonstrated that normality need not be reached to reduce the use of ARBT, but that improvement of preoperative HB levels could reduce the number of patients who would need ARBT. This is substantiated by the finding that for every 1 g/dl rise in preoperative HB, the need for ARBT was reduced by approximately 40%. Ideally an HB level in excess of 12 g/dl appears to reduce the risk of ARBT to a level more comparable with non-anaemic patients.

It could be argued the increased ARBT use is secondary to the increased age and ASA of the anaemic group. Although it is possible that the clinical threshold for administration of ARBT would have been lower within this group, the relationship between HB levels and transfusion use remained stable when cofounders such as ASA and age of patient were accounted for, implying a key role for preoperative HB levels. Furthermore, transfusion rates were not different between procedures for Left and Right sided malignancy, indicating operative factors were also of lesser importance.

Treatment of anaemia:

It is therefore relevant that only 44% of anaemic patients received some form of iron supplementation. The clinical effect of OI would initially appear to be minimal due to a non-significant rise in HB of only 0.1 g/dl from diagnosis to surgery in non-transfused anaemic patients. Such an observation parallels previous studies reviewing the role of OI in preoperative CRC patients, which indicated that OI does not increase HB in this context, but merely reduces the natural decline in HB in the preoperative period (Lidder et al., 2007).

Despite the apparent lack of efficacy of OI, two key limitations must be acknowledged. It can only be assumed that these patients were iron deficient based on the clinical context and reduced Mean Corpuscular Volume (MCV) values, yet MCV is recognised to have limitations in the diagnosis of iron deficiency (Goddard et al., 2011). Also no record of dosing or adherence to medication treatment protocols was available. Studies have demonstrated high variability in compliance and absorption of OI which may have affected the efficacy (Macdougall, 1999) . Secondly, although the difference in HB change with OI was non-significant over the preoperative time period, the difference was significant when

compared to a "control" group of untreated anaemic patients, potentially implying a larger treatment effect.

Figure 7 illustrates striking differences in ARBT use with variations in preoperative HB levels at increments of 1 g/dl- a factor of 10 greater than the observed treatment effect of OI. This could indicate that more efficacious iron treatments are required in order to increase HB levels to this degree. Intravenous iron has been trialled in this setting with limited success at low dose (Edwards et al., 2009) but with associated HB rises in excess of 1g/dL at higher doses (Simpson et al., 2010a).

Implications of operative access:

In the current study, it would appear that laparoscopic surgery does have a clinical impact upon ARBT use. Notably, transfusion rates were significantly lower in laparoscopic cases across the entire cohort and also in the anaemic subgroup, yet was not noted in the non-anaemic patients. This would indicate that the degree to which operative losses are reduced by minimal access surgery was of particular clinical relevance in anaemic patients who were particularly vulnerable to further losses.

This difference was unlikely to be secondary to the higher proportion of open surgical cases for left sided lesions. Although it is conceivable that left sided operations are associated with higher intraoperative losses which could prompt increased ARBT use, the fact that the transfusion rate was similar between those undergoing surgery for left and right sided malignancy would discredit this confounding link.

Complications:

There was no association between preoperative HB levels and LOS and also with postoperative complication frequency, rate or severity. The same is true for perioperative ARBT use. This is unexpected given several studies which have linked these factors in colorectal (Leichtle et al., 2011, Acheson et al., 2012) and non-cardiac surgery (Beattie et al., 2009).

LOS is a multifactorial outcome, encompassing social, surgical and medical factors. Due to the complex nature of this variable, a far greater sample size may be required to identify this relationship.

A key finding relating to postoperative outcomes was noted in infective complications. It would be expected that infective

complications would have been higher in anaemic patients both directly through the anaemia (Madbouly et al., 2006) and also indirectly by the associated increased administration of ARBT (Acheson et al., 2012). Although this was not the case, a significantly increased rate of infective complications was noted in those receiving iron supplementation compared to those not.

This relationship may have been apparent due to the anaemic status of those patients receiving iron, although this is contested by comparison with the low rate evident in the untreated anaemic group. Consequently, it is possible that a causal link exists between iron use and infection. It has been proposed that during infection, both host and infective organism compete for iron, and thus the subsequent inflammatory response induced blockade of iron mobilisation and absorption is a protective mechanism to overcome this (Pieracci and Barie, 2005).

Animal studies have suggested that available iron is utilised by pathogens to increase virulence (Telang et al., 2001a) which has also been translated into clinical studies whereby increased infection rates and severity have been seen with iron supplementation in malaria (Sazawal et al., 2006), dialysis (Brookhart et al., 2013) and also in general clinical practice (Litton

et al., 2013). This potential risk should be balanced with any potential benefits that may be seen.

2.5 Conclusions:

In summary anaemia is common in CRC surgical patients, particularly in those with large or right sided malignancy. Early identification of anaemia is important to allow attempted treatment which is important for several reasons. Firstly, HB levels continue to fall from the point of diagnosis to surgery if untreated. Secondly, anaemia is associated with increased ARBT requirement, but even small increases in HB levels can have potentially dramatic effects upon ARBT use.

Current local practices would indicate that oral iron can be regarded as "standard care" for anaemia, although detection and treatment initiation is extremely low. This may account for the high requirement of ARBT for patients, although high uptake of laparoscopic surgery may help to reduce this need.

In the current study, oral iron did not appear to be efficacious in terms of treatment of anaemia or prevention of ARBT. Furthermore, preoperative ARBT administration appeared ineffective at preventing subsequent perioperative ARBT use. IVI was utilised too infrequently to comment upon.

Further investigation into the efficacy of intravenous iron is therefore warranted. The preoperative diagnostic phase in the current study was 50 days. This provides a potential treatment window to correct anaemia which could have significant benefits for PBM.

Chapter 3:

The feasibility and clinical efficacy of intravenous iron administration in the preoperative setting of colorectal cancer associated anaemia.

Abstract:

Aims:

The study aimed to analyse the feasibility and efficacy of administration of a single, 15 minute intravenous iron infusion (IVI) in the preoperative optimisation of anaemic colorectal cancer patients.

Methods:

20 patients were recruited at least 14 days prior to the planned date of surgery. A single 1000mg dose of Ferric Carboxymaltose (FERINJECT®) was administered as an out-patient procedure. Blood samples were taken at Recruitment prior to drug administration (REC), on the day of surgery prior to any intervention (DOS) and on the first post-operative day. Allogeneic red blood cell transfusions (ARBT) and outcomes were recorded from recruitment throughout the study period.

Results:

There was a significant median rise in haemoglobin levels (HB) from REC to DOS of 1.8 g/dL (IQR 0.75-2.45 g/dL, $P<0.001$) for the entire cohort. Two patients received ARBT preoperatively, and for those not transfused preoperatively ($n=18$), this incremental

HB rise remained significant (P<0.001, median 1.65 g/dL, IQR 0.5-2.3 g/dL). Of these patients, those that responded to IVI had higher Erythropoietin (EPO) levels at recruitment (P<0.01), and lower recruitment HB values, Transferrin-Saturation (TSAT) and C-Reactive Protein (CRP) levels (P<0.05). REC HB (R_s=-0.62, P<0.01), REC TSAT levels (R_s=-0.67, P<0.01) and REC EPO (R_s=0.69, P<0.01) correlated with the magnitude of treatment change in HB levels. Five patients received ARBT until the fourth post-operative day, which was significantly lower than predicted (P<0.05).

Conclusion:

IVI can be feasibly administered in the preoperative out-patient setting to anaemic colorectal cancer patients with associated reductions in ARBT use and increases in HB levels.

3.1 Introduction:

Anaemia is found in approximately 40% of patients diagnosed with colorectal cancer (CRC) (Ludwig et al., 2004). Perioperative anaemia is associated with adverse postoperative outcomes (Leichtle et al., 2011, Carson et al., 1996) and increased utilisation of allogeneic red blood cell transfusion (ARBT) (Dunne et al., 2002b). Furthermore, the perioperative use of ARBT in CRC surgery has also been associated with deleterious effects on short and long term outcomes (Acheson et al., 2012).

The relationship between preoperative anaemia and adverse outcomes has generated interest in the use of preoperative iron supplementation. Although IVI has been demonstrated to increase haemoglobin (HB) levels in preoperative orthopaedic surgical patients (Khalafallah et al., 2012), a clear role for iron supplementation has not been established in anaemic colorectal cancer patients. Clinical trials have indicated oral iron to be both effective at raising HB levels preoperatively (Quinn et al., 2010) and reducing the preoperative fall in HB levels between diagnosis and surgery (Lidder et al., 2007) whilst reducing perioperative blood transfusions in this patient group (Lidder et al., 2007, Quinn et al., 2010). Despite a presumed superiority in efficacy, IVI has

been demonstrated to be ineffective in both these endpoints in randomised trials (Edwards et al., 2009).

This disparity may be in part due to hepcidin, a hormone that has been identified as a key regulator of iron handling, which reduces enteric iron absorption and systemic iron mobilisation (Zhang and Rovin, 2013). This hormone is of particular relevance in this context as CRC tumours are thought to induce hepcidin production (Ward et al., 2008a) and serum hepcidin levels are proportional to CRC stage (Roberts et al., 2008).

Drug development has resulted in IVI preparations that lend themselves to use within the colorectal surgical pathway. Test doses are no longer required, the incidence of anaphylaxis has been reduced, and the drugs can be given in single, short infusions (Auerbach and Ballard, 2010) which do not require prolonged monitoring.

This pilot study aimed to examine the feasibility of administration of a single 15 minute IVI infusion given in an out-patient setting comparable to a pre-operative assessment, within the constraints of target operating waiting-list times. Secondly, the study aimed to review if this infusion would result in a significant rise in patient HB

levels and a subsequent decrease in observed ARBT use. Furthermore, we aimed to identify potential predictors of response to therapy and hence propose potential methods of selection of candidates for treatment.

3.2 Methods:

Ethical approval was granted by Nottingham Research Ethics Committee (Ref No. 09/H0408/67) and the study was approved by the Medicines and Healthcare products Regulatory Agency, UK (MHRA), Clinical.trials.Gov reference NCT02057471.

Patient population:

Anaemia was defined according to the WHO definition (<12g/dL Females, <13g/dL Males) (WHO, 2011). Twenty adult patients were identified and recruited from local colorectal cancer multidisciplinary meetings with histologically confirmed colonic or rectal adenocarcinoma with surgery planned as the primary treatment, who were also anaemic on recent blood tests. Patients were excluded if they had any contraindication to IVI therapy, as were patients given a date for surgery less than 14 days after treatment would start. It was felt that a treatment effect may not be seen below this treatment period, and ethically could not be justified. Patients with previous or current haematological disease, current prisoners and those unable to provide informed consent were also excluded. Written consent was obtained prior to any study intervention.

Intervention:

Enrolled patients attended the hospital for one out-patient visit where recruitment blood tests were performed and the IVI administered. If patients were taking oral iron supplements at recruitment, this was discontinued and the General Practitioner informed. The IVI preparation infused over 15 minutes was 1000mg Ferric Carboxymaltose (Ferinject®, Vifor Pharma) diluted in 250ml of 0.9% normal saline. The IVI was given under supervision of a clinician, with a single blood pressure measurement taken before infusion to exclude pre-existing hypotension. No other monitoring was performed, and a test dose was not required.

Blood collection:

Blood was collected at three time points: at recruitment (REC) prior to the iron infusion, on the day of surgery prior to any intervention (DOS) and on the first post-operative day (D1). Haematological testing of the blood was performed including Full Blood Count to yield Haemoglobin (HB) and Mean Cell Volume (MCV). Biochemical analysis included serum ferritin, Transferrin Saturation (TSAT), C-Reactive Protein (CRP) and Erythropoietin levels (EPO). These tests were performed in the local NHS Trust

laboratory, with the exception of EPO, which was tested in line with local policy by transfer to NHS biochemistry laboratories in Dundee, UK.

Blood samples for hepcidin assay were centrifuged one hour after collection and the serum stored at minus 80^0C until the end of the study, whereby the samples were analysed using mass spectrometry at the University of Birmingham UK using the SELDI-TOF-MS method previously described (Ward et al., 2008b).

Additional Recording:

Patient demographics, operative details, tumour pathological staging, blood transfusions until discharge, complications and hospital length of stay were recorded as was the Cumulative Iron Deficit (CID). This is an estimation of required dose of iron required to treat IDA as calculated simply using the Ganzoni Equation (see Figure 8).

Cumulative Iron Deficit =

[Weight(kg) x (Target HB -Current HB(g/dL)) x 2.4] + Iron Store* (mg)

**Iron store =500mg if body weight > 35kg, 15 mg/kg if body weight < 35kg*
Where HB=Haemoglobin

Figure 8 - The Ganzoni equation for calculation of Cumulative Iron Deficit (CID).

Patients were also assigned a transfusion "trigger" point in line with local transfusion guidelines which are based upon the current UK Blood Transfusion and Tissue Transplantation Guidelines on the Appropriate use of Blood (UKBT, 2012). This decision was based upon patients' associated comorbidity, and resulted in 2 transfusion trigger groups – the first "stringent" group (Group A) with a target of maintaining HB above 7 g/dL, and a second group (Group B) for those patients with associated cardiac or respiratory disease, with a target of 9 g/dL. The ultimate decision to administer blood transfusions remained at the discretion of the clinical team.

Using these transfusion points, a transfusion prediction model was employed to identify if subjects could have received ARBT had IVI not been administered. This theoretical model was based on the assumption that the post-operative decrease in HB between Surgery and Day 1 was a constant nadir for each patient. Consequently, by subtracting the increase in HB evident following IVI treatment from the acquired HB levels after recruitment, it was possible to postulate the HB values of study subjects until D1 had IVI not been administered, and hence if assigned transfusion thresholds would have been met without the IVI . These calculations are summarised in Figure 9.

Predicted Day of Surgery HB without IVI =

Actual Day of Surgery HB – Treatment change in HB with IVI

Predicted Post Operative Day 1 HB without IVI =

Actual Day 1 Post Operative HB – Treatment change in HB with IVI

Where HB=Haemoglobin g/dL and IVI=Intravenous Iron.

Figure 9 - Calculation of Predicted Haemoglobin levels without intravenous iron treatment.

Statistical Analysis:

Response to treatment was defined as a 1.5g/dL rise in HB from recruitment to the day of surgery. This increment was selected on the basis of epidemiological studies which have indicated that over 80% of anaemic patients diagnosed with CRC have an HB between 10 and 11.9 g/dL (Ludwig et al., 2004) hence correction of HB by 1.5 g/dL will restore normal values in the majority of these patients. The statistical level of significance for all tests was defined as P=0.05. Independent samples test was used to compare the significance of the non-parametric blood parameters measured between responders and non-responders. Non-parametric data was otherwise compared with Wilcoxon signed rank test. Fisher's exact test was used to assess differences between predicted and actual ARBT administration. Correlations between variables and HB change with IVI treatment were estimated with Spearman's rank

test and Pearson’s product-moment method for non-parametric and parametric data respectively. Statistical analysis was performed using SPSS® version 21 (SPSS, Chicago, Illinois, USA).

3.3 Results:

Twenty patients were recruited (14 male, 6 female) with a median age of 77 years [IQR 73.5-79.8]. All patients received 1000mg of Ferric Carboxymaltose, and there were no immediate adverse events associated with drug administration. Patient demographics, tumour details and operative data are illustrated in Table 10.

Gender:	14 Male (70)	6 Female (30)
Age (Years):	77 (36-85)	
Height (m):	1.7 (1.52-1.88)	
Weight (Kg):	75 (56.2-105)	
BMI (kg/m^2):	25.9 (20.6-38.6)	
Recruitment HB (g/dL):	9.25 (6.7-11.9)	10.2 (4.6-11.7)
Recruitment MCV (fl):	79.5 (67-94)	85.5 (68-97)
Recruitment CID (mg):	1583.7 (1043-1934)	1399.4 (1194-1740)
Patients taking oral iron at Recruitment:	11 (55)	
Operation:	Right Hemicolectomy:	11 (55)
	Anterior Resection:	6 (30)
	Extended Right Hemicolectomy:	1 (5)
	Right Hemicolectomy & Sigmoid colectomy:	1 (5)
	Panproctocolectomy:	1 (5)
Access:	Laparoscopic:	10 (50)
	Open:	10 (50)
Tumour Stage:	T4	2 (10)
	T3	12 (60)
	T2	5 (25)
	T1	1 (5)
Nodal Status:	N2	1 (5)
	N1	3 (15)
	N0	16 (80)
Total Length of stay (days):	7 (2-112)	

Table 10 - Patient Demographics and operative details.
Where: Patient details expressed as medians & ranges (parenthesis), with categorical details expressed as frequencies & percentages (parenthesis).

The median IVI treatment duration was 27.5 days [IQR 16-43 days]. Five patients had documented postoperative complications, including two respiratory infections, one anastomotic leak, one port site hernia and a prolonged postoperative ileus. The median post-operative stay was 7 days [IQR 4.3-12 days].

Overall, 6 patients received ARBT from recruitment until post-operative discharge, which totalled 24 units transfused. Two patients were transfused preoperatively (5 units total), three patients were transfused on the day of surgery (7 units total), and three patients received ARBT postoperatively, all of which were on or after post-operative day 4 associated with complications (12 units). The details of all ARBT administered are highlighted in Table 11. There was no association between transfusion rates and mode of operative access (P=0.303).

Eight patients were assigned to "stringent" transfusion Group A, and 12 to "liberal" Group B. The rationale for assignment to these groups is available in Table 12. Within Group A, one patient received ARBT from recruitment until the fourth post-operative day, with two predicted to require ARBT. Four patients within Group B were transfused over this period, whilst eleven patients were predicted to have required ARBT. Comparison of actual

patients (*n*=5) who received ARBT to those predicted to require ARBT without IVI (*n*=13) was significantly different (*P*<0.05).

Patient No.	REC HB g/dL	DOS HB g/dL	Op	Access	Day Transfused	Reason for Transfusion
5	6	9.2	RH	Open	P (2)	HB taken prior to iron infusion & warranted transfusion when available
8	4.6	8.5	RH	Open	P (3)	HB taken prior to iron infusion & warranted transfusion when available
13	10.6	10.5	AR	Open	DOS (3) PO+ (3)	2.5L intraoperative blood loss
16	8.3	10.8	RH	Open	DOS (2)	Blood loss coupled with comorbidity
19	8.2	9.9	RH & SC	Open	PO+ (5)	Post-operative HB drop associated with sepsis
20	9.6	9.8	RH	Lap	DOS (2), PO+ (4)	Intraoperative losses with post-operative HB drop associated with anastomotic leak and sepsis

Table 11 - Patient and operative details of patients that received allogeneic red blood transfusions (ARBT) during the operative admission.

REC= Recruitment, DOS = Day of Surgery, HB= Haemoglobin, Lap=Laparoscopic, P=Preoperatively, DOS=Day of Surgery, PO= Postoperatively <day 4, PO+= Postoperatively ≥day 4, RH= Right hemicolectomy, AR= Anterior resection, SC= Sigmoid colectomy. Number of units of ARBT administered are within parenthesis

"Stringent" Group A:

Number	8	Male: 4	Female:4

	Age	**Relevant Cardio-respiratory comorbidities**
Patient 3:	36	Nil
Patient 4:	70	Nil
Patient 9:	80	Nil
Patient 11:	77	Nil
Patient 13:	78	Nil
Patient 14:	77	Nil
Patient 15:	68	Nil
Patient 17:	76	Nil

"Liberal" Group B:

Number	12	Male: 10	Female:2

	Age	**Relevant Cardio-respiratory comorbidities**
Patient 1:	77	Angina, PVD, NIDDM
Patient 2:	75	MI, CABG
Patient 5:	77	AF, HT
Patient 6:	78	HT, NIDDM, Cardiac valvular disease
Patient 7:	85	AF, HT
Patient 8:	73	Cardiac valvular disease
Patient 10:	84	Age, general medical status
Patient 12:	79	AF, Hypertension
Patient 16:	80	Age, general medical status
Patient 18:	73	HT, NIDDM diabetes
Patient 19:	84	AF
Patient 20:	76	NIDDM with HT

Table 12 - The comorbidity rationale for assignment of each patient to transfusion trigger groups.

Where PVD= Peripheral vascular disease, MI=Previous Myocardial Infarction, CABG=Previous Coronary artery bypass grafting, HT= Hypertension, AF=Atrial fibrillation, NIDDM= Non-insulin dependent diabetes.

Within the entire cohort, the median rise in HB was 1.8 g/dL [IQR 0.75-2.45 g/dL]. In patients not transfused preoperatively (*n*=18), this increase was 1.65g/dL and remained statistically significant [IQR 0.5-2.3 g/dL], *P*<0.001). There were associated increases in HB (*P*<0.001) and TSAT (*P*<0.001), and reciprocal fall in EPO (*P*<0.001) between recruitment and the day of surgery for those not transfused preoperatively. The HB changes are illustrated in Figure 10 for those patients who were not transfused over the period illustrated.

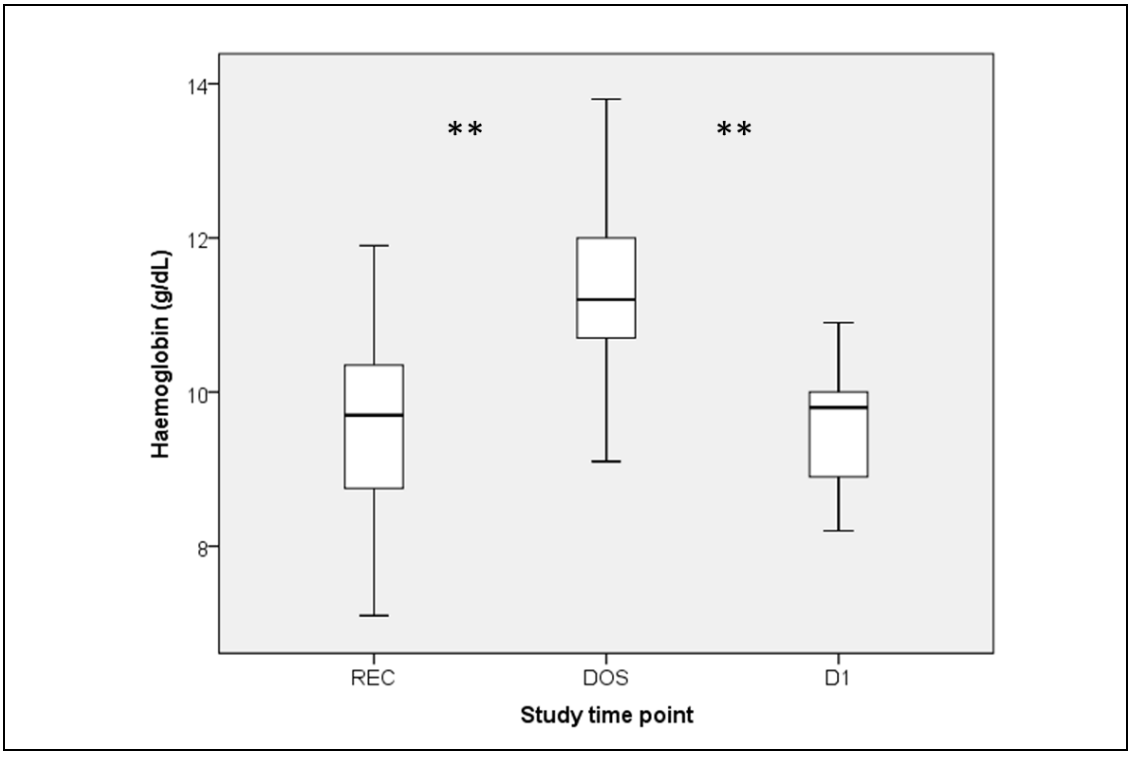


Figure 10 – Box-Plot representation of Haemoglobin (HB) changes across the study.

Where*: *REC= Recruitment, DOS= Day of Surgery, D1= Day 1 postoperatively, and **denotes statistical significance to P<0.01.

For those patients not transfused ARBT preoperatively, 11 patients responded to IVI treatment, and responders had significantly higher EPO levels at recruitment ($P<0.01$). Lower recruitment CRP levels ($P<0.05$), TSAT values ($P<0.05$) and HB values ($P<0.05$) were also evident in the responder group. There was no difference between the hepcidin levels of those responsive and unresponsive to treatment.

On further review of patients not transfused preoperatively, there was a positive correlation between change in HB with calculated CID ($R_s=0.61$, $P<0.01$) and also with REC EPO ($R_s=0.69$, $P<0.01$). Conversely, an inverse relationship was seen between change in HB and REC HB (R_s =-0.62, P<0.01) and REC TSAT (R_s=-0.67, $P<0.01$) as illustrated in Figure 11 and Figure 12. Recruitment levels of CRP, Hepcidin and ferritin were not correlated with a change in HB.

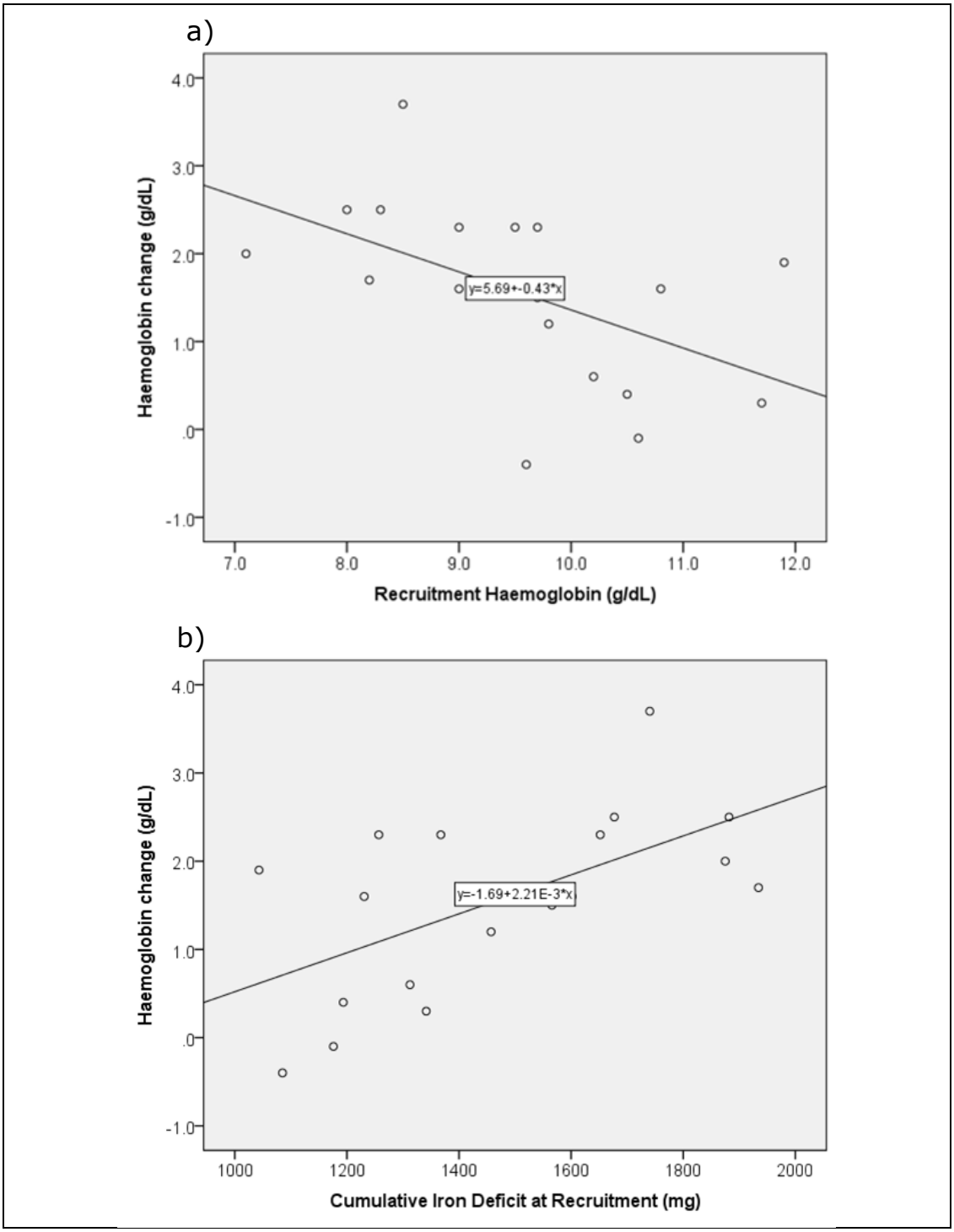


Figure 11 - Scatterplot graphs to illustrate the proportional change in Haemoglobin levels (HB) following therapy versus predictive parameters: a) Recruitment Haemoglobin b) Cumulative Iron Deficit at Recruitment (CID).

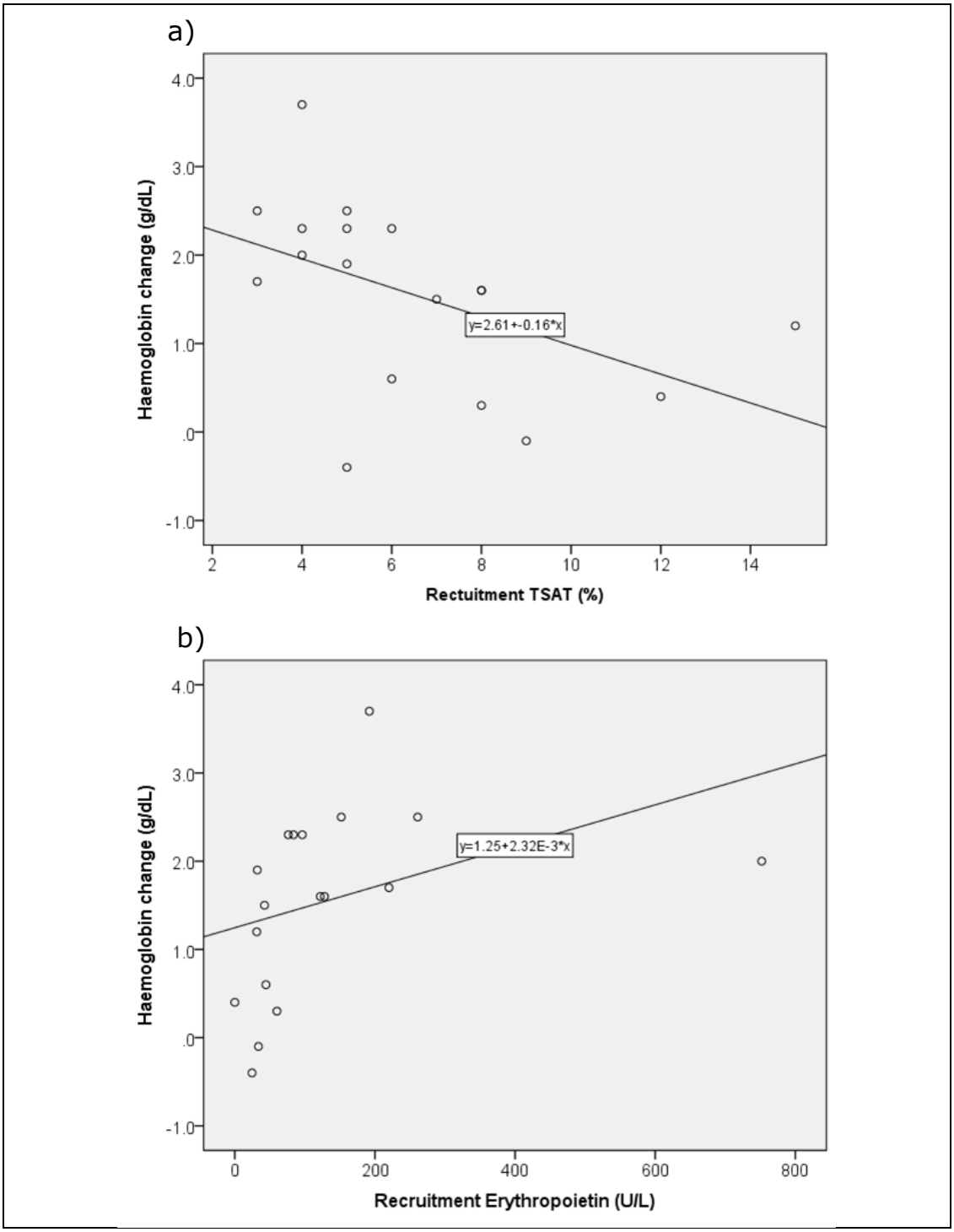


Figure 12 - Scatterplot graphs to illustrate the proportional change in Haemoglobin levels (HB) following therapy versus predictive parameters: a) Recruitment Transferrin Saturation (TSAT) and b) Recruitment Erythropoietin (EPO) levels.

3.4 Discussion:

The results from this study indicate that IVI therapy is a useful treatment modality in the preoperative optimisation of anaemic patients with CRC. The drug was safely administered in the outpatient, preadmission clinic setting without any associated adverse events, whilst conforming to patient treatment pathways and target waits. IVI therapy successfully raised patient HB with a median rise which would be expected to normalise the HB of the majority of anaemic patients diagnosed with CRC and was comparable to results from previous studies (Khalafallah et al., 2012). Furthermore, it appears that the IVI may have reduced the need for perioperative ARBT.

The transfusion prediction model employed does have limitations. The model assumes that without treatment the DOS HB would at best equal the REC HB. This potentially overestimates the actual DOS HB as, without treatment, HB levels would actually be expected to fall until the point of surgery (as illustrated in Chapter 2). Furthermore, this assumption does not take into account the impact of oral iron supplementation which 55% of the patients were taking at the point of recruitment, and may be efficacious had it been continued (Quinn et al., 2010).

All patients in this study could have received a further dose of IVI based on the Ganzoni equation and manufacturer's guidance, hence a larger response could potentially be achieved. Despite this dose reduction, it appears that IVI does increase HB within the timeframes of this study. Of note, the median treatment duration may be longer than feasible at other Centres. From other large studies using IVI to treat anaemia, it appears that the maximal HB response to IVI is seen after 4-6 weeks (Evstatiev et al., 2011). Similarly, data suggests that patient's with IDA will utilise 91-99% of the iron from Ferinject® by 24 days (Beshara et al., 2003), which needs to be factored in optimising treatment duration.

Furthermore, a maximum dose of 1g of Ferinject® can be given per week, and it appears that most patients in the current context will require more than 1 dose to achieve maximal effect. The importance of dose may be illustrated by the greater response to IVI seen in the present study than similar trials which used a smaller 600mg dose of Iron Sucrose (Edwards et al., 2009).

Consequently, an optimal treatment period is required in clinical practice, which will allow adequate dosing and a significant clinical response to justify the possible risks of IVI, but not delay operative intervention. Given the factors and logistical issues discussed, this

is unlikely to be less than the 2 weeks employed in this study but does warrant further investigation.

This study also sought to review potential predictive biomarkers which may predict the magnitude of response to IVI in raising HB levels from dose administration to the day of surgery. It is interesting to note that HB levels at recruitment were predictive of subsequent response to IVI, given historical data which has demonstrated similar findings with oral iron (Freire, 1989). As CID is calculated from this value, the correlation between CID and change in HB is also a logical association, as is the inverse relationship evident with TSAT.

In this study, hepcidin failed to identify responders to treatment which contrasts findings from similar studies using oral iron (Bregman et al., 2013). This may be either reflective of the smaller numbers involved in this study, or be a consequence of multifactorial influences on hepcidin levels which include both inflammation and CRC (Ward et al., 2008a, Roberts et al., 2008). As hepcidin influences both enteric iron absorption and iron mobilisation from the reticuloendothelial system, it is possible that hepcidin levels are more relevant in predicting the absorption and subsequent response to oral iron rather than systemic mobilisation

of IVI, or that levels are more reflective of tumour biology and stage than iron status. The predictive role of hepcidin still needs further evaluation.

Endogenous EPO levels at recruitment were also significantly higher in those that respond to IVI, and also correlated with HB response to IVI. Co-administration of exogenous EPO with IVI has been proposed in other preoperative settings (Cladellas et al., 2012) but is not clearly advocated in the context of malignancy, partly as a result of data suggesting EPO administration stimulates CRC recurrence (Pascual et al., 2013).

3.5 Conclusions:

IVI can be administered effectively in the preoperative setting of colorectal cancer and appears to both increase HB levels significantly, and may subsequently reduce ARBT requirements. This increase in HB appears to be greater than increments seen in similar studies using oral iron in this context. However, given the cost difference and risks associated with IVI, a randomised controlled trial is required to compare the efficacy of these iron supplementation modalities in terms of a clinical endpoint.

Chapter 4:

An open label study to determine the efficacy of ferric carboxymaltose in preoperative colorectal cancer related anaemia, and to develop biomarkers to predict response to this treatment strategy: The IVICA Study.

4.1 Introduction:

In light of the need for a randomised control trial comparing the efficacy of intravenous and oral iron in the preoperative management of anaemia associated with colorectal cancer (CRC) a study was designed to address the questions raised. Intravenous ferric carboxymaltose (IVI) was administered in the treatment arm, with oral ferrous sulphate (OI) given to the control group. The aims of the study were as follows:

4.1.1 Primary Aim:

To determine if the use of IVI in patients with colorectal adenocarcinoma related anaemia can reduce the need for allogeneic blood transfusion compared to OI. The primary endpoints were thus the mean number of transfused ARBT units in each group (mean transfusion volume), and the number of patients who received an ARBT (transfusion rate) from recruitment to outpatients 8-12 weeks following postoperative discharge (section 4.3).

4.1.2 Secondary Aims:

1. To compare haemoglobin (HB) and haematinic marker response to intravenous ferric carboxymaltose (IVI) and oral ferrous sulphate (OI) (section 4.3)
2. To compare peri- and postoperative outcomes in each arm of the study (section 4.4).
3. To determine any postoperative length of stay and quality of life differences (section 4.4).
4. To identify biomarkers which may predict response to each therapy (section 4.5).

4.2 Methods:

4.2.1 General Methodology:

Ethical approval was granted for the study by the National Research and Ethics Service (NRES, Nottingham2) prior to initiation, and the study was undertaken in line with the declaration of Helsinki. The study was registered with both the Medicines and Healthcare Products Regulatory Agency (MHRA) and Clinical Trials.Gov (reference NCT01701310) prior to commencing. The IVICA Trial was funded by the National Institute for Health Research (Research for Patient Benefit Programme).*

The study was a multicentre randomised controlled trial run across 7 sites within the United Kingdom, including both tertiary teaching hospitals (Nottingham University Hospitals NHS Trust, University of Birmingham Hospitals NHS Trust, Royal Derby Hospitals NHS Trust, University of Bristol Hospitals NHS Trust and St.James' University Hospitals NHS Trust) and district general hospitals (Wolverhampton Hospitals NHS Trust, Yeovil Foundation Hospital NHS Trust). Each Site followed the same preapproved Study protocol, and adhered to the principles of Good Clinical Practice (GCP).

***Footnote:**
Consequently, the views expressed are those of the author and not necessarily those of the NHS, the NIHR or the Department of Health.

4.2.2 **Patient identification:**

All patients diagnosed with CRC between 25th May 2012 and 2nd June 2014 were screened for eligibility. The initial screening decision was based upon the most recent HB value available. HB was tested either as the patient was referred to the outpatient department (OPD) for investigation, or following endoscopic evaluation of a suspicious lesion, or tested with analysis of renal function to ensure suitability for intravenous contrast during radiological staging assessment.

The HB inclusion criterion was defined as 1g/dL below the gender specific WHO definition (WHO, 2011) to ensure all included patients were anaemic at recruitment – thus below 11 g/dL for females and 12 g/dL for males. Patients with metastatic disease, pre-existing haematological disease, renal failure and those undergoing current chemotherapy were excluded to minimise the risk of a non-iron deficient aetiology to the anaemia. A full list of inclusion and exclusion criteria is outlined in Table 13 and Table 14 respectively, with the CONSORT flowchart illustrated in Figure 14 (end of the section 4.2).

Eligible patients were then sent an invitation to participate in the trial together with a patient information sheet. This was performed

at a point after they had been made aware of their diagnosis by the clinical team.

Criterion	Rationale
Participant willing and able to give informed consent for participation in the study.	To comply with the declaration of Helsinki.
Medically fit for surgery.	To ensure the study evaluates efficacy of the treatments in the preoperative period.
Date of planned surgery is ≥ 14 days from date of planned initiation of study intervention.	To allow a treatment effect to be evident. This is a realistic and clinically relevant time period between completion of diagnosis and staging, and operative date. It is in line with previous similar studies (Keeler et al., 2014).
Able (in the Investigators opinion) and willing to comply with study requirements.	To minimise participant drop-out.
Willing to allow his/her General Practitioner and Consultant, to be notified of participation in the study.	To ensure safety by involving all members of the clinical team.

Table 13 - Inclusion Criteria and their rationale in the study design.

Criterion	Rationale
Female participants who are pregnant, lactating or planning a pregnancy during the course of the study.	In rat models, intravenous iron has been demonstrated to be teratogenic in the first trimester (at high dose).
Patients with evidence of iron overload or disturbances in utilisation of iron as stated in the product SPC.	A contraindication for intravenous iron.
Previous allergy to intravenous iron or related iron products.	
Previous gastric, small bowel or colorectal surgery (where ≥50% of stomach or terminal ileum has been resected).	To prevent patients who may have impaired iron or B12 absorption, hence may be unresponsive to iron or may not be iron deficient.
Current chemotherapeutic treatment.	May be anaemic by non-iron deficient mechanisms.
Known previous anaemia not attributable to colorectal carcinoma (i.e. anaemia in patients with well established, inflammatory disorders or chronic renal disease).	
Known haematological disease.	
Significant renal or hepatic impairment.	
Features necessitating urgent surgery (e.g. obstructive symptoms).	Insufficient treatment duration to observe effect.
Significant symptomatic anaemia necessitating urgent transfusion (e.g. cardiovascular compromise).	The correct treatment to administer should be ARBT and this should not be delayed to start iron supplementation.
Patients who are unable to consent.	To comply with the declaration of Helsinki.
Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the result of the study, or the participant's ability to participate in the study.	
Donation of blood during the study.	This would affect the HB levels irrespective of treatment
Participants who have participated in another research study involving an investigational product (IMPs) in the past 12 weeks.	To avoid overburden of patient, and minimise risks of interaction of IMPs.

Table 14 -Exclusion Criteria and their rationale in the study design.

4.2.3 **Sample Size:**

A sample size calculation was performed using GraphPad StatMate™ version 2 (*GraphPad Software, Inc., California USA* to achieve a Power of 90% with an α level of $P<0.05$. A clinically significant effect was defined as a 1 unit difference in mean transfused volume. This was selected as it represents the minimum volume of an individual transfusion, and previous studies have identified a dose-dependent increase in mortality and serious morbidity as each single perioperative unit is administered (Ferraris et al., 2012).

Relevant studies in CRC have published the Standard Deviation* in perioperative transfusion rates to be in the order of 1.6 units (Christodoulakis et al., 2005). This control figure was used as the basis for the sample size calculation.

It was therefore calculated that 55 patients would be required in each arm of the study. Due to a predicted preoperative drop-out rate of 5%, it was decided that 116 patients would be recruited.

Footnote:
****Mean 1.35 units, transfusion rate 52%, HB 9-12g/dL***

4.2.4 Recruitment Visit:

Patients who agreed to participate in the trial were invited to attend the hospital for their Recruitment visit (REC). At this point, the consent process was completed and the consent form signed.

A full medical, medication and social history was then taken, which was checked against hospital records to ensure no contraindications to study inclusion were identified and ascertain important background information for the study.

Height and weight were recorded on all patients, a physical examination undertaken, and a baseline blood pressure recorded to exclude pre-drug hypotension. A urine pregnancy test was performed on females of child bearing age, i.e. all females under 55 years of age or those currently menstruating.

Three quality of life (QOL) questionnaires were completed by all participants. These included the SF-36 version 1 (modified), EQ-5D-5L and FACT-AN, and are discussed further in section 4.4. The SF-36 (Short-Form 36) is a widely used QOL questionnaire which asks 36 questions aiming to review facets of QOL within eight "dimensions" including psychological functioning, role limitations due to physical and emotional problems, pain, general health

perceptions, energy and vitality, social functioning and mental health (Brazier et al., 1992). This tool is widely used and has been validated in a variety of post-surgical and chronic health conditions (Ware Jr and Gandek, 1998).

EQ-5D-5L is versatile, generic assessment of QOL that provides a useful overview of general health related QOL by reviewing 5 individual items in conjunction with a visual-analogue scale. This tool has been validated as an assessment of QOL in multiple disease states including malignancy (Pickard et al., 2007) and chronic disease (Hurst et al., 1997, Culleton et al., 2007, Calvert et al., 2005).

FACT-An (Functional Assessment of Cancer Therapy-Anaemia) provides a useful contrast to the "generality" of the EQ-5D-5L. This QOL measure is designed for use specifically in the context of malignancy, and in particular, focusses on the impact of cancer related anaemia on QOL. FACT-AN has therefore been validated at evaluating the impact of cancer related anaemia associated symptoms on QOL (Yellen et al., 1997, Yoshimura et al., 2004).

4.2.5 Randomisation:

Randomisation between the control group of OI and the treatment group of IVI was performed in a 1:1 fashion. Randomisation used a web-based system using variable block allocation. The system was designed, set up and run by a unit independent to the study (Research Design Service East Midlands, University of Nottingham), although patient details were entered by the research team to obtain an un-blinded allocation.

Randomisation stratification was performed on the variables of patient gender and age. This was undertaken to ensure maximal homogeneity between groups within the constraints of the sample size.

4.2.6 Blood Analyses:

At the recruitment visit, a complete set of trial blood tests were sent. In the IVI group, these blood tests were performed as intravenous access was secured, whilst venepuncture was performed in the patients randomised to OI.

Blood samples were collected using the same Vacutainer ® systems (BD Vacutainer ®, New Jersey, United States) irrespective

of whether by venepuncture or cannulation. Blood was collected in BD Vacutainer ® vials (BD Vacutainer ®, New Jersey, United States) as outlined in Table 15. All samples (except stored serum "Red" vial) were processed and analysed in the same manner as any standard clinical test through appropriate National Health Service (NHS) hospital laboratories with full Clinical Pathology Accreditation (CPA).

Colour	Additive	Volume of blood collected	Test
Red	Silicon coating	6ml	Serum stored: -Hepcidin -H Pylori -CaGA
Yellow	Gel clot activator	5ml	Biochemistry: -Ferritin -TSAT -CRP
Green	Lithium Heparin	6ml	Erythropoietin
Lavender	Ethylene-Diamine-Tetra-Acetic acid (EDTA)	4ml	Full Blood Count: -Haemoglobin level -Mean Corpuscular Volume -Platelet Count

Table 15 – Description of vial colours used, blood volume collected listed in desired order of blood draw for study blood tests.

The Red vial was initially processed in a Good Laboratory Practice (GLP) accredited laboratory at each individual site. A specific study SOP was adhered to for the centrifuge and storage of serum which involved:

1. Equilibration of vial at room temperature for 60mins +/- 10 mins.
2. Centrifuge of vial at 2000g for 10mins at room temperature.
3. Transfer of the supernatant into 200µl aliquots.
4. Storage at -80^{o} Celsius.

At the end of the study, the stored serum was transferred on dry ice from each site to Department of Gastrointestinal Surgery, University of Nottingham. The subsequent analysis of these specimens is outlined in 4.5.2.

4.2.7 Treatments:

If randomised to the IVI group the dose was calculated based upon the patient body weight and inclusion HB value. The dose administered followed that used in the FERGIcor trial (Evstatiev et al., 2011), which is also now advised in the summary of product characteristics (SmPC) illustrated in Table 16. The product SmPC limits a maximum dose of 1000mg per week. Consequently, when patients required two doses, the initial dose of 1000mg was administered at this recruitment visit, and the patient invited to attend a second visit for the subsequent dose at least one week

later. Attempts were made to coincide this with the patients' standard Preoperative Assessment Clinic (PAC visit) which routinely occurred 1 to 2 weeks preoperatively.

	Body weight < 70 Kg	**Body weight > 70 Kg**
Haemglobin > 10 g/dL	1000mg (i.e. 1 dose)	1500mg (i.e. 2 doses)
Haemglobin < 10 g/dL	1500mg (i.e. 2 doses)	2000mg (i.e. 2 doses)

Table 16 - Calculation of dosing regimen for ferric carboxymaltose (FCM)

The IVI was prepared and administered as advised in the product SmPC (available online). This involved the aseptic addition of the drug to 250ml of normal saline, which was infused over 15 minutes. Blood pressure was recorded prior to administration.

In the control group, patients were provided with a 2 week supply of oral ferrous sulphate (OI) and advised to take 200mg twice a day without food. Advice was given regarding ingestion with liquid high in ascorbic acid (e.g. orange juice) to improve absorption (Hallberg et al., 1989). Patients were instructed to visit their GP for further supply if their date of operation was more than 14 days in advance, and the GP was sent a letter to inform them of this and the patient's involvement in the trial.

4.2.8 Day of Surgery:

Patients were interviewed on the day of surgery (DOS), and the QOL questionnaires, and all blood tests were repeated. Blood tests were processed in the same protocol as outlined in section 4.2.6. Direct questioning was performed to ascertain if any adverse events had occurred since previous review, including documentation of any drug side effects, any alterations to OI dosing and the rationale for this, any changes of medication since previous review and any other details of note. Hospital blood transfusion records were also reviewed to identify any preoperative ARBT use, which was also verified with the patient.

Intraoperatively, a discussion was held with the theatre staff including the anaesthetist, the operating surgeon and the scrub nurse, to ascertain the volume of blood lost during the procedure. The agreed value based on recorded losses measured in the suction devices, and also from the weight change of swabs used. This value was documented along with the volume and type of intravenous fluids infused during the procedure.

Tumour location was determined by the final histology report together with intraoperative findings. Right sided tumours were defined as tumours located within the colon from the caecum to

distal transverse colon, with Left sided tumours defined as tumours located within the colon from the splenic flexure to distal sigmoid colon, and rectal tumours located in the distal 15cm of large bowel proximal to the anal canal.

4.2.9 Day 2 Post-operative review:

On the second post-operative day (D2) a further patient review was performed together with assessment of the patient case-notes and hospital databases. This review was aimed at identifying any early postoperative ARBT use, early complications and also provided the opportunity to repeat the patient post-operative blood tests. Complication definitions are further defined in section 4.4.1.3.

The blood tests performed were the same as the previous visits, with the exception of hepcidin which was not retested at this point given the known acute phase response of this hormone (Nemeth et al., 2003b)

The QOL questionnaires were not repeated at this time point due to the multiple potential confounding variables, such as adequacy of postoperative analgesia and perceived "success" of the operation

such (e.g. the need for a stoma or conversion to an open procedure).

4.2.10 Outpatient review:

The final review was performed at the patients' first planned outpatient clinic department (OPD) appointment following discharge from surgery which corresponded to a period 6-12 weeks post discharge. If this appointment was expedited due to a complication, this appointment was not used for trial purposes, and review was delayed until the subsequent appointment falling within correct 8-12 week post-operative period. This was to ensure that all reviews occurred at a comparable postoperative time point, and so that the final outcome of any postoperative complications was known.

This final review served to document the details of any ARBT administered, postoperative complications encountered, length of hospital stay, final tumour stage and chemotherapy use over the entire duration of the study. These were confirmed with the patient, and by review of case-notes and all hospital electronic records (including transfusion). This visit also served to act as a

failsafe for any details missed on the D2 review which occurred on the second postoperative day, after the review had taken place.

At this review, the QOL questionnaires were also completed and a final full set of blood tests collected.

4.2.11 Trial completion criteria:

Completion of the trial was ultimately defined by attendance at the final OPD visit. However, patients were also deemed to have completed the trial in the following circumstances. Firstly, if a patient was recruited to the study and failed to undergo resectional surgery, the patient completed the trial at the point of surgery. All data obtained to this point was included in analysis.

Secondly, in patients who died prior to OPD follow up, or were lost to follow up (e.g. due to moving out of area), end of trial was defined as the date of death or date of postoperative hospital discharge respectively. All data acquired until the patient reached these points was recorded and analysed.

4.2.12 Statistical Analysis:

The statistical level of significance for all tests was defined as $P<0.05$. Paired non-parametric data was compared with Wilcoxon signed rank test. Non-parametric independent data was compared with Mann-U Whitney test, and with Kruskal-Wallis test when group numbers exceeded two. Two tailed Chi-squared test was used to assess differences in categorical data between groups. Correlations between non-parametric variables were estimated with Spearman's rank test and Pearson's correlation coefficient for parametric data. Statistical analysis was performed using SPSS® version 21 (SPSS, Chicago, Illinois, USA). More specific statistical methodology is included in each relevant section.

4.2.13 Summary:

A summary of the patient pathway is illustrated in Figure 13. In the following sections more specific methodology, focussed results and discussion is documented in the following subsections:

4.3 – Treatment of anaemia and Blood transfusion use.

4.4 – Patient experience: Quality of life and complications.

4.5 – Investigation of biomarkers to predict treatment response.

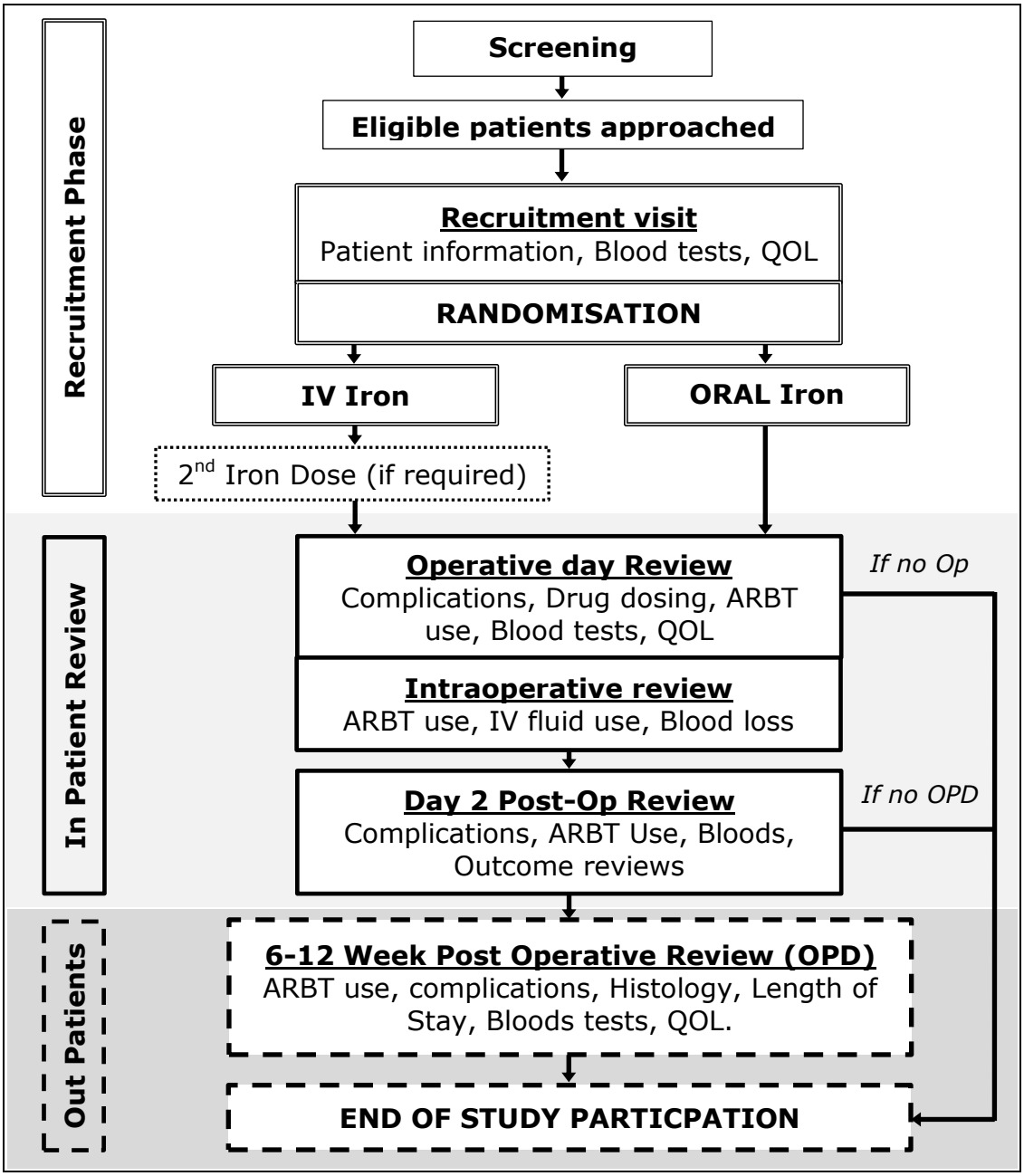
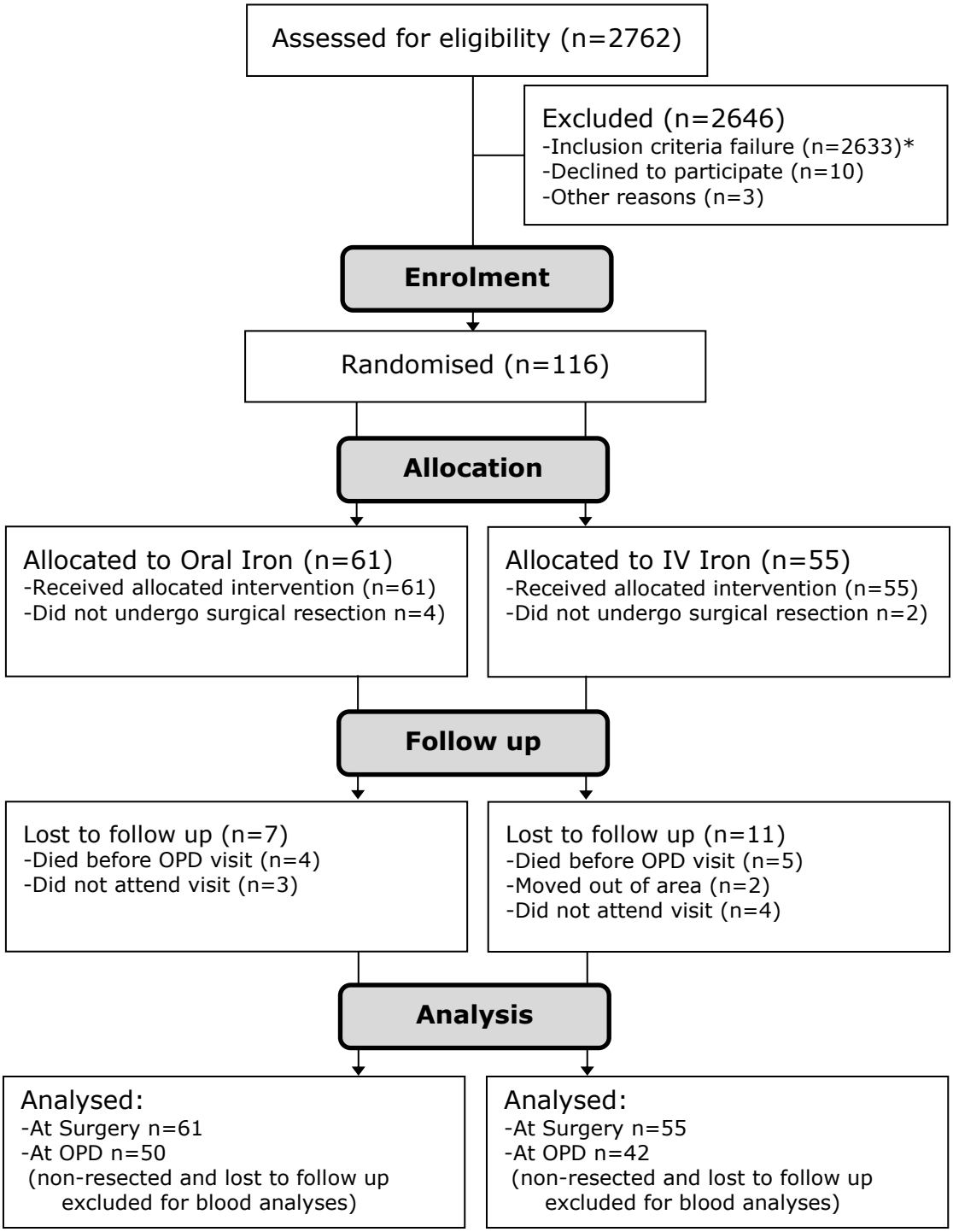


Figure 13 – A summary of the IVICA trial patient pathway.

Where ARBT= Allogeneic red blood cell transfusion, Op=Operation, QOL= Quality of Life assessment, IV= Intravenous.



**Exclusion(excl) details:*

Haemoglobin level normal (n=1807)	*Metastatic disease (n=240)*
Not for surgery/palliative (n=192)	*No HB available at diagnosis (n=124)*
Not adenocarcinoma (n=108)	*Chemo-/Radiotherapy as primary therapy(n=73)*
Operation date too soon (n=38)	*Medical comorbidity exclusion criteria(n=35)*
Endoscopic resection/observe (n=8)	*Clinical team deemed unsuitable (n=3)*
Prisoner (n=3)	*Unable to consent (n=2)*

Figure 14 - CONSORT 2010 Flowchart representation of randomisation and analysis in the IVICA Trial.

Chapter 4.3:

Treatment of anaemia and blood transfusion use.

4.3.1 Results:

4.3.1.1 General Demographics:

116 patients were recruited to the study, of which 110 underwent surgery to resect the primary malignancy. Four patients had their operation cancelled on the day of surgery due to deterioration in their clinical condition, 1 patient died during induction of anaesthesia, and the operation was abandoned at initial laparotomy in one patient due to finding inoperable disease.
Both groups were comparable in terms of demographic and operative details. Demographic and operative data is summarised in Table 17.

Median duration of study iron treatment was 21 days in both groups (OI, IQR 15-33; IVI IQR 15-34, *P*=0.75), although 11 patients failed to meet the desired 14 day treatment period. Of these patients (OI, *n*=6; IVI, *n*=5, *P*=0.87), 3 had their date of operation moved due to a change in clinical condition, and 8 due to an earlier date becoming available after recruitment to the study.

	Oral Iron	IV iron	P
n	61	55	
General Demographics			
Males	37	35	0.741
Females	24	20	
Age years (IQR)	76.5 (68.2-81.5)	73.8 (67.4-78.6)	0.594
Height m (95%CI)	1.67 (1.64-1.7)	1.68 (1.66-1.71)	0.599
Weight kg (95%CI)	72.78 (68.7-76.9)	79.01 (74.9-83.23)	0.101
Screening details:			
Inclusion HB g/dL (95%CI)	9.9 (9.7-10.2)	9.6 (9.3-10)	0.155
Median time in days from inclusion HB to REC (IQR)	20 (5-34)	26 (10-36)	0.205
Patients receiving oral ion at recruitment	30	25	0.688
Median number of days of iron pre-treatment if applicable (IQR)	20 (6-34)	26.5 (13-37)	0.11
Median number of days of study treatment (IQR)	21 (15-33)	21 (15-34)	0.748
Preoperative Risk Assessment:			
ASA ≤2	43	30	0.076
ASA ≥3	18	25	
Operative patients with significant cardiac or respiratory comorbidity*	12	21	<0.05
Median CR Possum mortality score at Recruitment % (IQR)	3.58 (2.58-9.29)	3.48 (2.58-6.62)	0.824
Median Adjusted Charlson Score at Recruitment (IQR)	5 (4-6)	5.5 (4-7)	0.425
Operative Information:			
No operation performed	4	2	0.478
Laparoscopic	30	26	
Converted laparoscopic	4	5	0.880
Open	23	22	
Right Colonic Tumour	41	35	
Left Colonic Tumour	12	11	0.552
Rectal Tumour	4	7	
Tumour T Stage: T≤ 2	5	8	0.305
Tumour T Stage:T3 & 4	52	45	
Median Tumour size mm (IQR)	45 (35-60)	41 (34-55)	0.485
Median Blood Loss ml (IQR)	100 (65-200)	100 (55-390)	0.321
Median Intraoperative fluid L (IQR)	2.5 (2-3)	2 (2-3)	0.826

Table 17 - Comparison of IVICA group composition.

Where: Interquartile range (IQR) or 95%Confidence Interval (95%CI) represented in parenthesis when specified. *denotes statistical differences between groups to P<0.05.

4.3.1.2 Iron Supplementation Dosing:

Study OI treatment protocol was adhered to by over 90% of patients (*n*=50) who did not have the date of surgery moved (*n*=55). Two patients reduced the dose due to drug side effects (dyspepsia and constipation), 2 patients increased the dose on clinician request, and 2 changed the drug oral formulation after 14 days. No patients randomised to OI went on to receive IVI.

In the IVI group, 82% of patients received 2 doses of IVI based on their recruitment weight and HB (*n*=45). The total dose administered was thus 2000mg in 27% (*n*=15), 1500mg in 55% (*n*=30) and 1000mg in 18% (*n*=10). Of those receiving 1000mg, 4 were calculated as needing a second dose but either had their operation date moved forward (*n*=2) or were unable to attend for the appointment (*n*=2).

Post-infusion (<24h) headache was the most frequent complication of IVI reported (*n*=3). Only one significant adverse drug reaction (ADR) was experienced; a rash which required intervention in the form of oral antihistamine medication.

4.3.1.3 Treatment of anaemia:

The lowest HB at REC within the IVI group was 7.3g/dL and 8.3g/dL within the OI group. The mean HB in OI at REC was 10.42 g/dL [95%CI 10.1-10.7] which was comparable to IVI 10.2g/dL [95%CI 9.8-10.5, *P*=0.24]. This rose significantly to DOS (*P*<0.01) with treatment in both groups to a mean of 11.0g/dL [95%CI 10.6-11.4] with OI and 11.9g/dL [95%CI 11.5-12.3] for IVI in those patients who did not receive preoperative ARBT over this period. This equated to a median treatment rise in HB for OI of 0.5g/dL [IQR -0.125-1.325, P<0.01] and 1.5g/dL [IQR 0.9-2.6, *P*<0.01] for IVI. HB levels remained significantly higher in the IVI group until OPD, which occurred a median 101 days [IQR 62-193] after REC in OI, and 91 days [IQR 61-135, P=0.98] for IVI. The HB changes across the study time points are illustrated in Table 18.

At REC, 60 patients (98%) were anaemic in OI group compared to 52 patients (95%) in IVI (*P*=0.54). At DOS, 55 patients (90%) in OI remained anaemic, which was significantly higher compared to the 41 patients (75%) in IVI (*P*<0.05). Furthermore, 30 patients within the OI group required iron supplementation after surgery which was significantly higher than the IVI group (*n*=4, *P*<0.01).

4.3.1.4 Treatment of Iron deficiency:

Median ferritin and TSAT levels were comparable between groups at REC (*P*=0.22) but were significantly higher in IVI at DOS. These changes are illustrated in Table 18.

Variable	Time	Oral	IVI	*P* value
HB (g/dL)	REC	10.4 (10.1-10.7)	10.2 (9.8-10.5)	0.25
	DOS**	11 (10.6-11.4)	11.9 (11.5-12.3)	<0.01
	D2**	10 (9.6-10.3)	10.8 (10.4-11.3)	<0.01
	OPD	12.6 (12.2-13)	13.2 (12.7-13.8)	0.05
Ferritin (µg/L)	REC	26 (15-50)	21 (8-46)	0.22
	DOS**	27.5 (17-51.5)	558 (329.8-1085.3)	<0.01
	D2**	78 (46-127.5)	587 (364-921.3)	<0.01
	OPD**	44 (22.9-78)	201 (68.6-313.5)	<0.01
TSAT (%)	REC*	8 (6-20)	7 (5-15)	<0.05
	DOS**	9 (5-14)	19 (16-29)	<0.01
	D2**	5 (3-8)	12 (8-16)	<0.01
	OPD	22 (14.5-33)	25 (15-33)	0.14

Table 18 - Blood value changes across each time point.

NB patient results excluded if received ARBT in 2 weeks prior to study time point. Where HB=Haemoglobin, TSAT= Transferrin Saturation, REC=Recruitment, DOS=Day of Surgery, D2=Day 2 postoperative, OPD=Outpatient visit. Parentheses display Interquartile Ranges for Ferritin & TSAT, and 95%Confidence Intervals for HB. *denotes statistical differences between groups to P<0.05, and **denotes statistical differences between groups to P<0.01.

Furthermore, there was no significant change in ferritin levels between REC and DOS (*P*=0.572), and between DOS and OPD (*P*=0.13) in the OI group, which was also true for TSAT levels from REC to DOS (P=0.285). TSAT levels did, however, increase significantly from DOS to OPD in this group (*P*<0.01).

In contrast, ferritin levels increased significantly in the IVI group from REC to DOS (*P*<0.01) and decreased significantly from DOS to OPD (*P*<0.01). Over these same time frames, TSAT significantly increased from REC to DOS only (*P*<0.01; DOS-OPD, *P*=0.249).

4.3.1.5 Differences in Blood Transfusion use:

A total of 73 units were transfused to 24 patients across the entire cohort during the study (OI, *n*=14; IVI *n*=10; *P*=0.53). Two patients received a total of 3 units ARBT preoperatively, both within the OI group. Most transfused patients received blood on DOS (*n*=12).

Of those undergoing surgery, 14 patients in the OI group were transfused a total of 36 units (mean 0.632u [95%CI 0.258-1.006]) until both discharge and OPD. This was not significantly different from the IVI group, whereby 9 patients received a total of 33 units

at a mean 0.623u [95%CI 0.09-1.156] per patient until discharge, and 10 patients received a total of 37 units (mean 0.698u [95%CI 0.151-1.246]) until OPD. There was no difference in ARBT use for all operative patients (n=110) across the study period as illustrated in Table 19.

Time point	End Point	Oral Iron (*n*=57)	IV Iron (*n*=53)	*P* value
Until end DOS∞	Mean Units	0.351 (0.016-0.685)	0.226 (0.027-0.426)	0.52
	Patients	7	6	0.876
Until end Day 7	Mean Units	0.596 (0.232-0.961)	0.340 (0.044-0.635)	0.275
	Patients	14	7	0.130
Until end Day 14	Mean Units	0.596 (0.232-0.961)	0.453 (0.088-0.818)	0.578
	Patients	14	8	0.215
Until end Day 28	Mean Units	0.632 (0.258-1.006)	0.472 (0.107-0.837)	0.542
	Patients	14	9	0.329
Until Discharge	Mean Units	0.596 (0.232-0.961)	0.623 (0.09-1.156)	0.935
	Patients	14	9	0.329
Until OPD	Mean Units	0.632 (0.258-1.006)	0.698 (0.151-1.246)	0.841
	Patients	14	10	0.470

Table 19 - Mean volume of blood transfused and number of patients transfused from Recruitment into study.

Where: ∞denotes all study patients included, otherwise comparisons include patients who underwent resectional surgery only. 95%CI expressed in parenthesis.

Of the 36 units administered in OI group, 94% (*n*=32) were administered up until the 7th postoperative day. In comparison, only 49% (*n*=18) of the total 37 units administered in the IVI group were given over this period, which was significantly lower (*P*<0.01). The variation in time point when ARBT were administered is illustrated in Figure 15.

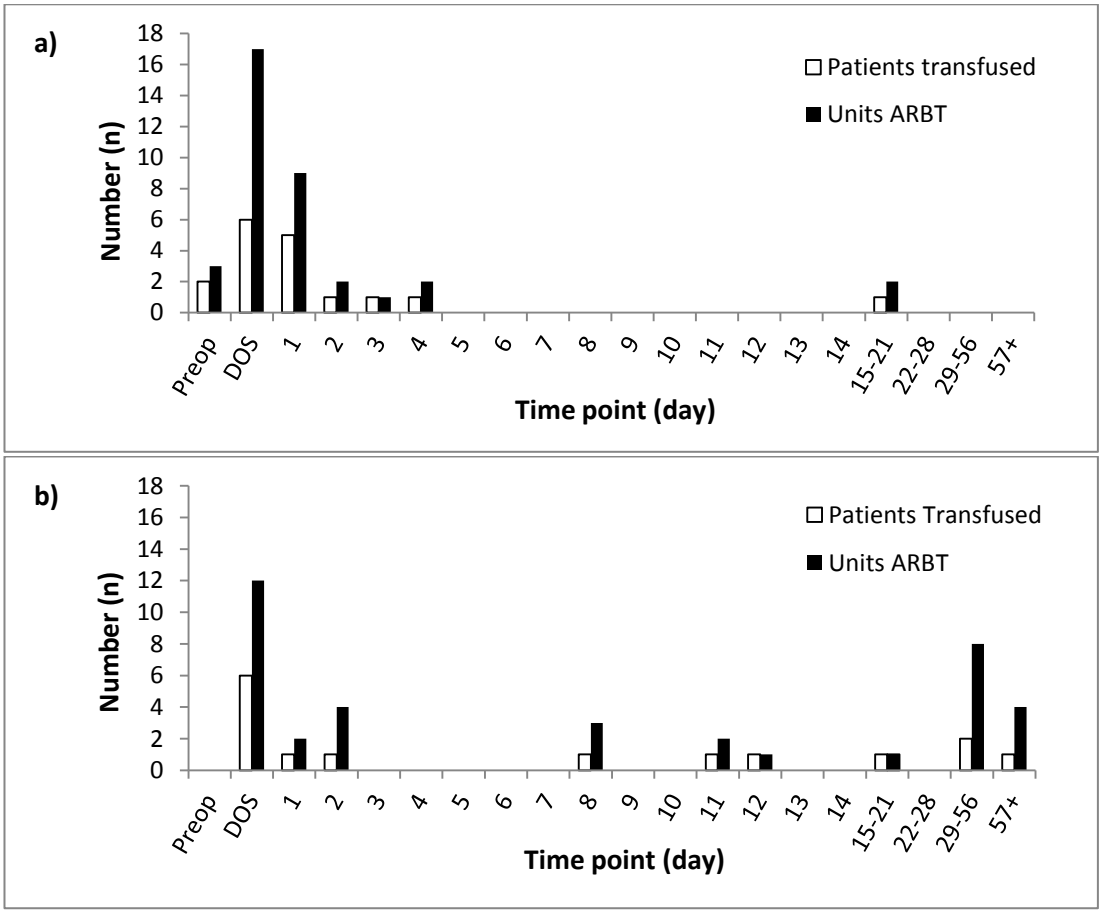


Figure 15 - **Graph illustrating time points of transfusion in surgical study patients and volumes of allogeneic blood administered in a)Oral iron Group, and b)IV iron Group.**

Where: Preop= From recruitment to Day of Surgery; DOS= Day of Surgery; numerical X axis represents postoperative days; ARBT= Allogeneic red blood transfusion.

The mean HB on the DOS for patients transfused over the course of the trial was 10.07g/dL [95%CI 9.3-10.8] which was significantly lower ($P<0.01$) than those untransfused patients [11.8g/dL 95%CI 11.5-12.1]. Although the mean HB of patients who were transfused within the IVI group was higher on DOS than OI [10.8g/dL 95%CI 9.6-12.1 vs 9.5g/dL 95%CI 8.5-10.5], the difference was non-significant ($P=0.08$). The mean HB rise attributable to iron supplementation in the preoperative period in transfused patients was significantly higher in the IVI group than OI [1.56g/dL 95%CI -0.296-3.42 vs -0.43g/dL 95%CI -1.51-0.64; $P<0.05$]. All transfused patients in the OI group were consequently anaemic on DOS, whilst 2 in the IVI group were not.

Two patients experienced severe intraoperative blood loss in excess of 1.5L, both within the IVI group. No other patient blood losses fell within 2 interquartile ranges of these values. The HB on DOS of these patients was 13.1g/dL and 12.5g/dL, with preoperative HB rises of 4.8g/dL and 1.7g/dL respectively. On subgroup analysis of all operative patients who did not experience severe haemorrhage, there was a significant reduction in ARBT use perioperatively in IVI group. Up to day 7 there was a significant reduction in both patients transfused ($P<0.05$) and mean units transfused per group ($P<0.05$) as illustrated in Table 20.

Over half the units transfused in the IVI group were administered to 2 patients. One had a prolonged and complicated stay after an anastomotic leak and received a total of 10 units, 6 units of which were administered more than a month postoperatively. The other patient received 8 units following an upper gastrointestinal bleed on Day 8 postoperatively. In comparison, only one patient in the OI group received a large transfusion of more than 4 units, 6 units of which were administered on DOS and 2 unit preoperatively.

Time Point	End Point	Oral Iron (*n*=57)	IV Iron (*n*=51)	*P Value*
Until end Day 3	Mean Units*	0.561 (0.232-0.961)	0.157 (0.015-0.299)	0.044
	Patients*	14	5	0.044
Until end Day 7	Mean Units*	0.596 (0.232-0.961)	0.157 (0.015-0.299)	0.027
	Patients*	14	5	0.044
Until end Day 10	Mean Units	0.596 (0.232-0.961)	0.216 (0.035-0.396)	0.073
	Patients	14	6	0.087
Until end Day 14	Mean Units	0.596 (0.232-0.961)	0.275 (0.004-0.545)	0.158
	Patients	14	6	0.087
Until end Day 28	Mean Units	0.632 (0.258-1.006)	0.294 (0.023-0.566)	0.146
	Patients	14	7	0.155
Until Discharge	Mean Units	0.596 (0.232-0.961)	0.333 0-0.674	0.296
	Patients	14	7	0.155
Until OPD	Mean Units	0.632 (0.258-1.006)	0.412 (0.042-0.781)	0.405
	Patients	14	8	0.253

Table 20- Subgroup analysis of transfusion use in operative patients without severe blood loss.

Where*: * *denotes statistical significance to P<0.05, 95%CI expressed in parenthesis.

4.3.1.6 Other factors and Blood Transfusion:

Mode of operative access:

Laparoscopic surgery was attempted in 65 with 9 converted to open procedures (IVI *n*=5; OI *n*=4). Median blood loss in the laparoscopic and converted group was 85ml (IQR 50-100) which was significantly lower than the open group of 300ml (IQR 180-410) (P<0.01). There was no difference in tumour location between modes of operative access (P=0.27), nor was there any difference in T stage (P=0.64) or tumour size (P=0.56). The transfusion rate of 11% (n=7, 65 patients total) in laparoscopic and converted group was significantly lower than the open group (31%, n=17, 45 patients total; P<0.01). This equated to a mean transfusion volume from REC to OPD of 0.2 units (95%CI 0.03-0.36) in the laparoscopic group compared to 1.33 units (95%CI 0.61-2.06) in open surgery (P<0.01). Table 21 illustrates the differences in transfusion use within each operative subgroup.

a) Laparoscopic/Converted

Time point	End Point	Oral Iron (*n*=34)	IV Iron (*n*=31)	*P* value
Until end Day 7	Mean Units	0.176 (0-0.36)	0.097 (0-0.24)	0.493
	Patients	4	2	0.460
Until end Day 28	Mean Units	0.176 (0-0.36)	0.097 (0-0.24)	0.493
	Patients	4	2	0.460
Until Discharge	Mean Units	0.176 (0-0.36)	0.097 (0-0.24)	0.493
	Patients	4	2	0.460
Until OPD	Mean Units	0.176 (0-0.36)	0.226 (0-0.52)	0.773
	Patients	4	3	0.786

b) Open

Time point	End Point	Oral Iron (*n*=23)	IV Iron (*n*=22)	*P* value
Until end Day 7	Mean Units	1.217 (0.38-2.05)	0.682 (0-1.37)	0.312
	Patients	10	5	0.140
Until end Day 28	Mean Units	1.304 (0.46-2.15)	1.000 (0.15-1.84)	0.600
	Patients	10	7	0.420
Until Discharge	Mean Units	1.217 (0.38-2.05)	1.364 (0.11-2.62)	0.840
	Patients	10	7	0.420
Until OPD	Mean Units	1.304 (0.61-2.05)	1.364 (0.11-2.62)	0.935
	Patients	10	7	0.420

Table 21 - Subgroup analysis of transfusion differences between oral and IV iron groups with respect to operative access for a)Laparoscopic/Converted and b)Open approaches.

Where: OPD=Outpatient Department; 95%CI expressed in parenthesis.

Site of colorectal malignancy:

Across the entire surgical cohort, there was no difference in transfusion rates (OPD, *P*=0.76, D28; *P*=82; D7; *P*=0.94) or mean units transfused (OPD, *P*=0.48; D28; *P*=0.66; D7; *P*=0.48) with respect to colonic or rectal surgery. This remained true on subgroup analysis of iron groups within each location type.

Moderate and severe anaemia vs Mild Anaemia at REC:

In the operative cohort, there were no OI patients with severe anaemia and 61% (*n*=35) of patients had moderate anaemia at REC. This compared with 9% of patients (*n*=5) who had severe anaemia and 58% (*n*=31) with moderate anaemia in the IVI group. Only 3 patients (IVI, n=2; OI, n=1) with HB at REC above 11g/dL received ARBT over the course of the study, hence there was no difference in ARBT use in the mild anaemia subgroup. Conversely, differences were evident in ARBT use from REC until the 7th postoperative day in patients with moderate or severe anaemia at REC (see Table 22).

Time point	**End Point**	**Oral Iron** (*n*=35)	**IV Iron** (*n*=36)	***P* value**
Until end Day 7	Mean Units	0.914 (0.35-1.48)	0.306 (0.02-0.6)	0.054
	Patients*	13	5	0.024
Until end Day 28	Mean Units	0.971 (0.39-1.55)	0.500 (0.07-0.93)	0.188
	Patients	13	7	0.097
Until Discharge	Mean Units	0.914 (0.35-1.48)	0.722 (0.001-1.44)	0.673
	Patients	13	7	0.097
Until OPD	Mean Units	0.971 (0.39-1.55)	0.833 (0.09-1.58)	0.768
	Patients	13	8	0.168

Table 22 - Subgroup analysis of transfusion use in patients with moderate or severe anaemia (HB ≤10.9g/dL) at recruitment.

***Where* * denotes statistical significance to *P*<0.05.**

4.3.1.7 Control comparison:

The trial data used to calculate the sample size was performed in a similar anaemic colorectal cancer cohort (Christodoulakis et al., 2005). If this is used as a baseline for comparison in the perioperative period, both IVI and OI are seen to reduce the transfusion rate and volume in operative patients with CRC (see Table 23a) compared to non-intervention. Similarly, the same remains true when compared with historical data presented in Chapter 2 (see Table 23b).

a)

	Control*	**OI**		**IVI**	
n	68	57		53	
Mean +/-SD (units)	1.35 +/-1.58	0.632 +/-1.41	*P<0.01*	0.472 +/-2.166	*P<0.01*
Patients transfused (*n*)	36	14	*P<0.01*	10	*P<0.01*

b)

	Chapter 2	**OI**		**IVI**	
n	28	57		53	
Mean +/-SD (units)	1.6 +/-2.16	0.632 +/-1.41	*P<0.01*	0.472 +/-2.166	*P<0.01*
Patients transfused (*n*)	13	14	*P<0.01*	10	*P<0.01*

Table 23 - Comparison of IVICA transfusion data for surgical patients over the entire study with a) Published data* and b) Comparable data from Chapter 2.

Where: *Published data= Christodoulakis, M et al. Preoperative epoetin alfa in colorectal surgery: A randomized, controlled study. Annals of Surgical Oncology, 12, 718-725)

4.3.2 Discussion:

The main aim of this trial was to compare the efficacy of IVI and OI in the preoperative management of anaemia in patients with CRC using mean volume of ARBT administered as the primary endpoint. The current data failed to identify any difference between groups in this measure, or the number of patients transfused, when considered at key time points from Surgery to outpatient follow-up.

It is important to recognise that despite the IVI and OI groups being very similar (Table 17), a notable difference was seen in the number of patients with significant cardiac or respiratory comorbidity. This is a major factor frequently used in the clinical decision to administer ARBT (Shander et al., 2013), hence it is possible that the patients in the IVI group were more likely to receive ARBT. This is exemplified by the fact that the mean HB of those transfused in the IVI group was higher than the OI group. Consequently, parity in ARBT use between groups may indicate IVI had reduced ARBT requirement to a greater degree than illustrated.

Furthermore, although the mean HB levels at REC were comparable between groups, the number of patients with more

severe anaemia was higher in the IVI group. Again, when considering the findings illustrated in Chapter 2 (Table 9), this may further indicate that there were more patients at high risk of requiring ARBT in the IVI group which compounds the previous argument.

It is also of importance that subgroup analysis did identify transfusion differences between groups. The mean transfusion volume and number of patients transfused from REC to the end of the 7th postoperative day was lower in the IVI group when reviewing operative patients who experienced less than 1.5L intraoperative blood loss. It could be argued that ARBT use in patients exceeding such losses is not a treatment failure as it is likely most patients in such circumstances would require ARBT irrespective of HB at DOS (JPAC, 2015). This is exemplified by the fact that one of these patients was no longer anaemic on DOS in the IVI group, and the other was only borderline anaemic after a nearly 5g/dL preoperative rise in HB with IVI treatment.

It is probable that the immediate perioperative period, ie the first 7 post days, represents the most important period in which ARBT should be avoided. It is hypothesised that ARBT have adverse effects on short term surgical and long term oncological outcomes

due the immunomodulatory effects of mediators which they contain (Cata et al., 2013). The effect of these is probably of most clinical relevance when compounded by the relative immunodeficient state induced by surgery (Ben-Eliyahu, 2002) which is most likely to exist within the first 7 postoperative days at which point immune function normalises following trauma (Brochner and Toft, 2009).

Furthermore, in clinical practice, the majority of patients are discharged home within the first 7 days following CRC surgery (Simpson et al., 2015). This was evident in the current study, with a median LOS of 6 days in both groups (see section 4.4.2.5). It therefore follows that if ARBT could be avoided in this period up to the end of the 7th postoperative day, most patients will be discharged from hospital, with the associated reduced risk of morbidity, and also be less susceptible to the "pro-tumour" effect of ARBT.

It is of note that certain subgroups of patient also benefitted from IVI over this period. There was a reduction in the transfusion rate of patients with moderate and severe anaemia at REC in the IVI group. Previous studies have indicated that the lower the HB is on the DOS, the higher the likelihood of ARBT requirement (Chapter

2, (Dunne et al., 2002b)). It is possible that IVI is more effective at reducing the severity of anaemia, and thereby placing patients into an HB range that carries a "standard" risk of ARBT use which is comparable to non-anaemic patients. All patients undergoing CRC surgery are at baseline potential risk of requiring ARBT irrespective of starting HB (Chapter 2).

The differences in HB changes between groups in the current study would appear to further support this argument. Although both IVI and OI resulted in significant increases in preoperative HB levels, the incremental rise seen with IVI was significantly greater than OI, and at a level more likely to have clinical benefit. The consequence of this was a significant reduction in the number of patients who were anaemic at surgery in the IVI group, which is a known risk factor for perioperative transfusion (Dunne et al., 2002b).

Furthermore, the HB levels at surgery of those transfused within the study were lower than those patients not transfused irrespective of study treatment. If IVI is more efficacious at raising HB levels preoperatively, it would indicate that the *risk* of transfusion requirement could be reduced to a greater degree with this treatment.

It is possible that the study failed to identify transfusion differences over a longer period due to the randomisation of more patients to the OI group. The study had been powered to recruit a minimum of 55 patients to each arm which was achieved at recruitment. However, when considering patients undergoing surgery, the IVI group did fall below this threshold by 2 patients due to cancellation of operations. Consequently, the IVI group was potentially underpowered for the primary endpoint. This vulnerability is exemplified by the fact that over half the units of ARBT transfused to this group were to just 2 patients with postoperative complications.

It is also possible that the duration of preoperative therapy was insufficient to allow IVI an optimal time period to take effect. Although, the current study identified a significant improvement with IVI in the treatment of iron deficiency measured in terms of TSAT and ferritin (see Table 18), it is possible that the median 21 day treatment period was insufficient for a maximal effect. Preclinical (Beshara et al., 2003) and clinical studies (Evstatiev et al., 2011) have indicated that IVI utilisation takes longer than this period. In clinical practice, if IVI could be administered earlier, it is possible that the reduction of ARBT use could be greater. Similarly, the attempt to coincide any secondary dose of IVI with

preoperative assessment visits may have caused the second dose to have insufficient time to take effect.

In the current study, the use of laparoscopic surgery (LS) was high reflecting changes in trend within CRC surgery. It is relevant, that although the use of LS was comparable between groups, the overall blood loss was lower across the study in patients undergoing LS, as was the use of ARBT. Such findings are consistent with previous trials (Kiran et al., 2004), hence it is possible that the high LS uptake reduced overall ARBT use rendering any inter-group differences less evident.

As illustrated in Table 23, the transfusion rate and mean volume of ARBT administered to both groups was lower than control data from Chapter 2 and previously published studies (Christodoulakis et al., 2005) . This raises 2 possibilities. Firstly, it is possible that both OI and IVI are effective at reducing ARBT requirement compared to no treatment. Secondly, as the overall mean transfusion for both OI and IVI in the open surgery subgroup (see Table 21) was similar to previously published data, it may indicate that the high utilisation of LS in the current trial has indeed minimised the potential effect of iron supplementation by reducing blood loss. This emphasises a need to further investigate potential

biomarkers which may predict response to iron treatment and thus improve patient selection for treatment (see Chapter 4.5).

4.3.3 Conclusions:

In conclusion, IVI appears to be superior to OI at treating anaemia and iron deficiency in anaemic CRC surgical patients. However, this did not translate to overall differences in the use of ARBT between groups. Both treatments were well tolerated in this clinical setting, and hence should be more widely used as part of preoperative PBM.

For more definitive evidence to guide more widespread use of IVI, a larger RCT remains needed, addressing some of the issues raised in this Chapter. The design of this is discussed in Chapter 6.

Chapter 4.4:

Patient Experience - Quality of life & Complications.

4.4.1 Specific Methodology for assessment of QOL:

4.4.1.1 Scoring each Quality of Life Tool:

FACT-AN:

This QOL measure utilised responses to 48 questions (each scored 1 to 4) to generate "subscale" results in Physical Well-Being (PWB), Social/Family Well-Being (SWB), Emotional Well-Being (EWB), Functional Well-Being (FWB), and Anaemia Subscale (ANS). These values were then used to calculate 3 further composite scores: FACT-An Trial Outcome Index (TOI), Fact-G and Fact-AN total (TOTAL) values (see Figure 16).

FACT-AN TOI =	PWB + FWB + ANS
FACT-G =	PWB + SWB + EWB + FWB
FACT-An TOTAL =	PWB + SWB + EWB + FWB + ANS

Figure 16 - Calculation of the composite scores for FACT-AN.

Where PWB=Physical Well-Being, SWB=Social/Family Well-Being, EWB= Emotional Well-Being, FWB= Functional Well-Being, ANS= Anaemia Subscale and TOI= FACT-An Trial Outcome Index.

In accordance with FACT-AN administration guidelines, subscale scores were prorated if more than 50% of the data was available (ie greater than 4 of the relevant 7 questions answered), but

responses were excluded if they failed to meet this level (Cella, 1997, Lind et al., 2002). Derived values (
Figure **16**) were only calculated if all the component subscale values were available (Cella, 1997, Lind et al., 2002). Additional missing data was therefore not imputed.

EQ-5D:

This tool requires patients to assign scores of between one and five to five specific questions relating to Mobility (MOB), Self-Care (SC), Usual Activity (US), Pain and Disability (PD) and Anxiety and Depression (AD). This is augmented by the use of a single 100 point Visual Analogue Scale (VAS) which acts a general overview of QOL. The component scores were thus recorded as integer values, and the VAS score converted to a numeric value with a maximum of 100. Missing data was not imputed as values obtained were not utilised for generation of further scores.

SF-36:

This tool collects responses to 36 questions and was assessed using validated software provided by questionnaire developers (QualityMetric Health Outcomes™, SF-36 Scoring Software version 4.0). This generated scores for 7 dimensions areas: Physical

Functioning (PF), Role Limitation due to pain (RP), Bodily Pain (BP), General Health (GH), Vitality (VT), Social Functioning (SF), Role limitation due to emotion (RE) and Mental Health (MH). Two further summary scores were also created to provide overviews of physical (PCS) and mental (MCS) facets.

4.4.1.2 Validation of QOL process:

The relationship between HB levels and selected components of each QOL tool was investigated to ensure HB change was a key causal factor underlying changes in QOL. This has been described in previous studies for components including SF-36 VT (Lind et al., 2002), SF-36 PCS (Boogaerts et al., 2003), FACT-TOI (Bremberg et al., 2007), ANS and FACT-G scores (Lind et al., 2002) and also VAS equivalents (Case et al., 1993).

These components were therefore used as markers of validity, and were tested using paired HB and QOL scores at REC and DOS. OPD values were excluded from this process due to the potential confounding of adjuvant chemotherapy. Validity was indicated by significant positive correlations between HB and QOL scores (Pearson's 2 Tailed), and further assessed to ensure increased mean QOL scores with reducing graded anaemia severity. Anaemia

was graded using the WHO definition (WHO, 2011)(see Table 2, Chapter 1). Analysis was performed using one way ANOVA and subsequent Post-Hoc Bonferroni correction for multiple analyses.

4.4.1.3 Definitions:

Minimum Clinical Difference:

Such a definition is required as a statistically significant change between QOL scores may exist which does not translate into a notable difference in patient experience. As a consequence, the notion of a Minimal Clinical Difference (MCD) exists, which is defined as:

> *"…the smallest difference in score in the domain of interest which patients perceive as beneficial and which would mandate, in the absence of troublesome side effects and excessive cost, a change in the patient's management…"*
>
> *(Jaeschke et al., 1989)*

Several methods have been described for determining such a MCD (Copay et al., 2007) which involve either passing a minimum threshold or being graded as an effect size. The benefit of assessment of effect size is that it would appear to yield more

information on which to base a risk:benefit judgement. Consequently, this grading system was utilised as measure of MCD, and was calculated using recognised methods (Copay et al., 2007) illustrated in Figure 17.

$$\textbf{Effect Size} = \frac{[\text{DOS QOL score}] - [\text{REC QOL score}]}{\text{SD REC QOL scores}}$$

Where:

Effect size :	<0.2	No significant clinical effect
	≥0.2 to <0.5	Mild effect*
	≥0.5 to <0.8	Moderate effect*
	≥0.8	Large effect*

Figure 17 - Calculation of effect size and the minimum clinical difference.

Where DOS= Day of surgery, REC= Recruitment, QOL score= quality of life component assessed, SD= Standard deviation, and * denotes Minimum Clinical Difference exceeded.

4.4.1.4 Complication Definitions:

Postoperative complications were defined and graded using the validated Clavien-Dindo (CD) classification (Dindo et al., 2004). These were further categorised as major if the CD grade exceeded 3 (Clavien et al., 2009). Complications were recorded as documented in the patient casenotes.

Major haemorrhage was defined as a recorded intraoperative blood loss in excess of 1500ml, as at acute blood losses above this level will invariably require blood transfusion (JPAC, 2015).

Infective complications were defined as any reported clinical diagnosis of an infection affecting any organ system. In this context, the site, grade and treatment administered were recorded to enable CD grading.

4.4.2 Patient Experience Results:

Completed quality of life questionnaire responses for analysis was high, in the order of 89% for EQ5D-5L, 86% for SF36, and 71% for FACT-AN across the entire study.

Of note, significantly more patients received adjuvant chemotherapy in the OI group (n=23 vs IVI n=12, P<0.05).

4.4.2.1 Anaemia vs QOL:

On review of the entire cohort at REC and DOS, the HB level at each time point was positively correlated with FACT-TOI (R=0.416; P<0.01), FACT-G (R=0.234; P<0.01), FACT-An ANS (R=0.279; P<0.01), EQ5D VAS (R=0.251; P<0.01), SF36 PCS (R=0.227; P<0.01) and SF36 VT (R=0.252; P<0.01). One way ANOVA comparison of graded anaemia severity and component scores revealed significant differences for FACT-An ANS (F=4.612;P<0.01), FACT-G (F=4.78;P<0.01), EQ5D VAS (F=6.099; P<0.01), SF36 PCS (F=2.946;P<0.05) and SF36 VT (F=3.976; P<0.01), Bonferroni Post Hoc correction for multiple analyses identified significant differences in FACT-AN TOI scores between mild and moderate anaemia (Mean difference 15.235 SE 3.6, P<0.01), between non-anaemia and moderate anaemia (Mean

difference 22.425 SE5.5, P<0.01) and between non-anaemia and severe anaemia (Mean difference 34.066 SE 10.6, *P*<0.01). Significant differences were also evident between FACT-An ANS scores in mild and moderate anaemia (Mean Difference 12.1 SE 4.03, P<0.05) and also FACT-G (Mean Difference 9.17, SE 2.59, *P*<0.01). SF-36 VT scores between mild and severe anaemia (Mean Difference 25.01 SE 9.2, P<0.05) and non-anaemic with severe anaemia SF36 VT scores (Mean Difference 29.33 SE 10.2, *P*<0.05) were also significant.

4.4.2.2 QOL score changes across the study:

FACT-An scores:

A general trend of increases in each component score were noted for both groups throughout the study period. Despite this, the mean score changes were only significant in the OI group between EWB scores on the day of surgery (DOS) and at final follow up (OPD) (*P*<0.05, see Figure 18). In the IVI group, however, all components increased significantly during at least one inter-visit period with the exception of SWB, and six of the 8 components increased from both recruitment (REC) to DOS and from DOS to OPD (see Figure 18b).

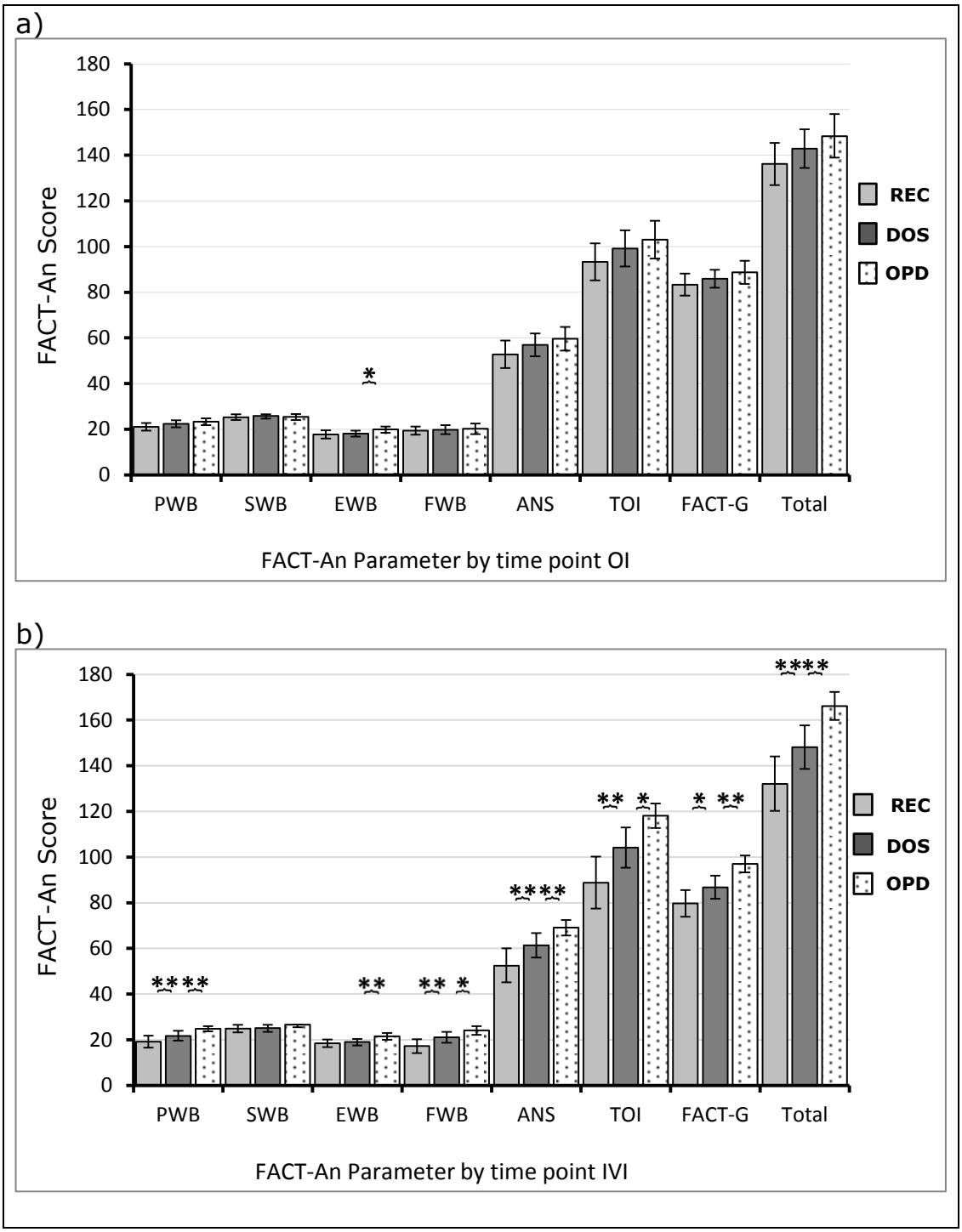


Figure 18 - FACT-An Mean scores at each time point for a) Oral Iron (OI) and b) Intravenous Iron (IVI).

Where: REC= Recruitment, DOS=Day of Surgery, OPD= Outpatient Department appointment. PWB= Physical Wellbeing, SWB= Social/Family Wellbeing, EWB= Emotional Wellbeing, FWB=Functional Wellbeing, ANS=Anaemia Subscale, TOI= FACT-An Trial Outcome Index, Fact-G= Fact G total score, FACT Total= FACT-An Total Score. * Denotes significance to P<0.05, and ** to P <0.01. 95%CI represented by error bars.

Despite the differences in intragroup responses, there remained little intergroup variation with only 4 of the components being significantly higher in the IVI group that OI at OPD (see Table 24).

Field	Time	Oral Iron	IV Iron	*P*
PWB [Max 28]	REC	21.1 (19.4-22.7)	19.2 (16.5-21.8)	0.896
	DOS	22.4 (20.8-23.9)	21.7 (19.6-23.9)	0.847
	OPD	23.3 (21.8-24.8)	24.8 (23.7-26)	0.213
SWB [Max 28]	REC	25.2 (24-26.5)	24.9 (23.2-26.5)	0.485
	DOS	25.9 (24.6-26.6)	25.1 (23.5-26.6)	0.378
	OPD	25.4 (24.1-26.7)	26.6 (25.5-27.7)	0.148
EWB [Max 24]	REC	17.7 (15.9-19.5)	18.5 (16.8-20.1)	0.553
	DOS	18.1 (16.8-19.4)	19 (17.5-20.4)	0.209
	OPD*	19.9 (18.5-21.2)	21.5 (20-23)	0.033
FWB [Max 28]	REC	19.4 (17.6-21.2)	17.23 (14.2-20.3)	0.329
	DOS	19.8 (17.9-21.8)	21.1 (18.7-23.4)	0.363
	OPD**	20.2 (17.9-22.5)	24.1 (22.2-26)	0.001
ANS [Max 80]	REC	52.8 (46.8-58.9)	52.4 (45.2-60)	0.466
	DOS	57 (51.9-62)	61.4 (56-66.7)	0.08
	OPD**	59.6 (54.4-64.8)	69.1 (65.7-72.5)	0.002
TOI [Max 136]	REC	93.3 (85.2-101.4)	88.8 (77.5-100.2)	0.905
	DOS	99.2 (91.3-107.1)	104.1 (95.3-113)	0.273
	OPD**	103 (94.7-111.3)	118.1 (112.7-123.4)	0.003
Fact G [Max 108]	REC	83.3 (78.5-88.2)	79.7 (73.9-85.5)	0.896
	DOS	85.9 (82-89.9)	86.8 (81.7-91.9)	0.285
	OPD**	88.7 (83.7-93.8)	97 (93.3-100.7)	0.003
FACT Total [Max 188]	REC	136.2 (126.9-145.4)	132.1 (120.2-144.1)	0.073
	DOS	142.9 (134.4-151.3)	148.1 (138.6-157.7)	0.172
	OPD**	148.3 (139-158)	166.1 (160-172.3)	0.005

Table 24 - FACT-An Mean scores for each group at each time point.

***Where*: IV= Intravenous, REC= Recruitment, DOS=Day of Surgery, OPD= Outpatient Department appointment. PWB= Physical Wellbeing, SWB= Social/Family Wellbeing, EWB= Emotional Wellbeing, FWB=Functional Wellbeing, ANS=Anaemia Subscale, TOI= FACT-An Trial Outcome Index, Fact-G= Fact G total score, FACT Total= FACT-An Total Score. * Denotes significance to *P*<0.05, and ** to *P* <0.01. 95%CI represented within parentheses, Maximum total score for each parameter indicated within box parentheses.**

EQ5D scores:

Individual component scores were comparable between both groups at each time point as illustrated in Table 25. VAS scores were significantly higher at DOS and at OPD in the IVI group.

Field	Time	Oral Iron	IV Iron	*P*
Mobility	REC	4.33 (4.1-4.6)	4.18 (3.8-4.5)	0.921
	DOS	4.64 (4.4-4.9)	4.26 (3.9-4.6)	0.299
	OPD	4.36 (4.1-4.6)	4.23 (3.8-4.6)	0.57
Self-Care	REC	4.84 (4.7-5)	4.89 (4.7-5)	0.945
	DOS	4.98 (4.9-5)	4.97 (4.9-5)	0.38
	OPD	4.81 (4.6-5)	4.89 (4.7-5)	0.742
Usual Activity	REC	4.26 (4-4.5)	3.97 (3.6-4.3)	0.717
	DOS	4.4 (4.2-4.8)	4.29 (4-4.6)	0.749
	OPD	4.26 (4-4.5)	4.41 (4.2-4.7)	0.27
Pain and Disability	REC	4.02 (3.7-4.3)	3.71 (3.3-4.1)	0.841
	DOS	4.24 (4-4.5)	4.18 (3.8-4.5)	0.134
	OPD	4.45 (4.2-4.7)	4.47 (4.2-4.7)	0.93
Anxiety and Depression	REC	4.29 (4.1-4.5)	4.21 (3.9-4.5)	0.348
	DOS	4.38 (4.2-4.6)	4.4 (4.2-4.8)	0.941
	OPD	4.6 (4.4-4.8)	4.79 (4.6-5)	0.117
Visual Analogue Score	REC	65.1 (58.4-71.8)	64.89 (57.6-72.1)	0.829
	DOS*	68.9 (63.9-74)	73.29 (67.8-78.8)	0.013
	OPD**	70.9 (65.1-76.8)	82.53 (77.8-87.2)	0.001

Table 25 - EQ5D mean score comparisons between groups.

Where: IV= Intravenous, REC= Recruitment, DOS=Day of Surgery, OPD= Outpatient Department appointment. 95%CI represented within parenthesis. *denotes significance P<0.05, ** denotes significance P<0.01

On review of intragroup changes in each component, the only significant incremental changes within the OI group was in Mobility scores, with a significant rise evident between REC and DOS, and decrease from DOS to OPD (P<0.01) (see Figure 19a & Table 27).

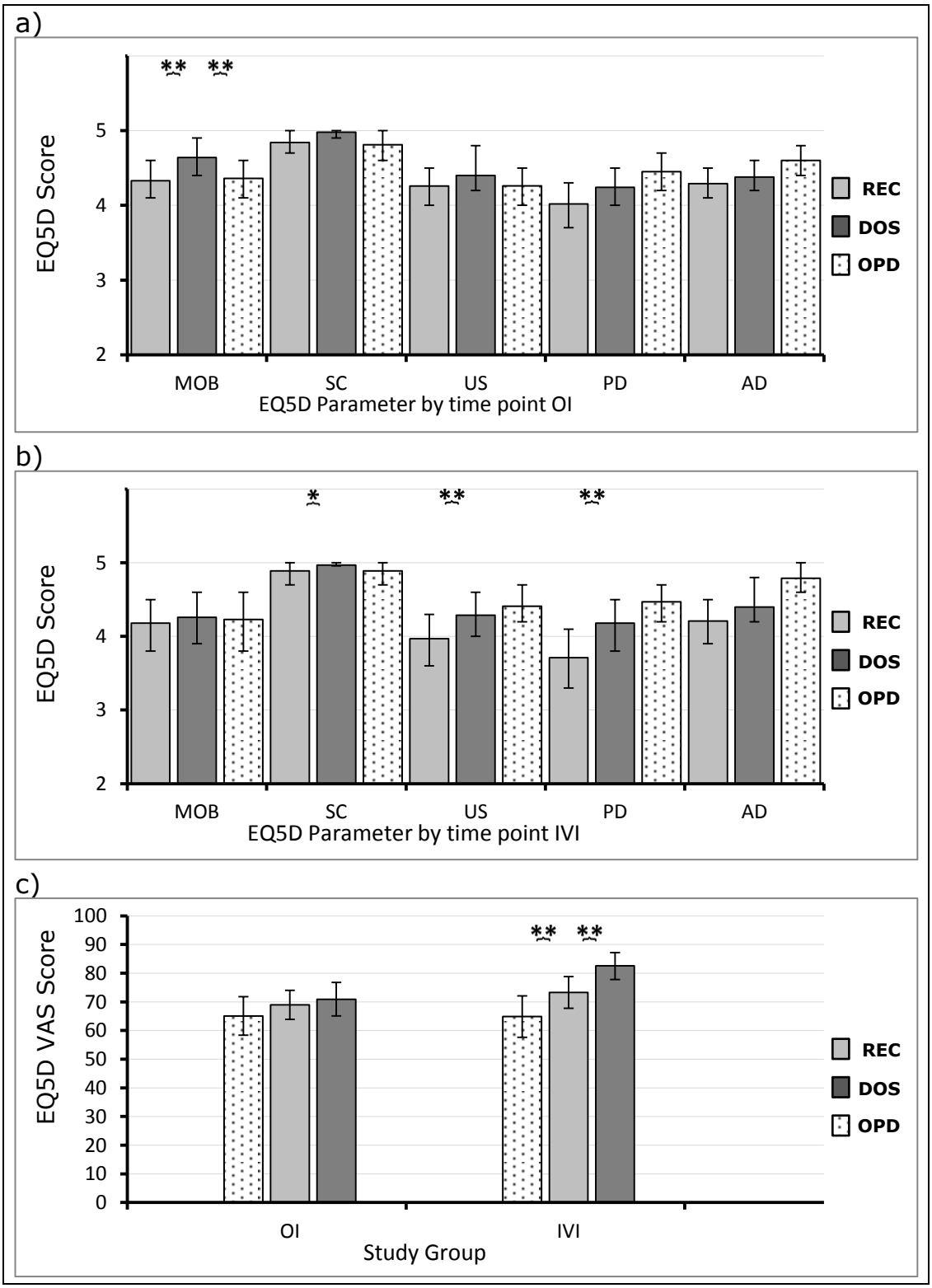


Figure 19 - Changes in EQ5D component scores at each time point for a) Oral Iron (OI) and b) Intravenous Iron (IVI) and c) Comparison of Visual Analogue Scores (VAS) for both groups.

Where: MOB= Mobility, SC= Self Care, US= Usual Activity, PD=Pain and Disability, AD= Anxiety and Depression, *denotes significant change of P<0.05 and ** of P<0.01. Error bars display 95%CI.

In contrast, significant increases were seen in the IVI group in 3 of the specific components (Self-care, $P<0.05$; Usual activities, $P<0.01$, Pain/Disability $P<0.01$) between REC and DOS. VAS also increased significantly between each time point (see Figure 19).

SF-36 scores:

Significant differences were evident between groups in all components at OPD with the exception of Bodily Pain (BP) and the Physical Component Summary (PCS). Additionally, significant differences were noted in Vitality scores at DOS (see Table 26).

Within the OI group, only the Mental Component Summary (MCS) score between DOS and OPD showed a significant increase (see Table 26). In contrast, within the IVI group, all factors significantly increased between DOS and OPD. Furthermore, General Health (GH, $P<0.05$), Vitality (VT, $P<0.01$), Social Functioning (SF, $P<0.01$) and MCS ($P<0.05$) increased significantly from REC to DOS (see Table 26). Figure 20 illustrates the changes in the SF-36 summary components across the study time points.

Field	Time	Oral Iron	IV Iron	*P*
Physical Functioning	REC	61.35 (51.4-71.2)	60.1 5 (48.1-72.2)	0.589
	DOS	63.88(54-73.7) *P=0.735*	61.43 (49.9-72.9) *P=0.154*	0.887
	OPD	64.11 (54-74.2) *P=0.237*	71.95 (62.2-81.7) *P<0.05**	0.377
Role Limitation due to pain	REC	41.91 (26.2-57.7)	45.83 (27.6-64)	0.713
	DOS	38.97 (23-54.9) *P=0.924*	44.17 (27.8-60.6) *P=0.297*	0.34
	OPD**	48.37 (48.4-50) *P=0.502*	77.5 (63.3-917) *P<0.01***	0.01
Bodily Pain	REC	66.88 (58.2-75.6)	61.77 (51.4-72.2)	0.981
	DOS	68.03 (58.8-77.2) *P=0.641*	65.67 (55.5-75.9) *P=0.056*	0.3
	OPD	75.71 (67.4-84.1) *P=0.053*	81.23 (73.1-89.4) *P=0.001***	0.229
General Health	REC	62.74 (58.2-67.3)	61.7 (54.3-69.1)	0.776
	DOS	64.88 (59.7-70.1) *P=0.112*	64.95 (59-70.9) *P<0.05**	0.758
	OPD**	64.79 (58.8-70.8) *P=0.652*	73.63 (68.4-78.8) *P<0.01***	0.002
Vitality	REC	46.91(38.8-55)	43.61 (34.9-52.3)	0.625
	DOS*	53.89 (45.3-61) *P=0.177*	56.83 (48.1-65.6) *P=0.000***	0.048
	OPD**	60.49 (53.7-67.3) *P=0.065*	74.83 (69.2-80.5) *P<0.01***	0.00
Social Functioning	REC	68.02 (59.4-76.6)	65.83 (53.9-77.8)	0.159
	DOS	69.49 (61-78) *P=0.716*	72.92 (61.3-84.6) *P<0.01***	0.349
	OPD*	76.47 (66.6-86.3) *P=0.391*	90 (83.7-96.3) *P<0.01***	0.03
Role limitation due to emotion	REC	50 (34.5-65.5)	63.33 (45.6-81)	0.849
	DOS	58.82 (43.1-74.5) *P=0.631*	61.67 (44.7-78.6) *P=0.202*	0.261
	OPD*	67.65 (51.8-83.5) *P=0.230*	85.56 (73.9-97.2) *P<0.05**	0.03
Mental Health	REC	73.65 (68.8-78.5)	76.8 (71.8-81.8)	0.947
	DOS	74.35 (68.5-80.2) *P=0.539*	80.53 (75.4-85.7) *P=0.080*	0.178
	OPD**	81.77 (77.4-86.1) *P=0.093*	91.33 (88-94.7) *P<0.01***	0.00
Physical Component Summary	REC	43.38 (39.8-46.9)	41.5 (36.9-46.1)	0.71
	DOS	43.4 (39.9-46.9) *P=0.915*	42.26 (38.3-46.3) *P=0.060*	0.678
	OPD	44.04 (408-47.3) *P=0.889*	47.96 (44.5-51.4) *P<0.01***	0.119
Mental Component Summary	REC	45.62 (42.3-48.9)	47.9 (44.4-51.5)	0.773
	DOS	47.69 (43.9-51.5) *P=0.352*	50.75 (47.4-54.2) *P<0.05**	0.1
	OPD**	51.9 (48.5-55.3) *P<0.05**	58.11 (56-60.2) *P<0.01***	0.001

Table 26 - SF36 mean scores for each group at each time point.

Where: IV= Intravenous, REC= Recruitment, DOS=Day of Surgery, OPD= Outpatient Department appointment. 95%CI represented within parenthesis. P values within group columns represent change from previous time point. *denotes significance to P<0.05, and ** to P<0.01

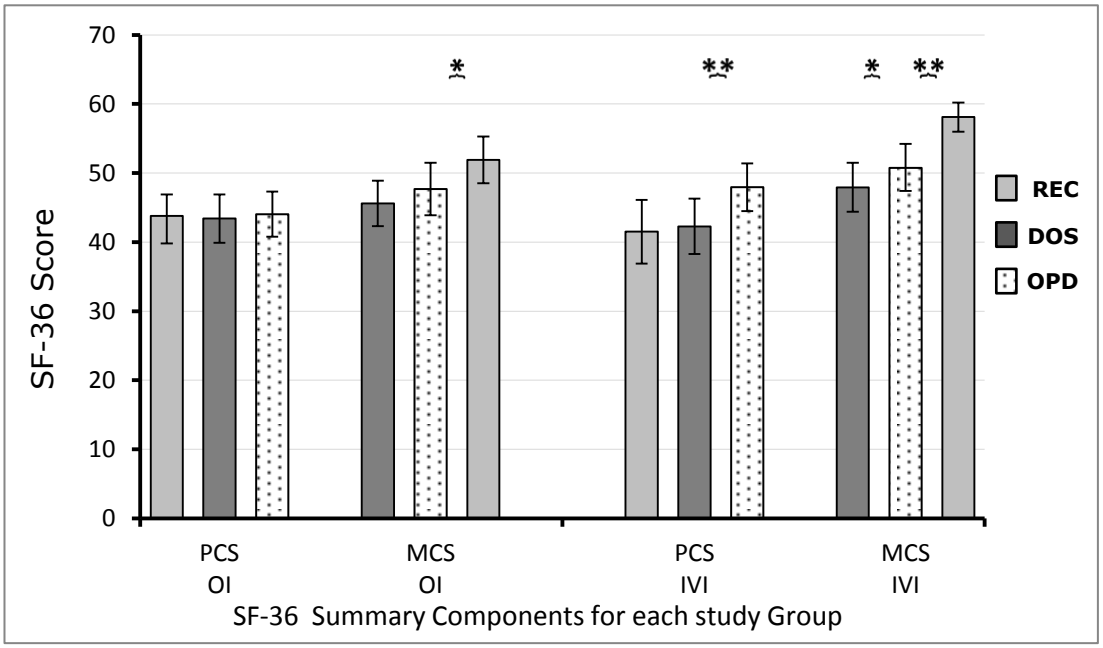


Figure 20 - Changes in mean SF36 summary scores between Recruitment (REC) and Day of Surgery (DOS) and Outpatients (OPD) for Oral Iron (OI) and Intravenous Iron (IVI).

Where PCS= Physical Component Summary, MCS= Mental Component Summary. *denotes significant change of P<0.05 and ** of P<0.01. Error bars display 95%CI.

4.4.2.3 Magnitude of Clinical Effect:

On comparison of scores for all components which showed a significant intragroup change from REC to DOS, only changes seen within the IVI group were of a magnitude to meet a MCD (see Table 27).

QOL	Component	Group	REC to DOS change (u)	*SD*	Effect Size	Effect Grade
FACT -AN	Physical Well Being	IVI	2.5	5.47	0.46	Small
	Functional Well Being	IVI	3.87	6.52	0.59	Moderate
	Anaemia Subscale	IVI	9	16.14	0.56	Moderate
	Trial Outcome Index	IVI	15.3	24.96	0.61	Moderate
	FACT-G	IVI	7.1	13.01	0.55	Moderate
	FACT-Total	IVI	7.1	27.24	0.26	Small
EQ 5D	Mobility	OI	0.31	1.08	0.29	Small
	Self-Care	IVI	0.08	0.51	0.16	NCD
	Pain and Disability	IVI	0.47	1.01	0.47	Small
	Visual Analogue Score	IVI	8.4	19.93	0.42	Small
SF36	General Health	IVI	3.25	20.26	0.16	NCD
	Vitality	IVI	13.22	23.79	0.56	Moderate
	Social Functioning	IVI	7.09	29.49	0.24	Small
	Mental Component Summary	IVI	2.85	9.34	0.31	Small

Table 27 - Evaluation of magnitude of clinical effect for component scores which increased significantly between Recruitment (REC) and Surgery (DOS).

Where: OI= Oral Iron, IVI=Intravenous Iron, SD=Standard Deviation of REC scores, and NCD=No clinical difference.

4.4.2.4 Complications:

There were 127 complications experienced by the entire cohort who had undergone surgery (*n*=110). In the OI group, 30 patients (52.6%) experienced a complication until the 7th postoperative day (D7), 33 patients (57.9%) until the end of postoperative day 28 (D28), and 40 patients (70.2%) until OPD. This equated to a mean of 0.93 complications [95%CI 0.63-1.23] per patient by D7, 1.05 complications [95%CI 0.75-1.36) by D28 and 1.28 [95%CI 0.98-1.58] complications by OPD.

This did not significantly differ in comparison with the IVI group in either patient number (D7, *n*=25[47.2%], *P*=0.567; D28, *n*=32[60.4%], *P* =0.791; OPD *n*=33[62.3%], *P*=0.38) or mean complication number (D7 mean 0.68 95%CI 0.63-1.22, *P*=0.199; D28 mean 1.03 95%CI 0.69-1.39, *P*=0.748; OPD mean 0.98 95%CI 0.66-1.31, *P*=0.585).

There were 9 deaths in the entire cohort over the duration of the study period (OI *n*=4; IVI *n*=5) which was not statistically different between groups (*P*=0.87). The same was true of 90 day mortality (*P*=0.91; OI *n*= 2; IVI *n*=3). There was no difference in grade of complication severity between groups (D7 *P*=0.692; D28 *P*=0.599;

OPD P=995). Table 28 illustrates the comparison of major complications between groups.

Time Point	Oral Iron	Intravenous iron	P value
Up to D7	3	4	0.624
Up to D28	3	5	0.400
Up to OPD	5	8	0.305

Table 28 - Patient numbers experiencing major complications from surgery to specified time period.

Where: D= Post- operative day, OPD= Outpatients Department final follow up.

Infective complications were more frequent in the IVI group, with 15 patients (28%) experiencing complications by D7 and 21 (39.6%) patients by D28. This compared with by 9 patients (15.8%) by D7 and 14 patients (24.6%) by D28 for OI. This difference, however, was non-significant (*P*=0.112 and *P*=0.09 respectively), as was the grade of these complications (D7 *P*=0.106; D28 *P*=0.083). Comparison of the most common infective complications is illustrated in Table 29.

Infection site	Oral Iron	IV Iron	*P* value
Wound	11	15	0.47
Lower Respiratory Tract	7	11	0.43
Urinary Tract	9	6	0.53
Sepsis of unknown origin	4	4	0.94

Table 29 - Percentage frequencies of commonest infective complications across the entire study.

Haemorrhage:

At REC platelet levels were similar between group (OI, 334 $x10^9$/L [IQR 269-375]; IVI 326 $x10^9$/L [IQR 243-389], *P*=0.65) and remained unchanged to DOS for OI (322 $x10^9$/L [IQR 248-393], *P*=0.21). In the IVI group, platelet count did significantly decrease (259 $x10^9$/L [IQR 220-337], *P*<0.01) such that at DOS, platelet count was significantly lower with IVI (*P*<0.05).

Despite this blood loss remained comparable between groups (see Table 17, Chapter 4.3). There were only 2 patients who experienced major haemorrhage (both IVI). These patients both had Platelet counts within the 1st centile for the entire cohort on the DOS, but were not statistical outliers and were within the local laboratory "normal" range. One was also taking oral anticoagulants preoperatively. Furthermore, Platelet count on the DOS did not correlate with blood loss (*Rs*=-0.056, *P*=0.613).

Risk modification:

CR-Possum scores improved from REC to DOS in 14 patients (23%) with OI compared to 24 patients in IVI (44%, *P*<0.05).

4.4.2.5 Length of hospital stay:

The median postoperative LOS for all patients successfully discharged from hospital was 6 days for both groups (OI [IQR 4-9] days; IVI [IQR 5-10], *P*=0.95). This did not change when including in-patient deaths as point of discharge (OI, 6 days [IQR 4-10]; IVI 6 days [IQR 5-10], *P*=0.86).

4.4.3 Discussion:

4.4.3.1 Quality of Life:

Comparison of QOL scores in non-blinded clinical trials is potentially vulnerable to patient perception on which treatment is more efficacious. The correlation of key component scores with HB levels and anaemia severity would indicate that any pre-conceived opinion was not a key confounder, and indicated a stronger causal link with HB changes. This essential observation therefore ensures a higher degree of validity for subsequent analysis and linking of QOL change with treatment intervention.

The 3 QOL measures utilised in the present study were selected to provide validated assessment of general health (EQ-5D and SF36) and more specific measurement of QOL related to malignancy and anaemia (FACT-An). It was noted that QOL scores across these were generally comparable between OI and IVI groups at REC and DOS with only 2 of the 24 measures assessed differing significantly at DOS (VT and VAS). However, by the point of OPD, intergroup differences were noted for 13 parameters (EQ5D: VAS; FACT-AN: EWB, FWB, ANS, TOI, FACT-G, FACT-Total; SF36: GH, VT, SF, RE, MH, MCS).

It is possible that no difference existed between groups at DOS as the 21 day median interval between REC and DOS was insufficient to allow a difference to manifest. This short period may be exacerbated by the measurement period that each QOL tool assesses over. The FACT-An instructs patients to report based on the *"past 7 days"* and the SF-36 on the "*past 4 weeks"*. Consequently, this reporting period may overlap time prior to treatment and entry into the trial, and thus reduce the magnitude of any change in QOL scoring. The consequence of this may have been to reduce differences seen between groups at DOS which only became evident at OPD.

Previous studies have described improvement in QOL in anaemic patients following treatment of anaemia with OI and IVI (Gisbert et al., 2009). However, this change was not evident with OI before 90 days in a study of anaemic patients with heart failure (Manjunath, 2013) and was seen at 43 days in patients with renal failure receiving IVI (Agarwal et al., 2006).

It is important to note that significantly more patients received adjuvant chemotherapy in the OI group. Such treatments are recognised to have deleterious impacts upon QOL (Chen et al., 2015), hence may have confounded any differences evident at

OPD. This possibility is substantiated by the finding of comparable HB levels between groups at OPD (see Chapter 4.2) hence it remains possible that these differences in QOL were secondary to other factors.

In the present study, significant intragroup changes between REC and DOS which met a MCD were only evident within the IVI group (Table 27). *Small* improvements were seen in 7 components across broad aspects of QOL, with *moderate* improvements seen in components more specific to anaemia. Some authors have argued that an effect size in excess of 0.5[SD] is required to demonstrate significant clinical change in QOL (Norman et al., 2003, Copay et al., 2007), a value based on both psychological assessment of the confines of human discrimination (Miller, 1956) and also by clinical validation (Norman et al., 2003). This increase would equate to treatment effect grade of *moderate* in the current study. Measures of Vitality (SF-36), Functional Well-Being and specific scores of anaemia symptomatology (ANS, FACT-AN) still met this higher threshold of MCD and hence changed significantly even over the short period from REC to DOS. These components would appear to be closely linked to anaemia and thus also HB levels, hence such a finding is congruous with the observations from Chapter 4.2 whereby increased changes in HB level were apparent from REC to

DOS within the IVI group. Similarly, the modest rises in HB within the OI group could account for the limited improvements in QOL.

Overall, the findings of the present study would indicate that IVI is more efficacious than OI at improving aspects of QOL. Although intergroup differences were minimal, this was potentially masked by a general trend to increase scores between each time point for each treatment arm. These increases were only clinically significant within the IVI group implying an increased efficacy compared to OI. Employing the highest threshold for a minimum clinical difference, this improvement is limited to QOL aspects most directly linked to HB levels, but does appear to have smaller clinical effects on several measures of physical and mental well-being.

4.4.3.2 Complications:

Previous studies have indicated that increasing severity of anaemia is associated with increasing frequency and severity of postoperative complications in colorectal patients (Gu et al., 2013, Leichtle et al., 2011). As a consequence, the current study hypothesised that IVI may be more efficacious at treating anaemia and thus reduce complication rates in comparison with OI.

Such a relationship did not appear to exist as similar numbers of complications and number of patients affected by complications were observed between groups. Furthermore, there was no difference in the severity of these complications.

It is possible that this is secondary to a Type II error, as on the day of surgery, 87% of surgical patients across the entire group remained anaemic (see Chapter 4.2). Although the risk of complications has been previously demonstrated to increase with increasing severity of anaemia, if most patients were in the mild anaemia category, then the increased risk would not be of sufficient magnitude to result in any clinical change within the sample size of this study.

The parity in complications is potentially reassuring for clinical IVI use as a demonstration of non-inferiority to OI. Concerns have been raised that IVI may increase post-surgical complication rates, particularly those related to infection. Iron has been shown to increase bacterial virulence (Telang et al., 2001b, Lee et al., 1979) and proliferation which is believed to be secondary to increasing the bioavailability of iron for pathogenic organisms and not just the host patient (Nairz et al., 2010).

In the current study, the rate of infective complications were slightly higher in the IVI group, albeit not-significantly. However, the definition used for "infective complications" was potentially problematic. It was not appreciated during the design of the study that so many patients would receive empirical antibiotic therapy without laboratory confirmation of infection. A clear example of this is wound infection, which was recorded and included within the study but was often diagnosed solely on clinical grounds before treatment initiated. This occurred mainly for infections which were managed by primary care following discharge from hospital, which adds a level of complexity for trial design. As a consequence this issue requires further investigation.

4.4.3.3 Mortality and Risk:

The increase in HB levels resulted in more patients having a reduced CR-Possum operative risk score. This theoretical model would imply that IVI was more efficacious at reducing perioperative mortality risk than OI, despite similar overall risk scores. This reduced risk did not translate into clinical practice, with similar mortality rates between groups at both 90 days and study cessation.

Previous investigation of the link between anaemia and post-operative mortality has indicated that this is both a direct relationship in the short term (Carson et al., 1996) but also indirect in the longer term as a consequence of increased ARBT requirements and their associated deleterious effects (Acheson et al., 2012). Follow up of the current study cohort 2 years post recruitment would therefore be of interest to further investigate whether the use of IVI has an effect on mortality.

4.4.3.4 Haemorrhage:

It was interesting to note that IVI was associated with reductions in platelet count on DOS, which mirrors observations from recent studies (Hazara and Bhandari, 2015, Yessayan et al., 2014). The mechanism behind this is poorly understood (Hazara and Bhandari, 2015) but hypotheses include improved treatment of IDA, thus reducing a potential drive for thrombocytosis (Dan, 2005). This may account for the observed differences between the OI and IVI group, due to differences in the efficacy of the drugs in treating iron deficiency (see Chapter 4.3).

Irrespective, this observed difference is on unproven clinical effect in the current study. Platelet count was unrelated to overall blood

loss, which is not unexpected as it is platelet *function* is an essential component of coagulation and not just absolute platelet *count* (Page et al., 2007, Rubak et al., 2015). Such an area is investigated further in Chapter 5.

Another facet to this issue relates to the proposed extended role in inflammation and cellular immunity that platelets may perform. Platelets have been demonstrated to be involved in the immune response to bacterial infection (Cox et al., 2011) and as important modulators of the inflammatory response (Morrell et al., 2014, von Hundelshausen and Weber, 2007, Speth et al., 2013). If such relationships are true, then this raises more issues for debate regarding potential detrimental effects of IVI, and in particular, increased risk of infective complications.

4.4.4 Conclusions:

Intravenous iron appears to be more efficacious at improving QOL scores of anaemic patients undergoing CRC surgery, particular when measuring aspects reflective of anaemia related symptoms. These results must be interpreted with caution due to the unblinded nature of the trial, but indicate another possible advantage of IV Iron

IV iron also appeared to be more effective at reducing preoperative risk prediction scores (CR-POSSUM). This occurred as a consequence of increased incremental rises in HB levels than resulting from OI use. However, this reduced risk prediction did not translate to any differences in postoperative complication rates or severity.

Chapter 4.5:

Investigation of potential biomarkers and prediction of response to iron therapy.

4.5.1 Background:

Biomarkers are defined as:

"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".

(Atkinson et al., 2001)

There are several different types including diagnostic, prognostic, pharmacodynamic and predictive (Sawyers, 2008).

Diagnostic markers use a measureable criterion with which to diagnose a disease. The most relevant example in the current study would be the use of Transferrin Saturation (TSAT) to diagnose iron deficiency anaemia. Prognostic biomarkers allow the prediction of a disease outcome, differentiating between individuals with an expected good and poor outcome (Sawyers, 2008) and include tests such as oestrogen receptor status in breast cancer (Kok et al., 2012). Pharmacodynamic biomarkers are of main importance in drug design and development (Mizuarai et al., 2009).

Predictive biomarkers are of most relevance in the current context as they are measured tests which may be used to estimate a response to an intervention or drug (Gallo et al., 2011). A secondary aim of the current trial was to assess such potential biomarkers.

4.5.1.1 Hepcidin:

The important link between CRC and hepcidin has been previously discussed in Chapter 1. In summary, CRC is an inflammatory state which may increase hepcidin levels as an acute phase protein (Pusatcioglu et al., 2014) or by direct production of the hormone (Ward et al., 2008a, Roberts et al., 2008). As hepcidin has been identified as a key hormone controlling iron absorption and mobilisation (Zhang and Rovin, 2013, Nakanishi et al., 2012, Deschemin and Vaulont, 2013), changes in these levels secondary to CRC may influence response to iron supplementation.

Firstly, the hormone could reduce the efficacy of oral iron by reducing absorption from the gut. Such a theory is consistent with healthy volunteer studies, whereby serum hepcidin levels showed an inverse relationship with iron absorption (Young et al., 2009). Similarly, in patients with dysmetabolic iron overload syndromes,

elevated levels of hepcidin were associated with reduced oral iron absorption (Ruivard et al., 2009). Consequently, the predictive potential of hepcidin levels has been demonstrated in patients with IDA treated with oral iron (Bregman et al., 2013).

This role does remain disputed as in pathological states, such as the anaemia associated with renal disease, hepcidin levels were not predictive of response to oral iron (Coyne, 2011).

It is possible that patients may not respond to OI but still respond adequately to IVI (Bregman and Goodnough, 2014) if the impact of elevated levels hepcidin is greater on iron absorption than iron mobilisation. Similarly, if hepcidin exerts a greater effect on iron mobilisation, the converse could be true and high levels could be predictive of non-response to IVI. In patients with renal failure, this predictive role was found to exist (Chand et al., 2014) which would substantiate this hypothesis.

The complex role of hepcidin in iron metabolism remains evident, as documented in Chapter 3, whereby hepcidin levels where not associated with response to IVI in the context of CRC related anaemia (Keeler et al., 2014). This indicates a need for further evaluation, and if it is demonstrated that an inverse relationship

between hepcidin and response to OI exists in CRC, then hepcidin may prove a useful biomarker to predict which patients would benefit from initiation of IVI as first line therapy (Nemeth, 2010), providing elevated levels did not also predict non response to IVI.

4.5.1.2 Erythropoietin:

Erythropoietin (EPO) is a glycoprotein hormone released from the kidney in response to hypoxia (Bunn, 2013) with the primary function of maintaining a constant, appropriate HB levels in normal and haemorrhagic states (Jelkmann, 2011). As HB levels rise, negative feedback mechanisms reduce EPO release, which in turn reduces the proliferation of colony-forming units-erythroid (CFU-E) which would normally differentiate producing proerythroblasts, normoblasts, and eventually erythrocytes (Jelkmann, 2011). Conversely, elevated EPO levels are expected in IDA with the aim of restoration of normal HB levels and oxygen delivery (Ward et al., 1971, Miller et al., 1990).

In the current study, it was hypothesised that the magnitude of response to iron therapy would be dependent on EPO levels as evident in the Pilot study (Chapter 3). It was thought that replenishment of the iron deficit alone would be insufficient to treat

anaemia if EPO levels were insufficient to drive erythroid production and prompt utilisation of these stores.

4.5.1.3 Helicobacter Pylori:

Helicobacter Pylori (HP) is a gram-negative spiral bacillus which is able to infect and remain lifelong within the mucosa of the host stomach (Blaser and Atherton, 2004). The prevalence of infection within the population is highly varied, affected by factors such as age, socioeconomic status and geographical location (Robinson and Atherton, 2009, Logan and Walker, 2001). For patients in the western world between the ages of 50 to 70 years, the infection rates are up to 40-60% (Logan and Walker, 2001).

Links between HP Infection, CRC and IDA have been proposed. Gastric inflammation caused by chronic HP infection can cause increased stimulation of gastric G-cells to secrete gastrin (Robinson and Atherton, 2009). This is accentuated by the simultaneous reduction in local somatostatin release which would normally inhibit gastrin release (Robinson and Atherton, 2009). Gastrin has been implicated as a stimulus for colonic epithelial proliferation in both *in vitro* and animal studies (Sirinek et al., 1985, Chu et al., 1992), hence indicates a potential stimulus for neoplastic mucosal

transformation. Such hypothesis has prompted investigations which have demonstrated a positive association between HP infection and colonic neoplasia (Zumkeller et al., 2006, Zhao et al., 2008).

The association of IDA with HP infection (Lee, 2007) is also of direct relevance to the current study. The mechanism behind this is thought to be a combination of 3 factors. Firstly, HP induced gastritis is thought to reduce gastric acid and ascorbic acid secretion, thereby raising intraluminal pH (Annibale et al., 2003). The importance of acidic gastric secretions in keeping ingested iron in the more readily absorbed Ferrous form is recognised to optimise iron bioavailability (Fuqua et al., 2012). Consequently, HP infection may reduce iron absorption and cause IDA.

This absorption may be further exacerbated by iron sequestration by HP within the stomach. A novel study identified behavioural differences between the HP organisms of patients with and without IDA, whereby the HP in IDA sufferers was shown to have increased iron uptake and iron utilisation (Yokota et al., 2008). This would imply that there was less iron available for the host to absorb.

Other mechanisms are potentially less relevant in the asymptomatic patient, and include chronic blood loss from HP

associated peptic ulceration (Majumdar et al., 2011) or gastric adenocarcinoma (Robinson and Atherton, 2009).

Overall HP seems to reduce iron absorption and increase losses causing IDA. Combining this concept with the principal of hypergastrinaemia, it is possible to formulate two hypotheses. Firstly, it is possible that the prevalence of HP infection will be higher in the CRC cohort recruited to this study than compared to the general population, as a result of selection of patients with both anaemia and CRC. Secondly, it is possible that HP infection may be a predictive biomarker of non-response to oral iron.

4.5.1.4 C-Reactive Protein:

C-Reactive Protein (CRP) is an acute phase protein that is released in response to inflammation and trauma and hence is a sensitive marker of these processes (Pepys and Baltz, 1983). As established, malignancy may generate a systemic inflammatory response, and hence CRP may become elevated in this setting (Wang and Sun, 2009). CRP levels were found to correlate with hepcidin levels in studies of surgical inflammation (Park et al., 2012), and this same relationship was noted in the Pilot trial (Chapter 3). This may imply

that CRP is a more readily available, cheaper alternative to hepcidin as a biomarker.

4.5.1.5 Summary Hypotheses:

It is postulated that:

- Hepcidin will be a predictive biomarker for response to oral and potentially IV iron.
- CRP levels will be associated with hepcidin levels, hence will be indicative of response in the same manner.
- Threshold erythropoietin levels will be needed to be reached to mount a response to iron therapy.
- H. Pylori serology positivity will predict non-response to oral iron, but not IV.

4.5.2 Specific Methodology:

Stored samples of serum collected at REC and DOS were used for analysis (see section 4.2.6 for collection and storage methodology).

4.5.2.1 Hepcidin Assay:

Hepcidin serology was analysed using the S-1328 Hepcidin-25 (human) Enzyme-Linked Immunosorbent Assay (ELISA) Test Kit (BACHEM™, Peninsula Labaratories, USA). All manufacturer recommended protocols (number III) were followed and are outlined below as described in the product protocol which is summarised below:

Preparation of components:

1. All kit components were left at room temperature for 1 hour to equilibrate.
2. The Enzyme Immuno-Assay Buffer (EIA) was diluted in 1L of sterile deionised water.
3. 1ml of Standard diluent buffer was added to the supplied vial of lyophilised standard peptide and mixed with a vortex agitator.

4. Serial dilutions of the standard were performed to cover the range required as set out in the kit protocol.
5. The antiserum was prepared by diluting the kit sample with 5ml EIA buffer.
6. The lyophilised biotinylated peptide tracer (BT-tracer) was prepared by addition of the sample provided in the kit to 5ml of the EIA buffer.
7. The EIAF substrate was created by mixing 90% FS1 Substrate solution and 10% FS2 substrate solution.

Serum Extraction:

1. Equal measures of Buffer A (1% trifluoroacetic acid) was added to the same volume of thawed serum samples, and centrifuged at 10000g for 20 minutes at 4^0C.
2. The supernatant was transferred to a new tube and any pellet formed was discarded.
3. A SEP-column was equilibrated by washing with 1ml of Buffer B, followed by three washes with 3ml of Buffer A.
4. The serum solution was then loaded into the SEP-column, and the column washed twice with 3ml of Buffer A.
5. 3ml of Buffer B was added to elute the peptide and collected in a polypropylene tube

6. The eluent was then freeze dried for 1.5-2 days in a freeze drier and the residue dissolved in EIA buffer for analysis.

Sample analysis:

1. 50µl of diluent and 25µl Enzyme Immuno-Assay Buffer (EIA) was added to blank wells
2. 50µl of "standard" in diluent and 25µl of antiserum in EIA buffer was then added to designated sample wells.
3. 50µl of test sample in diluent and 25µl of antiserum in EIA buffer was then added to test sample wells.
4. The test plate was then incubated at room temperature for 60 minutes.
5. 25µl of rehydrated BT-tracer was added to each well and left for a further 2 hours to incubate at room temperature.
6. The immunoplate was then washed with EIA buffer as directed.
7. 60µl of centrifuged streptavidin-HRP was then diluted in 12ml of EIA buffer and mixed. 100µl aliquots of this solution were then added to each well, before incubation at room temperature for a further hour. At the end of this period, the wells were washed a further 5 times with EIA buffer.

8. 100µl of the kit TMB solution was added to each well followed by a further 60 minutes of incubation at room temperature.
9. 100µl of 2N hydrochloric acid was added to each well to terminate the reaction and the microplate was then taken immediately to a 450nm light reader to measure the absorbance level. Each well was tested, with the mean value used from each duplicate test sample. From this value, the enzyme immunounit (EIU) value was calculated.

4.5.2.2 H Pylori assay:

H Pylori serology was analysed by ELISA using the Helicobacter Pylori IgG BIOHIT™ Test kit (BIOHIT™, Finland). This was performed using the following procedure as outline in the kit protocol described below:

1. The subject serum was mixed using a vortex agitator for 10 seconds, and 5 microlitres of each serum sample was added to 995 microlitres of test Diluent Buffer (containing blocking Protein, the non-ionic detergent Tween® 20, the preservative 0.1% ProClin 300, and red dye). This solution

was then mixed again using the Vortex agitator for 10 seconds.

2. 100 microlitre aliquots of this mixture were then pipetted into the test Microplate wells in duplicate. This was a framework coated with partially purified *H. Pylori* bacterial antigen. Dedicated wells were also filled with 100 microlitres of plain Diluent Buffer (thus a Blank sample to provide a baseline reading), 100 microlitres of Positive Control (pre-prepared IgG solution for positive value assurance), 100 microlitres of Negative Control (pre-prepared IgG solution for negative value assurance) and also 100 microlitres of pre-prepared Calibration liquid for comparator purposes. The plate was then incubated at 37^0C for 30 minutes.
3. Upon removal from the incubator, the Microplate wells were washed with Washing Buffer (Phosphate buffered saline containing Tween® 20 and 0.1% ProClin 300) which had been 10-fold diluted with distilled water.

   This process would remove all material from the wells, with the exception of any *H. Pylori* IgG antibodies which were present in the sample and had bound to the antigen coating of the Microplate wells.

4. 100 microlitres of the Conjugation solution were then added to each Microplate well, and the Microplate again incubated at 37^0C for 30 minutes. This Conjugation solution was a stabilising buffer of 0.02% methylisothiazolone, 0.02% bromonitrodiaxone, and 0.002% active isothiazolone preservatives, containing Horse Radish Peroxidase (HRP) conjugated monoclonal anti-human IgG which would bind to the *H Pylori* IgG antibodies which were present in the sample, and were now bound to the *H Pylori* antigen coating the well.
5. Following this period of incubation, the excess, unbound solutions were washed from the Microplate wells using the Washing Buffer. 100 microlitres of the test Substrate, an aqueous solution containing tetramethylbenzidine, was then added to the wells. Any HRP conjugated to the monoclonal anti-human IgG present within the wells would then oxidise this solution causing a blue discoloration. This process was allowed to occur in a dark environment for 30 minutes.
6. At the end of this period, the enzymatic reaction was halted with the addition of 100 microlitres of the Stop Solution (0.1 mol/L sulphuric acid) to each Microplate well, turning all "positive" samples yellow.

7. The Microplate was then taken immediately to the Microplate reader and tested using 450nm and 590nm light wavelengths. Each well was tested, with the mean value used from each duplicate test sample. From this value, the enzyme immunounit (EIU) value was calculated as illustrated in
8. Figure **21**. Serum H. Pylori positivity was equated to values in excess or equal to 30 EIU, indicating the presence of *H. Pylori* antibodies within the sample serum.

$$\frac{\text{Mean Sample AV} - \text{Blank AV}}{\text{Calibrator AV- Blank AV}} \times 100 = \text{Sample EIU}$$

Where: AV denotes Absorbance value and EIU denotes enzyme immunounit.

Figure 21 - **Calculation of the enzyme immunounit (EIU) value.**

4.5.2.3 <u>Erythropoietin and CRP Assay:</u>

These were performed in line with local NHS laboratory protocols, with CRP tested locally and EPO transferred to regional NHS laboratory testing centres. All test laboratories had GLP accreditation.

4.5.2.4 Statistical Analysis:

Statistical analysis was performed in line with methodologies described in section 4.2.1.1. Additional analyses were also performed using SPSS® version 21 (SPSS, Chicago, Illinois, USA).

Non-parametric data was transformed into normally distributed data by calculating the Log^{10} of the variable to allow one way ANOVA with Post Hoc Bonferroni calculation to be applied.

Multivariate linear regression analysis was performed for relevant variables including Gender, H Pylori status, iron formulation, duration of therapy, HB, MCV, Ferritin, TSAT, EPO, Hepcidin and CRP at REC in relation to HB changes from REC to DOS. This was performed for the entire cohort, and for each iron formulation individually. Potential biomarkers were identified as those which were significant to $P<0.05$ in multivariate analysis.

For assessment of potential biomarker sensitivity and specificity, Receiver Operating Characteristic curves (ROC) were produced assessing individual variables in relation to a positive response in HB from REC to DOS exceeding 1.5g/dL. This threshold has been used in previous studies as a marker of clinically significant HB response to iron therapy (Keeler et al., 2014).

The generated area under the curve (AUC) was assessed for accuracy using the following recognised ranges:

≥0.90-1 = Excellent
≥0.80-<0.90 = Good
≥0.70-<0.80 = Fair
≥0.60-<0.70 = Poor
0.50-<0.60 = Fail

ROC curves were only examined further if scoring at least "fair" for accuracy and reaching statistical significance of $P<0.05$. If this was achieved, the coordinates of the curve were examined to identify predictive thresholds which would achieve optimal specificity and specificity. This threshold was then applied to study patients and the following calculations performed:

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive + False Negative}}$$

$$\text{Specificity} = \frac{\text{True Negative}}{\text{False Positive + True Negative}}$$

$$\text{Positive Predictive Value (PPV)} = \frac{\text{True Positive}}{\text{True Positive + False Positive}}$$

$$\text{Positive Predictive Value (NPV} = \frac{\text{True Negative}}{\text{False Negative + True Negative}}$$

4.5.3 Results:

4.5.3.1 Biomarker Overview:

EPO, hepcidin and CRP levels were comparable between groups at REC and DOS (see Table 30) with the exception of EPO which was significantly lower on DOS in the IVI group (*P*<0.01).

Test	Time	Oral Iron	Intravenous Iron	*P* value
EPO mu/ml	REC	29.9 (19.1-49.9)	28.6 (16.5-71)	0.719
	DOS*	21.2 (13.7-35)	11 (8.6-16.6)	<0.01
Hepcidin ng/ml	REC	2.66 (1.29-6.37)	0.822 (0.328-4.94)	0.564
	DOS	3.43 (1.24-9.24)	7.08 (1.26-29.72	0.098
CRP g/ml	REC	8.5 (0-32.5)	6 (0-21.5)	0.617
	DOS	12.5 (0-37.8	9 (0-26.5)	0.822

Table 30 – A table illustrating changes in serum levels of Erythropoieitn (EPO), Hepcidin and C-Reactive Protein (CRP) between Recruitment (REC) and Day of Surgery (DOS).

Where: *denotes intergroup statistical differences to P<0.05.

EPO levels did reduce from REC to DOS within each group (OI, *P*<0.01; IVI, *P*<0.01)(see Figure 22a). Only hepcidin levels within the IVI group changed significantly from REC to DOS (*P*<0.01)(see Figure 22b)

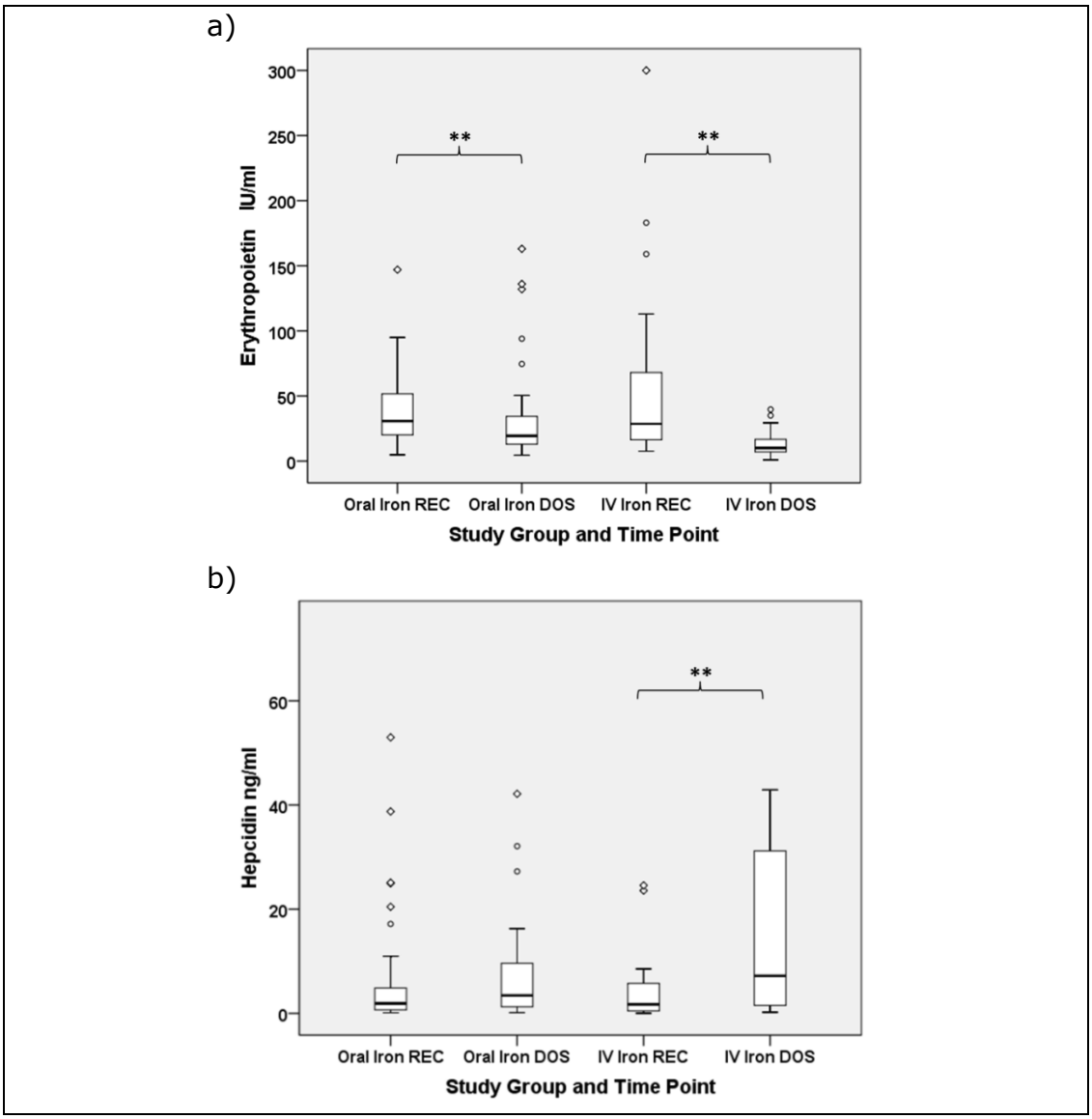


Figure 22 – Erythropoietin (a) and Hepcidin (b) level changes following treatment with either oral iron (OI) or intravenous iron (IV).

Where: REC=Recruitment, DOS=Day of surgery, and **denotes statistical significance to P<0.01.

Across the entire cohort, Log^{10} EPO (F=19.801, P<0.01), Log^{10} Hepcidin (F=4.37, P<0.01) and Log^{10} TSAT (F=20.639, P<0.01) levels were significantly different with increasing severity of anaemia. Post Hoc Bonferroni correction demonstrated significant differences between all grades of anaemia for EPO, and with severe anaemia for Hepcidin and TSAT (see Table 31).

Variable	Grade Anaemia	Grade Anaemia	Mean Diff	Std. Error	*P*	95% CI	
						Lower	Upper
Log^{10} EPO	Non	Mild*	-.22398	.0752	.020	-.4244	-.0236
		Mod**	-.44419	.0741	.000	-.6416	-.2468
		Sev**	-.82208	.1427	.000	-1.202	-.4418
	Mild	Non*	.22398	.0752	.020	.0236	.4244
		Mod**	-.22020	.0500	.000	-.3536	-.0868
		Sev**	-.59810	.1318	.000	-.9494	-.2468
	Mod	Non**	.44419	.0741	.000	.2468	.6416
		Mild**	.22020	.0500	.000	.0868	.3536
		Sev*	-.37790	.1312	.026	-.7275	-.0283
	Sev	Non**	.82208	.1427	.000	.4418	1.2023
		Mild**	.59810	.1318	.000	.2468	.9494
		Mod*	.37790	.1312	.026	.0283	.7275
Log^{10} HEP	Non	Mild	.25250	.1955	1.000	-.2694	.7744
		Mod	.38364	.1922	.285	-.1295	.8968
		Sev**	1.40303	.4096	.005	.3098	2.4963
	Mild	Non	-.25250	.1955	1.000	-.7744	.2694
		Mod	.13114	.1191	1.000	-.1868	.4491
		Sev*	1.15053	.3808	.017	.1342	2.1669
	Mod	Non	-.38364	.1922	.285	-.8968	.1295
		Mild	-.13114	.1191	1.000	-.4491	.1868
		Sev*	1.01940	.3791	.047	.0075	2.0313
	Sev	Non**	-1.4030	.4096	.005	-2.496	-.3098
		Mild*	-1.1505	.3808	.017	-2.166	-.1342
		Mod*	-1.0194	.3791	.047	-2.031	-.0075
Log^{10} TSAT	Non	Mild	.10161	.0793	1.000	-.1097	.3129
		Mod**	.38859	.0775	.000	.1821	.5951
		Sev**	.78807	.1463	.000	.3981	1.1780
	Mild	Non	-.10161	.0793	1.000	-.3129	.1097
		Mod**	.28698	.0512	.000	.1505	.4235
		Sev**	.68647	.1343	.000	.3287	1.0443
	Mod	Non**	-.38859*	.0775	.000	-.5951	-.1821
		Mild**	-.28698*	.0512	.000	-.4235	-.1505
		Sev*	.39948*	.1332	.018	.0445	.7545
	Sev	Non**	-.78807*	.1463	.000	-1.178	-.3981
		Mild**	-.68647*	.1343	.000	-1.044	-.3287
		Mod*	-.39948*	.1332	.018	-.7545	-.0445

Table 31 - Bonferroni Post Hoc comparison of Biomarkers levels in each WHO severity grade of anaemia using 1 way ANOVA.

Where: Log10=Logarithmic variable value, EPO= Erythropoietin, HEP= Hepcidin, TSAT= Transferrin Saturation, Non= Non anaemia, Mod=Moderate anaemia, Sev=Severe anaemia, Mean Diff= Mean Difference, Std Error= Standard Error, P= level of significance between intergroup comparisons, 95%CI= 95% Confidence Interval.*denotes significant difference to P<0.05, ** denotes significant difference to P<0.01.

Relationships between biomarkers at Recruitment:

Across the entire cohort there was a positive correlation between CRP and Hepcidin levels (R_S=0.323, *P*<0.01) and also ferritin (R_S=0.299, *P*<0.01). CRP was inversely related to TSAT (R_S=-0.245, *P*<0.05). Hepcidin levels were not associated with TSAT (R_S=0.159, *P*=0.129) but were associated with ferritin (R_S=0.53, *P*<0.01).

Prediction of Responses across the entire cohort:

Linear regression analysis of the change in HB, ferritin and TSAT from REC to DOS in relation to key variables (listed in section 4.5.2.4) at REC identified iron formulation as the most significant variable associated with positive changes (see Table 32)

Despite this, ROC analysis of REC Hepcidin levels and positive HB responses showed an AUC 0f 0.7 [95%CI 0.591-0.81] (*P*<0.01) (see Figure 23). The optimal coordinates from this indicated a hepcidin threshold of 1.2495 ng/ml, which yielded a sensitivity of 69%, specificity of 72%, PPV 57%, NPV of 81% for prediction of response.

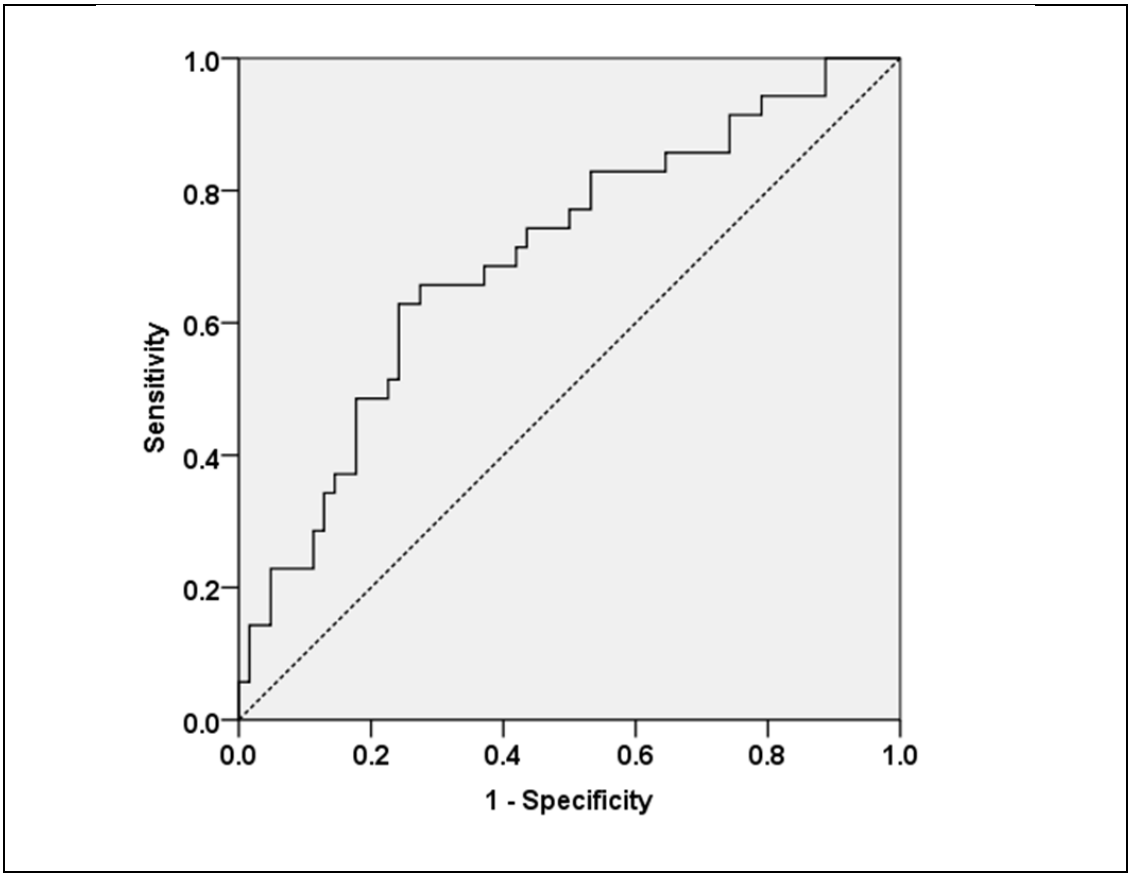


Figure 23 - Receiver Operating Characteristics curve assessing Recruitment Hepcidin levels for the entire cohort with a preoperative Haemoglobin rise of 1.5g/dL.

	Haemoglobin Change			Ferritin Change			TSAT change		
Variable	Unadjusted	Adjusted	*P*	Unadjusted	Adjusted	*P*	Unadjusted	Adjusted	*P*
Entire Cohort:									
Iron type	1.20 [0.69; 1.7]	0.90 [0.44;1.37]	<0.01*	690 [519; 862]	742 [573; 912]	<0.01*	16.5 [8.7; 24.3]	8.72 [2.5; 14.9]	<0.01*
Intravenous Iron Group:									
Duration	0.05 [0.02; 0.08]	0.03 [0.01; 0.06]	<0.01*	-8.64 [-22.1; 4.85]	-5.35 [-19.3; 8.67]	0.440	0.01 [-0.30; 0.33]	-0.11 [-0.43; 0.21]	0.486
EPO	0.17 [0.09; 0.2]	0.01 [0.002;0.02]	<0.01*	-1.16 [-4.82;2.5]	-1.63 [-5.3;2.03]	0.371	0.06 [-0.01;0.15]	0.07 [-0.01; 0.15]	0.112
Oral Iron Group:									
HB	-0.21 [-0.49; 0.08]	-0.64 [-1.27;-0.02]	<0.05*	-4.59 [-36.7; 27.5]	-34.3 [-106; 38.0]	0.336	-1.67 [-7.87; 4.52]	-6.21 [-16.6; 4.20]	0.231
CRP	-0.01 [-0.02; 0.01]	-0.01 [-0.02;-.002]	<0.05*	0.25 [-0.90; 1.41]	0.21 [-0.91; 1.34]	0.696	0.02 [-0.15; 0.21]	-0.16 [-0.31; 0.01]	<0.05*

Table 32 - Linear regression analysis demonstrating significant Recruitment variables with preoperative changes in Haemoglobin, Ferritin and Transferrin Saturation.

Where: unadjusted=univariate model, adjusted=multivariate model, EPO=Erythropoietin, HB=Haemoglobin, TSAT= Transferrin Saturation. Model Coefficient displayed with 95% Confidence Interval in Parenthesis.

4.5.3.2 Predictors of Haemoglobin change for IV Iron:

Linear regression highlighted duration of therapy and EPO levels at REC to be significantly associated with HB change within the IVI group (Table 32).

Duration of treatment:

On review of the impact of duration of IVI therapy and magnitude of HB response, a positive correlation was noted between duration of treatment and absolute HB change between REC and DOS (R_S=0.608, *P*<0.01, see Figure 24). Consequently increasing numbers of responders were evident with increasing duration of therapy (P<0.01), such that 77% of patients exceeding the 21 day median treatment duration responded to therapy (see Table 33).

Duration	Non Responders	Responders	*P value*
<14 days	9 (100)	0 (0)	*P*<0.01*
14-21 days	11(58)	8 (42)	
>21 days	6 (23)	20 (77)	

Table 33 – A table illustrating the number of patients who responded to intravenous iron therapy in relation to duration of treatment.

Where: Response was defined as an HB change of greater than 1.5g/dL between recruitment and surgery. Absolute patient numbers are illustrated with percentages in parenthesis. *Denotes Chi-squared statistical significance for both overall association and linear by linear association.

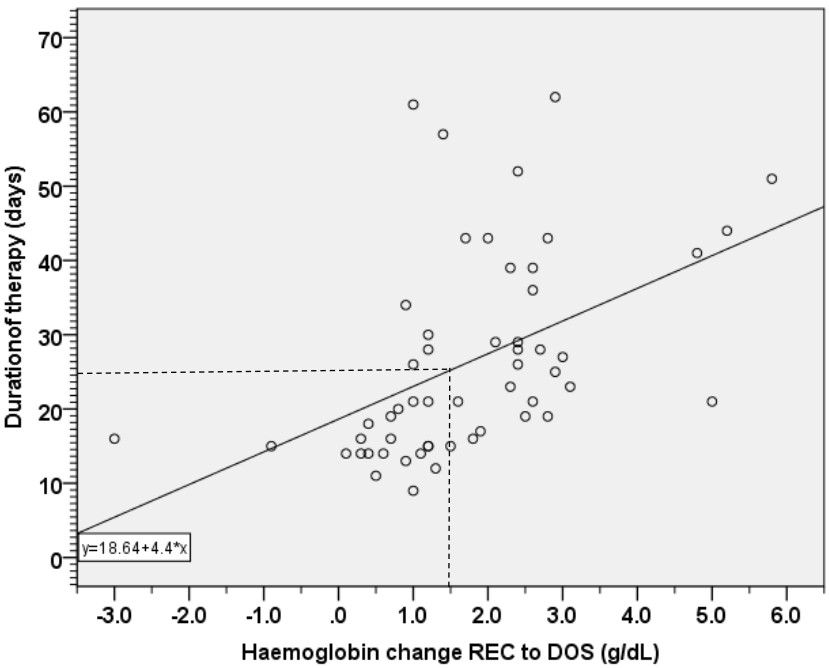


Figure 24 – Scattergraph illustrating the correlation between duration of intravenous iron therapy and haemoglobin change between recruitment and surgery (NB broken line indicates threshold for response).

As evident in Figure 24, it appeared that the duration of therapy required to achieve the threshold HB response (1.5g/dL) was between 20 and 30 days. This value was slightly higher than the median treatment period within the study (21 days [IQR 15-34] days).

Erythropoietin levels:

Twenty six patients exceeded 21 days of treatment within the study. ROC analysis of the EPO levels at REC in these patients in association with a 1.5g/dL treatment induced rise in HB demonstrated an AUC of 0.787 [95%CI 0.596-0.978] (P<0.05), and indicated that a threshold of 24iU/L would provide highest sensitivity and specificity as a predictor of response to IVI. This threshold provided a sensitivity of 72%, specificity of 83%, with a PPV of 93% and NPV of 50%. Of note, only 15% of patients (n=3) in the same oral iron subgroup responded to oral iron with EPO levels above 24IU/L.

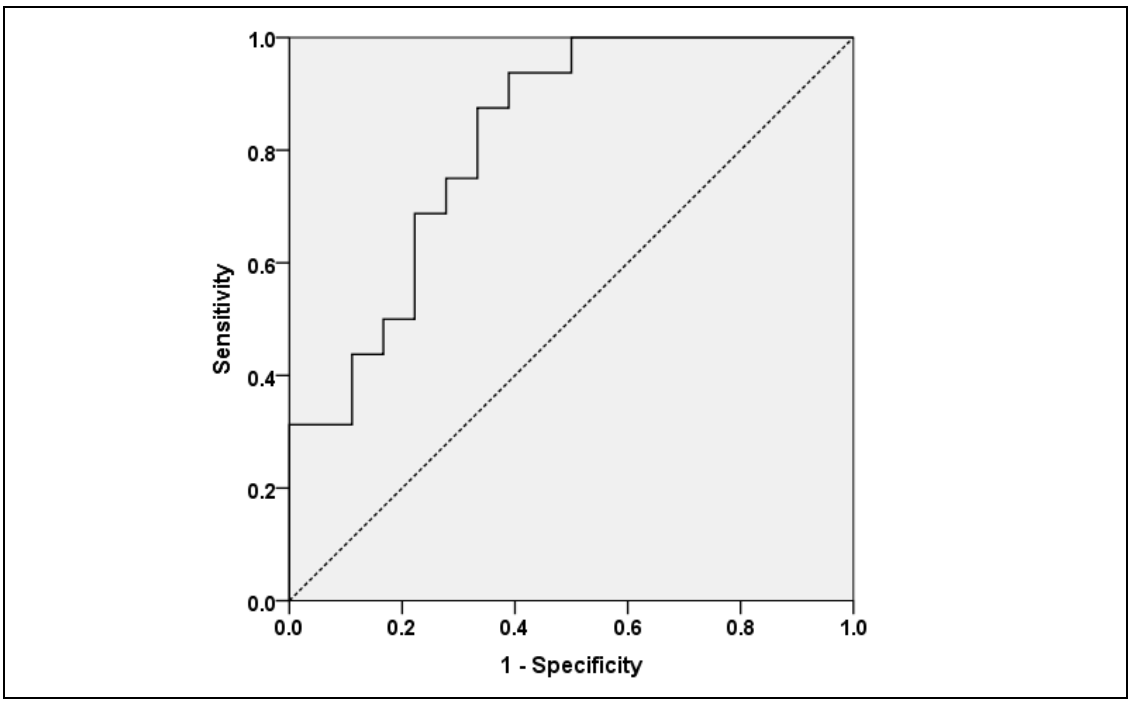


Figure 25 - Receiver Operating Characteristics curve assessing Recruitment Erythropoietin levels in those receiving IVI treatment for over 21 days in relation to threshold Haemoglobin response.

4.5.3.3 Predictors of Haemoglobin change for Oral Iron:

Linear regression analysis (see Table 32) of variables associated with positive changes in HB from REC to DOS showed significance for HB levels at REC ($P<0.05$) and CRP ($P<0.05$). Of note, only 14 patients (23%) experienced a treatment rise in HB of 1.5g/dL in the OI group. Two of these patients received a preoperative blood transfusion, hence were excluded from biomarker analyses.

When REC HB levels were considered in quartile ranges, there was no linear relationship between this and absolute response (overall association $P=0.18$; linear by linear relationship, $P=0.073$; see Figure 26a).

When patients with HB levels in the upper quartile were excluded, ROC analysis of REC CRP levels and HB response showed an AUC of 0.752 ($P<0.05$). Coordinate analysis of this indicated that CRP levels below 10 mg/ml were associated with HB response with a sensitivity of 80% and specificity of 63%. Thirty three patients across the entire OI group had CRP levels below this CRP threshold. When the REC HB levels of these patients were considered in relation to HB response a linear relationship became evident (overall association and linear relationship both $P<0.05$, see Figure 26b).

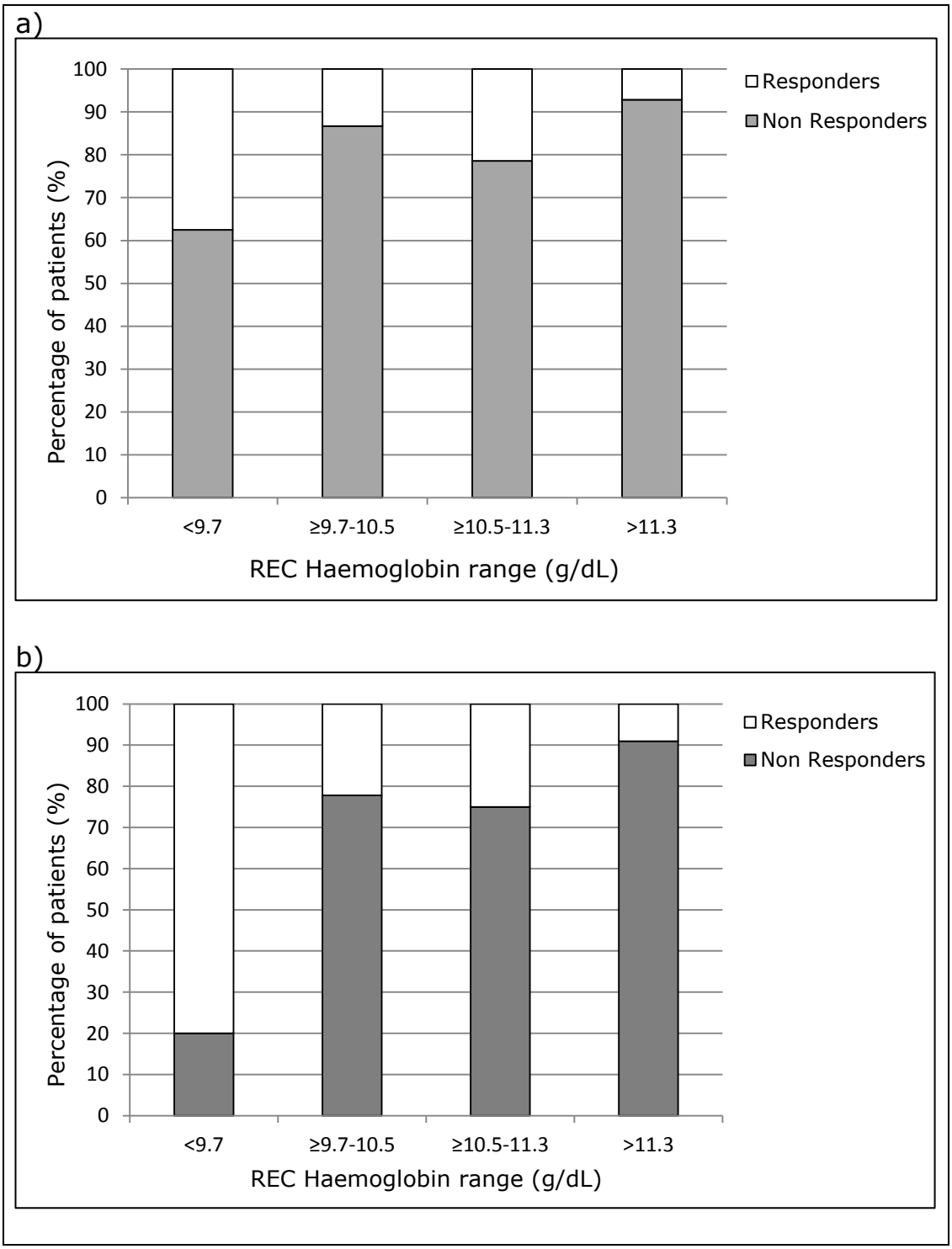


Figure 26 – Bar charts illustrating the percentage of patients within each Haemglobin centile who responded to oral iron treatment for a) All patients (*n*=61), and b) Those patients with CRP levels below 10mg/ml (*n*=33).

Other Biomarker results:

Across the entire cohort the prevalence of H Pylori infection was 27% (*n*=31), though there was no significant difference between infection rates in the OI (*n*=13) and IVI (*n*=18) groups (*P*=0.239).

In linear regression analysis for OI, there was no association with H Pylori infection and HB change (*P*=0.531). Furthermore, infection rates were comparable to those who responded to OI and those who did not (*P*=0.615)

4.5.4 Discussion:

The results from this study have demonstrated the complexities of biomarker evaluation. As identified, when reviewing any particular outcome it is possible to identify multiple influential variables. Distillation of the exact individual causal relationship is extremely challenging and vulnerable to significant error. Consequently, the findings of this part of the study must be interpreted with caution.

One key area of concern relates to the small number of patients involved. The sample size of each study arm were initially small, yet the forced exclusion of many of these patients, together with the relative infrequency of patients achieving the desired treatment threshold, further limited this study size. This must be recognised, and thus the findings should be interpreted as inferences noteworthy of further investigation rather than as robust determinates of clinical practice.

Section 4.3 highlighted that IVI was more effective at treating preoperative anaemia and iron deficiency in the current study setting. This was further reflected in the current results by measurement of the physiological responses to anaemia. It was expected that with more efficacious treatment of anaemia, the

normal hormonal drives to overcome such anaemia, such as EPO, would be reduced, whilst hepcidin levels would rise to prevent further iron absorption and mobilisation. Although both OI and IVI were seen to reduce EPO levels, IVI did so to a greater degree. This is potentially due to the more efficacious treatment of the anaemia, as EPO levels were themselves closely associated with the severity of anaemia (see Table 31).

The reduction in EPO levels did not return levels to a baseline "normal" level, which has recently been proposed as 7.6IU/L [IQR 5.8-9.9] in males and 7.9IU/L [6.0-10.6] in females (Grote Beverborg et al., 2015). This may be reflective of the comorbidity of the current study population but is more likely due to a failure of treatment to return HB levels to "normal" as IVI patient EPO levels returned closer to this normal level than those in the OI group.

Hepcidin levels were also seen to increase with treatment, and to a greater degree with IVI. This would be expected as a normal physiological response to "shutting off" iron absorption and mobilisation (Arezes and Nemeth, 2015). Furthermore, the positive correlation between hepcidin and ferritin levels reflected an expected response to treatment of iron deficiency. Such a relationship has been identified in other studies of IBD

(Mecklenburg et al., 2014) and kidney disease (van der Weerd et al., 2015).

It was therefore interesting to note that hepcidin was not associated with TSAT levels. It would be expected that TSAT would increase as severity of iron deficiency anaemia reduced, hence hepcidin levels would also increase as a result. It is possible that this was not evident due to the influence of inflammation on hepcidin, ferritin and TSAT levels.

Whilst ferritin and hepcidin rise with inflammation (Arezes and Nemeth, 2015, Kell and Pretorius, 2014), TSAT levels generally decrease (Coyne, 2006, Wish, 2006) which is due to relative increases in transferrin levels (Wish, 2006). Such an observation was noted in the present study as TSAT levels were negatively correlated with CRP.

Consequently, it is possible that in the current cohort of patients, the malignancy induced inflammatory response contributed to increases hepcidin and ferritin levels whilst simultaneously decreasing TSAT levels, but affecting each to a differing degree and uncoupling the direct relationship between TSAT, ferritin and hepcidin as a result.

This possibility is reinforced by the two concepts. Firstly, as CRP is known to increase with inflammation (Pepys and Baltz, 1983), it would be expected that as levels of CRP rose, then so too would hepcidin and ferritin levels, whilst TSAT levels would decrease. This was indeed the case in the present study indicating that all three variables were significantly influenced by inflammation independent to iron status. The degree of this influence could conceivably differ between variables.

Secondly, it is believed that hepcidin may be produced from CRC tumours (Ward et al., 2008, Roberts et al., 2008). This may provide a non-physiologically responsive source of hepcidin, such that regardless of anaemic status (and hence TSAT) levels are artificially elevated.

Such observations raise key issues with the use of hepcidin as a predictor of response. Firstly, even if it was a perfect predictive biomarker to predict response to iron treatment, then the close association with far cheaper, more simple and readily accessible tests in the form of CRP or ferritin would make these more preferable in clinical use.

Moreover, despite ROC analysis indicating that it may be a potential tool for use across the entire cohort, the clinical transferability of this is minimal. As established, the main predictor of changes in HB, TSAT and ferritin was in fact iron formulation. The responses to both IVI and OI appear to be significantly different, hence one biomarker will probably be ineffective at predicting a response to "iron".

Intravenous Iron response prediction:

In light of this, a linear regression model was employed to highlight potential areas for focus. Within the IVI group, it appeared that a combination of duration and EPO levels were important. It is unsurprising that duration was essential, given findings documented in Chapter 3 (Keeler et al., 2014) whereby treatment periods of nearly 4 weeks were associated with significant clinical effects. Furthermore, studies have indicated that maximal IVI utilisation takes in the region of 24 days (Beshara et al., 2003). These findings appeared to support the desired treatment period of over 3 weeks that was seen in the present study.

Similarly, the association of increasing endogenous EPO levels with HB response is in line with findings in Chapter 3 (Keeler et al., 2014) and also with other studies evaluating changes in HB in

response to EPO (Goodnough et al., 1994). Furthermore, as recombinant EPO, either as monotherapy or co-administered with IVI supplements, has been advocated for the treatment of preoperative anaemia (Cladellas et al., 2012, Doodeman et al., 2013), it would appear logical for endogenous EPO levels to be influential on HB response.

EPO levels cannot be assumed to be elevated in the context of anaemia, as renal failure (Mercadal et al., 2012), malignancy (Miller et al., 1990), and chemotherapy (Glaspy et al., 2005) are all associated with abnormal endogenous levels. In the current study context, the latter two are of particular relevance.

The predictive threshold proposed for patients treated in excess of 21 days would initially appear to be of satisfactory sensitivity and specificity for purpose. Similarly, the AUC would suggest the model to be fair and statistically significant in this group.

However, the model employed is heavily limited by only reviewing approximately half of the IVI patients (i.e. those over the median 21 day treatment period). This is exemplified by retrospective review of the patients in Chapter 3, whereby the threshold would have only correctly predicted HB response in 65% of the patients.

Oral Iron response prediction:

It is of interest that duration of therapy was not as important in OI treatment as IVI. It is possible that this is because the absorbed OI is of such a small amount that it is difficult to overcome ongoing losses. As demonstrated in Chapter 2, the natural history of untreated HB levels in CRC patients is a gradual decline in the preoperative period. It is thus possible that a balance must be struck between an adequate treatment period to allow HB to rise, yet simultaneously not be excessive allowing ongoing haemorrhage and further depletion of iron stores. This could render patients receiving OI far more susceptible to ongoing losses as a result of the smaller HB responses induced by this treatment.

Previous studies evaluating the response of patients with IDA to OI have indicated that worsening severity of anaemia was associated with increased response (Freire, 1989). Although this was mirrored in the present study to a degree, this finding was limited by the potential influence that inflammation may have on oral iron handling. It has been discussed that patients in the present study are anaemic due to a mixed mechanism of absolute iron deficiency (AID) due to chronic haemorrhage and functional iron deficiency (FID) secondary to inflammation (Ludwig et al., 2013). It is the

FID that distinguishes CRC patients from the AID patients in the aforementioned study.

Consequently, the finding that any predictive model in CRC patients must incorporate not only severity of anaemia but also degree of inflammation is noteworthy. CRP is a recognised measure of inflammation (Pepys and Baltz, 1983), but the use of this as a biomarker has vulnerabilities. CRP is affected by several factors other than inflammation which are as diverse smoking (Ohsawa et al., 2005), nutritional state (Nienaber-Rousseau et al., 2014) and the individual ability to mount an inflammatory response.

Conclusions from the current data set are severely limited by the numbers included for analysis and the specificity of the model proposed. However, it does further indicate a potential group who may benefit from selection for IVI as a treatment for their anaemia, identified by a routinely available test.

The significance of H Pylori infection on response?:

Recent UK based prevalence studies of H Pylori infection in dyspeptic patients have identified infection rates of under 10%.

The prevalence of positive serology was greater in the present study (27%) which is in line with the suggestion that infection rates are higher in patients with CRC (Zumkeller et al., 2006, Zhao et al., 2008). Despite this higher rate, the limited patient numbers limits robust evaluation of H Pylori infection, although it did not appear to influence OI response.

One key mechanism by which H Pylori has been proposed to reduce enteric iron absorption is via reduction in gastric acid secretion (Annibale et al., 2003). It is possible that H Pylori was not found to influence OI response in the present study due to the different patterns of infection that can occur within the stomach. Whilst it is true that H Pylori may reduce gastric acid secretion if infection is pangastric, if the infection is limited to the gastric antrum, then it may actually increase acid secretion (Blaser and Atherton, 2004). Without endoscopic confirmation of the location of infection, it may not be possible to predict which effect H Pylori will have on gastric secretions, and thus on iron absorption.

4.5.5 Conclusions:

Hepcidin has been proposed as a biomarker to predict response to iron treatment (Bregman et al., 2013).The rationale for this is that the hormone has been heavily implicated in all aspects of iron homeostasis. The results from the current study could imply that in the context of IVI, as the absorption of iron is not a limiting factor, then prediction of utilisation is more likely to forecast subsequent response. Utilisation of iron appears both a time dependent process, and is driven by the hormones controlling erythropoiesis, the most essential of which is EPO. These are therefore perhaps the most economical and accurate factors to consider in prediction of treatment response.

Similarly, when predicting response to oral iron, absorption of the drug is probably the most variable factor influencing response. Absorption of iron is governed by hepcidin and inflammation. As inflammation is linked with hepcidin levels, then directly measuring the degree of inflammation may be more efficient. This could then be further considered in relation to the severity of anaemia as governed by the HB value alone.

Consequently, the main conclusion that can be drawn regarding the prediction of response of HB to iron supplementation, is that in *most* patients IVI will be more efficacious than OI. This can be increased further by allowing treatment periods in excess of three weeks, and is more likely to be effective in patients with high endogenous EPO levels. In patients whereby the risk benefit of administration would render IVI unsuitable, such as those with limited time before surgery or known allergy, then OI could be considered. This is more likely to be successful with worsening anaemia, and in the context of normal inflammatory markers. These findings do however allow focus of future work in this field.

4.6 Overall Study Conclusion:

On review of all the findings from the study documented in this section, it would seem that IVI is more efficacious than oral iron at treating anaemia and iron deficiency, and improves key components of the quality of life of patients undergoing colorectal without significant side effects of treatment. These benefits may translate to significant reductions in blood transfusion requirements in selected patient populations in the particularly high risk perioperative period.

Although a definitive proven clinical benefit was not established, when the findings are considered in the context of published literature, particularly data regarding the perioperative risks of anaemia, the study would appear to produce more evidence in favour of preoperative IVI for the management of preoperative anaemia in CRC patients. Unfortunately to reliably delineate which treatment patients should receive requires further study, and is discussed further in Chapter 6.

Chapter 5:

An observational study investigating the effect of platelet function on outcome after colorectal surgery.

Abstract

Introduction

Previous studies have assumed patients have uniform responses to aspirin, yet significant numbers are occult *hypo-* or *hyper-* responders. A new validated test of platelet function measures platelet P-selectin expression, which rises with increased platelet activity. This study investigated the measured perioperative changes in platelet function in response to aspirin, and subsequently whether quantitative variations in platelet activity affected perioperative complication severity and frequency.

Methods

107 patients undergoing major colorectal surgery were recruited and assigned to either control (no antiplatelet therapy) or aspirin groups. P-selectin was measured following platelet stimulation at recruitment prior to cessation of medication, and at surgery before intervention. Perioperative complications, haemoglobin changes and blood transfusions were also recorded.

Results

Platelet function was higher in control (*n*=87) than aspirin group (*n*=20) at recruitment (median 1303u [IQR 1102-1499] vs 77u

[IQR 63.5-113.5], $P<0.01$) and surgery (median 1224u [IQR 944-1496] vs 281.5u [IQR 106.8-943], $P<0.01$). There was a positive correlation between length of aspirin cessation and platelet function at surgery ($R^S=0.66$, $P<0.01$). Complication rates and haemorrhagic complication rates ($P<0.05$) were higher with aspirin than control, although complication severity was not increased. Platelet function of the entire cohort at surgery was not associated with complication rate, severity or transfusion use.

Discussion

Although complication rates were higher in aspirin group, impaired platelet function within ranges seen with aspirin continuation did not affect complication severity or rate or blood transfusion use. Consequently, aspirin continuation may not affect clinical outcome in patients undergoing major colorectal surgery and requires further investigation with a large randomized trial.

5.1 Introduction:

Epidemiological studies have indicated that over 60% of patients over 65 years regularly take aspirin (Ajani et al., 2006). Consequently, many patients due to undergo elective surgery are taking this drug, yet debate still exists regarding if and when aspirin should be discontinued preoperatively.

The argument in favor of cessation relates to the perceived risk of increased operative blood loss. A 0.5 fold increase in haemorrhage associated complications has been proposed when low-dose aspirin is continued until surgery (Burger et al., 2005). Quantitative increases in surgical blood loss have also been demonstrated in this context (Sun et al., 2008) as has increased allogeneic red blood cell transfusion (ARBT) requirements [reviewed in (Ferraris et al., 2005)]. Much of this work has been performed in the context of cardiac surgery, and more recent, small, observational studies in laparoscopic urological (Mortezavi et al., 2013) and gastrointestinal surgery (Ono et al., 2013) have not identified this risk.

Conversely, a "*rebound*" prothrombotic state is thought to transiently occur when aspirin is stopped (Beving et al., 1996).

This may in part contribute to the increase in cardiavascular events which have been evident when aspirin is discontinued (Oscarsson et al., 2010, Biondi-Zoccai et al., 2006).

Current recommendations for surgical practice remain complicated. The American College of Chest Physicians Evidence-Based Clinical Practice Guidelines advocate continuation of aspirin in moderate to high cardiac risk patients in non-cardiac surgery and discontinuation for 7-10 days in low risk patients (Douketis et al., 2012). This Grade 2c recommendation, however, was predominantly based on studies which are limited by the assumption that response to aspirin is uniform and constant. In reality, *hypo-response* to aspirin could be potentially as high as 25% (Hovens et al., 2007) with *hyper-response* in the order of 15% (Coakley et al., 2005, Fiore et al., 1990). Consequently, recent attempts to generate best practice guidance in abdominal surgery were hampered by a lack of quality data (Sahebally et al., 2014)

In response, a new selective test has been developed which measures platelet function and specifically assesses the anti-platelet effect of aspirin and clopidogrel (Fox et al., 2009). This validated blood test measures P-Selectin (PS), a marker

proportionally expressed on the surface of platelets upon activation, such that the higher the PS expression, the higher the platelet function (Fox et al., 2009).

The study aimed to investigate the measured perioperative changes in platelet function in response to aspirin, and subsequently whether quantitative variations in platelet activity across the cohort affected perioperative outcomes including complication rates and severity following major colorectal surgery. It was hypothesized that impaired platelet function at surgery would be associated with increased complication rates.

5.2 Materials and Methods:

Full ethical approval for this observational study was granted from the National Research Ethics Service, Nottingham UK (REF 06/Q2403/137), and was undertaken in accordance with the Declaration of Helsinki.

Patient selection and eligibility:

Consecutive patients were approached for recruitment in 3 discrete four-month periods between September 2011 and January 2014. Patients were identified from preadmission clinic records and inclusion was determined based on a plan to undergo major colorectal surgery. All patients were considered eligible irrespective of age, gender and comorbidity. Patients taking clopidogrel as their antiplatelet regimen or warfarin at recruitment were excluded. Full written consent for participation was confirmed prior to any study interventions.

Study procedures:

Blood samples were collected at three time-points over the course of each patient's treatment. The first recruitment sample (REC) was taken at the preassessment clinic appointment 7-10 days prior to surgery. The second sample was taken on the day of surgery

(DOS) prior to intervention. The final sample was taken on the first postoperative day (D1) coinciding with when the clinical team had requested blood samples for clinical purposes. Haemoglobin (HB) values were obtained at REC and D1and PS values were obtained at REC and DOS.

P-Selectin testing:

A 5ml blood sample was collected in a citrate blood tube. One milliliter aliquots were then transferred to two pre-warmed (37^0C) PS Test vials: "A" and "B". Vial A contained arachidonic acid and epinephrine, which stimulate the cyclooxygenase-1 activation pathway of platelets. This is the pathway that is inhibited by aspirin, hence the vial is used to measure aspirin efficacy. Vial B vial contained no stimulant and acted as the control measure of baseline activation and is used for quality control purposes only.

After 5 minutes incubation the contents of each vial were then transferred to labelled fixing tubes containing PAMFix (Platelet Solutions, Cardiovascular Medicine, University of Nottingham, UK). Once mixed with this solution, the level of platelet activation was arrested and would remain unchanged for up to nine days (Fox et

al., 2009). These fixed samples were stored at room temperature and transferred for Flow Cytometry analysis within this period. Flow Cytometry analysis was performed using previously described techniques (Fox et al., 2009) to yield the median fluorescence value for each sample. Median fluorescence is proportional to PS expression on the platelet surface and thus is a measure of platelet function (Fox et al., 2009).

Additional recording:

Patient demographics, medical history and current medications were recorded at recruitment, including details of aspirin cessation. Operative details were also recorded from relevant logs, including intravenous fluid administration. Blood transfusion records were reviewed to delineate the use of any blood products and patient records were reviewed for the details of any perioperative adverse events and to ascertain the length of hospital admission.

Complications:

Major Adverse Cardiac Events (MACE) were defined as acute myocardial infarction, cardiac arrest, severe arrhythmia, or cardiovascular death within the first 30 postoperative days (Oscarsson et al., 2010). All observed complications were graded

using the validated Clavien-Dindo (CD) scoring system (Dindo et al., 2004). Complications were thus Graded from 1 (minor deviation from normal postoperative course without the need for intervention) to Grade 5 (death) (Dindo et al., 2004). Haemorrhagic complications were defined as any overt signs of abnormal bleeding from any source. These were further defined as "Major" if the associated CD score was 3 or greater (Clavien et al., 2009), i.e. necessitated surgical, endoscopic or radiological intervention; or resulted in organ failure or death, or required intensive care support.

Statistical Analysis:

Reduced platelet function was defined as a median fluorescence below the 75th centile of the recruitment values for the aspirin group. The statistical level of significance for all tests was defined as $P<0.05$. Paired non-parametric data was compared with Wilcoxon signed rank test. Non-parametric independent data was compared with Mann-U Whitney test, and with Kruskal-Wallis test when group numbers exceeded two. Fisher's exact and Chi-squared test was used to assess qualitative differences between groups. Correlations between non-parametric variables were estimated with Spearman's rank test. Binary logistic regression

was used to explore complication occurrence in relation to platelet function accounting for confounders. Statistical analysis was performed using SPSS® version 21 (SPSS, Chicago, Illinois, USA).

5.3 Results:

Patient demographics and antiplatelet use:

Figure 27 illustrates the numbers of patients screened and recruited to the study. One hundred and seven patients were included for review (males=63, females=44).

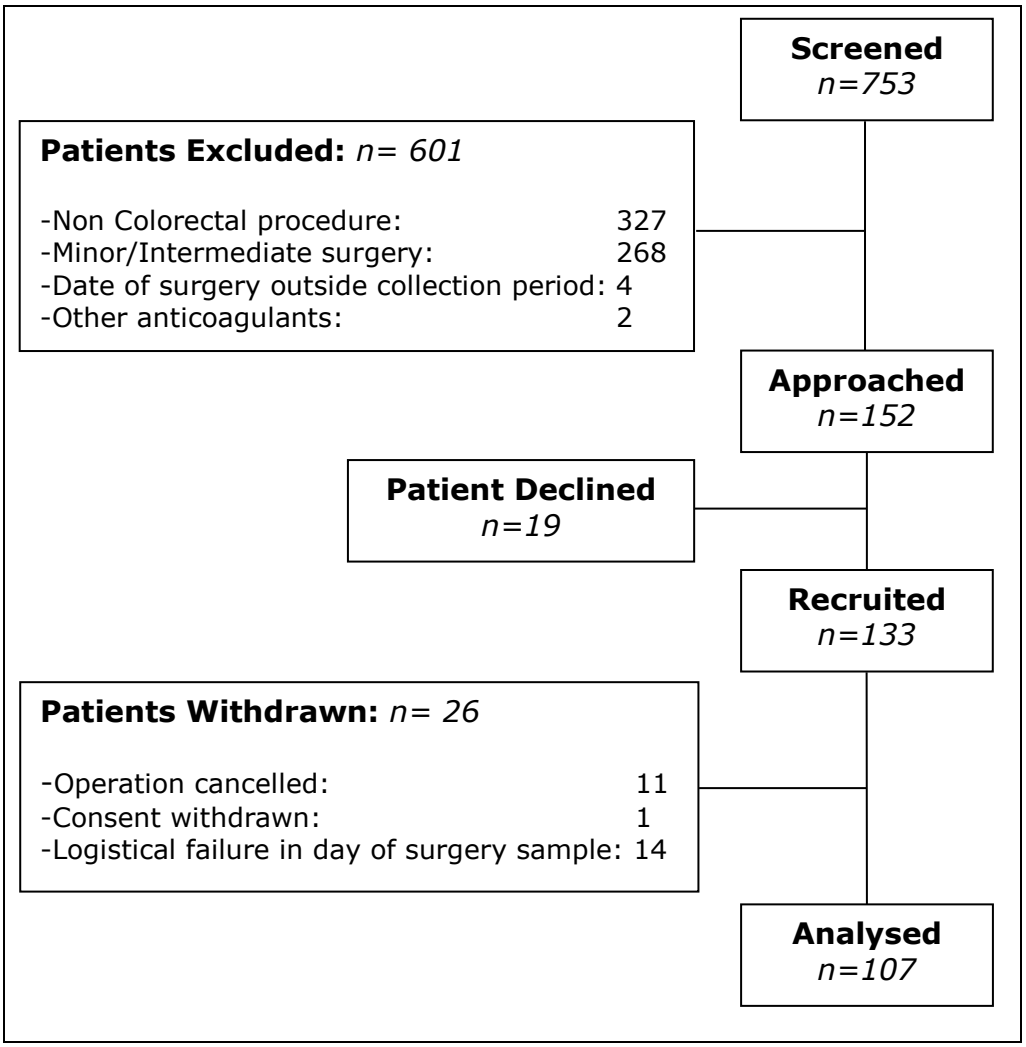


Figure 27 - Flow diagram illustrating patient screening and exclusion details.

Where: n= patient number

Of the entire cohort, 87 were not receiving antiplatelet agents and composed the control group, whilst 20 patients were within the aspirin group. Table 34 illustrates the patient demographics and operative details within each group.

Demographics:	**Control**	**Aspirin**	***P***
Number	87	20	
Males	49 *(56)*	14 *(70)*	0.32
Females	38 *(44)*	6 *(30)*	
Age (median, years)	67.2 *IQR 55.6-75.5*	73.9** *IQR 69-79.7*	*<0.05*
ASA	2 Range 1-3	2 Range 1-3	0.08
Operation:			
Right sided colonic†:	32 *(37)*	6 *(30)*	
Right hemicolectomy	26	6	
Extended Right hemicolectomy	5	-	
Transverse colectomy	1	-	
Left sided colonic†:	19 *(22)*	6 *(30)*	
Left Hemicolectomy	1	1	
Sigmoid colectomy	5	5	
Hartmann's Procedure	9	-	0.71†
Subtotal colectomy	4	-	
Recto-Anal†:	36 *(41)*	8 *(40)*	
Anterior Resection	24	4	
Abdominoperineal excision rectum	2	4	
Panproctocolectomy	4	-	
Reversal of Hartmann's	4	-	
Restorative proctectomy	2	-	
Access:			
Laparoscopic	42 *(48)*	10 *(50)*	0.8
Converted	6 *(7)*	-	
Open	39 *(45)*	10 *(50)*	
Length of Stay			
Days	7 *IQR 4-12*	7 *IQR 6-8*	0.81

Table 34 - Patient demographics and operative details.

Values expressed as medians unless specified, with percentages within parenthesis. All statistical analysis is compared to control group, **denotes significance P<0.05, †denotes analysis assessed between Right sided colonic, Left sided colonic and Recto-Anal groups.

Platelet function variation in each study subgroup:

Of the patients in the aspirin group, 7 continued therapy until DOS, 3 stopped within 5 days of the operation, and 10 stopped at least 5 days prior to surgery. The median cessation period was 4.5 days (IQR 0-7 days). There was a significant correlation between length of cessation and platelet function at DOS (R^S=0.66, P<0.01) which is illustrated in Figure 28.

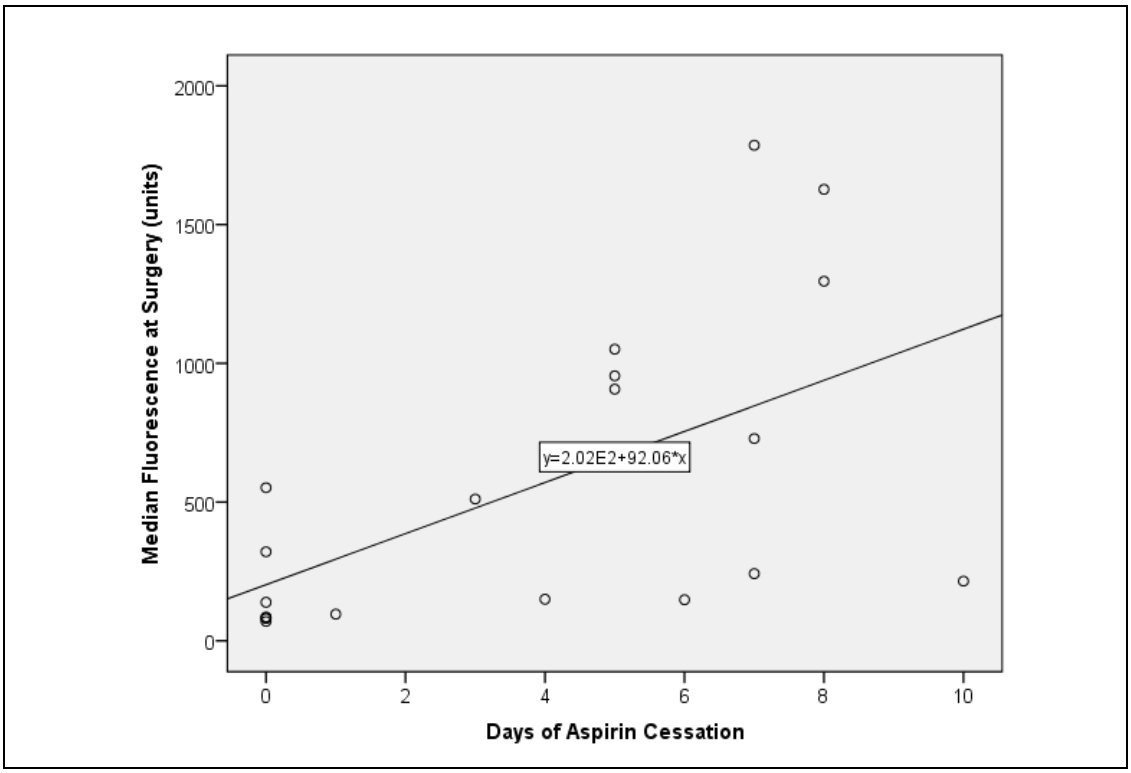


Figure 28 - Platelet function variation in response to duration of aspirin cessation.

At recruitment, control platelet function (median fluorescence 1303u [IQR 1102-1499]) was significantly higher than the aspirin

group (median fluorescence 77u [IQR 63.5-113.5], *P*<0.01). From Recruitment to the DOS, there was no significant change in platelet function in the control group (*P*= 0.11), although platelet function did increase in the aspirin group over this period (*P*<0.05). Platelet function remained significantly higher in the control group at DOS (median fluorescence 1224u [IQR 944-1496] vs 281.5u [IQR 106.8-943], *P*<0.01), irrespective of whether the aspirin had been stopped (*P*<0.05). These changes are illustrated in Figure 29.

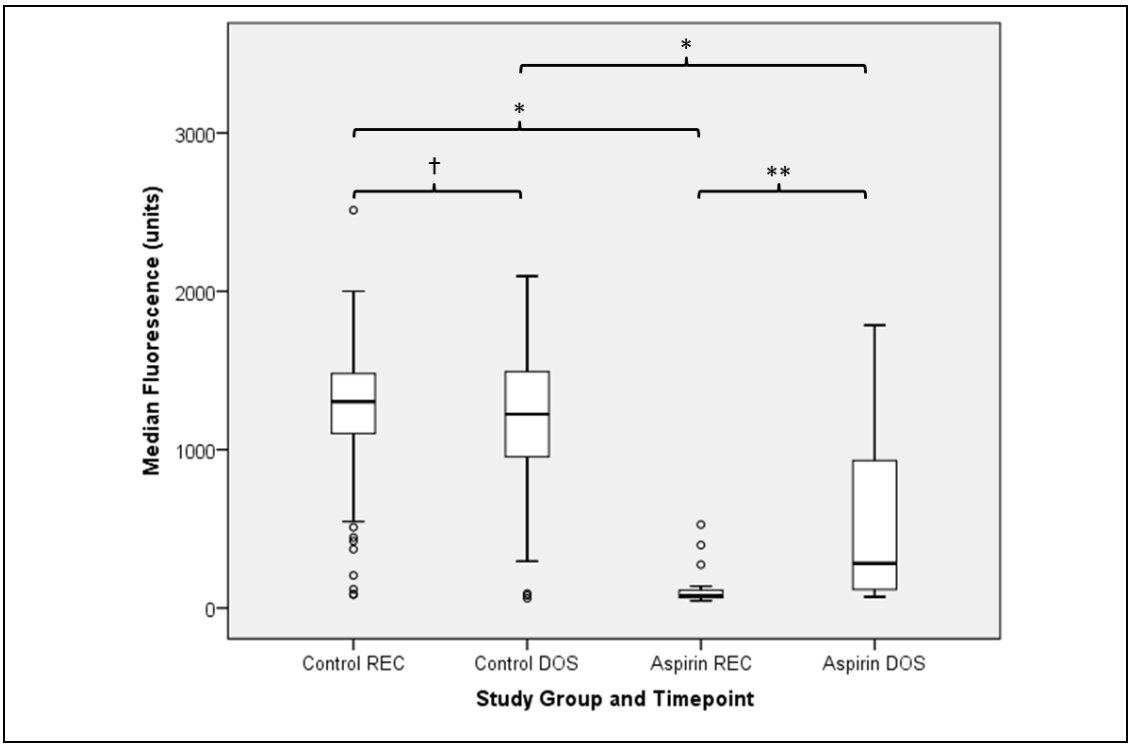


Figure 29 - Box-plot graphs illustrating median fluorescence changes between recruitment (REC) and day of surgery (DOS).

Where: *Denotes significance to P<0.01, ** denotes significance to P<0.05, and † denotes non significance.

Perioperative complications:

Overall, 61 patients experienced 120 complications (see Table 35). The complication rate was significantly higher in the aspirin group compared to control ($P<0.05$). Despite this, there was no difference in severity of complications between groups ($P=0.32$).

	Control	Aspirin	*P*
Overall Complication number	92	28	0.28
Patients with complications	45 (52)	16 (80)	<0.05**
Complication Severity:			0.32
CD Grade 1	9 (10)	6 (30)	
CD Grade 2	20 (23)	7 (35)	
CD Grade 3	5 (6)	2 (10)	
CD Grade 4	11 (13)	1 (5)	
Patients with haemorrhagic complications	3 (3)	4 (20)	<0.05*
Patients with Major haemorrhagic complications	1 (1)	1 (5)	0.34

Table 35 - Complication comparison between groups.

Values expressed are patient numbers with integer percentage in parenthesis, where: CD= Clavien-Dindo contracted scale and ** denotes statistical significance to P<0.05

Within the aspirin group, platelet function on DOS was unrelated to severity ($P=0.46$) or number of complications ($P=0.97$) which was also true for the entire cohort (severity $P=0.18$, number $P=0.31$) (see Figure 30). Regression analysis of platelet function at DOS and occurrence of postoperative complications was non-significant

(OR 0.99 [95%CI 0.99-1], *P*=0.1). The magnitude of this was not materially altered when accounting for confounders ASA, age and gender (adjusted OR 0.99 [95%CI 0.998-1], *P*=0.18).

There was no difference in complication rates (*P*=0.46), complication number (*P*=0.26) and complication grade (*P*=0.47) between those with normal and those with reduced platelet function on DOS.

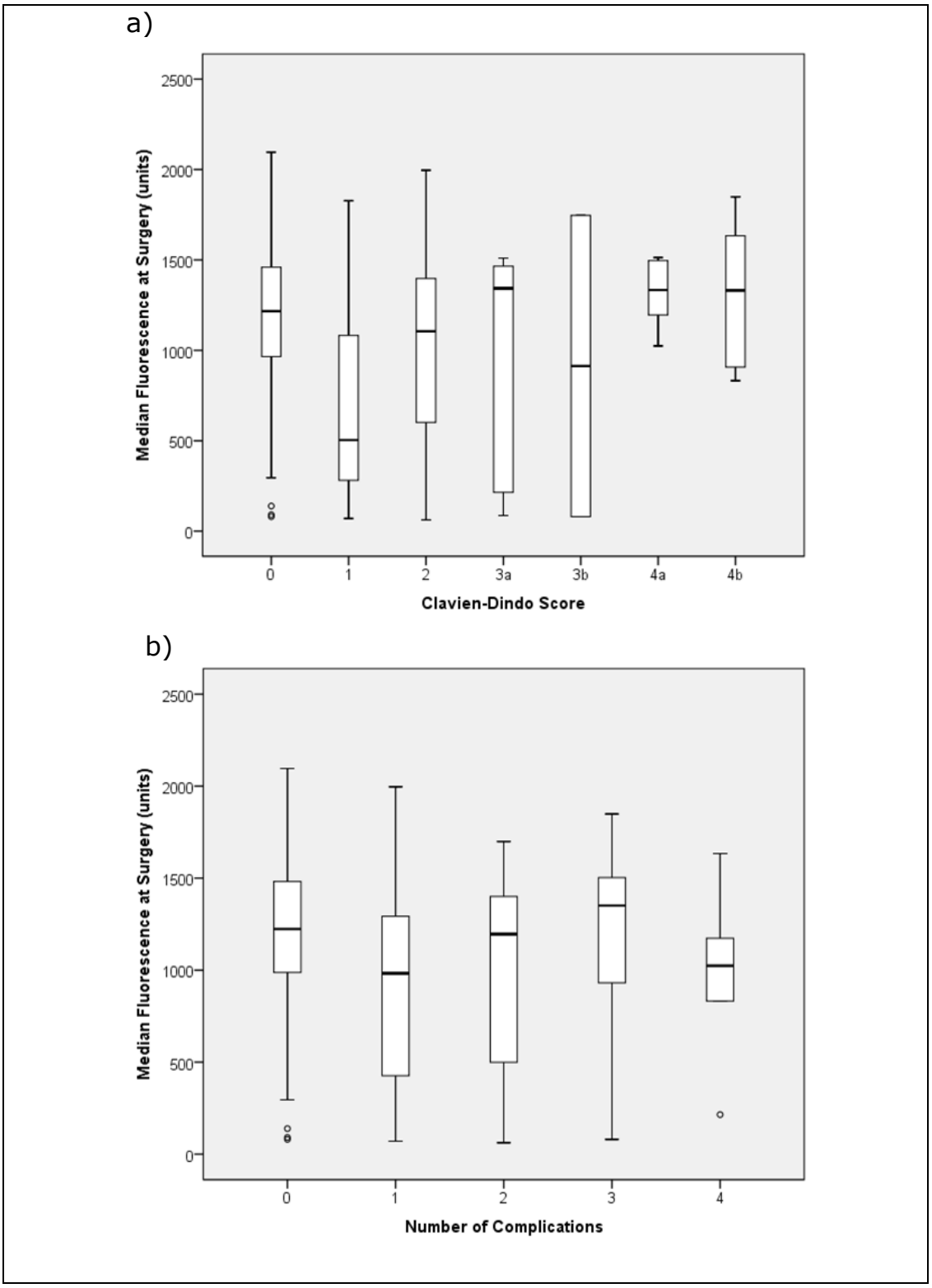


Figure 30 - Box-plot graphs illustrating platelet function on the day of surgery categorised by a) Severity of perioperative complications as graded by the Clavien-Dindo Score b) Frequency of perioperative complications.

There were 3 MACE within the control group, all of which were significant arrhythmias warranting intensive care therapy. The remaining 2 cardiac complications were also within the control group, as were the only 2 thromboembolic events. There were 8 perioperative complications related to haemorrhage in 7 individual patients, 5 of which were within the aspirin group (4 patients), and 3 in the control group (3 patients). All of those patients within the aspirin group with haemorrhagic complications had stopped aspirin for 5 days or less.

Although this increased frequency of haemorrhage related complications within the aspirin group was statistically significant ($P<0.05$), the severity of these events did not differ between groups ($P=0.34$). Importantly, there was no difference in the actual platelet function of patients experiencing perioperative haemorrhagic events compared to those that did not ($P=0.17$)

Perioperative allogeneic red blood cell transfusions:

Eleven patients received ARBT from surgical admission to the fifth postoperative day, 5 of which were transfused intraoperatively, and 3 on the first postoperative day. There was no difference between transfusion rates between the groups ($P=0.43$). There was no difference between platelet function on DOS of those

patients who were transfused compared to those that were not (P=0.74). Of those patients transfused, 8 underwent open procedures and 3 underwent laparoscopic procedures (2 converted). The HB levels of those patients that received ARBT over this period were significantly lower preoperatively than those patients who did not receive ARBT (P<0.01).

Perioperative haemoglobin changes:

For the entire cohort of patients who were did not receive ARBT between REC and D1, the perioperative HB drop increased with reduced platelet function though was non-significant (R^S=-0.123, P=0.227) (see Figure 31). There was no correlation between the volume of intravenous fluid administered intraoperatively and the perioperative HB drop (R^S=0.087, P=0.44).

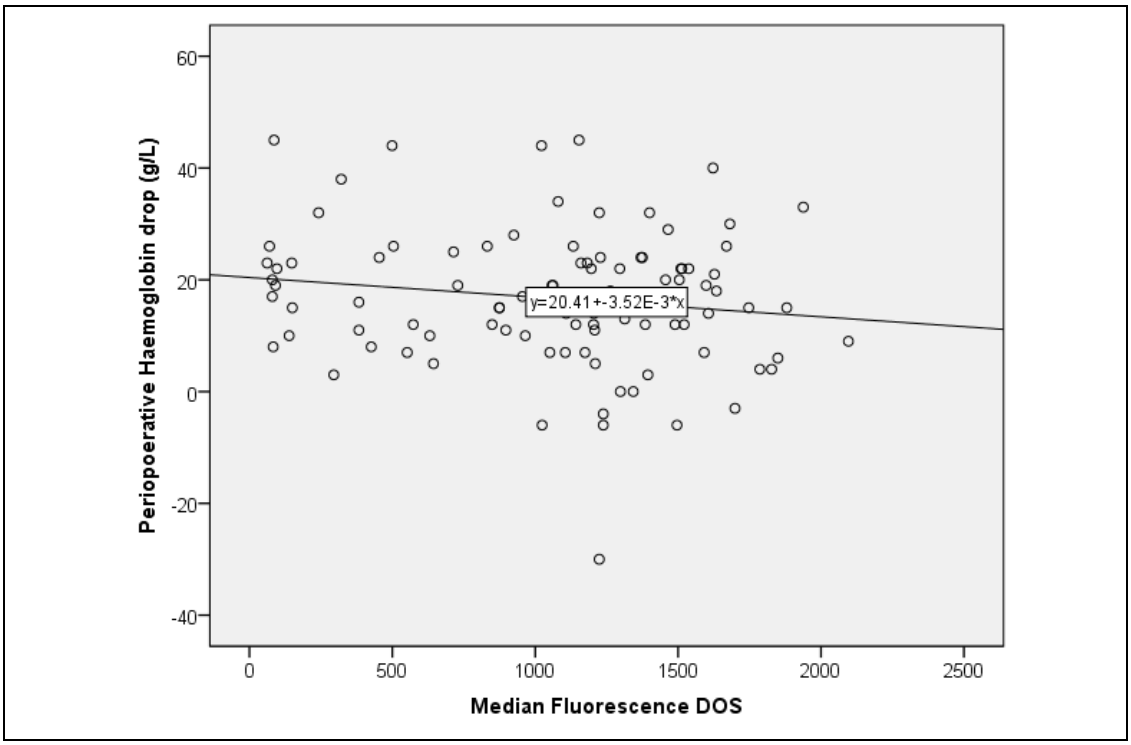


Figure 31 - Graph illustrating the change in haemglobin levels from surgery to day 1 post operatively in relation to platelet function on the day of surgery.

Within the Control group, 19 patients received iron supplements in the preoperative period (oral iron *n*=15, intravenous iron *n*=4). In this same timeframe, 6 patients in the Aspirin group received iron supplementation (oral iron *n*=3, intravenous iron *n*=3).

In the control group there was no difference in the platelet function of those patients receiving any form of iron supplementation compared to those not (REC *P*=0.69, DOS *P*=0.7). Similarly, there was no significant difference in the platelet function at REC

(*P*=0.88) or DOS (*P*=0.38) for those receiving OI or IVI compared to No Iron (see Figure 32).

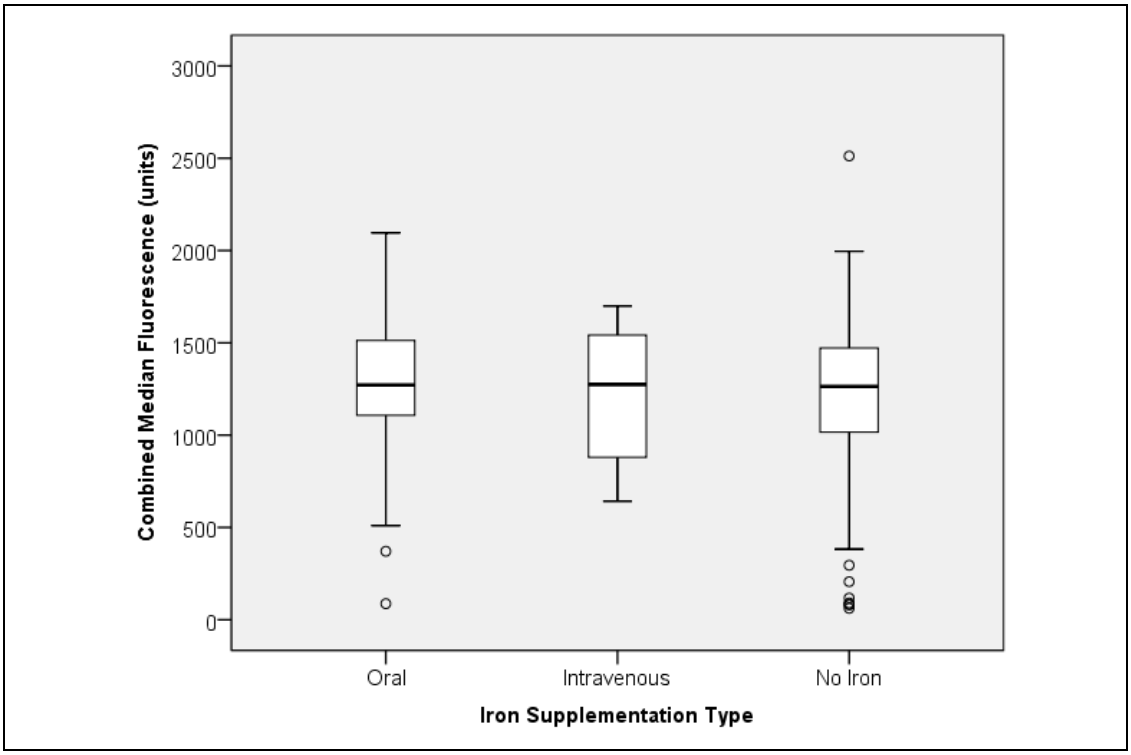


Figure 32 – Box plot comparison of platelet function within the control group in relation to iron supplementation. Median Fluorescence values are combined from both Recruitment and Day of Surgery.

Anaemia and Platelet function in the Control group:

There was no difference in platelet function between anaemic and non-anaemic patients at Recruitment (*P*=0.281). However, at DOS platelet function was significantly higher in patients who were anaemic at recruitment (*P*<0.05) (see Figure 33).

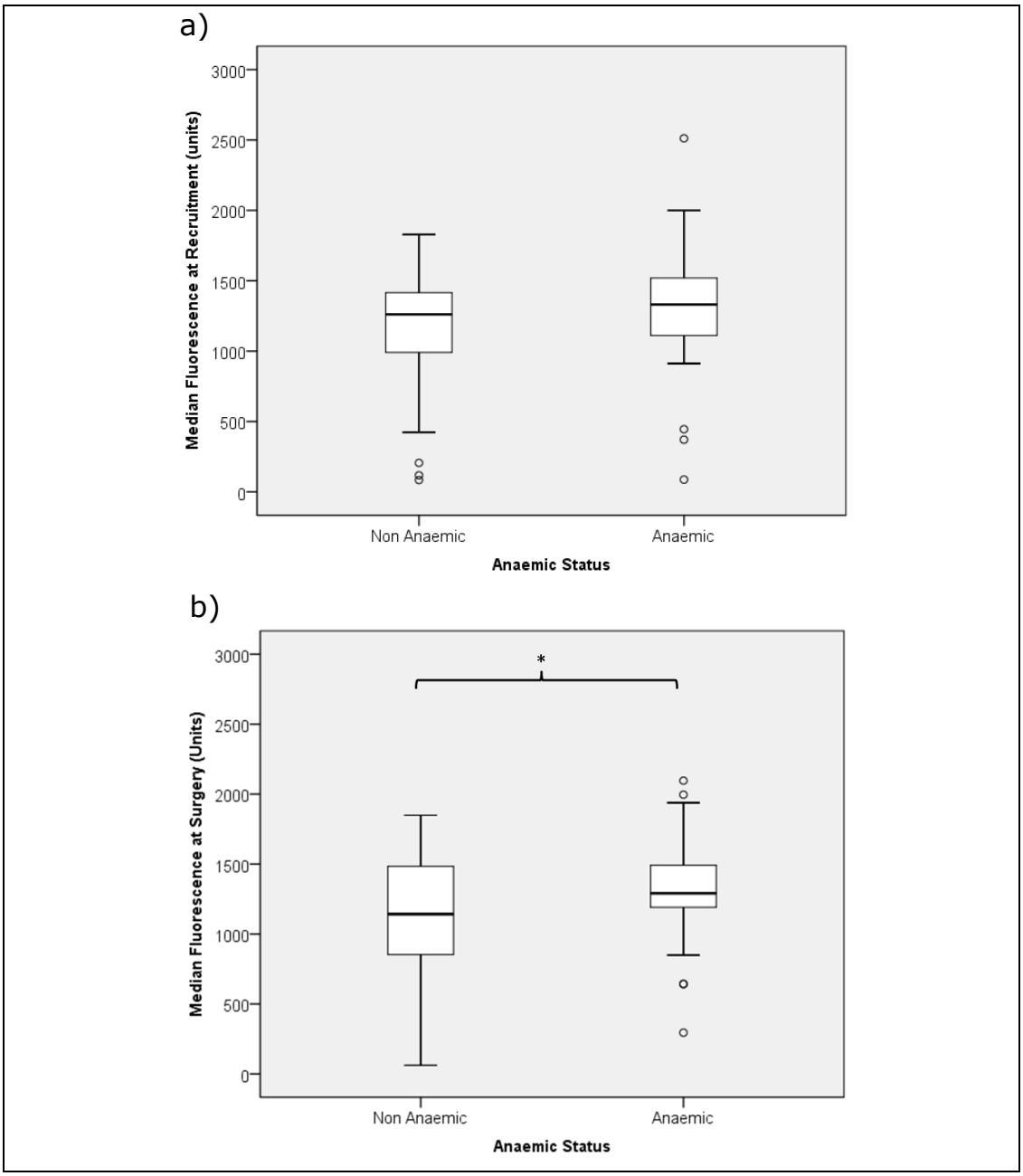


Figure 33 – Platelet function variation at a) Recruitment and b)Surgery in relation to anaemic status in control patients.

Where: * denotes significance to P<0.05.

5.4 Discussion:

Platelet function and Outcome:

There is still debate surrounding the correct practice for perioperative antiplatelet use (Oscarsson et al., 2010, Sahebally et al., 2014). A balance must be made between perioperative cardiovascular and haemorrhagic risks (Oscarsson et al., 2010). This study investigated this concept in patients undergoing major colorectal surgery by reviewing changes in postoperative complications in relation to changes in platelet activity as measured by a newly developed test of platelet function.

In the current study, it was evident that the anti-platelet effect of aspirin persisted beyond the 5-7 day wash-out period often used in clinical practice. This resulted in lower than normal levels of platelet function on the day of surgery. As illustrated in Figure 28, a period in the region of 10 days is required for baseline levels to be reached, which is similar to the normal lifespan of platelets (Leeksma and Cohen, 1955) reflecting the irreversible mechanism of action of aspirin. This poses difficulty in light of evidence which suggests that the cardiovascular risk of cessation of aspirin increases after the 8^{th} day of discontinuation (Burger et al., 2005)

and rightly raises concerns about undertaking this in clinical practice.

It would initially appear that this aspirin related reduction in platelet function is relevant following major colorectal surgery as the frequency of all complications and haemorrhagic complications was increased in this group. This association is in line with previous studies reviewing continued aspirin use within a variety of surgery (Neilipovitz et al., 2001, Nielsen et al., 2000). However, it is relevant to note that the severity of complications was not increased as graded using the validated Clavien-Dindo scoring system, which was also true for complications related to perioperative bleeding. Furthermore, the length of hospital stay was unchanged between groups which further questions whether the increase in complications is of any clinical consequence.

Consensus on this association has not been reached (Burger et al., 2005), and the present study provides a unique perspective upon the issue by measuring the actual platelet function (in the form of P-selectin) rather than assuming platelet function is impaired when taking aspirin. If aspirin were responsible for differences in complication rates, it would be expected that an association between platelet function and adverse outcome would be evident in the present study, i.e. the lower the platelet function on the day of

surgery, the higher the complication rates. The absence of this relationship further questions the causality of aspirin. It is possible that aspirin served as an indicator of generalized comorbidity and hence was a marker of patients more susceptible to complications, as reflected by the increased age of patients in the aspirin group.

In this study we used the perioperative drop in HB levels as a surrogate marker of blood loss. The authors recognize that such a value is influenced by several factors and not solely blood loss, but arguably the most important confounder, perioperative fluid administration, was not correlated with HB change in this study, implicating blood loss as the major causal link. This technique has been employed in similar observational studies evaluating the impact of aspirin in emergency general surgery (Chechik et al., 2011).

It is of interest that reducing platelet function at surgery was associated with only modest, non-significant increases in perioperative HB decreases, which questions the importance of minor impairment of platelet function. Furthermore, as the use of ARBT and severity of complications was unrelated to platelet function, this further indicates that this may be of minimal clinical consequence even at the level of platelet impairment seen when

aspirin is continued until surgery. Such a finding is consistent with a previous meta-analysis which proposed that the increase in bleeding seen with continued perioperative aspirin use was insufficient to have significant clinical ramifications (Burger et al., 2005).

The current study is limited by a relatively small number of patients within the aspirin group. Furthermore, due to the relative infrequency of perioperative cardiovascular and haemorrhagic complications, very large recruitment numbers would be required to accurately detect a difference between perioperative continuation and cessation of aspirin. Despite this, we feel that the use of validated measures of platelet activity provides a unique perspective by comparison of actual platelet activity and operative outcomes. The findings from this study appears to provide more evidence in favor of continuation of aspirin and may indicate that the degree to which aspirin reduces platelet function is unlikely to adversely affect outcomes in the majority of patients. Specific patient groups may be more vulnerable, in particular anemic patients in whom a slight increase in blood loss may increase ARBT requirement. As surgical practices and equipment change, so too must perioperative care, and with increasing numbers of major colorectal surgical procedures being undertaken via minimal access

approaches, it is possible that the reduced blood loss associated with laparoscopic surgery (COLOR, 2005) may compensate for the level of inhibition of platelet function seen with aspirin use.

Platelet function, Anaemia and Iron supplementation:

Previous studies in IBD have indicated that iron supplementation is associated with reduced P-selectin expression (Kulnigg-Dabsch et al., 2013). Although such an association was not observed in the present study it is possible indirect relationships exist between iron supplementation and platelet activation secondary to Iron Deficiency Anaemia (IDA)(Kulnigg-Dabsch et al., 2013).

As discussed previously (Chapter 1), IDA in colorectal surgical patients is often due to both chronic haemorrhage and the systemic inflammatory response, both of which are known to also increase platelet numbers and platelet activation. It is therefore possible that treatment of anaemia, in part, reverses these increases, hence iron supplementation could merely reduce the over activation of platelets as an indirect consequence of treating IDA rather than directly reducing platelet function. Such a hypothesis is consistent with the findings of the present study,

whereby anaemia was identified with increased platelet activation at surgery (Figure 33b).

Such a causal link is far from established in the context of published literature. A study investigating platelet function in females with menorrhagia indicated that IDA was associated with impaired platelet function (Akay et al., 2008) which normalised with iron supplementation. Furthermore a study in children found IDA was associated impaired platelet function independent of platelet P-selectin expression (Yildirim et al., 2011).

The inconsistency in findings from these studies could be reflective of the different aetiologies of IDA. As discussed in Chapter 1, the inflammatory response induced by IBD causes IDA by different mechanisms to the IDA experienced by females with chronic haemorrhage and children with dietary deficiency. Consequently, the findings from these studies may not be directly transferrable to all clinical scenarios. Indeed in Chapter 4, intravenous iron was associated with reduced platelet count as IDA was treated. Although absolute platelet numbers do not reflect platelet function (Page et al., 2007, Rubak et al., 2015), such findings in the context of the present study do further highlight the complex relationship between platelet function, IDA and iron

supplementation and does require further investigation (see Chapter 7).

5.5 Conclusions:

In conclusion, aspirin continuation may not affect clinical outcome in patients undergoing major colorectal surgery, but a large-scale randomized trial remains essential to definitively answer this important clinical conundrum. A potential design of such a study could include comparison of aspirin continuation with a group who had discontinued for less than 7 days, allowing near normal platelet function to be achieved preoperatively and still permit re-instigation before cardiovascular risk rises.

Chapter 6:

Planning a Clinical Trial to investigate the role of iron supplementation in colorectal cancer.

6.1 Introduction:

The design, set-up and completion of the clinical trials documented in Chapters 3, 4 and 5 has provided valuable experience and understanding of the Trials process. This final chapter will document the extrapolation of this understanding in order to design a final study with the aim of definitively establishing the role of iron supplementation in colorectal cancer (CRC).

Several limitations to IVICA were highlighted in Chapter 4. These represented flaws in the design of the study, which could not have been known during inception of the study. If this IVICA is regarded as a Pilot study, then these limitations can be addressed in order to produce the most robust follow-up study possible.

6.2 Determination of Outcome:

Design of a clinical trial is not necessarily a linear process. Once it has been established what intervention is being evaluated and in which general patient population, it is first necessary to determine the outcome measure which will be used to compare the intervention. The intricacies of the study pathway and design will then follow from this essential framework, designed in order to allow comparison of that outcome in a relevant setting.

Clearly the patient population and interventions for the future study have been established as anaemic patients with CRC and iron supplementation respectively. Consequently the outcome must be delineated.

6.2.1 Why blood transfusion may not be the answer:

A multitude of potential study end-points exist which could be used to compare the clinical efficacy of iron supplementation in this patient population. For the reasons outlined in previous sections, allogeneic red blood cell transfusion use seems important and relevant to all potential stakeholders. However, despite large differences in treatment effects seen in terms of Haemoglobin levels (HB), correction of anaemia and Quality of Life scores (QOL), IVICA I failed to definitively establish if such a difference existed or not. Furthermore, anecdotal verbal feedback received from the scientific community during discussion of IVICA I has raised the possibility that other outcomes should perhaps be assessed. Consequently, such qualitative opinion needs formal review and consideration before any future trial is conceived. The tool selected to undertake this was the Delphi Process.

6.2.2 Gauging expert opinion- Delphi Survey:

The Delphi process has been developed over the last 60 years since initial inception in the 1950s as a means for "*think-tanks*" to forecast and predict potential military defence issues and strategies (Yousuf, 2007). Over this period it has been recognised that the technique can be utilised in research when there is incomplete knowledge on a particular topic, or multiple opinions and perspectives exist about a solution to a problem (Skulmoski et al., 2007, Okoli and Pawlowski, 2004).

The process is designed to collect, refine and reach consensus on a subject by surveying the opinions of a group of experts by facilitating communication between individuals (Hsu and Sandford, 2007, Custer et al., 1999). Although minor variations to the exact methodology exist (Custer et al., 1999), the general structure of the process remains uniform. The methodology conformed to in the current survey follows that most commonly employed (Day and Bobeva, 2005).

General Taxonomic Design:

The options available for the methodological design of Delphi Inquiries has been summarised (Day and Bobeva, 2005) and are

illustrated in Table 36. This framework was employed to design the current Delphi survey.

Criteria	**Delphi Design Option**
Purpose of the study	Building, Exploration, Testing, Evaluation
Number of rounds	2 – 10
Participants	Homogenous or Heterogenous
Mode of operation	Face to Face or Remote Access
Anonymity of expert panel	Full or Partial
Communication media	Paper/Pen, Telephone/Fax, Face-to-Face, Computerised
Concurrency of Rounds	Sequential or Real-Time conference

Adapted from Day & Bobeva (2005), A Generic Toolkit for the successful management of Delphi Studies, E-Journal of Business Research Methodology; 3(2) 103-116.

Table 36 - Taxonomy of Delphi Inquiry Designs.

Purpose of the Survey:

The survey was considered as "Explorative", i.e. with the aim of investigating options and opinions regarding potential outcome measures which could be used for IVICA II.

Number of Rounds to be performed:

The number of rounds was not pre-defined, and hence the survey would only be terminated when group agreement ("consensus") had been reached.

The definition of consenus in the Delphi process remains ill-defined (von der Gracht, 2012). The main reason for this relates to differing expert opinion between whether consenus or stability is a more optimal endpoint (Holey et al., 2007). Although the main focus of a Delphi Survey is to acquire a uniform group opinion, it has been proposed that statistical measures of distribution may indicate a consensus has been reached between rounds, but may not account for the stability of such consensus (Dajani et al., 1979). It is possible that a large swing in respondents' opinion has occurred around a similar central tendency within a similar distribution which may indicate consensus has been reached, yet due to markedly changed opinions across one round, consenus is vastly unstable. It is for this reason that the statistical methodology employed remains variable between research groups undertaking Delphi Surveys, and is recognised as a limitation of the technique (Ju and Jin, 2013)

In light of this, the current Delphi Process would only be terminated when both consensus and stability was reached. Non-parametric statistical tests have been proposed as the most accurate tools when expert panel numbers are less than 30 hence were used in this context (Shah and Kalaian, 2009, Kalaian and Kasim, 2012). Analysis of the Interquartile Range (IQR) is

regarded as a robust measure of consensus (von der Gracht, 2012) and has been validated in the context of Likert-scale tests over consecutive Delphi rounds (von der Gracht and Darkow, 2010, de Vet et al., 2005). These studies assessed Likert-scales similar to those used in the present survey and defined consensus as an IQR of less than or equal to 2. This definition of consensus is widely accepted (von der Gracht, 2012) hence was adopted in the current study.

IQR has also been used to aid measure of stability between rounds, by using the values to assess convergence of responses as denoted by the Relative Interquartile Range (RIQR) (Landeta, 2006, Ray and Sahu, 1990). Compression and stability of responses can be indicated by reductions in RIQR between rounds hence was used as the definition in the present survey. One method for calculation of RIQR is illustrated in Figure 34 (Siegel, 2012).

$$\textbf{Relative Interquartile Range (RIQR)} = \frac{Q^3 - Q^1}{\text{Median}}$$

Where:

Q^1 = *First quartile*
Q^3 = *Third quartile*

Figure 34 - Calculation of the Relative Interquartile Range.

Panel Participants:

An heterogenous participant group was selected as it has been suggested that this has provided the highest quality outcomes in previous Delphi surveys (Delbecq et al., 1975, Rowe et al., 1991, Murphy et al., 1998). It has been recommended (Powell, 2003) that suitable "experts" should be chosen based upon their:

"…work in the appropriate area and credibility with the target audience…"

Similarly Day and Bobeva (Day and Bobeva, 2005) outlined key credentials of the Panel as being:

1. *Knowledgeable about the area of research.*
2. *Motivated to engage with the process.*
3. *Able to articulate judgements.*

Consequently, an expert panel was constructed to comprise individuals meeting these criteria. Clinicians from across all relevant medical sub-specialities including Colorectal Surgery, Gastroenterology, Anaesthetics, Intensive Care, and Surgical Academia were invited.

The panel was assembled using the principles ("steps") outlined by Delbecq *et al* (Delbecq et al., 1975). This started with identification of the expertise and knowledge base required of the expert panel member and identifying potential individuals who would fit such criteria (steps 1 & 2). These individuals were contacted and asked to nominate other potential candidates, before a list of potential experts was generated in rank order of suitability (Steps 3 & 4).

There is no consensus on the "optimal" panel size but it is recommended that panel sizes are larger than 10 (Akins et al., 2005), with larger groups for more heterogenous panels (Hsu and Sandford, 2007). Review of typical panel size shows most Delphi Surveys to employ 15-20 experts (Ludwig, 1997).

An uptake of over 80% was expected for each round, and as most surveys run over 3 Rounds (Skulmoski et al., 2007), an initial panel of 20 was selected. This would ensure that the panel size would remain above the threshold of 10 participants across three potential rounds. The first 20 experts ranked on the candidate list were then invited to participate in the study (Step 5).

Other considerations:

Anonymity is a key component of the Delphi Technique (Yousuf, 2007, von der Gracht, 2012) as this allows each panel member to freely express their own views and opinions. In order to preserve this to the highest degree possible, the survey was conducted via anonymised email responses, which were not divulged to other participants, with the exception of the lead researcher compiling the survey.

Specific Methodology:

Delphi Planning Stage:

In order to streamline the process, a core panel of three experts was initially created to outline potential options in response to 2 core questions:

1. Which patients should be included in a study evaluating the role of iron supplementation in colorectal cancer?
2. How important are each of the following options at assessing the efficacy of iron supplementation in the treatment of colorectal cancer?

Following this meeting, the list of options generated was used to form the basis of the Questionnaire for Round 1 of the Survey and was subsequently distributed to the expert panel (see Table 37).

Speciality of Expertise	**Number of Participants Invited**
Colorectal Surgery	11
Gastroenterology	3
Academic Surgical Research	3
Intensive Care	1
Anaesthesia (with interest in Preoperative Care)	1

Table 37 - Expert Panel Composition by Speciality.

Delphi Survey Round 1:

The Round 1 questionnaire (see appendix 1), was distributed to all panel members with the same covering information. This included reference to the purpose of the Survey and re-emphasis of the anonymous nature of the study. Instructions on how to complete the questionnaire were outlined, along with an explanation of how the 10 point Likert scale should be graded (Likert, 1932). This standard scale asked each respondent to rate each option on a scale of 1 to 10, with *"1"* corresponding to *"unimportant"* and *"10"* indicating *"extremely important"* in a graduated fashion.

Each panel member was asked to return their completed questionnaire and the responses pooled. Each expert was encouraged to propose additional outcome measures that they believed to be of merit which were not included in the list. A forum to make additional comments and raise any relevant issues was also included in the Questionnaire.

After 4 weeks, each expert who had failed to send a response was sent a reminder. After a subsequent 4 week period had elapsed, the round was closed. The mean, median, IQR and RIQR were then calculated for the collection of group responses to each Survey question.

Delphi Survey Round 2:

The Survey principles outlined in Round 1 were adhered to in Round 2. The Questionnaire was modified in several key ways and distributed only to those experts who completed the previous Round. Firstly, the order of appearance of each outcome option was altered as this has been suggested to minimise bias (Hallowell and Gambatese, 2010). Secondly, group feedback was added to each questionnaire, to include the median and 25^{th} and 75^{th} centile ranges. Each questionnaire was also individualised to show the

response that had been given by that particular expert to each question in Round 1.

Finally, the additional outcome measures that had been proposed by panel members in Round 1 were added for subsequent evaluation.

Panel members were then encouraged to complete the new survey reconsidering their previous rating of each option with new knowledge of the overall group responses. Explanation for any modification in response was requested on a voluntary basis.

Inter-Round Analysis

Following closure of Round 2 an assessment was made of each option, reviewing whether group consensus had been reached, and if so, whether this was stable in nature as per the criteria described in section 6.2.2. Options that had attained both of these measures were removed from the Survey.

Delphi Survey Round 3:

The third Round questionnaire was thus constructed using the remaining options. These were again reordered in a random

fashion and distributed to each panel member who had completed Round 2, and documented both group and individualised feedback.

Inter-Round Analysis

Following Round 3, the remaining responses were assessed for consensus and stability. At this point consensus and stability had been met for all remaining outcomes.

Results of the Delphi Survey:

Completed questionnaires were received from 16 experts in Round 1, 13 in Round 2 and 10 in Round 3. Consensus and stability were met in 19 of the questions at the end of Round 2, and in all components at the end of Round 3 hence the survey was terminated at that point. The overall group response for each Round is illustrated in Table 38.

Table 39 illustrates the rank order of each outcome measure option, illustrating values obtained at the point of achieving both consenus and stability.

Statement		Round 1			Round 2			Round 3		
		Median	**IQR**	**RIQR**	**Median**	**IQR**	**RIQR**	**Median**	**IQR**	**RIQR**
Which patients should be included in a study evaluating the role of iron supplementation in colorectal cancer?										
	Surgical Patients only	8	4	0.5	6	3	0.5	7	1.75	0.25
	Patients due to undergo surgery or chemotherapy (including chemo-radiation) as the primary treatment modality	8	4.25	0.53	8	1	0.13	-		
How important are each of the following options at assessing the efficacy of iron supplementation in the treatment of colorectal cancer?										
	Blood Transfusion use – average units transfused per patient	8	3	0.38	9	2	0.22	-		
	Blood Transfusion use– number of patients transfused	6.5	3.25	0.5	7	1	0.14	-		
	Blood Transfusion use – Recruitment to Day 2 post operatively	5	3.25	0.65	5	1	0.2	-		
	Blood Transfusion use – Recruitment to Day 4 post operatively	6	4.75	0.79	6	4	0.67	6	1	0.17
	Blood Transfusion use – Recruitment to Day 30 post operatively	8	2.25	0.28	8	1	0.13	-		
	Blood Transfusion use – Recruitment to 8 weeks post recruitment	5	2.5	0.5	5	2	0.4	-		
	Blood Transfusion use – Recruitment to day 5 post-operatively	NA	NA	NA	7	5	0.71	7	1.75	0.25
	Blood Transfusion use – Recruitment to discharge	NA	NA	NA	6	4	0.67	8	1.25	0.16
	Cancer free survival – 1 year	6	2.5	0.42	6	2	0.33	-		
	Cancer free survival – 2 year	7	3	0.43	6	3	0.5	8	1.5	0.18
	Length of postoperative stay	5.5	3.5	0.64	5	2	0.4	-		
	Return to work evaluation	5	3.25	0.65	6	3	0.5	5	1.5	0.3
	Health Economics evaluation	8	3	0.38	7	1	0.14	-		
	Complication severity – Recruitment to Day 2 post operatively	3.5	4	1.14	3	2	0.67	-		
	Complication severity – Recruitment to Day 4 post operatively	5	4	0.8	5	3	0.6	3	1.5	0.38
	Complication severity – Recruitment to Day 30 post operatively	7.5	1.25	0.17	8	1	0.13	-		
	Complication severity – Recruitment to 8 weeks post recruitment	6	2.5	0.42	5	2	0.4	-		
	A composite complication outcome – Recruitment to Day 2 post op	3	3	1	3	2	0.67	-		
	A composite complication outcome – Recruitment to Day 4 post op	4	3.5	0.88	4	3	0.75	3	1.5	0.5
	A composite complication outcome – Recruitment to Day 30 post op	8	2.5	0.31	8	1	0.13	-		
	A composite complication outcome – Recruitment to 8 weeks later	7	3.5	0.58	6	3	0.5	7	2	0.28
	Infective complication rates – Recruitment to Day 2 post operatively	3	2	0.67	3	1	0.33	-		
	Infective complication rates – Recruitment to Day 4 post operatively	4	2.25	0.56	4	2	0.5	-		
	Infective complication rates – Recruitment to Day 30 post operatively	7	1.25	0.18	7	1	0.14	-		
	Infective complication rates – Recruitment to 8 weeks post recruitment	6	3	0.5	6	2	0.33	-		
	Mortality – Recruitment to Day 30 post operatively	6	3.25	0.54	7	1	0.14	-		
	Mortality – Recruitment to 8 *weeks* post recruitment	5	2.25	0.45	5	3	0.6	6	1.5	0.25
	Mortality – Recruitment to 2 *years* post recruitment	6	2.5	0.42	7	2	0.29	-		
	Quality of life – Recruitment compared to Day of Surgery	4.5	3.75	0.83	3	2	0.67	-		
	Quality of life – Recruitment compared to day of Discharge	4.5	2.5	0.56	4	2	0.5	-		
	Quality of life – Recruitment compared to Day 30 postoperatively	7	2.25	0.32	7	2	0.29	-		
	Quality of life – Recruitment compared to 8 weeks post recruitment	7	2	0.29	8	1	0.13	-		

Table 38 – A Table illustrating Delphi Scores for each statement across each survey round. *Likert-Scale used (1 unimportant to 10 Extremely important), IQR=Interquartile Range, RIQR=Relative Interquartile Range, NA=Not tested in Round, -=Consensus and stability met in previous round.*

Rank*	Outcome component	Score+
1	Blood Transfusion use – average units transfused per patient	9
2	Blood Transfusion use – Recruitment to Day 30 post operatively	8
3	Blood Transfusion use – Recruitment to discharge	8
4	A composite complication outcome – Recruitment to Day 30 post op	8
5	Cancer free survival – 2 year	8
6	Quality of life – Recruitment compared to 8 weeks post recruitment	8
7	Health Economics evaluation	7
8	Complication severity – Recruitment to Day 30 post operatively	7
9	Mortality – Recruitment to 2 years post recruitment	7
10	Blood Transfusion use – Recruitment to day 5 post-operatively	7
11	Quality of life – Recruitment compared to Day 30 postoperatively	7
12	Infective complication rates – Recruitment to Day 30 post operatively	7
13	A composite complication outcome – Recruitment to 8 weeks later	7
14	Blood Transfusion use– number of patients transfused	7
15	Mortality – Recruitment to Day 30 post operatively	7
16	Infective complication rates – Recruitment to 8 weeks post recruitment	6
17	Cancer free survival – 1 year	6
18	Mortality – Recruitment to 8 weeks post recruitment	6
19	Blood Transfusion use – Recruitment to Day 4 post operatively	6
20	Complication severity – Recruitment to 8 weeks post recruitment	5
21	Length of postoperative stay	5
22	Return to work evaluation	5
23	Blood Transfusion use – Recruitment to Day 2 post operatively	5
24	Blood Transfusion use – Recruitment to 8 weeks post recruitment	5
25	Quality of life – Recruitment compared to day of Discharge	4
26	Infective complication rates – Recruitment to Day 4 post operatively	4
27	Complication severity – Recruitment to Day 4 post operatively	4
28	Complication severity – Recruitment to Day 2 post operatively	3
29	Quality of life – Recruitment compared to Day of Surgery	3
30	A composite complication outcome – Recruitment to Day 4 post op	3
31	A composite complication outcome – Recruitment to Day 2 post op	3
32	Infective complication rates – Recruitment to Day 2 post operatively	3
Patient study group		
1	Patients due to undergo surgery or chemotherapy (including chemo-radiation) as the primary treatment modality	8
2	Surgical Patients only	7

Table 39 - Delphi Survey Final Scores.

NB *denotes that Rank ordered by median score and further ordered by mean score if median values equal. +denotes median value acquired in the Round where that outcome reached consensus and stability.

Discussion of Delphi Results:

As illustrated in Table 4, this Survey has provided a sequential list of valued outcomes that could potentially be employed in IVICA II. It was necessary to use the mean score in order to differentiate between several outcomes due to similar median values, which is a limitation of the Likert-scale employed, rather than the Delphi process. Despite this, the results provided a valuable gauge of expert opinion to assist in the design of IVICA II.

The results of the Delphi Survey must be interpreted within the scope of the postulated limitations of the technique. The tool has been reported as diluting opinion in an attempt to gain consensus (Sackman, 1975, Powell, 2003). Furthermore, fundamental principles of the technique, such as anonymity, have also been criticised (Sackman, 1975).

However, it would appear that most of the current opinion relating to the weaknesses of the technique relates to poor execution of the process, such as inappropriate panel selection and insufficient number of rounds (Powell, 2003). It was thus intended that the current Survey was undertaken using the most robust design possible to overcome such issues. In retrospect, the definition of stability utilised in the current survey may not have been the most

optimal. By selecting the definition of RIQR employed, the subsequent definition of stability was perhaps too similar to that of consensus. Consequently, other methods of calculation of the RIQR, such as that described by Ray and Sahu (Ray and Sahu, 1990) may have provided a more robust measure of stability.

It was possible to identify several themes from the responses acquired from the Survey. Firstly, the general propose of IVICA II, i.e. the investigation of the use of iron supplementation in CRC, appears to be a valid, relevant and an important clinical issue. This was indicated by the high final scores assigned to both potential patient study groups.

Secondly, it would appear that IVICA II should review iron supplementation in all patients undergoing treatment for CRC, (and not just surgical patients). The expert panel appear to favour utilising the primary endpoint of mean units blood transfusion per patient. The popularity of this endpoint was reiterated by featuring in the top 3 outcome options.

The time period over which this should be measured remains more complex. The highest scoring options used the operation itself to

define the time period, which is clearly not appropriate for a study investigating a population of potential non-surgical patients.

If these findings are extrapolated, it would appear that very short time periods were unpopular within the Panel. This is exemplified by the 2 and 4 day time periods which consistently scored lowest across Rounds for each potential outcome. The important inference was that such time periods should be avoided in the event that additional outcomes had to be utilised in the final trial design.

Despite this, it is essential that these findings be adopted within the context of patient opinion and feasible trial design. Any clinical trial will only be successful if there is significant patient approval of the study itself.

For this reason, it was also essential to assess patient perspective prior to final construction of a final trial framework which could incorporate both patient and expert opinion.

6.2.3 Gauging patient opinion:

Introduction to PPIE:

The concept of Patient and Public Involvement and Engagement (PPIE) is increasingly advocated for clinical research, and should occur at every stage of the research cycle (Research, 2014). This strategy ensures that the study remains relevant and credible to potential participants, and reviews the best possible outcome for the target population from a patient perspective (Research, 2014).

The importance of PPIE in research is exemplified by the "INVOLVE" project initiated by the National Institute for Health Research (NIHR). This outlined the key difference between *participation* in research, i.e. merely being a subject within a trial, and *involvement* in the study itself. Involvement is indicated by patients:

- Aiding in the identification of research priorities as members of a project advisory or steering group.
- Assisting in the development of patient information leaflets or other research materials.
- Acting as joint grant holders or co-applicants on research projects.

(INVOLVE, 2013)

In light of the above, it was therefore necessary to ensure that PPIE was initiated during the design phase of IVICA II.

6.2.4 PPIE methodology:

Step 1:Preparation and Aims of PPIE:

The aims of the initial PPIE were threefold in parallel to the definition of patient involvement listed above:

1. Obtain patient perspective on potential study outcomes and pathway designs.
2. Create an interested patient representative group who could assist in the development of subsequent patient information literature.
3. Identify at least one lead representative to be more involved in subsequent grant application.

An initial meeting was thus held with the local PPIE Facilitator for the Nottingham Digestives Diseases Centre and Biomedical Research Unit (NDDBRU) who agreed to be an independent participant in the process. The aims of the PPIE meeting were thus discussed, and potential group members identified. These included 4 patients who had previously volunteered an interest to the Facilitator to be involved in PPIE, thus were logged within the NDDBRU PPIE database. A further 2 patients who had participated in the IVICA trial had also expressed an interest to be involved in

such meetings, hence were also identified as potential candidates. It was decided that 4 patient representatives were required for optimal function of the focus group.

A lay abstract of the research question was then produced. This outlined the study background, importance, potential methodological designs and a list of potential study outcomes similar to those used in the Delphi Survey.

Step 2: Participant contact:

All potential participants were then contacted in writing. This included literature on the PPIE process and a prepaid answer slip to return if they were interested in involvement. This was intended to ensure there was no feeling of obligation to participate.

Positive responses were received from two of the previous volunteers, along with both of the IVICA trial participants. The lay abstract was then circulated for review prior to the meeting, along with the date and location of the meeting itself.

Step 3: PPIE meeting:

This was chaired by the lead Researcher with input from the Facilitator who also recorded minutes from the meeting. A summary of the meeting agenda is illustrated in Table 40.

Component	Topics covered
What is PPIE?	Role of Researcher in PPIE
	Role of the Patients in PPIE / Personal experiences of each patients research
	Collective role of the group
IVICA I:	Why is anaemia important in colorectal cancer
	IVICA I design and pathway
	Limitations of IVICA I
IVICA II:	Potential Pathway / Design
	Potential outcomes for the study
	Potential treatments
Future PPIE	Request for Support in creation of subsequent study literature
	Request for a group member to be act as a co-applicant for any grant applications

Table 40 - Summary of PPIE Focus Group Agenda.

Step 4: PPIE follow up:

At the end of the meeting, consent was sought from each member to be contacted again for future involvement in the trial, and contact details recorded.

The facilitator subsequently produced a draft minutes of the meeting, which was then circulated via electronic–mail to each

member of the Focus Group for approval. The date for a follow-up meeting was also proposed.

6.2.5 Focus Group Results:

The PPIE meeting yielded important feedback on several issues which were invaluable to the design of IVICA II. Ensuring that the study ethos was in alignment with patient opinion and perspectives was essential to ensure future patient engagement (and thus enrolment) in IVICA II.

Potential study outcomes:

The Focus Group (FG) consenus scores (using the same Likert-scale as employed in the Delphi Survey) as follows:

Blood transfusion – 8:

The group understood the importance of this and assigned this a high score accordingly. Limitations to the choice included a perception that Blood Transfusion was:

> *"…one of many risks of surgery…"*
> *"…only used if absolutely necessary…"*
>
> *(Focus Group Comments)*

The FG also implied that although they comprehended the risks associated with perioperative Blood Transfusions following the group discussions, that some of the general population would still perceive them as "safe" and only used when required, hence avoidance was potentially less important. The Group stressed that a detailed explanation would have to be provided to each potential participant to effectively outline the true value of the outcome.

Length of stay in hospital - 9:

The FG attached significant importance to this outcome, recognising that a short hospital stay was optimal for each individual patient, and that this was also beneficial to society by minimising treatment costs. The FG felt that such an endpoint would also entice patient recruitment.

Complications - 8:

This was universally regarded with high value. The FG sentiment was that complications had direct implications on length of hospital stay. Less importance was placed on this outcome, as it was felt that "minor" complications could be included in the measure, which did not significantly impact upon patient "wellbeing".

Infection Rates – 0:

The FG believed that the perceived "low" impact that infection had upon them was insufficient to warrant potential study involvement to the degree that IVICA II would require. This sentiment persisted despite explanation that such infections were not merely wound infections, but potentially serious intrabdominal and respiratory infections.

Mortality rate and Cancer Survival- 9:

These two options were ultimately the FG favourite. They explained that if they underwent treatment for malignancy, their primary goal would be to *"survive"* or *"beat"* cancer, and hence the study should measure the impact that iron supplementation had upon this endpoint.

Of note, the FG enquired if there was a quantifiable benefit in these measures that patients could expect to experience if the treatments were successful. The group maintained their stance despite an explanation that cancer related mortality, disease free survival and perioperative mortality are complex and difficult to predict due to the multifactorial importance of factors including comorbidity, tumour stage, location and biology etc.

"…If patients are being asked to enter a study which might make them live cancer-free for longer, they need to know how much this may benefit them on a case-by case basis so they can be realistic about the risks and benefits…"

"…They might think it will change things much more than it will…"
(Focus Group Comments)

Quality of Life – 9:

This outcome was very popular within the FG, which was strengthened as the patients reported their own experiences of the symptomatology of anaemia. The FG highlighted that such an outcome measure would encourage high levels of patient recruitment –

"…it shows the study is really about how patients feel..."
"…in this situation we [will] explore every avenue to feel better…"
(Focus Group Comments)

Cost-benefit analysis 6:

This met a degree of disapproval among the FG. Although they unanimously appreciated that cost of a treatment must be considered, that to have this a primary study endpoint would alienate patients. They felt such an option would:

"…make it all feel it was about cost and not care…"
"…The study should be about patient benefit first, and cost benefit second…"
Focus Group Comments

Potential study pathway:

The next key area discussed within the FG related to the design of the study patient pathway. The FG reported they believed recruitment should be performed as early as possible to allow timely initiation of treatment. When the FG reviewed data from the IVICA study, they concluded that the swift treatment of anaemia could have several beneficial outcomes for patients within the study. Firstly, a clinical effect of the iron treatment could become evident sooner, thereby reducing the symptomatology of anaemia at an earlier stage. Secondly, the duration of treatment would be longer, thereby maximising the potential treatment effect of iron supplementation before cancer therapy. Finally, the FG stated that entering the study at an early stage would empower patients in their cancer treatment. They explained that during the "staging" process of CRC, patients my feel that little is being done to treat the underlying disease, and entering a clinical trial which may ultimately improve their outcome would help to alleviate this.

The FG suggested that this should happen at the point that CRC was suspected, such as when the suspected malignancy was first identified at initial colonoscopy or Computed Tomography scan. It was then explained to the FG that recruitment at this early stage would result in patients being recruited to the study who would

subsequently undergo several different treatment primary modalities for their CRC. It was explained that this may entail surgery, chemotherapy, neoadjuvant chemoradiotherapy, and even no subsequent treatment if malignancy was excluded or the patient deemed unfit for any treatment.

The FG reported that this should be overcome by the trial design if possible. They stated that withdrawal of any patient from the study by the research team if malignancy was excluded following recruitment would be acceptable:

"...no one would mind that they were removed from the study if they found out they didn't have cancer – they would be delighted not to be needed!..."

(Focus Group Comments)

The final area covered within the FG related to the choice of medication to be administered to the study subjects. Topics discussed related to the number of hospital visits that would be acceptable to each participant, and the use of a placebo as treatment.

The FG stated that the study should be designed to ensure as few visits to hospital would be required of each participant. However,

as multiple attendances were required on clinical grounds, these visits could easily be coincided and hence should not be the major factor in selection of potential study drugs. The FG concluded that this decision should be based upon the perceived efficacy of treatments, and thus, if more effective treatments would require more visits, this should still be selected.

When this concept was discussed further, the FG were asked whether they felt that administration of a placebo would be acceptable to patients, emphasising that no treatment would be administered to this group. They explained that patients would be happy to be enrolled with this possibility, providing certain criteria were met:

1) Potential participants were fully informed of the possibility before recruitment.
2) That the potential for allocation to placebo treatment was less than allocation to a treatment, i.e. it was more likely that patients would receive a treatment than not.
3) Participants remain unaware of their allocation so they may benefit from the placebo effect.
4) The allocation could be revealed in an emergency.

The FG summarised that patients would understand the rationale and need for the placebo arm, and that this would not reduce the

ease of recruitment to the study. The perception that the research team would review and manage the anaemia was sufficient to encourage recruitment.

The FG understood the importance of the placebo group and recognised the unease which the Researchers may hold towards this. As a consequence, an "observational" recruitment arm was proposed by the FG, which would involve reviewing the outcome data for all eligible patients who declined recruitment to the study, thus acting as a non-interventional comparator group. The FG were then informed of the problematic nature of this, in relation to ethical considerations and a need to conform with Good Clinical Practice. This discussion further reaffirmed the FG acceptance of the Placebo group.

Other outcomes from the Focus Group:

The PPIE meeting had also aimed to create a FG who were prepared to be involved in the subsequent creation and review of study patient information sheets and literature. All members of the FG expressed a desire to remain part of this process and be further involved as required. Furthermore, one member also volunteered to act as a Lay applicant in subsequent Grant applications.

6.3 Construction of IVICA II design and pathway:

The results from the Delphi and PPIE, together with findings from Chapters 2, 3, 4 and 5 must all be considered in the design of IVICA II. This would ensure the study was valuable to clinicians and patients alike, and also as robust as possible to meet the aim of the study.

6.3.1 Aim of IVICA II:

IVICA II should investigate the efficacy of iron supplementation in the management of anaemia associated with colorectal cancer. This is a highly valued by patients and clinical experts, and hence will include any potential patient diagnosed with CRC. Furthermore, the FOXTROT study is currently underway which is believed may show a benefit for neoadjuvant chemotherapy to some patients with CRC (Reibetanz and Germer, 2013b). If this is indeed true, then the treatment pathway for CRC will drastically alter in the next few years, and could render a Trial reviewing only surgical patients obsolete.

Consequently, eligible patients will be approached upon radiological or endoscopic detection of suspected malignancy. Patients who subsequently have CRC excluded by histological assessment will be

withdrawn from the study, and their GP informed of the most recent blood results and what treatment they have received. This would allow the longest duration of iron treatment possible thus permit the greatest potential effect upon anaemia. This was highlighted as important by IVICA I, and is in alignment with the patient perspectives obtained from the PPIE group.

Patients with confirmed malignancy would then receive one of the following cancer treatments as determined by the clinical team:

1) Surgery for primary disease as first treatment.
2) Surgery for metastatic disease as primary treatment.
3) Neoadjuvant therapy as first treatment.
4) Primary palliative chemotherapy.
5) No treatment – palliation of symptoms only.

This collection of heterogenous treatments clearly poses challenges for selection of a valid endpoint. Timing of cancer intervention will vary from 0 days after recruitment for group 5 above, to potentially 16 weeks later for groups 1 and 2. Similarly, group 1 may have "completed" their cancer treatment after as short as 2-3 days after surgical discharge, whilst group 3 could undergo surgical intervention over 4 months after recruitment.

Overcoming such obstacles within one trial is clearly difficult. Despite this, the Delphi Survey illustrated that endpoints which involved a longer time-frame marked from "surgery" were favourable, particularly the 30 day mark. Such a time period will not be feasible given the current setting, but acts as a possible guide. If the time period measured was from the start of cancer treatment, or decision not to treat, and in the order of 6 weeks, this could overcome this issue. The surgical patients would have undergone their operative intervention but unlikely to have started adjuvant therapy. Similarly, those receiving neoadjuvant or primary chemotherapy would be nearing completion of this treatment, and not yet undergone surgery. The study would then be able to assess individual efficacy within each of these subgroups.

Furthermore, the clinical transferability of the study would be of the highest order. In essence the potential cancer treatments can be considered as either surgical, oncological or best supportive care. If a benefit to iron supplementation was seen in each of these three generalised groups, this could be employed in future clinical practice across multiple timepoints for the same patient. An example would be a patient who undergoes surgical resection, then adjuvant therapy, and finally subsequent palliative chemotherapy.

IVICA II could be used to guide the management of anaemia across all these treatment stages.

Selection of blood transfusion as the primary endpoint would be in concordance with the Delphi Survey and scored highly in PPIE. It remains a logical choice as the problematic relationship between anaemia and adverse outcomes is closely related to the need for blood transfusion and is essential in PBM. The implications of this in surgical patients has been covered throughout this Thesis, and although the need for blood transfusion during chemotherapy for CRC is perhaps lower, it remains a significant risk factor for adverse outcome in this patient group also (Laffer et al., 1997). The magnitude of the importance of this merits the use of ARBT as the main endpoint for IVICA II, measured in terms of mean units administered per patient as suggested by the expert Delphi panel.

6.3.2 Secondary Endpoints:

Quality of life (QOL) was valued extremely highly in this context by the PPIE group, and would ensure that the study remains patient centred and engaged with. This also scored very highly with the Delphi Panel. This could therefore be measured at recruitment, at start of "cancer therapy", and then 6 weeks later.

From IVICA I (section 4.4), it appeared that the FACT-AN QOL tool demonstrated the largest differences in treatment effect between groups hence should be employed in IVICA II. The demonstration of "equality" between other QOL component scores between groups was not a failing of IVICA I and yielded important information about how both OI and IVI were similar at changing general aspects of QOL. Consequently, it is essential to utilise a generic QOL tool to augment the more anaemia and cancer specific reporting of the FACT-AN (Yellen et al., 1997, Yoshimura et al., 2004).

Experience from the IVICA trial indicated that employing both the SF-36 and EQ-5D did increase the trial burden on participants significantly. Furthermore, the only significant clinical effect improvement for OI was seen within the EQ-5D questionnaire. For these reasons, selection of one of these tools would minimise participant workload, and if the EQ-5D was used, would still provide essential evaluation between groups based on the findings of IVICA I, whilst still providing a reflection of the general aspects of QOL (Pickard et al., 2007). FACT-AN and EQ-5D should therefore be used in IVICA II.

Complications were valued highly by both the PPIE and Delphi groups. In particular a "composite" score was ranked highly by the latter. Composite outcome scores have advantages such as demonstrating a generalised efficacy of a treatment (Freemantle et al., 2003), but are also regarded as potentially misleading when incorrectly used as a primary endpoint (Prieto-Merino et al., 2013). As this composite outcome would feature as a secondary endpoint, and given the heterogeneity of the various patients within the trial, inclusion seems valid.

The measure itself, could include events common to both potential pathways. A prime example would be that of infection, which occurs in both immunocompromised chemotherapy patients, and also postoperative patients. Such a factor, although not regarded highly by the PPIE group, was well regarded by the expert panel, and also has been proposed as essential in recent literature (Litton et al., 2013).

A potential composite outcome been used in several key Studies (discussed in Chapter 1) which investigated the impact of anaemia on post-surgical outcomes (Karkouti et al., 2008) including colorectal surgery (Leichtle et al., 2011). These included events such as:

1. Myocardial infarction
2. Cerebrovascular accident (any persistent new neurological deficit after surgery).
3. Acute kidney injury (>2-fold increase in creatinine concentration to above normal levels of 100 μmol/L in women and 110 μmol/L in men or the need for dialysis support)
4. Death occurring within 30 days from start of treatment.

Although these events are more major than a potential wound infection, composite outcomes can be weighted to reflect this. It would thus be possible to include these 5 events in this secondary endpoint.

Additional secondary endpoints:

As anaemia is a laboratory test based definition, it seems prudent to include an endpoint that reflects the efficacy of iron treatment in relation to this. Although previous trials have been criticised for using non-clinical endpoints in assessing the efficacy of iron supplementation in anaemia (McIntyre et al., 2013), it seems valuable to review HB changes as an additional marker of response to treatment given the findings of Chapters 3 and 4.

The final endpoint that should be measured is that of a longer term mortality assessment. The Delphi Survey scored this as marginally

less important than cancer free survival, but due to the variability of the participant population, such a measure may be inappropriate.

A two year mortality assessment would be of value to the study, as it would incorporate the benefits that the drugs may have in the short and also the long term. The avoidance of ARBT is essentially a surrogate marker of this as ARBT are thought to increase complications, morbidity and adversely affect cancer survival. Comparing 2 year mortality would arguably supercede this as a measure of efficacy and could easily be evaluated as a longer-term follow after the trial has closed.

In order to conform with the PPIE findings, it must be stressed to potential patients that this is not the key aim of the study, and not the reason for entering the trial, but is a postulated additional benefit.

Summary of Endpoints:

A recent metanalysis reviewing the use of IVI in anaemia (McIntyre et al., 2013) proposed that a randomised clinical trial was required which should:

1. Define whether IVI should be used as a first line treatment to reduce allogeneic red blood cell transfusions in patients in hospital.
2. Include well defined infection endpoints
3. Include patient centred endpoints including mortality and major morbidity.

The use of the endpoints discussed would fulfil these recommendations and also satisfies the findings of expert opinion from the Delphi Survey, and patient opinion from the PPIE Focus Group.

6.3.3 Treatment selection:

The comparison of intravenous iron (IVI) with oral iron (OI) in IVICA I may have been too simplistic. The study indicated that both were equally efficacious in terms of minimising overall transfusion requirements. A limitation of this study is that although comparison with historical data (Chapter 4, Table 23) indicated both treatments were of patient benefit, within the scope of the trial participants, it was not possible to definitively ascertain whether iron supplementation was of benefit compared to no treatment.

Offering "no" treatment to a study group does raise ethical concerns. Despite this, on review of the retrospective data presented in Chapter 2, although OI is referred to as "standard care", less than half of anaemic CRC patients receive iron supplementation at diagnosis. In clinical practice it thus appears that "no" treatment is frequently utilised.

Furthermore, the clinical efficacy of both OI and IVI have be questioned in Chapters 1,2 and 4, which remains true in the wider context of published literature.

The ethical consideration of diagnosing anaemia, recognising the clinical implications of this diagnosis in both patients undergoing surgical and chemotherapy based care, and subsequently opting against treatment should not be ignored. However, given the arguments above, it seems that there is a paucity of evidence to say which treatments are effective, and as most anaemic CRC patients remain untreated, a placebo group should be considered as ethically sound. Furthermore, studies do exist which have compared a single iron supplement with either a placebo or no treatment (Lidder et al., 2007, Edwards et al., 2009) in this same setting.

This was discussed with the PPIE group who were in agreement that such a group would be acceptable to patients. Consequently the ideal study design for IVICA II would include such a placebo group and would also improve the quality of the trial (Jadad et al., 1996).

Which Iron Supplements should be evaluated?

Ferric Carboxymaltose (FCM) should be used IVI trial formulation in IVICA II. Not only has IVICA I shown a potential for benefit, a good safety profile in the relevant population, but more NHS Trusts have FCM on the formulary than other preparations and use it as first line IVI in non-renal patients. Consequently, this will help ensure the clinical transferability of the study findings and also improve ease of identification of potential recruiting centres to the Trial.

IVICA I highlighted the main limitation of the preparation: most patients would require 2 doses, necessitating 2 additional hospital visits. Other IVI formulations could administer the dose on 1 attendance so were considered. However, as covered within PPIE, this was not considered to be problematic, and hence additional visits could be coincided with clinical attendances.

The choice of OI supplement is less simple. Although IVICA used ferrous sulphate (OFS), it was noted that the most commonly used OI at the point of recruitment was ferrous fumarate (OFF). The rationale behind the increased use of OFF is unknown, but could be speculated to be cost related, with OFS being the most expensive at present. Secondly, OFF has the highest elemental iron content per tablet, i.e. the highest amount of iron available for absorption (Goldberg, 2013).

Despite this, OFS has two distinct theoretical advantages. Firstly, the absorption of OFS has been demonstrated to be the highest of all iron preparations during *in vitro* studies (Zariwala et al., 2013). Furthermore, OFS has the lowest incidence of side effects for all the OI non-modified release tablet preparations (Cancelo-Hidalgo et al., 2013) so could be postulated to have the highest treatment adherence rates. High adherence rates were evident in IVICA I, and efficacy was comparable to FCM, which further argues for study of this drug in IVICA II.

6.3.4 Other study considerations:

Blinding of allocation:

IVICA II should be a randomised double blinded, placebo controlled trial with appropriate methods for ensuring blinding and random allocation (Jadad et al., 1996). This will require each patient to be randomised to one of three groups:

1. Intravenous iron and oral placebo tablets.
2. Intravenous placebo and oral iron tablets.
3. Intravenous placebo and oral placebo.

Blinding of OI is limited due to the associated darkening of stool (Lidder et al., 2007, Rimon et al., 2005) but should still be attempted. Blinding of IVI has been performed in previous studies by administration of the drug behind a curtain and using opaque tubing for the infusion. The infusion would have to be prepared and administered by team members independent to the collection and analysis of the data.

Randomisation stratification:

IVICA I and Chapter 2 indicated that key factors associated with transfusion use include HB level and mode of planned operative access. These could be combined with the stratification variables used in IVICA of gender and age to ensure more homogeneity between groups.

Stratification based on location of tumour should also be considered in IVICA II despite not being associated with transfusion use in either IVICA I or Chapter 2. This would be required to ensure similar numbers of patients with rectal and colonic malignancies within each group, which is essential due to the different treatment strategies employed.

Inclusion criteria:

The inclusion criteria should mirror those of IVICA I, with selected exceptions, such as the inclusion of patients with metastatic disease and any reference to surgical intervention. The most notable alteration would be that of the inclusion HB, which will be set higher in IVICA II. Not only has the need for a HB "buffer" been removed because patients will be recruited at an earlier time point, but three key issues were established in Chapter 2. Firstly, most patients do not receive treatment for their anaemia preoperatively hence such a margin to account for potential pre-recruitment treatment is not required. Secondly, patients with even very mild anaemia are at significantly increased risk of needing ARBT and hence may benefit from early treatment. Finally, HB levels fall from diagnosis to surgery, hence even though IVICA I implied that most benefit to iron supplementation would be seen in patients with moderate/severe anaemia, these patients may have had more mild

anaemia at presentation. As a consequence patients should be included if they are anaemic by the WHO definition.

Withdrawal Criteria:

IVICA I highlighted the problematic nature that unexpected massive blood loss poses when using ARBT as the main outcome. Consequently, patients with a documented blood loss in excess of 1.5L during the study period will be withdrawn from the study.

6.4 Conclusions:

This Chapter has documented the planning of a clinical trial which seeks to investigate key themes within this Thesis. There are areas of the design which have not been discussed, such as sample size calculation and estimation of number of Centres required. Unfortunately, the change in the HB threshold for inclusion in IVICA II means that the screening logs from IVICA I would not be able to provide the required information to aid this decision making. However, this could rectified with a short prospective review of MDT discussions at several sample sites to provide an estimation of how many patients would be eligible per year and would enter into each cancer treatment group.

The design is thus far from complete, but the main framework is now in place and a summary design is illustrated in Table 41. This trial design has been based upon findings from Chapters 1 to 4 of this Thesis, together with results from a Delphi Survey and PPIE Focus Group. However, there are additional components that could be investigated within IVICA II which were raised in Chapter 5. The prime example of this would be to include applications to investigate platelet function in the "surgical" arm of the study. IVICA II would be ideally placed to further elucidate potential links

between iron supplementation, anaemia and platelet function that were raised in the previous Chapter.

Component	Summary
Title	A double blind, randomised, placebo controlled study to investigate the efficacy of iron supplementation in the treatment of colorectal adenocarcinoma associated anaemia.
Primary Endpoint	Inter-group differences in the mean number of units of allogeneic red blood cells transfused in the first 6weeks following start of cancer treatment, or point of decision not to actively treat malignancy. Start of cancer treatment is defined as the day of either: first dose of chemotherapy, radiotherapy or surgery for CRCa
Secondary Endpoints	Intergroup differences in: -Composite outcome measure. -Quality of life scores measured by the FACT-AN and EQ-5D tools. -Haemoglobin and haematinics. -2 year mortality.
Inclusion criteria	Suspected adenocarcinoma of the colon or rectum by endoscopic or radiological appearances. Anaemic by WHO definition (males<13g/dl, females <12g/dL). Willing and able to give informed consent. Male or Female, aged over 18years. Able to comply with all study requirements. Willing to allow GP/Consultant to be notified.
Exclusion criteria	Absolute contraindications to oral or intravenous iron. Previous surgery where ≥50% of stomach/T.I has been resected. Known haematological disease. Features necessitating urgent surgery. Significant symptomatic anaemia necessitating urgent transfusion. Patients who are unable to consent. Significant renal or hepatic impairment. Deemed unsuitable by investigator. Donation of blood during the study. Prisoners and minors (<18 years).
Withdrawal	Patients who following further investigation have colorectal adenocarcinoma definitively excluded. Documented blood loss of >1.5L during study.
Arms of study	1. Oral Ferrous Sulphate (200mg twice daily for 4 weeks) Intravenous Placebo (0.9% sodium chloride solution) 2. Intravenous Ferinject (dosed as per IVICA study) Oral placebo (1 tablet twice daily for 4 weeks) 3. Oral placebo (1 tablet twice daily for 4 weeks) Intravenous Placebo (0.9% sodium chloride solution)
Visit 1	Consent and randomisation. Baseline assessments including blood tests and quality of life. Initiation of study therapies.
Visit 1b	Minimum 1 week after visit 1a for second intravenous infusion. This is for all patients regardless of randomisation, if dosing of intravenous iron would require second dose.
Visit 2	Start of Cancer treatment. Repeat of assessments including blood tests and quality of life. Assessment of treatment adherence, complications etc.
Visit 3	Final visit (6 weeks after visit 2). Final assessments – blood tests, quality of life, complications etc.
Final review	Assessment of mortality at 2 years (notes review only)

Table 41 - IVICA II trial summary.

Chapter 7:

Conclusions.

Allogeneic red blood cell transfusions carry risks to surgical patients in both the short and long term. In the short term they are thought to confer direct infective and immunological risks, and are also associated with increased complication rates and mortality. Furthermore, effects on immune function are thought to impair longer term outcomes which are particular evident in patients undergoing cancer surgery.

As a consequence of this, the notion of Patient Blood Management (PBM) has been introduced into clinical practice. The essence of this revolves around early identification and treatment of anaemia, preservation of red cell mass during surgery, and postoperative optimisation to minimise the physiological impact of anaemia. The net effect of these measures is to reduce the need for ARBT and thus minimise their associated risks.

This thesis has explored three aspects of PBM within the preoperative phase of patients undergoing colorectal surgery. Chapter 2 investigated current practices in the preoperative diagnosis of anaemia in patients due to undergo colorectal cancer surgery. This retrospective review identified that only two thirds of patients were tested prior to preoperative assessment review. At this point, over half of patients were anaemic, yet less than half of anaemic patients received some form of iron supplementation.

Furthermore, significant reductions in the haemoglobin levels of untreated patient were evident between diagnosis and surgery. The relevance of this was that increasing severity of anaemia at surgery was also found to be associated with marked increases in the need for perioperative ARBT use.

In Summary, this Chapter highlighted deficiencies in the detection and preoperative treatment of anaemia, which could have increased the risk of requiring ARBT in the immediate perioperative period. Chapter 3 then aimed to review if addressing the issue of early detection and treatment of anaemia with intravenous iron supplementation could indeed increase patient haemoglobin levels at surgery and postulate if this would reduce transfusion requirements. This small Pilot study demonstrated a median increase in haemoglobin attributable to intravenous iron of 1.65 g/dL, and this was associated with a significant reduction in the predicted transfusion rate of the cohort.

This Trial further therefore provided more weight to the hypothesis that early identification and treatment of preoperative anaemia in colorectal cancer patients may minimise the need for ARBT but had several key limitations. Firstly the sample size was small making observations vulnerable to statistical error. Secondly, there was no

comparator control group, hence the clinical end point of ARBT use could only be assessed with the use of a predictive model.

In order to overcome this and explore the optimal treatment modality for such patients, a much larger Randomised Control Trial was designed and undertaken as documented in Chapter 4. In this study, intravenous iron was identified as being more efficacious than oral iron at raising preoperative HB levels and treating anaemia. However, although the transfusion rates were lower in both groups compared to rates in untreated patients identified in Chapter 2, the transfusion rates and volumes administered did not significantly differ between study groups.

Subgroup analysis did indicate that intravenous iron may be more efficacious at reducing the need for ARBT in patients not experiencing massive intraoperative blood loss. Further benefits were also seen with regard to quality of life scores, particularly those components which were closely linked to HB levels.

Despite the inference of a potential superiority of intravenous iron, the trial failed to definitively identify a significant clinical benefit in favour of the intravenous formulation. Considering the findings of Chapter 2 (i.e. increased risk of ARBT use with increasing severity of preoperative anaemia), together with the findings from Chapter

3 and 4 that intravenous iron is effective at raising preoperative HB levels, it remains logical to believe that a clinical benefit in terms of ARBT use may exist but, as yet, has not been clearly outlined. This prompted some of the work in Chapter 6.

The third clinical trial (Chapter 5) investigated the final aspect of preoperative PBM concerning the perioperative management of antiplatelet drugs. This Chapter examined the effect of platelet function on perioperative blood loss following major colorectal surgery, and hence by measuring actual platelet function, examined whether the continuation of aspirin was likely to adversely affect patient outcomes including bleeding and ARBT use.

The study found that although complication rates were higher in the aspirin group, impaired platelet function within ranges seen with aspirin continuation did not affect complication severity or rate or blood transfusion use. The trial postulated that aspirin continuation was therefore unlikely to significantly affect clinical outcome in this context, but acknowledged key limitations. These included sample size, particularly in relation to the number of patients who were taking aspirin within the trial. As a result, the trial proposed a potential design of a larger randomised controlled trial which would be required to further elucidate the optimal

perioperative management of antiplatelet agents in this surgical group.

Crossover was evident within this trial with some of themes raised in the previous chapter relating to iron supplementation and platelet function. The possibility of impaired platelet number and function was discussed in this Chapter and in Chapter 4 and highlights a further area for future investigation.

The clinical trials within this thesis have therefore raised several pertinent issues. In light of this, Chapter 6 explored the design of a final clinical trial to examine these further. This Chapter used four facets to aid in the trial design; employing the findings from a Delphi Survey of expert opinion; analysing patient perspectives via focus groups; building on the researcher experiences from the IVICA trial and also utilising finings and questions raised in Chapters 2, 4 and 5. This subsequent design serves as a demonstration of how work from this Thesis should be carried forward, and forms the basis of a future application for Grant funding and subsequent large scale RCT.

In summary, the work documented within this thesis has inferred potential benefit in favour of several key practices in preoperative PBM. However, it has failed to provide definitive evidence for radical change in protocol. It has provided a sound grounding for

the design of several subsequent clinical trials which could be well suited to provide such data.

The framework provided by this Thesis should not be limited to investigation of the role of intravenous iron as part of PBM in colorectal cancer. Indeed, based upon the work undertaken for and around this Thesis, investigations in other contexts have already been established. This researcher has already designed and set up a clinical trial investigating intravenous iron in palliative oncology which is currently underway and heralds an innovative and relevant area to extend investigation into. Furthermore plans to follow a similar design of IVICA in preoperative oesophagogastric malignancy are already in place with the aim of opening a trial in the coming years.

List of abbreviations.

AD	Anxiety and Depression
AID	Absolute Iron Deficiency
APR	Abdominoperineal Resection
ARBT	Allogeneic Red Blood Cell Transfusion
ANOVA	Analysis of Variance
ANS	Anaemia Subscale
ASA	American Society of Anesthesiology grade
AUC	Area under Curve
BCSP	Bowel Cancer Screening Programme
BP	Bodily Pain
CD	Clavien-Dindo
CID	Cumulative Iron Deficit
CJD	Creutzfeld-Jakob disease
CPA	Clinical Pathology Accreditation
CRC	Colorectal Cancer
CRP	C-Reactive Protein
CRT	Chemoradiotherapy
CVD	Cardiovascular Disease
D1	Day 1 postoperative
D2	Day 2 postoperative
DOS	Day of Surgery
DMT1	Divalent Metal Transporter 1
EIA	Enzyme Immuno-Assay Buffer

ELISA	Enzyme-Linked Immunosorbent Assay
EPO	Erythropoietin
EQ5D	Euro-Quol 5 Dimension questionnaire
EWB	Emotional Wellbeing
FACT-AN	Functional Assessment of Cancer Therapy (Anaemia)
FCM	Ferric Carboxymaltose
FG	Focus Group
FID	Functional Iron Deficiency
FWB	Functional Wellbeing
GCP	Good Clinical Practice
g/dL	Grams per decilitre
GH	General Health
GI	Gastrointestinal
GLP	Good Laboratory Practice
GP	General Practitioner
HB	Haemoglobin
HP	Helicobacter Pylori
IDA	Iron Deficiency Anaemia
IS	Iron Supplementation
IQR	Interquartile Range
IV	Intravenous
IVI	Intravenous Iron
LOS	Length of Stay

LS	Laparoscopic Surgery
MACE	Major Adverse Cardiac Event
MCD	Minimum Clinical Difference
MCS	Mental Component Summary
MCV	Mean Corpuscular Volume
MOB	Mobility
NBOCAP	National Bowel Cancer Audit Programme
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NPV	Negative Predictive Value
LAR	Low Anterior Resection
OFF	Oral Ferrous Fumarate
OFS	Oral Ferrous Sulphate
OI	Oral Iron
OPA	Outpatient Appointment
OPD	Outpatient Department
OR	Odds Ratio
PAC	Preoperative Assessment Clinic
PBM	Patient Blood Management
PCS	Physical Component Summary
PPIE	Patient Participation, Involvement and Engagement
PPV	Positive Predictive Value
PS	P-Selectin

PD	Pain and Disability
PF	Physical Functioning
PWB	Physical Wellbeing
QOL	Quality of Life
RE	Role limitation due to emotion
REC	Recruitment
RIQR	Relative Interquartile Range
ROC	Receiver Operated Characteristic Curve
RP	Role Limitation due to pain
RR	Risk Ratio
SC	Self Care
SD	Standard Deviation
SEM	Standard Error of Mean
SF36	Short Form 36
SF	Social Functioning
SWB	Social/Family Wellbeing
TOI	Trial Outcome Index
TNM	Tumour Nodes Metastases Classification
TSAT	Transferrin Saturation
U	Units
UK	United Kingdom
US	Usual Activity
USA	United States of America

VAS	Visual Analogue Scale
VT	Vitality
WHO	World Health Organisation
5-FU	5 Fluoro-Uracil
95%CI	95% Confidence Interval

References.

AAPRO, M. 2009. An Update on Twenty Years of Anemia Management with Erythropoiesis-Stimulating Agents in Nephrology and Oncology/Hematology. *Oncologist,* 14**,** 1-5.
ABHOLD, E., RAHIMY, E., WANG-RODRIGUEZ, J., BLAIR, K. J., YU, M. A., BRUMUND, K. T., WEISMAN, R. A. & ONGKEKO, W. M. 2011. Recombinant human erythropoietin promotes the acquisition of a malignant phenotype in head and neck squamous cell carcinoma cell lines in vitro. *BMC Res Notes,* 4**,** 553.
ACHESON, A. G., BROOKES, M. J. & SPAHN, D. R. 2012. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Annals of Surgery,* 256**,** 235-44.
AGARWAL, R., RIZKALA, A. R., BASTANI, B., KASKAS, M. O., LEEHEY, D. J. & BESARAB, A. 2006. A randomized controlled trial of oral versus intravenous iron in chronic kidney disease. *Am J Nephrol,* 26**,** 445-54.
AJANI, U. A., FORD, E. S., GREENLAND, K. J., GILES, W. H. & MOKDAD, A. H. 2006. Aspirin use among US adults - Behavioral risk factor surveillance system. *American Journal of Preventive Medicine,* 30**,** 74-77.
AKAY, O. M., AKIN, E., MUTLU, F. S. & GULBAS, Z. 2008. Effect of Iron Therapy on Platelet Function among Iron-Deficient Women with Unexplained Menorrhagia. *Pathophysiol Haemost Thromb,* 36**,** 80-3.
AKINS, R. B., TOLSON, H. & COLE, B. R. 2005. Stability of response characteristics of a Delphi panel: application of bootstrap data expansion. *BMC Med Res Methodology,* 5.
ALLEYNE, M., HORNE, M. K. & MILLER, J. L. 2008. Individualized treatment for iron-deficiency anemia in adults. *Am J Med,* 121**,** 943-8.
ALY, E. H. 2009. Laparoscopic colorectal surgery: summary of the current evidence. *Ann R Coll Surg Engl,* 91**,** 541-4.
AMATO, A. & PESCATORI, M. 2006. Perioperative blood transfusions and recurrence of colorectal cancer. *Cochrane Database of Systematic Reviews.* http://onlinelibrary.wiley.com/doi/10.1002/14651858.CD005033.pub2/abstract: John Wiley & Sons, Ltd.
ANDREWS, N. C. & SCHMIDT, P. J. 2007. Iron Homeostasis. *Annual Review of Physiology,* 69**,** 69-85.
ANKER, S. D., COLET, J. C., FILIPPATOS, G., WILLENHEIMER, R., DICKSTEIN, K., DREXLER, H., LUSCHER, T. F., MORI, C., VON EISENHART ROTHE, B., POCOCK, S., POOLE-WILSON, P. A. & PONIKOWSKI, P. 2009. Rationale and design of Ferinject assessment in patients with IRon deficiency and chronic Heart Failure (FAIR-HF) study: a randomized, placebo-controlled study of intravenous iron supplementation

in patients with and without anaemia. *Eur J Heart Fail,* 11**,** 1084-91.
ANNIBALE, B., CAPURSO, G., LAHNER, E., PASSI, S., RICCI, R., MAGGIO, F. & DELLE FAVE, G. 2003. Concomitant alterations in intragastric pH and ascorbic acid concentration in patients with Helicobacter pylori gastritis and associated iron deficiency anaemia. *Gut,* 52**,** 496-501.
ARCELUS, J. I., VILLAR, J. M. & MUÑOZ, N. 2012. Should we follow the 9th ACCP guidelines for VTE prevention in surgical patients? *Thrombosis Research,* 130, Supplement 1**,** S4-S6.
AREZES, J. & NEMETH, E. 2015. Hepcidin and iron disorders: new biology and clinical approaches. *Int J Lab Hematol,* 37 Suppl 1**,** 92-8.
ARONSTAM, A. & ASTON, D. L. 1982. A Comparative Trial of a Controlled-Release Iron Tablet Preparation (Ferrocontin Continuous) and Ferrous Fumarate Tablets. *Pharmatherapeutica,* 3**,** 263-267.
ATKINSON, A. J., COLBURN, W. A., DEGRUTTOLA, V. G., DEMETS, D. L., DOWNING, G. J., HOTH, D. F., OATES, J. A., PECK, C. C., SCHOOLEY, R. T., SPILKER, B. A., WOODCOCK, J., ZEGER, S. L. & GRP, B. D. W. 2001. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics,* 69**,** 89-95.
AUERBACH, M. & BALLARD, H. 2010. Clinical use of intravenous iron: administration, efficacy, and safety. *Hematology Am Soc Hematol Educ Program,* 2010**,** 338-47.
BAER, A. N., DESSYPRIS, E. N. & KRANTZ, S. B. 1990. The pathogenesis of anemia in rheumatoid arthritis: a clinical and laboratory analysis. *Semin Arthritis Rheum,* 19**,** 209-23.
BEALE, A. L., PENNEY, M. D. & ALLISON, M. C. 2005. The prevalence of iron deficiency among patients presenting with colorectal cancer. *Colorectal Disease,* 7**,** 398-402.
BEATTIE, W. S., KARKOUTI, K., WIJEYSUNDERA, D. N. & TAIT, G. 2009. Risk Associated with Preoperative Anemia in Noncardiac Surgery A Single-center Cohort Study. *Anesthesiology,* 110**,** 574-581.
BEN-ELIYAHU, S. 2002. The price of anticancer intervention. Does surgery promote metastasis? *Lancet Oncol,* 3**,** 578-9.
BERIS, P., MUNOZ, M., GARCIA-ERCE, J. A., THOMAS, D., MANIATIS, A. & VAN DER LINDEN, P. 2008. Perioperative anaemia management: consensus statement on the role of intravenous iron. *Br J Anaesth,* 100**,** 599-604.
BERNARD, A. C., DAVENPORT, D. L., CHANG, P. K., VAUGHAN, T. B. & ZWISCHENBERGER, J. B. 2009. Intraoperative transfusion of 1 U to 2 U packed red blood cells is associated with increased 30-day mortality, surgical-site infection,

pneumonia, and sepsis in general surgery patients. *J Am Coll Surg,* 208**,** 931-7, 937 e1-2; discussion 938-9.
BESHARA, S., SORENSEN, J., LUBBERINK, M., TOLMACHEV, V., LANGSTROM, B., ANTONI, G., DANIELSON, B. G. & LUNDQVIST, H. 2003. Pharmacokinetics and red cell utilization of 52Fe/59Fe-labelled iron polymaltose in anaemic patients using positron emission tomography. *Br J Haematol,* 120**,** 853-9.
BEVING, H., ZHAO, C., ALBAGE, A. & IVERT, T. 1996. Abnormally high platelet activity after discontinuation of acetylsalicylic acid treatment. *Blood Coagulation & Fibrinolysis,* 7**,** 80-84.
BIONDI-ZOCCAI, G. G. L., LOTRIONTE, M., AGOSTONI, P., ABBATE, A., FUSARO, M., BURZOTTA, F., TESTA, L., SHEIBAN, I. & SANGIORGI, G. 2006. A systematic review and meta-analysis on the hazards of discontinuing or not adhering to aspirin among 50 279 patients at risk for coronary artery disease. *Eur Heart J,* 27**,** 2667-2674.
BLAJCHMAN, M. A. 2001. Bacterial contamination of blood products. *Transfus Apher Sci,* 24**,** 245.
BLASER, M. J. & ATHERTON, J. C. 2004. Helicobacter pylori persistence: biology and disease. *J Clin Invest,* 113**,** 321-333.
BOCK, M., MULLER, J., BACH, A., BOHRER, H., MARTIN, E. & MOTSCH, J. 1998. Effects of preinduction and intraoperative warming during major laparotomy. *Br J Anaesth,* 80**,** 159-63.
BONE, R. C., BALK, R. A., CERRA, F. B., DELLINGER, R. P., FEIN, A. M., KNAUS, W. A., SCHEIN, R. M. H., SIBBALD, W. J., ABRAMS, J. H., BERNARD, G. R., BIONDI, J. W., CALVIN, J. E., DEMLING, R., FAHEY, P. J., FISHER, C. J., FRANKLIN, C., GORELICK, K. J., KELLEY, M. A., MAKI, D. G., MARSHALL, J. C., MERRILL, W. W., PRIBBLE, J. P., RACKOW, E. C., RODELL, T. C., SHEAGREN, J. N., SILVER, M., SPRUNG, C. L., STRAUBE, R. C., TOBIN, M. J., TRENHOLME, G. M., WAGNER, D. P., WEBB, C. D., WHERRY, J. C., WIEDEMANN, H. P. & WORTEL, C. H. 1992. American-College of Chest Physicians Society of Critical Care Medicine Consensus Conference - Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis. *Critical Care Medicine,* 20**,** 864-874.
BONNAR, J., GOLDBERG, A. & SMITH, J. A. 1969. Do pregnant women take their iron? *Lancet,* 1**,** 457-8.
BOOGAERTS, M., COIFFIER, B., KAINZ, C. & GRP, E. B. Q. W. 2003. Impact of epoetin beta on quality of life in patients with malignant disease. *British Journal of Cancer,* 88**,** 988-995.
BOSCH, B., GULLER, U., SCHNIDER, A., MAURER, R., HARDER, F., METZGER, U. & MARTI, W. R. 2003. Perioperative detection

of disseminated tumour cells is an independent prognostic factor in patients with colorectal cancer. *British Journal of Surgery,* 90**,** 882-888.
BRAZIER, J. E., HARPER, R., JONES, N. M., O'CATHAIN, A., THOMAS, K. J., USHERWOOD, T. & WESTLAKE, L. 1992. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *BMJ,* 305**,** 160-4.
BREGMAN, D. B. & GOODNOUGH, L. T. 2014. Experience with intravenous ferric carboxymaltose in patients with iron deficiency anemia. *Ther Adv Hematol,* 5**,** 48-60.
BREGMAN, D. B., MORRIS, D., KOCH, T. A., HE, A. & GOODNOUGH, L. T. 2013. Hepcidin levels predict nonresponsiveness to oral iron therapy in patients with iron deficiency anemia. *Am J Hematol,* 88**,** 97-101.
BREMBERG, E. R., BRANDBERG, Y., HISING, C., FRIESLAND, S. & EKSBORG, S. 2007. Anemia and quality of life including anemia-related symptoms in patients with solid tumors in clinical practice. *Medical Oncology,* 24**,** 95-102.
BRENNER, H., HOFFMEISTER, M., ARNDT, V. & HAUG, U. 2007. Gender differences in colorectal cancer: implications for age at initiation of screening. *British Journal of Cancer,* 96**,** 828-831.
BREYMANN, C., GLIGA, F., BEJENARIU, C. & STRIZHOVA, N. 2008. Comparative efficacy and safety of intravenous ferric carboxymaltose in the treatment of postpartum iron deficiency anemia. *Int J Gynaecol Obstet,* 101**,** 67-73.
BROCHNER, A. C. & TOFT, P. 2009. Pathophysiology of the systemic inflammatory response after major accidental trauma. *Scandinavian Journal of Trauma Resuscitation & Emergency Medicine,* 17.
BROOKHART, M. A., FREBURGER, J. K., ELLIS, A. R., WANG, L., WINKELMAYER, W. C. & KSHIRSAGAR, A. V. 2013. Infection risk with bolus versus maintenance iron supplementation in hemodialysis patients. *J Am Soc Nephrol,* 24**,** 1151-8.
BROWNLIE, T. T., UTERMOHLEN, V., HINTON, P. S., GIORDANO, C. & HAAS, J. D. 2002. Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. *Am J Clin Nutr,* 75**,** 734-42.
BUNN, H. F. 2013. Erythropoietin. *Cold Spring Harbor Perspectives in Medicine,* 3.
BURGER, W., CHEMNITIUS, J. M., KNEISSL, G. D. & RUCKER, G. 2005. Low-dose aspirin for secondary cardiovascular prevention - cardiovascular risks after its perioperative withdrawal versus bleeding risks with its continuation - review and meta-analysis. *Journal of Internal Medicine,* 257**,** 399-414.

BUSCH, O. R., HOP, W. C., HOYNCK VAN PAPENDRECHT, M. A., MARQUET, R. L. & JEEKEL, J. 1993. Blood transfusions and prognosis in colorectal cancer. *N Engl J Med,* 328**,** 1372-6.
CALVERT, M. J., FREEMANTLE, N. & CLELAND, J. G. F. 2005. The impact of chronic heart failure on health-related quality of life data acquired in the baseline phase of the CARE-HF study. *Eur J Heart Fail,* 7**,** 243-251.
CAMPBELL, E. W. 1996. Hematology for primary care physicians. *Dm Disease-a-Month,* 42**,** 133-194.
CANCELO-HIDALGO, M. J., CASTELO-BRANCO, C., PALACIOS, S., HAYA-PALAZUELOS, J., CIRIA-RECASENS, M., MANASANCH, J. & PEREZ-EDO, L. 2013. Tolerability of different oral iron supplements: a systematic review. *Current Medical Research and Opinion,* 29**,** 291-303.
CAPPELL, M. S. & GOLDBERG, E. S. 1992. The relationship between the clinical presentation and spread of colon cancer in 315 consecutive patients. A significant trend of earlier cancer detection from 1982 through 1988 at a university hospital. *J Clin Gastroenterol,* 14**,** 227-35.
CARSON, J. L., DUFF, A., POSES, R. M., BERLIN, J. A., SPENCE, R. K., TROUT, R., NOVECK, H. & STROM, B. L. 1996. Effect of anaemia and cardiovascular disease on surgical mortality and morbidity. *The Lancet,* 348**,** 1055-1060.
CASE, D. C., BUKOWSKI, R. M., CAREY, R. W., FISHKIN, E. H., HENRY, D. H., JACOBSON, R. J., JONES, S. E., KELLER, A. M., KUGLER, J. W., NICHOLS, C. R., SALMON, S. E., SILVER, R. T., STORNIOLO, A. M., WAMPLER, G. L., DOOLEY, C. M., LARHOLT, K. M., NELSON, R. A. & ABELS, R. I. 1993. Recombinant-Human-Erythropoietin Therapy for Anemic Cancer-Patients on Combination Chemotherapy. *Journal of the National Cancer Institute,* 85**,** 801-806.
CASTILLO, J. J., DALIA, S. & PASCUAL, S. K. 2010. Association between red blood cell transfusions and development of non-Hodgkin lymphoma: a meta-analysis of observational studies. *Blood,* 116**,** 2897-2907.
CATA, J. P., WANG, H., GOTTUMUKKALA, V., REUBEN, J. & SESSLER, D. I. 2013. Inflammatory response, immunosuppression, and cancer recurrence after perioperative blood transfusions. *Br J Anaesth,* 110**,** 690-701.
CELLA, D. 1997. The functional assessment of cancer therapy-anemia (FACT-An) scale: A new tool for the assessment of outcomes in cancer anemia and fatigue. *Seminars in Hematology,* 34**,** 13-19.
CELLA, D. 1998. Factors influencing quality of life in cancer patients: Anemia and fatigue. *Seminars in Oncology,* 25**,** 43-46.

CHAND, S., WARD, D. G., NG, Z. Y., HODSON, J., KIRBY, H., STEELE, P., ROOPLAL, I., BANTUGON, F., IQBAL, T., TSELEPIS, C., DRAYSON, M. T., WHITELEGG, A., CHOWRIMOOTOO, M. & BORROWS, R. 2014. Serum hepcidin-25 and response to intravenous iron in patients with non-dialysis chronic kidney disease. *J Nephrol*.

CHAVES, P. H. M. 2008. Functional outcomes of anemia in older adults. *Seminars in Hematology,* 45**,** 255-260.

CHECHIK, O., INBAR, R., DANINO, B., LADOR, R., GREENBERG, R. & AVITAL, S. 2011. Anti-Platelet Therapy: No Association with Increased Blood Loss in Patients Undergoing Open or Laparoscopic Appendectomy. *Israel Medical Association Journal,* 13**,** 342-344.

CHEN, H. H., CHEN, W. T. L., LEE, H. C., LIN, J. K., FANG, C. Y., CHOU, Y. H., LIN, P. C., LIN, B. W., HUANG, C. C., YEH, C. H., HSU, H. H., CHEN, H. C., TING, W. C., YANG, M. C. & TAN, E. C. H. 2015. Health-related quality of life and cost comparison of adjuvant capecitabine versus 5-fluorouracil/leucovorin in stage III colorectal cancer patients. *Quality of Life Research,* 24**,** 473-484.

CHRISTODOULAKIS, M., TSIFTSIS, D. D. & PERIOPERATI, H. S. O. 2005. Preoperative epoetin alfa in colorectal surgery: A randomized, controlled study. *Annals of Surgical Oncology,* 12**,** 718-725.

CHU, M., REHFELD, J. F. & BORCH, K. 1992. Effects of gastric fundectomy and antrectomy on the colonic mucosa in the hamster. *Digestion,* 53**,** 28-34.

CLADELLAS, M., FARRE, N., COMIN-COLET, J., GOMEZ, M., MERONO, O., BOSCH, M. A., VILA, J., MOLERA, R., SEGOVIA, A. & BRUGUERA, J. 2012. Effects of Preoperative Intravenous Erythropoietin Plus Iron on Outcome in Anemic Patients After Cardiac Valve Replacement. *American Journal of Cardiology,* 110**,** 1021-1026.

CLAVIEN, P. A., BARKUN, J., DE OLIVEIRA, M. L., VAUTHEY, J. N., DINDO, D., SCHULICK, R. D., DE SANTIBANES, E., PEKOLJ, J., SLANKAMENAC, K., BASSI, C., GRAF, R., VONLANTHEN, R., PADBURY, R., CAMERON, J. L. & MAKUUCHI, M. 2009. The Clavien-Dindo Classification of Surgical Complications Five-Year Experience. *Annals of Surgery,* 250**,** 187-196.

COAKLEY, M., SELF, R., MARCHANT, W., MACKIE, I., MALLETT, S. V. & MYTHEN, M. 2005. Use of the platelet function analyser (PFA-100 (R)) to quantify the effect of low dose aspirin in patients with ischaemic heart disease. *Anaesthesia,* 60**,** 1173-1178.

CODY, J., DALY, C., CAMPBELL, M., DONALDSON, C., KHAN, I., RABINDRANATH, K., VALE, L., WALLACE, S. & MACLEOD, A. 2005. Recombinant human erythropoietin for chronic renal

failure anaemia in pre-dialysis patients. *Cochrane Database of Systematic Reviews*.
COLLINS & ANDERSON 2012. *Physiology of the gastrointestinal tract.,* New York, Elsevier.
COLOR 2005. Laparoscopic surgery versus open surgery for colon cancer: short-term outcomes of a randomised trial. *The Lancet Oncology,* 6**,** 477-484.
CONRAD, M. E. & UMBREIT, J. N. 1993. A concise review: iron absorption--the mucin-mobilferrin-integrin pathway. A competitive pathway for metal absorption. *Am J Hematol,* 42**,** 67-73.
COOK, J. D. 2005. Diagnosis and management of iron-deficiency anaemia. *Best Practice & Research Clinical Haematology,* 18**,** 319-332.
COOK, J. D. & REDDY, M. B. 2001. Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. *Am J Clin Nutr,* 73**,** 93-8.
COPAY, A. G., SUBACH, B. R., GLASSMAN, S. D., POLLY, D. W. & SCHULER, T. C. 2007. Understanding the minimum clinically important difference: a review of concepts and methods. *Spine Journal,* 7**,** 541-546.
COX, D., KERRIGAN, S. W. & WATSON, S. P. 2011. Platelets and the innate immune system: mechanisms of bacterial-induced platelet activation. *Journal of Thrombosis and Haemostasis,* 9**,** 1097-1107.
COYNE, D. 2006. Iron indices: what do they really mean? *Kidney Int Suppl***,** S4-8.
COYNE, D. W. 2011. Hepcidin: clinical utility as a diagnostic tool and therapeutic target. *Kidney International,* 80**,** 240-244.
CULLETON, B. F., WALSH, M., KLARENBACH, S. W., MORTIS, G., SCOTT-DOUGLAS, N., QUINN, R. R., TONELLI, M., DONNELLY, S., FRIEDRICH, M. G., KUMAR, A., MAHALLATI, H., HEMMELGARN, B. R. & MANNS, B. J. 2007. Effect of frequent nocturnal hemodialysis vs conventional hemodialysis on left ventricular mass and quality of life: a randomized controlled trial. *JAMA,* 298**,** 1291-9.
CUSTER, R. L., SCARCELLA, J. A. & STEWART, B. R. 1999. The Modified Delphi Technique- Arotational Modification. *Journal of Vocational and Technical Education* [Online], 15. Available: http://scholar.lib.vt.edu/ejournals/JVTE/v15n2/custer.html [Accessed October 2014].
DAJANI, J. S., SINCOFF, M. Z. & TALLEY, W. K. 1979. Stability and Agreement Criteria for the Termination of Delphi Studies. *Technological Forecasting and Social Change,* 13**,** 83-90.
DALLALIO, G., FLEURY, T. & MEANS, R. T. 2003. Serum hepcidin in clinical specimens. *Br J Haematol,* 122**,** 996-1000.

DALLALIO, G., LAW, E. & MEANS, R. T., JR. 2006. Hepcidin inhibits in vitro erythroid colony formation at reduced erythropoietin concentrations. *Blood,* 107**,** 2702-4.
DAN, K. 2005. Thrombocytosis in iron deficiency anemia. *Internal Medicine,* 44**,** 1025-1026.
DAY, J. & BOBEVA, M. 2005. A Generic Toolkit for the Successful Management of Delphi Studies. *The electronic journal of Business Research Methodology,* 3**,** 103-116.
DE CALIGNON, A., POLYDORO, M., SUAREZ-CALVET, M., WILLIAM, C., ADAMOWICZ, D. H., KOPEIKINA, K. J., PITSTICK, R., SAHARA, N., ASHE, K. H., CARLSON, G. A., SPIRES-JONES, T. L. & HYMAN, B. T. 2012. Propagation of tau pathology in a model of early Alzheimer's disease. *Neuron,* 73**,** 685-97.
DE OLIVEIRA, G. S., JR., SCHINK, J. C., BUOY, C., AHMAD, S., FITZGERALD, P. C. & MCCARTHY, R. J. 2012. The association between allogeneic perioperative blood transfusion on tumour recurrence and survival in patients with advanced ovarian cancer. *Transfus Med,* 22**,** 97-103.
DE VET, E., DE NOOIJER, J., DE VRIES, N. K. & BRUG, J. 2005. Determinants of forward stage transition from precontemplation and contemplation for fruit' consumption. *American Journal of Health Promotion,* 19**,** 278-285.
DEAN L 2005. Blood Groups and red cell antigens. *In:* BETHESDA (ed.) *Chapter 3, Blood transfusions and the immune system. .* National Center for Biotechnology Information (US).
DELBECQ, A. L., VAN DE VEN, A. H. & GUSTAFSON, D. H. 1975. *Chapter 4: The Delphi Technique, in Group Techniques for Program Planning: A Guide to Nominal and Delphi Processes,* Glenview, IL.
DESCHEMIN, J. C. & VAULONT, S. 2013. Role of Hepcidin in the Setting of Hypoferremia during Acute Inflammation. *PLoS One,* 8.
DHARMARAJAN, T. S., AVULA, S. & NORKUS, E. P. 2006. Anemia increases risk for falls in hospitalized older adults: An evaluation of falls in 362 hospitalized, ambulatory, long-term care, and community patients. *Journal of the American Medical Directors Association,* 7**,** 287-293.
DINDO, D., DEMARTINES, N. & CLAVIEN, P. A. 2004. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Annals of Surgery,* 240**,** 205-13.
DONALDSON, M. D., SEAMAN, M. J. & PARK, G. R. 1992. Massive blood transfusion. *Br J Anaesth,* 69**,** 621-30.
DOODEMAN, H. J., VAN HAELST, I. M. M., EGBERTS, T. C. G., BENNIS, M., TRAAST, H. S., VAN SOLINGE, W. W., KALKMAN, C. J. & VAN KLEI, W. A. 2013. The effect of a preoperative erythropoietin protocol as part of a multifaceted

blood management program in daily clinical practice (CME). *Transfusion,* 53**,** 1930-1939.
DOUKETIS, J. D., SPYROPOULOS, A. C., SPENCER, F. A., MAYR, M., JAFFER, A. K., ECKMAN, M. H., DUNN, A. S. & KUNZ, R. 2012. Perioperative Management of Antithrombotic Therapy Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence- Based Clinical Practice Guidelines. *Chest,* 141**,** E326s-E350s.
DUNNE, J. R., GANNON, C. J., OSBORN, T. M., TAYLOR, M. D., MALONE, D. L. & NAPOLITANO, L. M. 2002a. Preoperative anemia in colon cancer: assessment of risk factors. *Am Surg,* 68**,** 582-7.
DUNNE, J. R., MALONE, D., TRACY, J. K., GANNON, C. & NAPOLITANO, L. M. 2002b. Perioperative anemia: an independent risk factor for infection, mortality, and resource utilization in surgery. *J Surg Res,* 102**,** 237-44.
EDWARDS, T. J., NOBLE, E. J., DURRAN, A., MELLOR, N. & HOSIE, K. B. 2009. Randomized clinical trial of preoperative intravenous iron sucrose to reduce blood transfusion in anaemic patients after colorectal cancer surgery. *Br J Surg,* 96**,** 1122-8.
ESPALLARDO, C. D., MORALES, M. J. L., CALVET, C. C., LOPEZ, L. M., MARTINEZ, I. R. & MARIN, M. T. M. 2011. The multidisciplinary approach is useful for optimising preoperative haemoglobin in colorectal cancer surgery. *Cirugia Espanola,* 89**,** 392-399.
EVSTATIEV, R., MARTEAU, P., IQBAL, T., KHALIF, I. L., STEIN, J., BOKEMEYER, B., CHOPEY, I. V., GUTZWILLER, F. S., RIOPEL, L. & GASCHE, C. 2011. FERGIcor, a Randomized Controlled Trial on Ferric Carboxymaltose for Iron Deficiency Anemia in Inflammatory Bowel Disease. *Gastroenterology,* 141**,** 846-853.e2.
EXADAKTYLOS, A. K., BUGGY, D. J., MORIARTY, D. C., MASCHA, E. & SESSLER, D. I. 2006. Can anesthetic technique for primary breast cancer surgery affect recurrence or metastasis? *Anesthesiology,* 105**,** 660-664.
FAQUIN, W. C., SCHNEIDER, T. J. & GOLDBERG, M. A. 1992. Effect of Inflammatory Cytokines on Hypoxia-Induced Erythropoietin Production. *Blood,* 79**,** 1987-1994.
FEARON, K. C., JENKINS, J. T., CARLI, F. & LASSEN, K. 2013. Patient optimization for gastrointestinal cancer surgery. *Br J Surg,* 100**,** 15-27.
FERRARIS, V. A., DAVENPORT, D. L., SAHA, S. P., AUSTIN, P. C. & ZWISCHENBERGER, J. B. 2012. Surgical outcomes and transfusion of minimal amounts of blood in the operating room. *Arch Surg,* 147**,** 49-55.

FERRARIS, V. A., FERRARIS, S. P., MOLITERNO, D. J., CAMP, P., WALENGA, J. M., MESSMORE, H. L., JESKE, W. P., EDWARDS, F. H., ROYSTON, D., SHAHIAN, D. M., PETERSON, E., BRIDGES, C. R. & DESPOTIS, G. 2005. The Society of Thoracic Surgeons Practice Guideline Series: Aspirin and other antiplatelet agents during operative coronary revascularization (Executive summary). *Annals of Thoracic Surgery,* 79**,** 1454-1461.

FINBERG, K. E. 2013. Regulation of systemic iron homeostasis. *Curr Opin Hematol*.

FIORE, L. D., BROPHY, M. T., LOPEZ, A., JANSON, P. & DEYKIN, D. 1990. The Bleeding-Time Response to Aspirin - Identifying the Hyperresponder. *Am J Clin Pathol,* 94**,** 292-296.

FLEMING, M. D., ROMANO, M. A., SU, M. A., GARRICK, L. M., GARRICK, M. D. & ANDREWS, N. C. 1998. Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc Natl Acad Sci U S A,* 95**,** 1148-53.

FLEMING, M. D., TRENOR, C. C., 3RD, SU, M. A., FOERNZLER, D., BEIER, D. R., DIETRICH, W. F. & ANDREWS, N. C. 1997. Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet,* 16**,** 383-6.

FOX, S. C., MAY, J. A., SHAH, A., NEUBERT, U. & HEPTINSTALL, S. 2009. Measurement of platelet P-selectin for remote testing of platelet function during treatment with clopidogrel and/or aspirin. *Platelets,* 20**,** 250-9.

FRANK, R. E., SACLARIDES, T. J., LEURGANS, S., SPEZIALE, N. J., DRAB, E. A. & RUBIN, D. B. 1995. Tumor Angiogenesis as a Predictor of Recurrence and Survival in Patients with Node-Negative Colon-Cancer. *Annals of Surgery,* 222**,** 695-699.

FREEMANTLE, N., CALVERT, M., WOOD, J., EASTAUGH, J. & GRIFFIN, C. 2003. Composite outcomes in randomized trials - Greater precision but with greater uncertainty? *Jama-Journal of the American Medical Association,* 289**,** 2554-2559.

FREIRE, W. B. 1989. Hemoglobin as a predictor of response to iron therapy and its use in screening and prevalence estimates. *Am J Clin Nutr,* 50**,** 1442-9.

FUQUA, B. K., VULPE, C. D. & ANDERSON, G. J. 2012. Intestinal iron absorption. *Journal of Trace Elements in Medicine and Biology,* 26**,** 115-119.

GALLO, V., EGGER, M., MCCORMACK, V., FARMER, P. B., IOANNIDIS, J. P. A., KIRSCH-VOLDERS, M., MATULLO, G., PHILLIPS, D. H., SCHOKET, B., STROMBERG, U., VERMEULEN, R., WILD, C., PORTA, M. & VINEIS, P. 2011. STrengthening the Reporting of OBservational studies in

Epidemiology-Molecular Epidemiology (STROBE-ME): An Extension of the STROBE Statement. *Plos Medicine,* 8.
GANZ, T. 2011. Hepcidin and iron regulation, ten years later. *Blood* [Online].
GERA, T., SACHDEV, H. P., NESTEL, P. & SACHDEV, S. S. 2007. Effect of iron supplementation on haemoglobin response in children: systematic review of randomised controlled trials. *J Pediatr Gastroenterol Nutr,* 44**,** 468-86.
GIANNOULIS, C., DANIILIDIS, A., TANTANASIS, T., DINAS, K. & TZAFETTAS, J. 2009. Intravenous administration of iron sucrose for treating anemia in postpartum women. *Hippokratia,* 13**,** 38-40.
GISBERT, J. P., BERMEJO, F., PAJARES, R., PEREZ-CALLE, J. L., RODRIGUEZ, M., ALGABA, A., MANCENIDO, N., DE LA MORENA, F., CARNEROS, J. A., MCNICHOLL, A. G., GONZALEZ-LAMA, Y. & MATE, J. 2009. Oral and intravenous iron treatment in inflammatory bowel disease: hematological response and quality of life improvement. *Inflammatory Bowel Diseases,* 15**,** 1485-91.
GLASPY, J., HENRY, D., PATEL, R., TCHEKMEDYIAN, D., APPLEBAUM, S., BERDEAUX, D., LLOYD, R., BERG, R., AUSTIN, M. & ROSSI, G. 2005. Effects of chemotherapy on endogenous erythropoietin levels and the pharmacokinetics and erythropoietic response of darbepoetin alfa: A randomised clinical trial of synchronous versus asynchronous dosing of darbepoetin alfa. *European Journal of Cancer,* 41**,** 1140-1149.
GODDARD AF, JAMES MW, MACINTYRE AS & SCOTT BB. 2005. *Guidelines for the management of iron deficiency anaemia. BSG Guidelines 2005* [Online]. http://www.bsg.org.uk/clinicalguidelines/small-bowel-nutrition/guidelines-for-the-management-ofiron-deficiency-anaemia-2005.htm.
GODDARD, A. F., JAMES, M. W., MCINTYRE, A. S., SCOTT, B. B. & GASTROENTEROLOGY, B. S. 2011. Guidelines for the management of iron deficiency anaemia. *Gut,* 60**,** 1309-1316.
GOLDBERG, N. D. 2013. Iron deficiency anemia in patients with inflammatory bowel disease. *Clin Exp Gastroenterol,* 6**,** 61-70.
GOLUBOV, J., FLANAGAN, P. & ADAMS, P. 1991. Inhibition of iron absorption by omeprazole in rat model. *Dig Dis Sci,* 36**,** 405-8.
GOODNOUGH, L. T., PRICE, T. H., FRIEDMAN, K. D., JOHNSTON, M., CIAVARELLA, D., KHAN, N., SACHER, R., VOGLER, W. R., WISSEL, M. & ABELS, R. I. 1994. A Phase-Iii Trial of Recombinant-Human-Erythropoietin Therapy in Nonanemic

Orthopedic Patients Subjected to Aggressive Removal of Blood for Autologous Use - Dose, Response, Toxicity, and Efficacy. *Transfusion,* 34**,** 66-71.
GOODNOUGH, L. T. & SHANDER, A. 2007. Blood management. *Arch Pathol Lab Med,* 131**,** 695-701.
GREENBURG, A. G. 1996. Pathophysiology of anemia. *Am J Med,* 101**,** 7S-11S.
GREM, J. 2000. 5-Fluorouracil: Forty-Plus and Still Ticking. A Review of its Preclinical and Clinical Development. *Investigational New Drugs,* 18**,** 299-313.
GROTE BEVERBORG, N., VERWEIJ, N., KLIP, I. T., VAN DER WAL, H. H., VOORS, A. A., VAN VELDHUISEN, D. J., GANSEVOORT, R. T., BAKKER, S. J., VAN DER HARST, P. & VAN DER MEER, P. 2015. Erythropoietin in the General Population: Reference Ranges and Clinical, Biochemical and Genetic Correlates. *PLoS One,* 10**,** e0125215.
GU, J., STOCCHI, L., REMZI, F. & KIRAN, R. P. 2013. Factors associated with postoperative morbidity, reoperation and readmission rates after laparoscopic total abdominal colectomy for ulcerative colitis. *Colorectal Disease,* 15**,** 1123-1129.
GUILLOU, P. J., QUIRKE, P., THORPE, H., WALKER, J., JAYNE, D. G., SMITH, A. M., HEATH, R. M. & BROWN, J. M. 2005. Short-term endpoints of conventional versus laparoscopic-assisted surgery in patients with colorectal cancer (MRC CLASICC trial): multicentre, randomised controlled trial. *Lancet,* 365**,** 1718-26.
GUNSHIN, H., FUJIWARA, Y., CUSTODIO, A. O., DIRENZO, C., ROBINE, S. & ANDREWS, N. C. 2005. Slc11a2 is required for intestinal iron absorption and erythropoiesis but dispensable in placenta and liver. *J Clin Invest,* 115**,** 1258-66.
HABR-GAMA, A., SOUZA, P. S., RIBEIRO, U., JR., NADALIN, W., GANSL, R., SOUSA, A. S., JR., CAMPOS, F. & GAMA-RODRIGUES, J. 1998. Low rectal cancer. *Diseases of the Colon & Rectum,* 41**,** 1087-1096.
HALLBERG, L., BRUNE, M. & ROSSANDER, L. 1989. The role of vitamin C in iron absorption. *Int J Vitam Nutr Res Suppl,* 30**,** 103-8.
HALLOWELL, M. R. & GAMBATESE, J. A. 2010. Qualitative Research: Application of the Delphi Method to CEM Research. *Journal of Construction Engineering and Management,* 136**,** 99-107.
HAZARA, A. M. & BHANDARI, S. 2015. Intravenous iron administration is associated with reduced platelet counts in patients with chronic kidney disease. *Journal of Clinical Pharmacy and Therapeutics,* 40**,** 20-23.

HEBERT, P. C., WELLS, G., BLAJCHMAN, M. A., MARSHALL, J., MARTIN, C., PAGLIARELLO, G., TWEEDDALE, M., SCHWEITZER, I. & YETISIR, E. 1999. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. *N Engl J Med,* 340**,** 409-17.

HEISS, M. M., TARABICHI, A., DELANOFF, C., ALLGAYER, H., JAUCH, K.-W., HERNANDEZ-RICHTER, T., MEMPEL, W., BECK, K.-G., SCHILDBERG, F. W. & MESSMER, K. 1996. Perisurgical erythropoietin application in anemic patients with colorectal cancer: A double-blind randomized study. *Surgery,* 119**,** 523-527.

HENRY, D. H. 1992. Changing Patterns of Care in the Management of Anemia. *Seminars in Oncology,* 19**,** 3-7.

HO, C. H., YU, Y. B. & WU, P. H. 2008. The prevalence of iron deficiency anemia and its clinical implications in patients with colorectal carcinoma. *Journal of the Chinese Medical Association,* 71**,** 119-122.

HOCKEL, M. & VAUPEL, P. 2001. Tumor hypoxia: definitions and current clinical, biologic, and molecular aspects. *J Natl Cancer Inst,* 93**,** 266-76.

HOLEY, E. A., FEELEY, J. L., DIXON, J. & WHITTAKER, V. J. 2007. An exploration of the use of simple statistics to measure consensus and stability in Delphi studies. *Bmc Medical Research Methodology,* 7.

HOVENS, M. M. C., SNOEP, J. D., EIKENBOOM, J. C. J., VAN DER BOM, J. G., MERTENS, B. J. A. & HUISMAN, M. V. 2007. Prevalence of persistent platelet reactivity despite use of aspirin: A systematic review. *Am Heart J,* 153**,** 175-181.

HSU, C. & SANDFORD, B. 2007. The Delphi Technique; Making Sense of Consensus. *Practical Assessment, Research and Evaluation,* 12.

HURST, N. P., KIND, P., RUTA, D., HUNTER, M. & STUBBINGS, A. 1997. Measuring health-related quality of life in rheumatoid arthritis: validity, responsiveness and reliability of EuroQol (EQ-5D). *Br J Rheumatol,* 36**,** 551-9.

HUSTED, H., BLOND, L., SONNE-HOLM, S., HOLM, G., JACOBSEN, T. W. & GEBUHR, P. 2003. Tranexamic acid reduces blood loss and blood transfusions in primary total hip arthroplasty: a prospective randomized double-blind study in 40 patients. *Acta Orthop Scand,* 74**,** 665-9.

INUI, A. 1999. Cancer anorexia-cachexia syndrome: are neuropeptides the key? *Cancer Res,* 59**,** 4493-501.

INVOLVE 2013. What is public involvement in research? : National Institute for Health Research.

ISBISTER, J. P., SHANDER, A., SPAHN, D. R., ERHARD, J., FARMER, S. L. & HOFMANN, A. 2011. Adverse blood transfusion outcomes: establishing causation. *Transfus Med Rev,* 25**,** 89-101.

ITO, T. & JENSEN, R. T. 2010. Association of long-term proton pump inhibitor therapy with bone fractures and effects on absorption of calcium, vitamin B12, iron, and magnesium. *Curr Gastroenterol Rep,* 12**,** 448-57.

JADAD, A. R., MOORE, R. A., CARROLL, D., JENKINSON, C., REYNOLDS, D. J., GAVAGHAN, D. J. & MCQUAY, H. J. 1996. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials,* 17**,** 1-12.

JAESCHKE, R., SINGER, J. & GUYATT, G. H. 1989. Measurement of Health-Status - Ascertaining the Minimal Clinically Important Difference. *Control Clin Trials,* 10**,** 407-415.

JAMIL, K., KALYANI, P., PERIMI, R. & KAMESHWARI, S. 2009. Assessment of severity of anemia and its effect on the quality of life (QOL) of patients suffering with various types of neoplasia. *Biology and Medicine,* 1**,** 63-72.

JELKMANN, W. 2011. Regulation of erythropoietin production. *Journal of Physiology-London,* 589**,** 1251-1258.

JOHANNES, T., MIK, E. G., NOHE, B., UNERTL, K. E. & INCE, C. 2007. Acute decrease in renal microvascular PO2 during acute normovolemic hemodilution. *Am J Physiol Renal Physiol,* 292**,** F796-803.

JONGEN-LAVRENCIC, M., PEETERS, H., ROZEMULLER, H., ROMBOUTS, W., MARTENS, G., VREUGDENHIL, G., PILLAY, M., COX, P., BIJSER, M., BRUTEL, G., BREEDVELD, F. & SWAAK, A. 1996. IL-6-induced anaemia in rats: possible pathogenetic implications for anaemia observed in chronic inflammations. *Clin Exp Immunol,* 103**,** 328-334.

JPAC. 2014. *Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee - Patient Blood Management* [Online]. Available: http://www.transfusionguidelines.org.uk/uk-transfusion-committees/national-blood-transfusion-committee/patient-blood-management [Accessed December 2014].

JPAC. 2015. *Joint United Kingdom (UK) Blood Transfusion and Tissue Transplantation Services Professional Advisory Committee, 7.3.1: Red cell transfusion in major haemorrhage,* [Online]. http://www.transfusionguidelines.org.uk/transfusion-handbook/7-effective-transfusion-in-surgery-and-critical-care/7-3-transfusion-management-of-major-haemorrhage. [Accessed March 2015.

JU, B. & JIN, T. 2013. Incorporating nonparametric statistics into Delphi studies in library and information science. *Information Research-an International Electronic Journal,* 18.
KALAIAN, S. & KASIM, R. 2012. Terminating Sequential Delphi Survey Data Collection. *Practical Assessment, Research and Evaluation* [Online], 17. [Accessed November 2014].
KARKOUTI, K., WIJEYSUNDERA, D. N. & BEATTIE, W. S. 2008. Risk associated with preoperative anemia in cardiac surgery: a multicenter cohort study. *Circulation,* 117**,** 478-84.
KEELER, B. D., KRELL, J., ACHESON, A. G., BROOKES, M. J., STEBBING, J. & FRAMPTON, A. E. 2012. Is there a role for intravenous iron therapy in patients undergoing colorectal cancer resection? *Expert Rev Anticancer Ther,* 12**,** 1407-12.
KEELER, B. D., SIMPSON, J. A., NG, S., TSELEPIS, C., IQBAL, T., BROOKES, M. J. & ACHESON, A. G. 2014. The feasibility and clinical efficacy of intravenous iron administration for preoperative anaemia in patients with colorectal cancer. *Colorectal Disease,* 16**,** 794-800.
KELL, D. B. & PRETORIUS, E. 2014. Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. *Metallomics,* 6**,** 748-773.
KHALAFALLAH, A. A., ABDUL, M. A., CHAN, J., SUNG, M. F., BATES, G., AHUJA, K., BATTEN, J. C. & EINODER, B. 2012. A Prospective Randomized Controlled Trial to Assess the Effect of Intravenous versus Oral Iron Therapy in the Treatment of Orthopaedic Preoperative Anaemia. *J of Blood Disorders and Transfusion,* 3**,** 127-133.
KIDA, H., NISHIKAWA, N., MATSUNAMI, K., KATSUYAMA, R. & KAWAHITO, M. 1999. [The effect of epidural anesthesia on reducing blood loss during upper abdominal surgery]. *Masui,* 48**,** 265-70.
KIRAN, R. P., DELANEY, C. P., SENAGORE, A. J., MILLWARD, B. L. & FAZIO, V. W. 2004. Operative blood loss and use of blood products after laparoscopic and conventional open colorectal operations. *Archives of Surgery,* 139**,** 39-42.
KNIGHT, K., WADE, S. & BALDUCCI, L. 2004. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. *The American Journal of Medicine,* 116**,** 11-26.
KNUTSON, M. D., OUKKA, M., KOSS, L. M., AYDEMIR, F. & WESSLING-RESNICK, M. 2005. Iron release from macrophages after erythrophagocytosis is up-regulated by ferroportin 1 overexpression and down-regulated by hepcidin. *Proc Natl Acad Sci U S A,* 102**,** 1324-8.
KOK, M., KOORNSTRA, R. H., MOOK, S., HAUPTMANN, M., FLES, R., JANSEN, M. P., BERNS, E. M., LINN, S. C. & VAN 'T VEER, L. J. 2012. Additional value of the 70-gene signature and levels of ER and PR for the prediction of outcome in

tamoxifen-treated ER-positive breast cancer. *The Breast,* 21**,** 769-778.
KULNIGG-DABSCH, S., SCHMID, W., HOWALDT, S., STEIN, J., MICKISCH, O., WALDHOR, T., EVSTATIEV, R., KAMALI, H., VOLF, I. & GASCHE, C. 2013. Iron deficiency generates secondary thrombocytosis and platelet activation in IBD: the randomized, controlled thromboVIT trial. *Inflammatory Bowel Diseases,* 19**,** 1609-16.
KULNIGG, S. & GASCHE, C. 2006. Systematic review: managing anaemia in Crohn's disease. *Alimentary Pharmacology & Therapeutics,* 24**,** 1507-1523.
KUMAR, S., RANDHAWA, M., GANESAMONI, R. & SINGH, S. 2012. Tranexamic acid reduces blood loss in percutaneous nephrolithotomy: a prospective randomized controlled study. *Journal of Urology,* EPub ahead of print.
LAFFER, U., HARDER, F., JAGGI, P., METZGER, U., AEBERHARD, P., EGELI, R., ARMA, S., BARRAS, J. P., LAMPERT, V. D., MARTINOLI, S., MULLER, W., SCHRODER, R., VONHUBEN, R., WEBER, W., CASTIGLIONE, M., GOLDHIRSCH, A., MAIBACH, R., SCHMITZ, S. F. H., GLOOR, F., STAMM, B. & WALTZER, U. 1997. Association between blood transfusion and survival in a randomised multicentre trial of perioperative adjuvant portal chemotherapy in patients with colorectal cancer. *European Journal of Surgery,* 163**,** 693-701.
LANDETA, J. 2006. Current validity of the Delphi method in social sciences. *Technological Forecasting and Social Change,* 73**,** 467-482.
LEE, A. 2007. Early influences and childhood development. Does Helicobacter play a role? *Helicobacter,* 12**,** 69-74.
LEE, J. T., AHRENHOLZ, D. H., NELSON, R. D. & SIMMONS, R. L. 1979. Mechanisms of the Adjuvant Effect of Hemoglobin in Experimental Peritonitis .5. Significance of the Coordinated Iron Component. *Surgery,* 86**,** 41-48.
LEEKSMA, C. H. W. & COHEN, J. A. 1955. Determination of the Life of Human Blood Platelets Using Labelled Diisopropylfluorophosphonate. *Nature,* 175**,** 552-553.
LEHMANN, H. & CARRELL, R. W. 1969. VARIATIONS IN THE STRUCTURE OF HUMAN HAEMOGLOBIN: With Particular Reference to the Unstable Haemoglobins. *British Medical Bulletin,* 25**,** 14-23.
LEICHTLE, S. W., MOUAWAD, N. J., LAMPMAN, R., SINGAL, B. & CLEARY, R. K. 2011. Does Preoperative Anemia Adversely Affect Colon and Rectal Surgery Outcomes? *J Am Coll Surg,* 212**,** 187-194.
LI, H. & WANG, S. X. 2008. Intravenous iron sucrose in peritoneal dialysis patients with renal anemia. *Perit Dial Int,* 28**,** 149-54.

LI, Y.-Y., HSIEH, L.-L., TANG, R.-P., LIAO, S.-K. & YEH, K.-Y. 2009. Interleukin-6 (IL-6) released by macrophages induces IL-6 secretion in the human colon cancer HT-29 cell line. *Human Immunology,* 70**,** 151-158.

LIDDER, P. G., SANDERS, G., WHITEHEAD, E., DOUIE, W. J., MELLOR, N., LEWIS, S. J. & HOSIE, K. B. 2007. Pre-operative oral iron supplementation reduces blood transfusion in colorectal surgery - a prospective, randomised, controlled trial. *Ann R Coll Surg Engl,* 89**,** 418-21.

LIKERT, R. 1932. A technique for the measurement of attitudes. . *Archives of Psychology,* 22**,** 5-55.

LIND, M., VERNON, C., CRUICKSHANK, D., WILKINSON, P., LITTLEWOOD, T., STUART, N., JENKINSON, C., GREY-AMANTE, P., DOLL, H. & WILD, D. 2002. The level of haemoglobin in anaemic cancer patients correlates positively with quality of life. *British Journal of Cancer,* 86**,** 1243-1249.

LITTON, E., XIAO, J. & HO, K. M. 2013. Safety and efficacy of intravenous iron therapy in reducing requirement for allogeneic blood transfusion: systematic review and meta-analysis of randomised clinical trials. *BMJ,* 347**,** f4822.

LOCATELLI, F., COVIC, A., ECKARDT, K. U., WIECEK, A., VANHOLDER, R. & BOARD, E.-E. E. A. 2009. Anaemia management in patients with chronic kidney disease: a position statement by the Anaemia Working Group of European Renal Best Practice (ERBP). *Nephrology Dialysis Transplantation,* 24**,** 348-354.

LOGAN, R. P. H. & WALKER, M. M. 2001. Epidemiology and diagnosis of Helicobacter pylori infection. *BMJ,* 323**,** 920-922.

LOPEZ, R. M., ALADREN, B. S. & GARCIA, F. G. 2009. Use of agents stimulating erythropoiesis in digestive diseases. *World Journal of Gastroenterology,* 15**,** 4675-4685.

LUDWIG, B. 1997. Predicting the Future: Have you considered using the Delphi Methodology? *Journal of Extension* [Online], 35. Available: http://www.joe.org/joe/1997october/tt2.php [Accessed Nov 2014].

LUDWIG, H., MULDUR, E., ENDLER, G. & HUBL, W. 2013. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol,* 24**,** 1886-92.

LUDWIG, H., VAN BELLE, S., BARRETT-LEE, P., BIRGEGÅRD, G., BOKEMEYER, C., GASCÓN, P., KOSMIDIS, P., KRZAKOWSKI, M., NORTIER, J., OLMI, P., SCHNEIDER, M. & SCHRIJVERS, D. 2004. The European Cancer Anaemia Survey (ECAS): A large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. *European Journal of Cancer,* 40**,** 2293-2306.

MACCIO, A. & MADEDDU, C. 2012. Management of anemia of inflammation in the elderly. *Anemia,* 2012**,** 563251.
MACDOUGALL, I. C. 1999. Strategies for iron supplementation: oral versus intravenous. *Kidney Int Suppl,* 69**,** S61-6.
MACDOUGALL, I. C., TUCKER, B., THOMPSON, J., TOMSON, C. R. V., BAKER, L. R. I. & RAINE, A. E. G. 1996. A randomized controlled study of iron supplementation in patients treated with erythropoietin. *Kidney Int,* 50**,** 1694-1699.
MACRAE, F. A. & STJOHN, D. J. B. 1982. Relationship between patterns of bleeding and hemoccult sensitivity in patients with colorectal cancers or adenomas. *Gastroenterology,* 82**,** 891-898.
MADBOULY, K. M., SENAGORE, A. J., REMZI, F. H., DELANEY, C. P., WATERS, J. & FAZIO, V. W. 2006. Perioperative blood transfusions increase infectious complications after ileoanal pouch procedures (IPAA). *International Journal of Colorectal Disease,* 21**,** 807-13.
MAJUMDAR, D., BEBB, J. & ATHERTON, J. 2011. Helicobacter pylori infection and peptic ulcers. *Medicine,* 39**,** 154-161.
MANJUNATH, S. 2013. Impact of oral iron therapy on quality of life in patients with heart failure. *International Journal of Basic & Clinical Pharmacology,* 2**,** 43-46.
MAO, L., LIN, S. & LIN, J. 2013. The effects of anesthetics on tumor progression. *Int J Physiol Pathophysiol Pharmacol,* 5**,** 1-10.
MAXWELL-ARMSTRONG, C. A., ROBINSON, M. H. & SCHOLEFIELD, J. H. 2000. Laparoscopic colorectal cancer surgery. *Am J Surg,* 179**,** 500-7.
MAXWELL, M. J. & WILSON, M. J. A. 2013. Complications of blood transfusion. *Nephrology Dialysis Transplantation,* 28**,** 225-229.
MCCALLION, K., MITCHELL, R. M. S., WILSON, R. H., KEE, F., WATSON, R. G. P., COLLINS, J. S. A. & GARDINER, K. R. 2001. Flexible sigmoidoscopy and the changing distribution of colorectal cancer: implications for screening. *Gut,* 48**,** 522-525.
MCCLEMENTS, P. L., MADURASINGHE, V., THOMSON, C. S., FRASER, C. G., CAREY, F. A., STEELE, R. J. C., LAWRENCE, G. & BREWSTER, D. H. 2012. Impact of the UK colorectal cancer screening pilot studies on incidence, stage distribution and mortality trends. *Cancer Epidemiology,* 36**,** e232-e242.
MCCORMACK, M., NIAS, A. H. & SMITH, E. 1990. Chronic anaemia, hyperbaric oxygen and tumour radiosensitivity. *Br J Radiol,* 63**,** 752-9.
MCEVOY, M. T. & SHANDER, A. 2013. Anemia, Bleeding, and Blood Transfusion in the Intensive Care Unit: Causes, Risks, Costs,

and New Strategies. *American Journal of Critical Care,* 22**,** Es1-Es13.
MCINTYRE, L. A., TINMOUTH, A. & FERGUSSON, D. 2013. Intravenous iron therapy for treatment of anaemia. *BMJ,* 347.
MCKIE, A. T., BARROW, D., LATUNDE-DADA, G. O., ROLFS, A., SAGER, G., MUDALY, E., MUDALY, M., RICHARDSON, C., BARLOW, D., BOMFORD, A., PETERS, T. J., RAJA, K. B., SHIRALI, S., HEDIGER, M. A., FARZANEH, F. & SIMPSON, R. J. 2001. An Iron-Regulated Ferric Reductase Associated with the Absorption of Dietary Iron. *Science,* 291**,** 1755-1759.
MCLELLAN, S. & WALSH, T. 2004. Oxygen delivery and haemoglobin. *Continuing Education in Anaesthesia, Critical Care & Pain,* 4**,** 123-6.
MEANS JR, R. T. 2004. Hepcidin and anaemia. *Blood Reviews,* 18**,** 219-225.
MECKLENBURG, I., REZNIK, D., FASLER-KAN, E., DREWE, J., BEGLINGER, C. & HRUZ, P. 2014. Serum hepcidin concentrations correlate with ferritin in patients with inflammatory bowel disease. *J Crohns Colitis,* 8**,** 1392-7.
MERCADAL, L., METZGER, M., CASADEVALL, N., HAYMANN, J. P., KARRAS, A., BOFFA, J. J., FLAMANT, M., VRTOVSNIK, F., STENGEL, B. & FROISSART, M. 2012. Timing and determinants of erythropoietin deficiency in chronic kidney disease. *Clin J Am Soc Nephrol,* 7**,** 35-42.
MERCHANT, A. A. & ROY, C. N. 2012. Not so benign haematology: anaemia of the elderly. *British Journal of Haematology,* 156**,** 173-185.
MILLER, C. B., JONES, R. J., PIANTADOSI, S., ABELOFF, M. D. & SPIVAK, J. L. 1990. Decreased erythropoietin response in patients with the anemia of cancer. *N Engl J Med,* 322**,** 1689-92.
MILLER, G. A. 1956. The Magical Number 7, Plus or Minus 2 - Some Limits on Our Capacity for Processing Information. *Psychological Review,* 63**,** 81-97.
MIZUARAI, S., YAMANAKA, K., ITADANI, H., ARAI, T., NISHIBATA, T., HIRAI, H. & KOTANI, H. 2009. Discovery of gene expression-based pharmacodynamic biomarker for a p53 context-specific anti-tumor drug Wee1 inhibitor. *Molecular Cancer,* 8.
MORRELL, C. N., AGGREY, A. A., CHAPMAN, L. M. & MODJESKI, K. L. 2014. Emerging roles for platelets as immune and inflammatory cells. *Blood,* 123**,** 2759-2767.
MORTEZAVI, A., HERMANNS, T., HEFERMEHL, L., SEIFERT, B., SPAHN, D., SCHMID, D., SULSER, T. & EBERLI, D. 2013. Continuous Low-Dose Aspirin Therapy in Robotic-Assisted Laparoscopic Radical Prostatectomy Does Not Increase Risk

of Surgical Haemorrhage. *Journal of Urology,* 189**,** E415-E415.
MUNOZ, M., GOMEZ-RAMIREZ, S., MARTIN-MONTANEZ, E. & AUERBACH, M. 2014. Perioperative anemia management in colorectal cancer patients: a pragmatic approach. *World J Gastroenterol,* 20**,** 1972-85.
MURPHY, M. K., BLACK, N., LAMPING, D. L., MCKEE C.M., SANDERSON, C. F. B. & ASKHAM, J. 1998. Consensus development methods and their use in clinical guideline development. *Health Technology Assessment,* 2.
MUSALLAM, K. M., TAMIM, H. M., RICHARDS, T., SPAHN, D. R., ROSENDAAL, F. R., HABBAL, A., KHREISS, M., DANDALEH, F. S., KHAVANDI, K., SFEIR, P. M., SOWEID, A., HOBALLAH, J. J., TAHER, A. T. & JAMALI, F. R. 2011. Preoperative anaemia and postoperative outcomes in non-cardiac surgery: a retrospective cohort study. *Lancet,* 378**,** 1396-1407.
NAIRZ, M., SCHROLL, A., SONNWEBER, T. & WEISS, G. 2010. The struggle for iron - a metal at the host-pathogen interface. *Cellular Microbiology,* 12**,** 1691-1702.
NAKANISHI, T., KURAGANO, T., KAIBE, S., NAGASAWA, Y. & HASUIKE, Y. 2012. Should we reconsider iron administration based on prevailing ferritin and hepcidin concentrations? *Clinical and Experimental Nephrology,* 16**,** 819-826.
NAPOLITANO, L. M. & CORWIN, H. L. 2004. Efficacy of red blood cell transfusion in the critically ill. *Critical Care Clinics,* 20**,** 255-+.
NBTC. 2014. *National Blood Transfusion Commitee Guidelines* [Online]. Available: http://www.transfusionguidelines.org.uk/uk-transfusion-committees/national-blood-transfusion-committee/patient-blood-management 2015].
NCIN 2009. Colorectal Cancer Survival by Stage. *In:* NETWORK, N. C. I. (ed.) *Colorectal Cancer Survival by Stage - NCIN Data Briefing.* http://www.ncin.org.uk/publications/data_briefings/colorectal_cancer_survival_by_stage.aspx.
NEILIPOVITZ, D. T., BRYSON, G. L. & NICHOL, G. 2001. The Effect of Perioperative Aspirin Therapy in Peripheral Vascular Surgery: A Decision Analysis. *Anesthesia & Analgesia,* 93**,** 573-580 10.1097/00000539-200109000-00009.
NEMETH, E. 2010. Targeting the hepcidin-ferroportin axis in the diagnosis and treatment of anemias. *Adv Hematol,* 2010**,** 750643.
NEMETH, E., RIVERA, S., GABAYAN, V., KELLER, C., TAUDORF, S., PEDERSEN, B. K. & GANZ, T. 2004. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of

the iron regulatory hormone hepcidin. *J Clin Invest,* 113**,** 1271-6.
NEMETH, E., VALORE, E. V., TERRITO, M., SCHILLER, G., LICHTENSTEIN, A. & GANZ, T. 2003a. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood,* 101**,** 2461-3.
NEMETH, E., VALORE, E. V., TERRITO, M., SCHILLER, G., LICHTENSTEIN, A. & GANZ, T. 2003b. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. *Blood,* 101**,** 2461-2463.
NICE 2011. Colorectal cancer- The diagnosis and management of colorectal cancer. *In:* NICE (ed.) *National Institute for Health and Clinical Excellence (NICE) clinical guideline 131.* NHS Nice.
NIELSEN, J. D., HOLM-NIELSEN, A., JESPERSEN, J., VINTHER, C. C., SETTGAST, I. W. & GRAM, J. 2000. The effect of low-dose acetylsalicylic acid on bleeding after transurethral prostatectomy--a prospective, randomized, double-blind, placebo-controlled study. *Scand J Urol Nephrol,* 34**,** 194-8.
NIENABER-ROUSSEAU, C., SWANEPOEL, B., DOLMAN, R. C., PIETERS, M., CONRADIE, K. R. & TOWERS, G. W. 2014. Interactions between C-Reactive Protein Genotypes with Markers of Nutritional Status in Relation to Inflammation. *Nutrients,* 6**,** 5034-5050.
NIKOLOV, K., VIDELOV, D., LAZAROV, S., KALCHEVA, V. & ATANASOV, A. 1990. [A modified technic of preoperative acute normovolemic hemodilution--the advantages and economic effect of its use in planned colorectal surgery]. *Khirurgiia (Sofiia),* 43**,** 30-7.
NORMAN, G. R., SLOAN, J. A. & WYRWICH, K. W. 2003. Interpretation of changes in health-related quality of life - The remarkable universality of half a standard deviation. *Med Care,* 41**,** 582-592.
OHSAWA, M., OKAYAMA, A., NAKAMURA, M., ONODA, T., KATO, K., ITAI, K., YOSHIDA, Y., OGAWA, A., KAWAMURA, K. & HIRAMORI, K. 2005. CRP levels are elevated in smokers but unrelated to the number of cigarettes and are decreased by long-term smoking cessation in male smokers. *Preventive Medicine,* 41**,** 651-656.
OKOLI, C. & PAWLOWSKI, S. D. 2004. The Delphi method as a research tool: an example, design considerations and applications. *Information & Management,* 42**,** 15-29.
OKONKO, D. O., GRZESLO, A., WITKOWSKI, T., MANDAL, A. K., SLATER, R. M., ROUGHTON, M., FOLDES, G., THUM, T., MAJDA, J., BANASIAK, W., MISSOURIS, C. G., POOLE-WILSON, P. A., ANKER, S. D. & PONIKOWSKI, P. 2008. Effect of intravenous iron sucrose on exercise tolerance in anemic

and nonanemic patients with symptomatic chronic heart failure and iron deficiency FERRIC-HF: a randomized, controlled, observer-blinded trial. *J Am Coll Cardiol,* 51**,** 103-12.

OKUYAMA, M., IKEDA, K., SHIBATA, T., TSUKAHARA, Y., KITADA, M. & SHIMANO, T. 2005. Preoperative iron supplementation and intraoperative transfusion during colorectal cancer surgery. *Surg Today,* 35**,** 36-40.

ONO, K., IDANI, H., HIDAKA, H., KUSUDO, K., KOYAMA, Y. & TAGUCHI, S. 2013. Effect of Aspirin Continuation on Blood Loss and Postoperative Morbidity in Patients Undergoing Laparoscopic Cholecystectomy or Colorectal Cancer Resection. *Surgical Laparoscopy Endoscopy & Percutaneous Techniques,* 23**,** 97-100.

OSCARSSON, A., GUPTA, A., FREDRIKSON, M., JARHULT, J., NYSTROM, M., PETTERSSON, E., DARVISH, B., KROOK, H., SWAHN, E. & EINTREI, C. 2010. To continue or discontinue aspirin in the perioperative period: a randomized, controlled clinical trial. *Br J Anaesth,* 104**,** 305-312.

PAGE, L. K., PSAILA, B., PROVAN, D., HAMILTON, J. M., JENKINS, J. M., ELISH, A. S., LESSER, M. L. & BUSSEL, J. B. 2007. The immune thrombocytopenic purpura (ITP) bleeding score: assessment of bleeding in patients with ITP. *British Journal of Haematology,* 138**,** 245-248.

PARK, K. H., SAWADA, T., KOSUGE, T., KITA, J., SHIMODA, M., TOMOSUGI, N. & KUBOTA, K. 2012. Surgical inflammation induces hepcidin production after abdominal surgery. *World Journal of Surgery,* 36**,** 800-6.

PASCUAL, M., BOHLE, B., ALONSO, S., MAYOL, X., SALVANS, S., GRANDE, L. & PERA, M. Preoperative administration of erythropoietin stimulates tumor recurrence after surgical excision of colon cancer in mice by a vascular endothelial growth factor–independent mechanism. *Journal of Surgical Research*.

PASCUAL, M., BOHLE, B., ALONSO, S., MAYOL, X., SALVANS, S., GRANDE, L. & PERA, M. 2013. Preoperative administration of erythropoietin stimulates tumor recurrence after surgical excision of colon cancer in mice by a vascular endothelial growth factor-independent mechanism. *J Surg Res,* 183**,** 270-7.

PEPYS, M. B. & BALTZ, M. L. 1983. Acute Phase Proteins with Special Reference to C-Reactive Protein and Related Proteins (Pentaxins) and Serum Amyloid a-Protein. *Advances in Immunology,* 34**,** 141-212.

PERKINS, S. 2006. Diagnosis of Anemia. *In:* CARL R. KJELDSBERG (ed.) *Practical Diagnosis of Hematologic Disorders Fourth Edition* Chicago Illanois: ASCP Press.

PICKARD, A. S., WILKE, C. T., LIN, H. W. & LLOYD, A. 2007. Health utilities using the EQ-5D in studies of cancer. *Pharmacoeconomics,* 25**,** 365-384.
PIERACCI, F. M. & BARIE, P. S. 2005. Iron and the risk of infection. *Surg Infect (Larchmt),* 6 Suppl 1**,** S41-6.
POSTON, G. J., TAIT, D., O'CONNELL, S., BENNETT, A. & BERENDSE, S. 2011. Diagnosis and management of colorectal cancer: summary of NICE guidance. *BMJ,* 343**,** d6751.
POWELL, C. 2003. The Delphi technique: myths and realities. *Journal of Advanced Nursing,* 41**,** 376-382.
PRIETO-MERINO, D., SMEETH, L., VAN STAA, T. P. & ROBERTS, I. 2013. Dangers of non-specific composite outcome measures in clinical trials. *Bmj-British Medical Journal,* 347.
PUSATCIOGLU, C. K., NEMETH, E., FANTUZZI, G., LLOR, X., FREELS, S., TUSSING-HUMPHREYS, L., CABAY, R. J., LINZMEIER, R., NG, D., CLARK, J. & BRAUNSCHWEIG, C. 2014. Systemic and tumor level iron regulation in men with colorectal cancer: a case control study. *Nutrition & Metabolism,* 11.
QUINN, M., DRUMMOND, R. J., ROSS, F., MURRAY, J., MURPHY, J. & MACDONALD, A. 2010. Short course pre-operative ferrous sulphate supplementation is it worthwhile in patients with colorectal cancer? *Ann R Coll Surg Engl,* 92**,** 569-572.
RAFTERY, T. L. & SAMSON, N. 1980. CARCINOMA OF THE COLON - A CLINICAL CORRELATION BETWEEN PRESENTING SYMPTOMS AND SURVIVAL. *American Surgeon,* 46**,** 600-606.
RAJE, D., MUKHTAR, H., OSHOWO, A. & INGHAM CLARK , C. 2007. What Proportion of Patients Referred to Secondary Care with Iron Deficiency Anemia Have Colon Cancer? *Dis Colon Rectum.,* 50**,** 1211-1214.
RANGANATHAN, P. & PRAMESH, C. S. 2010. Letter 1: Randomized clinical trial of preoperative intravenous iron sucrose to reduce blood transfusion in anaemic patients after colorectal cancer surgery (Br J Surg 2009; 96: 1122–1128). *British Journal of Surgery,* 97**,** 297-298.
RAY, P. & SAHU, S. 1990. Productivity management in India: a Delphi study. *International Journal of Operations and Production Management,* 10**,** 25-51.
REIBETANZ, J. & GERMER, C. T. 2013a. Neoadjuvant Chemotherapy for locally-advanced Colon Cancer First Results from the FOxTROT-Study. *Chirurg,* 84**,** 1000-1000.
REIBETANZ, J. & GERMER, C. T. 2013b. [Neoadjuvant chemotherapy for locally advanced colon cancer : Initial results of the FOxTROT study.]. *Chirurg*.
RESEARCH, N. I. F. H. 2014. *Patient and Public Involvement (PPI)* [Online].

http://www.crncc.nihr.ac.uk/about_us/ccrn/ntw/Patient%20and%20Public%20Involvement%20%28PPI%29/Patient%20and%20Public%20Involvement%20%28PPI%29.
RIMON, E., KAGANSKY, N., KAGANSKY, M., MECHNICK, L., MASHIAH, T., NAMIR, M. & LEVY, S. 2005. Are we giving too much iron? Low-dose iron therapy is effective in octogenarians. *American Journal of Medicine,* 118**,** 1142-1147.
ROBERTS, K., WARD, D. G., BROOKES, M. J., NAQVI, M., ISMAIL, T., IQBAL, T. & TSELEPIS, C. 2008. Hepcidin expression relates to colorectal tumour stage. *Gastroenterology,* 134**,** A447-A447.
ROBINSON, K. & ATHERTON, J. C. 2009. *Sequelae and long-term consequences of infectious diseases, Chapter 7, Helicobacter Pylori. Editors: Fratamico, Pina M.;Smith, J. L;Brogden, Kim A. American Society for Microbiology.,* Washington, D.C., ASM Press.
ROGERS, G., BECKER, P., BLINDER, M., CELLA, D., CHANAN-KHAN, A., CLEELEAND C, COCCIA, P., DJULBEGOVIC, B., GILREATH, J., KRAUT EH, MATULONIS, U., MILLENSON, M., REINKE, D., ROSENTHAL, J., SCHWARTZ, R., SOFF, G., STEIN, R., VLAHOVIC, G., WEIR, A., A. GILREATH, E. H. K., URSULA A. MATULONIS, MICHAEL M. MILLENSON, DENISE, CHANAN-KHAN, C. C., PETER F. COCCIA, BENJAMIN DJULBEGOVIC, JEFFREY & GEORGE M. RODGERS III, P. S. B., MOREY BLINDER, DAVID CELLA, ASHER 2012. Cancer and Chemotherapy Induced Anemia. *J Natl Compr Canc Netw,* 10**,** 628-653.
ROWE, G., WRIGHT, G. & BOLGER, F. 1991. Delphi: a re-evaluation of
research and theory. *Technical Forecasting Social Change,* 39**,** 235–251.
ROY, C. N. & ENNS, C. A. 2000. Iron homeostasis: new tales from the crypt. *Blood,* 96**,** 4020-7.
RUBAK, P., NISSEN, P. H., KRISTENSEN, S. D. & HVAS, A. M. 2015. Investigation of platelet function and platelet disorders using flow cytometry. *Platelets***,** 1-9.
RUIVARD, M., LAINE, F., GANZ, T., OLBINA, G., WESTERMAN, M., NEMETH, E., RAMBEAU, M., MAZUR, A., GERBAUD, L., TOURNILHAC, V., ABERGEL, A., PHILIPPE, P., DEUGNIER, Y. & COUDRAY, C. 2009. Iron absorption in dysmetabolic iron overload syndrome is decreased and correlates with increased plasma hepcidin. *Journal of Hepatology,* 50**,** 1219-1225.
RYBO, G. & SOLVELL, L. 1971. Side-effect studies on a new sustained release iron preparation. *Scand J Haematol,* 8**,** 257-64.

SABM. 2013. *Society for the Advancement of Blood Management* [Online]. http://www.sabm.org/. Available: http://www.sabm.org/ [Accessed December 2014].
SACKMAN, H. 1975. *Delphi Critique*, Lexington Books, Boston, MA.
SADAHIRO, S., SUZUKI, T., TOKUNAGA, N., MUKAI, M., TAJIMA, T., MAKUUCHI, H. & SAITO, T. 1998. Anemia in patients with colorectal cancer. *Journal of Gastroenterology,* 33**,** 488-494.
SAHEBALLY, S. M., HEALY, D., COFFEY, J. C. & WALSH, S. R. 2014. Should patients taking aspirin for secondary prevention continue or discontinue the medication prior to elective, abdominal surgery? Best evidence topic (BET). *International Journal of Surgery,* 12**,** 16-21.
SAIF, M. W., KATIRTZOGLOU, N. A. & SYRIGOS, K. N. 2008. Capecitabine: an overview of the side effects and their management. *Anti-Cancer Drugs,* 19**,** 447-464 10.1097/CAD.0b013e3282f945aa.
SAKAMOTO, J., OHASHI, Y., HAMADA, C., BUYSE, M., BURZYKOWSKI, T. & PIEDBOIS, P. 2004. Efficacy of oral adjuvant therapy after resection of colorectal cancer: 5-year results from three randomized trials. *J Clin Oncol,* 22**,** 484-92.
SAWYERS, C. L. 2008. The cancer biomarker problem. *Nature,* 452**,** 548-552.
SAZAWAL, S., BLACK, R. E., RAMSAN, M., CHWAYA, H. M., STOLTZFUS, R. J., DUTTA, A., DHINGRA, U., KABOLE, I., DEB, S., OTHMAN, M. K. & KABOLE, F. M. 2006. Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomised, placebo-controlled trial. *Lancet,* 367**,** 133-43.
SEIDENFELD, J., PIPER, M., FLAMM, C., HASSELBLAD, V., ARMITAGE, J. O., BENNETT, C. L., GORDON, M. S., LICHTIN, A. E., WADE, J. L., WOOLF, S. & ARONSON, N. 2001. Epoetin treatment of anemia associated with cancer therapy: A systematic review and meta-analysis of controlled clinical trials. *Journal of the National Cancer Institute,* 93**,** 1204-1214.
SHAH, H. & KALAIAN, S. 2009. Which Parametric Statistical Method to Use For Analyzing Delphi Data. *Journal of modern applied statistical method,* 8**,** 226-232.
SHANDER, A., GROSS, I., HILL, S., JAVIDROOZI, M. & SLEDGE, S. 2013. A new perspective on best transfusion practices. *Blood Transfusion,* 11**,** 193-202.
SHANDER, A., HOFMANN, A., OZAWA, S., THEUSINGER, O. M., GOMBOTZ, H., SPAHN, D. R., SABM & MSBM 2010. Activity-based costs of blood transfusions in surgical patients at four hospitals. *Transfusion,* 50**,** 753-765.

SHANDER, A., VAN AKEN, H., COLOMINA, M. J., GOMBOTZ, H., HOFMANN, A., KRAUSPE, R., LASOCKI, S., RICHARDS, T., SLAPPENDEL, R. & SPAHN, D. R. 2012. Patient blood management in Europe. *Br J Anaesth,* 109**,** 55-68.

SIEGEL, J. S. 2012. *The Demography and Epidemiology of Human Health and Aging*, In consultation with SJ Olshansky, Publisher; Springer. p96.

SIEGEL, J. S. & OLSHANSKY, S. J. 2012. The Demography and Epidemiology of Human Health and Aging Introduction. *Demography and Epidemiology of Human Health and Aging***,** 3-17.

SIMPSON, J., NG, S., BROOKES, M. & ACHESON, A. 2010a. Single Dose Preoperative Administration of Intravenous Iron Corrects Iron Deficiency Anaemia in Colorectal Cancer. *Journal of blood disorders and transfusion,* 1.

SIMPSON, J. A., TSELEPIS, C., IQBAL, T., ACHESON, A. G. & BROOKES, M. J. 2010b. Letter 2: Randomized clinical trial of preoperative intravenous iron sucrose to reduce blood transfusion in anaemic patients after colorectal cancer surgery (Br J Surg 2009; 96: 1122-1128). *Br J Surg,* 97**,** 298-9; author reply 299.

SIMPSON, J. A. D., NG, S. L., BROOKES, M. J. & ACHESON, A. G. 2010c. Single Dose Preoperative Administration of Intravenous Iron Corrects Iron Deficiency Anaemia in Colorectal Cancer. *J Blood Disord Transfus,* 1:101 doi:10.4172/2155-9864.1000101.

SIMPSON, J. C., MOONESINGHE, S. R., GROCOTT, M. P., KUPER, M., MCMEEKING, A., OLIVER, C. M., GALSWORTHY, M. J. & MYTHEN, M. G. 2015. Enhanced recovery from surgery in the UK: an audit of the enhanced recovery partnership programme 2009-2012. *Br J Anaesth*.

SIRINEK, K. R., LEVINE, B. A. & MOYER, M. P. 1985. Pentagastrin stimulates in vitro growth of normal and malignant human colon epithelial cells. *Am J Surg,* 149**,** 35-9.

SKULMOSKI, G., HARTMAN, F. & KRAHN, J. 2007. The Delphi Method for Gradate Research. *Journal of Information Technology Education,* 6.

SOLLEVI, A. 1988. Hypotensive anesthesia and blood loss. *Acta Anaesthesiol Scand Suppl,* 89**,** 39-43.

SPETH, C., LOFFLER, J., KRAPPMANN, S., LASS-FLORL, C. & RAMBACH, G. 2013. Platelets as immune cells in infectious diseases. *Future Microbiology,* 8**,** 1431-1451.

SPIVAK, J. L. 1994. Cancer-related anemia: its causes and characteristics. *Semin Oncol,* 21**,** 3-8.

STRACHAN, L. & NOBLE, D. W. 2001. Hypoxia and surgical patients--prevention and treatment of an unnecessary cause of morbidity and mortality. *J R Coll Surg Edinb,* 46**,** 297-302.

SUN, J. C., WHITLOCK, R., CHENG, J., EIKELBOOM, J. W., THABANE, L., CROWTHER, M. A. & TEOH, K. H. 2008. The effect of pre-operative aspirin on bleeding, transfusion, myocardial infarction, and mortality in coronary artery bypass surgery: a systematic review of randomized and observational studies. *Eur Heart J,* 29**,** 1057-71.

TADROS, T., WOBBES, T. & HENDRIKS, T. 1992. Blood transfusion impairs the healing of experimental intestinal anastomoses. *Annals of Surgery,* 215**,** 276-81.

TELANG, S., VIMR, E., MAHONEY, J. R., LAW, I., LUNDQVIST-GUSTAFSSON, H., QIAN, M. & EATON, J. W. 2001a. Strain-specific iron-dependent virulence in Escherichia coli. *J Infect Dis,* 184**,** 159-65.

TELANG, S., VIMR, E., MAHONEY, J. R., LAW, I., LUNDQVIST-GUSTAFSSON, H., QIAN, M. W. & EATON, J. W. 2001b. Strain-specific iron-dependent virulence in Escherichia coli. *Journal of Infectious Diseases,* 184**,** 159-165.

TEUCHER, B., OLIVARES, M. & CORI, H. 2004. Enhancers of iron absorption: ascorbic acid and other organic acids. *Int J Vitam Nutr Res,* 74**,** 403-19.

THEWS, O., KELLEHER, D. K. & VAUPEL, P. 2001. Erythropoietin restores the anemia-induced reduction in cyclophosphamide cytotoxicity in rat tumors. *Cancer Res,* 61**,** 1358-61.

THEWS, O., KOENIG, R., KELLEHER, D. K., KUTZNER, J. & VAUPEL, P. 1998. Enhanced radiosensitivity in experimental tumours following erythropoietin treatment of chemotherapy-induced anaemia. *Br J Cancer,* 78**,** 752-6.

TINEGATE, H., CHATTREE, S., IQBAL, A., PLEWS, D., WHITEHEAD, J. & WALLIS, J. P. 2013. Ten-year pattern of red blood cell use in the North of England. *Transfusion,* 53**,** 483-9.

TITOS-ARCOS, J., SORIA-ALEDO, V., CARRILLO-ALCARAZ, A., VENTURA-LÓPEZ, M., PALACIOS-MUÑOZ, S. & PELLICER-FRANCO, E. 2012. Is Intravenous Iron Useful for Reducing Transfusions in Surgically Treated Colorectal Cancer Patients? *World Journal of Surgery,* 36**,** 1893-1897.

TOY, P., POPOVSKY, M. A., ABRAHAM, E., DANIEL, R., AMBRUSO, D. R., HOLNESS, L. G., KOPKO, P. M., MCFARLAND, J. G., NATHENS, A. B., SILLIMAN, C. C., STRONCEK, D. & INST, N. H. L. B. 2005. Transfusion-related acute lung injury: Definition and review. *Critical Care Medicine,* 33**,** 721-726.

UKBT. 2012. *United Kingdom Blood Transfusion and Tissue Transplantation Guidelines* [Online]. http://www.transfusionguidelines.org.uk/index.aspx [Accessed February 2014].

UKBT. 2014. *Red cell transfusion in surgery* [Online]. http://www.transfusionguidelines.org.uk/transfusion-

handbook/7-effective-transfusion-in-surgery-and-critical-care/7-1-transfusion-in-surgery. [Accessed May 2014].
ULRICH, C. M., ROBIEN, K. & SPARKS, R. 2002. Pharmacogenetics and folate metabolism -- a promising direction. *Pharmacogenomics,* 3**,** 299-313.
VAMVAKAS, E. C. & BLAJCHMAN, M. A. 2009. Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. *Blood,* 113**,** 3406-3417.
VAN DER WEERD, N. C., GROOTEMAN, M. P., NUB, A. M., TER WEE, P. M., SWINKELS, D. W. & GAILLARD, C. A. 2015. Hepcidin in chronic kidney disease: not an anaemia management tool, but promising as a cardiovascular biomarker. *Neth J Med,* 73**,** 108-18.
VANEK, V. W., WHITT, C. L., ABDU, R. A. & KENNEDY, W. R. 1986. COMPARISON OF RIGHT COLON, LEFT COLON, AND RECTAL-CARCINOMA. *American Surgeon,* 52**,** 504-509.
VARAT, M. A., ADOLPH, R. J. & FOWLER, N. O. 1972. Cardiovascular effects of anemia. *Am Heart J,* 83**,** 415-26.
VAUPEL, P. & MAYER, A. 2005. Hypoxia and anemia: effects on tumor biology and treatment resistance. *Transfusion Clinique et Biologique,* 12**,** 5-10.
VAUPEL, P., THEWS, O. & HOECKEL, M. 2001. Treatment resistance of solid tumors: role of hypoxia and anemia. *Med Oncol,* 18**,** 243-59.
VAZIRI, N. D. 1999. Mechanism of erythropoietin-induced hypertension. *American Journal of Kidney Diseases,* 33**,** 821-828.
VELENIK, V., ANDERLUH, F., OBLAK, I., STROJAN, P. & ZAKOTNIK, B. 2006. Capecitabine as a radiosensitizing agent in neoadjuvant treatment of locally advanced resectable rectal cancer: Prospective phase II trial. *Croatian Medical Journal,* 47**,** 693-700.
VINCENT, J. L., SAKR, Y., DE BACKER, D. & VAN DER LINDEN, P. 2007. Efficacy of allogeneic red blood cell transfusions. *Best Pract Res Clin Anaesthesiol,* 21**,** 209-19.
VON DER GRACHT, H. A. 2012. Consensus measurement in Delphi studies Review and implications for future quality assurance. *Technological Forecasting and Social Change,* 79**,** 1525-1536.
VON DER GRACHT, H. A. & DARKOW, I. L. 2010. Scenarios for the logistics services industry: A Delphi-based analysis for 2025. *International Journal of Production Economics,* 127**,** 46-59.
VON HUNDELSHAUSEN, P. & WEBER, C. 2007. Platelets as immune cells - Bridging inflammation and cardiovascular disease. *Circulation Research,* 100**,** 27-40.
WALTER, C. J., BELL, L. T. O., PARSONS, S. R., JACKSON, C., BORLEY, N. R. & WHEELER, J. M. D. 2013. Prevalence and

significance of anaemia in patients receiving long-course neoadjuvant chemoradiotherapy for rectal carcinoma. *Colorectal Disease,* 15**,** 52-56.
WANG, C. S. & SUN, C. F. 2009. C-reactive protein and malignancy: clinico-pathological association and therapeutic implication. *Chang Gung Med J,* 32**,** 471-82.
WARD, D. G., ROBERTS, K., BROOKES, M. J., JOY, H., MARTIN, A., ISMAIL, T., SPYCHAL, R., IQBAL, T. & TSELEPIS, C. 2008a. Increased hepcidin expression in colorectal carcinogenesis. *World J Gastroenterol,* 14**,** 1339-45.
WARD, D. G., ROBERTS, K., STONELAKE, P., GOON, P., ZAMPRONIO, C. G., MARTIN, A., JOHNSON, P. J., IQBAL, T. & TSELEPIS, C. 2008b. SELDI-TOF-MS determination of hepcidin in clinical samples using stable isotope labelled hepcidin as an internal standard. *Proteome Science,* 6.
WARD, H. P., KURNICK, J. E. & PISARCZYK, M. J. 1971. Serum level of erythropoietin in anemias associated with chronic infection, malignancy, and primary hematopoietic disease. *J Clin Invest,* 50**,** 332-5.
WARE JR, J. E. & GANDEK, B. 1998. Overview of the SF-36 Health Survey and the International Quality of Life Assessment (IQOLA) Project. *Journal of Clinical Epidemiology,* 51**,** 903-912.
WAYMACK, J. P., GUGLIUZZA, K., DONG, Y. L. & HERNDON, D. N. 1993. Effect of blood transfusion on immune function. IX. Effect on lymphocyte metabolism. *J Surg Res,* 55**,** 269-72.
WHITTAKER, M. & GOLIGHER, J. C. 1976. The prognosis after surgical treatment for carcinoma of the rectum. *Br J Surg,* 63**,** 384-8.
WHO 2011. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. World Health Organistaion
WISH, J. B. 2006. Assessing iron status: beyond serum ferritin and transferrin saturation. *Clin J Am Soc Nephrol,* 1 Suppl 1**,** S4-8.
WU, C. C., HO, W. M., CHENG, S. B., YEH, D. C., WEN, M. C., LIU, T. J. & P'ENG, F. K. 2006. Perioperative parenteral tranexamic acid in liver tumor resection - A prospective randomized trial toward a "blood transfusion"-free hepatectomy. *Annals of Surgery,* 243**,** 173-180.
YAMAGUCHI, K., TAKAGI, Y., AOKI, S., FUTAMURA, M. & SAJI, S. 2000. Significant detection of circulating cancer cells in the blood by reverse transcriptase-polymerase chain reaction during colorectal cancer resection. *Annals of Surgery,* 232**,** 58-65.
YAZER, M. H., PODLOSKY, L., CLARKE, G. & NAHIRNIAK, S. M. 2004. The effect of prestorage WBC reduction on the rates of

febrile nonhemolytic transfusion reactions to platelet concentrates and RBC. *Transfusion,* 44**,** 10-5.
YDY, L. R. A., SLHESSARENKO, N. & DE AGUILAR-NASCIMENTO, J. E. 2007. Effect of perioperative allogeneic red blood cell transfusion on the immune-inflammatory response after colorectal cancer resection. *World Journal of Surgery,* 31**,** 2044-2051.
YELLEN, S. B., CELLA, D. F., WEBSTER, K., BLENDOWSKI, C. & KAPLAN, E. 1997. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. *Journal of pain and symptom management,* 13**,** 63-74.
YESSAYAN, L., YEE, J., ZASUWA, G., FRINAK, S. & BESARAB, A. 2014. Iron repletion is associated with reduction in platelet counts in non-dialysis chronic kidney disease patients independent of erythropoiesis-stimulating agent use: a retrospective cohort study. *Bmc Nephrology,* 15.
YILDIRIM, Z. K., ORHAN, M. F. & BUYUKAVCI, M. 2011. Platelet function alterations and their relation to P-selectin (CD62P) expression in children with iron deficiency anemia. *Blood Coagul Fibrinolysis,* 22**,** 98-101.
YOKOTA, S., KONNO, M., MINO, E., SATO, K., TAKAHASHI, M. & FUJII, N. 2008. Enhanced Fe ion-uptake activity in Helicobacter pylori strains isolated from patients with iron-deficiency anemia. *Clinical Infectious Diseases,* 46**,** E31-E33.
YOSHIMURA, A., KOBAYASHI, K., FUMIMOTO, H., FUJIKI, Y., EREMENCO, S. & KUDOH, S. 2004. Cross-cultural validation of the Japanese Functional Assessment of Cancer Therapy-Anemia (FACT-An). *J Nippon Med Sch,* 71**,** 314-22.
YOUNG, M. F., GLAHN, R. P., ARIZA-NIETO, M., INGLIS, J., OLBINA, G., WESTERMAN, M. & O'BRIEN, K. O. 2009. Serum hepcidin is significantly associated with iron absorption from food and supplemental sources in healthy young women. *American Journal of Clinical Nutrition,* 89**,** 533-538.
YOUSUF, M. I. 2007. Using Experts' Opinions Through Delphi Technique. *Practical Assessment, Research and Evaluation* [Online], 12. [Accessed November 2014].
ZARIWALA, M. G., SOMAVARAPU, S., FARNAUD, S. & RENSHAW, D. 2013. Comparison study of oral iron preparations using a human intestinal model. *Sci Pharm,* 81**,** 1123-39.
ZHANG, X. & ROVIN, B. H. 2013. Beyond anemia: hepcidin, monocytes and inflammation. *Biol Chem,* 394**,** 231-8.
ZHAO, Y. S., WANG, F., CHANG, D., HAN, B. & YOU, D. Y. 2008. Meta-analysis of different test indicators: Helicobacter pylori infection and the risk of colorectal cancer. *International Journal of Colorectal Disease,* 23**,** 875-82.

ZIMMERMANN, M. B. & HURRELL, R. F. 2007. Nutritional iron deficiency. *The Lancet,* 370**,** 511-520.
ZUMKELLER, N., BRENNER, H., ZWAHLEN, M. & ROTHENBACHER, D. 2006. Helicobacter pylori infection and colorectal cancer risk: a meta-analysis. *Helicobacter,* 11**,** 75-80.