Preoperative blood management in colorectal cancer surgery: the controversial role of iron

M.J. Wilson

Preoperative blood management in colorectal cancer surgery: the controversial role of iron

M.J. Wilson

TRIP Promotiereeks

Stichting TRIP (Transfusie- en transplantatiereacties in patiënten) heeft ten doel het bevorderen van hemovigilantie en biovigilantie in Nederland. TRIP biedt promovendi die relevant onderzoek hebben verricht de mogelijkheid hun dissertatie te publiceren in de TRIP promotiereeks.

Printing of this thesis was financially supported by

Stichting TRIP (Transfusie- en transplantatiereacties in patienten)
Leiden University Medical Center
Chipsoft

Cover and layout

Roos Verhooren (Silver Arrows MultiMedia)

Printing

ProefschriftMaken

Preoperative blood management in colorectal cancer surgery: the controversial role of iron

Proefschrift

ter verkrijging van de graad van doctor aan de
Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 18 december 2018
klokke 16.15 uur

door

Michael Jordi Wilson
geboren te Delft in 1989

Promotor:	Prof. dr. J.J. Zwaginga	
Co-promotores:	Prof. dr. J. Jeekel	Erasmus Universiteit, Rotterdam
	Dr. M. Schipperus	HagaZiekenhuis, Den Haag
Leden promotiecommissie:	Prof. dr. A. Brand	
	Prof. dr. C. Verhoef	Erasmus Universiteit, Rotterdam
	Dr. J.W.T. Dekker	Reinier de Graaf Gasthuis, Delft
	Dr. B.A. Bonsing	

CONTENTS

Chapter 1	Introduction
Chapter 2	Long-term prognostic value of preoperative anemia in patients with colorectal cancer: a systematic review and meta-analysis
Chapter 3	The role of preoperative iron deficiency in colorectal cancer patients
Chapter 4	Short-term effect of preoperative intravenous iron therapy in colorectal cancer patients: results of a cohort study
Chapter 5	Patient blood management in colorectal cancer patients: a survey among Dutch gastroenterologists, surgeons and anesthesiologists
Chapter 6	Iron therapy as treatment of anemia: a potentially detrimental and hazardous strategy in colorectal cancer patients
Chapter 7	The effect of intravenous iron therapy on long-term survival in anemic colorectal cancer patients: results from a matched cohort study
Chapter 8	General discussion and future perspectives
Chapter 9	Summary and conclusions
Chapter 10	Dutch summary
	About the author
	List of publications
	Dankwoord

Chapter 1 // **General Introduction**

Anemia, defined by the World Health Organization as hemoglobin <8 mmol/L in males and <7.5 mmol/L in females, is highly prevalent among patients diagnosed with colorectal cancer.[1, 2] Typically, multiple factors contribute to the development of anemia in cancer patients, with iron deficiency as principal cause.[3] Iron deficiency can be induced by chronic tumor-induced blood loss, resulting in an absolute iron deficiency, and impaired iron homeostasis, caused by systemic inflammation with increased hepcidin levels and resulting in functional iron deficiency. Finally, surgery-induced blood loss further aggravates the severity of anemia.

In patients awaiting surgery, anemia is commonly observed and more and more considered as an important health problem.[4, 5] Anemia namely is found to be associated with increased postoperative morbidity and mortality, increased duration of hospitalization, and reduced quality of life.[6, 7] Regarding colorectal cancer patients, preoperative anemia is also an independent prognostic factor for impaired long-term overall and disease-free survival.[8-10] Correcting anemia, notwithstanding the fact that the observed association should not be held equivalent to causality, has therefore become of main interest, not only to improve quality of life but possibly also survival.

Blood transfusions in earlier days were the default therapy to correct such anemia. The overall goal of transfusion is to treat or prevent the deficiency in oxygen delivery to body tissues. The major benefit of blood transfusion, as compared to other treatment modalities for anemia, is a rapid increase in hemoglobin (Hb) levels. Hence, blood transfusion is the only option for patients who require immediate correction of anemia. The first blood transfusions were attempted in the 17th Century, shortly after the English physician William Harvey discovered the circulation of blood. Although successful blood transfusions between animals had been observed, when transfusion of animal blood into humans, mostly to treat psychiatric illnesses, proved fatal, a ban on transfusions was installed by the pope. It was not until 1818 when James Blundell, a British obstetrician, performed the first successful human-to-human blood transfusions for the treatment of postpartum hemorrhage. However, the undiscovered ABO blood group incompatibilities caused these blood transfusions to often show grave hemolytic transfusion reactions with severe morbidity and even mortality. Ever since, several vital discoveries, such as the ABO human blood groups by Karl Landsteiner in 1900, and the ability to anticoagulate and thus test and store blood, contributed largely to the present availability and safety of blood transfusions. Blood transfusions presently save many trauma and obstetric patients from exsanguination and enable complicated surgery and intensive hemato-oncologic treatments.

In modern transfusion medicine in developed countries, the nowadays' high level of safety in the transfusion chain, involving the entire process from donor recruitment to transfusion outcome, is evidenced by the low incidence of adverse events in the transfusion chain.[11] However, aside from this low risk for adverse events, growing evidence suggests that the correction of anemia by blood transfusion is associated with increased postoperative morbidity and mortality.[12, 13] In the

specific context of colorectal cancer surgery, the use of perioperative blood transfusion was not only found associated with increased short-term postoperative morbidity, but, importantly, also with impaired long-term overall and disease-free survival, as already demonstrated by Busch et al in 1993.[14] In a randomized controlled trial, Busch et al. demonstrated that regardless of their type (autologous or allogeneic), transfusions are associated with poor prognosis. Twenty years later, Harlaar et al. studied the long-term outcomes of this randomized controlled trial, demonstrating that the patients did not benefit from autologous as compared to standard allogeneic transfusion. On the contrary, the overall and colorectal-cancer specific survival rates were worse in the patients in the autologous group.[15] The causality of the association between blood transfusions and long-term prognosis in colorectal cancer, as well as the potential causal mechanism, is being questioned and is still a major topic of discussion.[16-18]

Red blood cell production is normally controlled by erythropoietin, a cytokine produced in the kidneys. Erythropoiesis-stimulating agents (ESAs) were therefore initially developed for the treatment of anemia in patients with chronic kidney disease. Later, in an attempt to avoid blood transfusion and eliminate the associated risks, ESAs were additionally used in cancer patients undergoing chemotherapy. ESAs indeed increased the Hb level in these patients, and, as a result, decreased the need for blood transfusions.[19, 20] However, aside from these short-term advantageous effects, thromboembolic risks were also found associated with ESA treatment.[21-24] In addition, numerous randomized studies with ESA therapy in various types of cancer have shown a decrease in overall and disease-free survival.[25-28] ESAs therefore are now contraindicated when the anticipated treatment outcome is cure. Hence, only in patients undergoing palliative treatment the use of ESAs may be considered.[29]

New approaches to optimize the preoperative hemoglobin level and thus reduce the blood transfusion requirement, however, remain of large interest and are collectively termed as patient blood management (PBM). In this regard, the effect of iron, and especially intravenous iron, is increasingly being explored.[30-32] While oral iron is the standard treatment for iron deficiency anemia since the 19th century, it also has significant disadvantages. It is known to be slow in terms of absorption rate, to potentially cause constipation, and, due to poor duodenal absorption caused by increased hepcidin production, to be largely ineffective in patients with inflammation and cancer. These side effects have led to the development of parenteral iron compounds, that indeed showed to be more effective in optimization of Hb level and to have less side effects. Presently, ferric carboxymaltose (Ferinject)[33-35] and iron isomaltoside 1000 (Monofer)[36] are most frequently used for intravenous iron administration. In the perioperative setting, the iron preparations can be administered as a single treatment of up to 1000 mg in a relatively short time, and the effect of such iron preparations is mostly studied in orthopedic and cardiac.[37-39] However, presently, also in cancer surgery perioperative intravenous iron therapy is more and more considered while anemia in cancer patients is most frequently associated with iron deficiency.[3] In the specific context of

colorectal cancer surgery, intravenous iron, as compared to oral iron, has been shown to be more effective in treating preoperative anemia and iron deficiency. However, most studies so far did not demonstrate intravenous iron to reduce the blood transfusion requirement and, more importantly, actually improve postoperative outcome.[40, 41] As a result, the advantages and use of preoperative intravenous iron remain matter of debate in colorectal cancer patients.

Whilst the short-term effects and safety of intravenous iron are increasingly reported, strikingly no data on the long-term oncological effects and safety are available. Possible long-term oncological effects of iron therapy, however, are of special interest for several reasons. First, the results of laboratory, epidemiological and animal studies have shown a crucial role of iron in promoting cancer development and cancer growth.[42-47] Second, anemia of inflammation is believed to be a potentially defense strategy of the human body to limit the growth of tumor cells.[48] Anemia of inflammation is characterized by both reduced duodenal iron uptake and the sequestration of iron into the reticuloendothelial system. As a result, there is a disturbance of iron homeostasis with subsequent limitation of the availability of iron for not only erythropoiesis, but also, and importantly, the growth of tumor cells. Third, and finally, corroborating evidence implicates that especially gastrointestinal cancer cells, likely by their original iron-absorbing nature, have an altered iron homeostasis.[49] This altered iron metabolism is characterized by increased iron import and decreased iron export proteins, resulting in enhanced proliferation.

OUTLINE OF THE THESIS

Against the background described above, the general aim of this thesis was to evaluate the role of iron in anemic patients with solid cancer, with special attention to the long-term oncological effects of iron therapy in the preoperative setting. In this thesis, this role of iron is specifically studied in the context of colorectal cancer.

Colorectal cancer is the second most common malignancy in the Western world after non-melanoma skin cancer.[50] Patients with TNM stage I-III colorectal cancer (i.e. no distant metastases) are considered for curative treatment by surgical resection of the primary tumor.[51] Partly because of advances in surgical techniques, coupled with effective (neo)adjuvant therapy), the five-year survival rate of colorectal cancer has increased to 64%.[52] The main reason to study the role of iron in the specific context of colorectal cancer is because the effects of both anemia and blood transfusion are already extensively studied in this patient group. As anemia and blood transfusion appear to be strongly associated with adverse short and long-term outcome following surgery, the use of iron therapy has gained increased attention in this patient group.[30, 40] Specifically in colorectal cancer patients awaiting elective surgery, this has led to an increased administration of iron, and specifically intravenous iron, with the aim of optimizing patient's condition and improving the postoperative outcome.

In **Chapter 2** the long-term prognostic value of preoperative anemia in colorectal cancer patients is assessed in a systematic review and meta-analysis. In **Chapter 3** data on the prevalence and type of iron deficiency are reported. In addition, the prognostic value of iron deficiency is presented. **Chapter 4** includes a national survey among gastroenterologists, surgeons, and anesthesiologists to assess the current preoperative blood management strategies in the Netherlands, and to identify preferences of different physicians in the treatment of preoperative anemia. In **Chapter 5**, the short-term effects of preoperative intravenous iron therapy, including change in hemoglobin level and postoperative complication and blood transfusion rate, are studied. In **Chapter 6** the hypothesis that iron therapy, as treatment of anemia, may impair long-term tumor prognosis is discussed. The effect of preoperative intravenous iron therapy on long-term survival and tumor prognosis is presented in **Chapter 7. Chapter 8** presents a general discussion of the overall results together with perspectives for further research. Finally, **Chapter 9 and 10** contain the respective English and Dutch summary of the main findings in this thesis.

REFERENCES

1. Knight K, Wade S, Balducci L. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. *Am J Med* 2004; 116 Suppl 7A:11S-26S.
2. Ludwig H, Van Belle S, Barrett-Lee P, et al. The European Cancer Anemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anemia in cancer patients. *Eur J Cancer* 2004; 40(15):2293-306.
3. Ludwig H, Muldur E, Endler G, et al. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol* 2013; 24(7):1886-92.
4. Beattie WS, Karkouti K, Wijeysundera DN, et al. Risk associated with preoperative anemia in noncardiac surgery: a single-center cohort study. *Anesthesiology* 2009; 110(3):574-81.
5. Butcher A, Richards T. Cornerstones of patient blood management in surgery. *Transfus Med* 2017.
6. Fowler AJ, Ahmad T, Phull MK, et al. Meta-analysis of the association between preoperative anemia and mortality after surgery. *Br J Surg* 2015; 102(11):1314-24.
7. Leichtle SW, Mouawad NJ, Lampman R, et al. Does preoperative anemia adversely affect colon and rectal surgery outcomes? *J Am Coll Surg* 2011; 212(2):187-94.
8. An MS, Yoo JH, Kim KH, et al. T4 stage and preoperative anemia as prognostic factors for the patients with colon cancer treated with adjuvant FOLFOX chemotherapy. *World J Surg Oncol* 2015; 13:64.
9. Lee H, Park HC, Park W, et al. Negative impact of pretreatment anemia on local control after neoadjuvant chemoradiotherapy and surgery for rectal cancer. *Radiat Oncol J* 2012; 30(3):117-23.
10. van Halteren HK, Houterman S, Verheij CD, et al. Anemia prior to operation is related with poorer long-term survival in patients with operable rectal cancer. *Eur J Surg Oncol* 2004; 30(6):628-32.
11. TRIP (Transfusion and Transplantation Reactions in Patients) *Annual Report Hemovigilance*. 2015.
12. Shander A, Cappellini MD, Goodnough LT. Iron overload and toxicity: the hidden risk of multiple blood transfusions. *Vox Sang* 2009; 97(3):185-97.
13. Vamvakas EC, Blajchman MA. Transfusion-related mortality: the ongoing risks of allogeneic blood transfusion and the available strategies for their prevention. *Blood* 2009; 113(15):3406-17.
14. Busch OR, Hop WC, Hoynck van Papendrecht MA, et al. Blood transfusions and prognosis in colorectal cancer. *N Engl J* Med 1993; 328(19):1372-6.
15. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.
16. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
17. Vamvakas EC, Blajchman MA. Transfusion-related immunomodulation (TRIM): an update. *Blood Rev* 2007; 21(6):327-48.
18. Vamvakas EC. Allogeneic blood transfusion and cancer recurrence: 20 years later. *Transfusion* 2014; 54(9):2149-53.
19. Littlewood TJ, Bajetta E, Nortier JW, et al. Effects of epoetin alfa on hematologic parameters and quality of life in cancer patients receiving nonplatinum chemotherapy: results of a randomized, double-blind, placebo-controlled trial. *J Clin Oncol* 2001; 19(11):2865-74.
20. Ludwig H, Aapro M, Bokemeyer C, et al. Treatment patterns and outcomes in the management of anemia in cancer patients in Europe: findings from the Anemia Cancer Treatment (ACT) study. *Eur J Cancer* 2009; 45(9):1603-15.
21. Bennett CL, Silver SM, Djulbegovic B, et al. Venous thromboembolism and mortality associated with recombinant erythropoietin and darbepoetin administration for the treatment of cancer-associated anemia. *Jama* 2008; 299(8):914-24.
22. Glaspy J, Crawford J, Vansteenkiste J, et al. Erythropoiesis-stimulating agents in oncology: a study-level meta-analysis of survival and other safety outcomes. *Br J Cancer* 2010; 102(2):301-15.
23. Tonelli M, Hemmelgarn B, Reiman T, et al. Benefits and harms of erythropoiesis-stimulating agents for anemia related to

cancer: a meta-analysis. *Cmaj* 2009; 180(11):E62-71.

24. Tonia T, Mettler A, Robert N, et al. Erythropoietin or darbepoetin for patients with cancer. *Cochrane Database Syst Rev* 2012; 12:CD003407.
25. Hedenus M, Adriansson M, San Miguel J, et al. Efficacy and safety of darbepoetin alfa in anemic patients with lymphoproliferative malignancies: a randomized, double-blind, placebo-controlled study. *Br J Haematol* 2003; 122(3):394-403.
26. Henke M, Laszig R, Rube C, et al. Erythropoietin to treat head and neck cancer patients with anemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial. *Lancet* 2003; 362(9392):1255-60.
27. Leyland-Jones B, Semiglazov V, Pawlicki M, et al. Maintaining normal hemoglobin levels with epoetin alfa in mainly nonanemic patients with metastatic breast cancer receiving first-line chemotherapy: a survival study. *J Clin Oncol* 2005; 23(25):5960-72.
28. Wright JR, Ung YC, Julian JA, et al. Randomized, double-blind, placebo-controlled trial of erythropoietin in non-small-cell lung cancer with disease-related anemia. *J Clin Oncol* 2007; 25(9):1027-32.
29. NCCN. *Cancer- and chemotherapy-induced anemia*. 2014.
30. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemia in colorectal cancer patients. *BMC Surg* 2015; 15:78.
31. Froessler B, Palm P, Weber I, et al. The Important Role for Intravenous Iron in Perioperative Patient Blood Management in Major Abdominal Surgery: A Randomized Controlled Trial. *Ann Surg* 2016.
32. Richards T, Clevenger B, Keidan J, et al. PREVENTT: preoperative intravenous iron to treat anemia in major surgery: study protocol for a randomised controlled trial. *Trials* 2015; 16:254.
33. Anker SD, Comin Colet J, Filippatos G, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med* 2009; 361(25):2436-48.
34. Kulnigg S, Stoinov S, Simanenkov V, et al. A novel intravenous iron formulation for treatment of anemia in inflammatory bowel disease: the ferric carboxymaltose (FERINJECT) randomized controlled trial. *Am J Gastroenterol* 2008; 103(5):1182-92.
35. Van Wyck DB, Mangione A, Morrison J, et al. Large-dose intravenous ferric carboxymaltose injection for iron deficiency anemia in heavy uterine bleeding: a randomized, controlled trial. *Transfusion* 2009; 49(12):2719-28.
36. Jahn MR, Andreasen HB, Futterer S, et al. A comparative study of the physicochemical properties of iron isomaltoside 1000 (Monofer), a new intravenous iron preparation and its clinical implications. *Eur J Pharm Biopharm* 2011; 78(3):480-91.
37. Cuenca J, Garcia-Erce JA, Martinez F, et al. Perioperative intravenous iron, with or without erythropoietin, plus restrictive transfusion protocol reduce the need for allogeneic blood after knee replacement surgery. *Transfusion* 2006; 46(7):1112-9.
38. Investigators I, Litton E, Baker S, et al. Intravenous iron or placebo for anemia in intensive care: the IRONMAN multicentre randomized blinded trial : A randomized trial of IV iron in critical illness. *Intensive Care Med* 2016; 42(11):1715-1722.
39. Theusinger OM, Leyvraz PF, Schanz U, et al. Treatment of iron deficiency anemia in orthopedic surgery with intravenous iron: efficacy and limits: a prospective study. *Anesthesiology* 2007; 107(6):923-7.
40. Keeler BD, Simpson JA, Ng O, et al. Randomized clinical trial of preoperative oral versus intravenous iron in anemic patients with colorectal cancer. *Br J Surg* 2017.
41. Edwards TJ, Noble EJ, Durran A, et al. Randomized clinical trial of preoperative intravenous iron sucrose to reduce blood transfusion in anemic patients after colorectal cancer surgery. *Br J Surg* 2009; 96(10):1122-8.
42. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1):12-31.
43. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.
44. Stevens RG, Jones DY, Micozzi MS, et al. Body iron stores and the risk of cancer. *N Engl J Med* 1988; 319(16):1047-52.
45. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.
46. Xue X, Shah YM. Intestinal iron homeostasis and colon tumorigenesis. *Nutrients* 2013; 5(7):2333-51.
47. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. *Protein Cell* 2015; 6(2):88-100.

48. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.

49. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5):646-74.

50. Siegel RL, Miller KD, Fedewa SA, et al. Colorectal cancer statistics, 2017. *CA Cancer J Clin* 2017; 67(3):177-193.

51. Sobin LH, Fleming ID. TNM Classification of Malignant Tumors, fifth edition (1997). Union Internationale Contre le Cancer and the American Joint Committee on Cancer. *Cancer* 1997; 80(9):1803-4.

52. Edge S, Byrd, DR, Compton, CC, et al. AJCC (American Joint Committee on Cancer) Cancer Staging Manual, 7th edition. Vol. 7th edtion. *New York: Springer*, 2010.

Chapter 2 // **Long-term prognostic value of preoperative anemia in patients with colorectal cancer: a systematic review and meta-analysis**

M.J. Wilson, M. van Haaren, J.J. Harlaar, Hee Chul Park, H.J. Bonjer, J. Jeekel, J.J. Zwaginga, M. Schipperus

ABSTRACT

Objective: To evaluate the long-term prognostic factor of preoperative anemia in colorectal cancer patients.

Background: Anemia is frequently observed in colorectal cancer patients, with a case incidence of 30 to 67 percent. Besides an indicator of tumor-induced blood loss and inflammation, anemia in cancer is also suggested to be a cause of inferior outcome, possibly via worsening of tumor hypoxia. As surgery is likely to enhance anemia, the long-term prognostic value of preoperative anemia seems most interesting.

Methods: Comprehensive searches were carried out in all relevant databases, including MEDLINE, Embase and Web-of-Science. To include studies addressing overall survival, follow-up had to be at least 24 months or till death. For pooling of survival results, a mixed-linear (fixed-effects) model was fit to the reported hazard ratios (HRs) to calculate a pooled estimate and confidence interval.

Results: We included 12 studies comprising 3588 patients to estimate the association between preoperative anemia and overall survival (OS) and disease-free survival (DFS). In a fixed-effects meta-analysis of eight studies, including both colon and rectal cancer, preoperative anemia was significantly associated with poor OS (HR 1.56; 95% CI 1.30 to 1.88; $p < 0.001$). A meta-analysis of seven studies also showed that preoperative anemia was significantly associated with poor DFS (HR 1.34; 95% CI 1.11 to 1.61; $p = 0.002$). Restricted to studies exclusively on colon cancer or rectal cancer, HRs for OS were 1.25 (95% CI 1.00 to 1.55; $p = 0.05$) and 2.59 (95% CI 1.68 to 4.01; $p < 0.001$), respectively, while HRs for DFS were 1.21 (95% CI 0.96 to 1.52; $p = 0.11$) and 1.61 (95% CI 1.18 to 2.21; $p = 0.003$).

Conclusion: The present meta-analysis reveals the long-term prognostic value of preoperative anemia in colorectal cancer patients, most distinct in in rectal cancer patients. However, this meta-analysis is mainly based on retrospective studies with high heterogeneity. These results justify raised awareness about the impact of preoperative anemia on long-term survival.

INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in men and second in women, accounting for more than 1.4 million new cases and 694 000 associated deaths per year worldwide.[1] The primary treatment for patients with colorectal cancer is surgical resection of the primary tumor. Partly because of advances in surgery, coupled with effective (neo)adjuvant therapy, the five- year survival rate of colorectal cancer has increased to 64 percent.[2]

Anemia, defined by the World Health Organization as hemoglobin <13 g/dL in males and <12 g/dL in females, is present in 30 to 67 percent of colorectal cancer patients at some point during the course of their disease.[3] Contributing mechanisms to the development of anemia include tumor-induced blood loss and reduced iron uptake and utilisation due to IL-6 driven overexpression of hepcidin, known as anemia of chronic disease.[4] Myelosuppressive chemotherapy and surgery-induced blood loss further aggravate the severity of the anemia.[5] Besides a marker of more advanced tumor stage and treatment intensity, anemia in cancer is also suggested to be a cause of inferior outcome, possibly via worsening of tumor hypoxia.[6] Hypoxia has been linked to radiotherapy and chemotherapy resistance, as oxygen is essential for the cytotoxic activities of these treatments.[7-9] Furthermore, by inducing proteomic and genomic changes, hypoxia may also increase the proliferative and metastatic potential.[7]

While surgical resection of the tumor, often the primary treatment for patients with colorectal cancer, is likely to abruptly intensify the anemia, we hypothesize that the long-term prognostic value of anemia in colorectal cancer patients is best studied with preoperative hemoglobin values. Several studies in patients undergoing surgery for colorectal cancer have described preoperative anemia to be a prognostic factor for decreased disease-free survival (DFS) and overall survival (OS),[10-13] but no quantitative and comprehensive review examining the correlation between preoperative anemia and long-term survival has been published. The purpose of this systematic review and meta-analysis is to confirm the long-term prognostic value of preoperative anemia in patients with primary colorectal cancer.

METHODS

All aspects of the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) were followed.[14]

Literature search strategy

Comprehensive searches were carried out by a medical librarian in MEDLINE, Embase, Web-of-science, Scopus, Cochrane, CINAHL, PubMed publisher, ProQuest, Lilacs, Scielo and Google scholar. The search was performed on articles published through February 2016 relevant to the long-term prognostic value of preoperative anemia in patients with colorectal cancer. No publication year

or publication language restrictions were applied. Our overall search strategies included terms and alternative spellings for anemia (anemia, hemoglobin), preoperative (preoperative, pretreatment, pre surgical, pre therapeutic), recurrence or survival (recurrence, survival, survival analysis, mortality, prognosis, risk factors, risk assessment, follow up, cohort), cancer (cancer, neoplasm, tumor, carcinoma, adenocarcinoma, malignancy), and colorectal (colorectal, large intestine, colon, rectum, bowel).

Study selection

Studies were evaluated for inclusion by two independent researchers (MvH, MJW) for relevance to the subject. Study selection was accomplished through three levels of study screening. In level 1, the following types of studies were excluded: reviews, case-reports, letters, editorial, poster abstracts editorials, papers studying non-human. In level 2, abstracts were reviewed for relevance and full-text articles were obtained. To be considered relevant, abstracts had to describe (1) preoperative anemia or anemia-related parameters (hemoglobin, hematocrit) in patients with colorectal cancer, and (2) survival-related parameters (disease-free survival, cancer-specific survival, overall survival, mortality). In articles addressing overall survival or mortality, follow-up had to be for at least 24 months or till death. In level 3, full text articles were reviewed for inclusion in qualitative and quantitative synthesis. Any discrepancies in exclusion were resolved by discussion between the reviewers with supervision by MS.

Critical appraisal and data extraction

The methodological quality of the included studies was assessed according to the 'Newcastle Ottawa Scale (NOS) for Cohort Studies', which score selection, comparability, and outcome.

The following study details were extracted: first author, study type, sample size, definition anemia, therapy anemia, time measurement hemoglobin level, follow up, results survival analysis and hazard ratio (HR) with 95% CI. If HR was not reported, or if HR could not be estimated from reported data, attempts were made to contact the study authors for individual patient data.

Statistical analysis

The main outcomes were OS and DFS, comparing colorectal cancer patients with preoperative anemia, to those with no preoperative anemia. For pooling of survival results, a mixed-linear (fixed-effects) model was fit to the reported HRs to calculate a pooled estimate and confidence interval. Pooled HRs were calculated for both colon and rectal cancer mixed, and for colon and rectal cancer separated. If the study reported both univariate and multivariate results, the latter was used in the analysis. If these statistical variables were not made available in the article reporting them, HR was estimated from reported or given data using methods reported by Tierney et al.[15] Tests of statistical significant were performed using the Z-test with α=0.05. Heterogeneity across

studies was tested using I2 statistics. An I2 value more than 50% is recognized as significant heterogeneity.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

RESULTS

The identification of eligible studies is shown in fig 1. A total of 803 studies were identified from the literature search and 431 studies remained after excluding duplicate articles. Three additional studies, agreed upon by both reviewers, were included after manually scrutinizing reference lists. Titles and abstracts of all identified studies were reviewed to exclude the clearly irrelevant ones. A total of 33 potentially relevant articles were read in full. Of 33 papers, 13 fell within the scope of the study and were included in the qualitative analysis.[10-13,] 16-24 The main characteristics of the 13 eligible publications are shown in table 1.

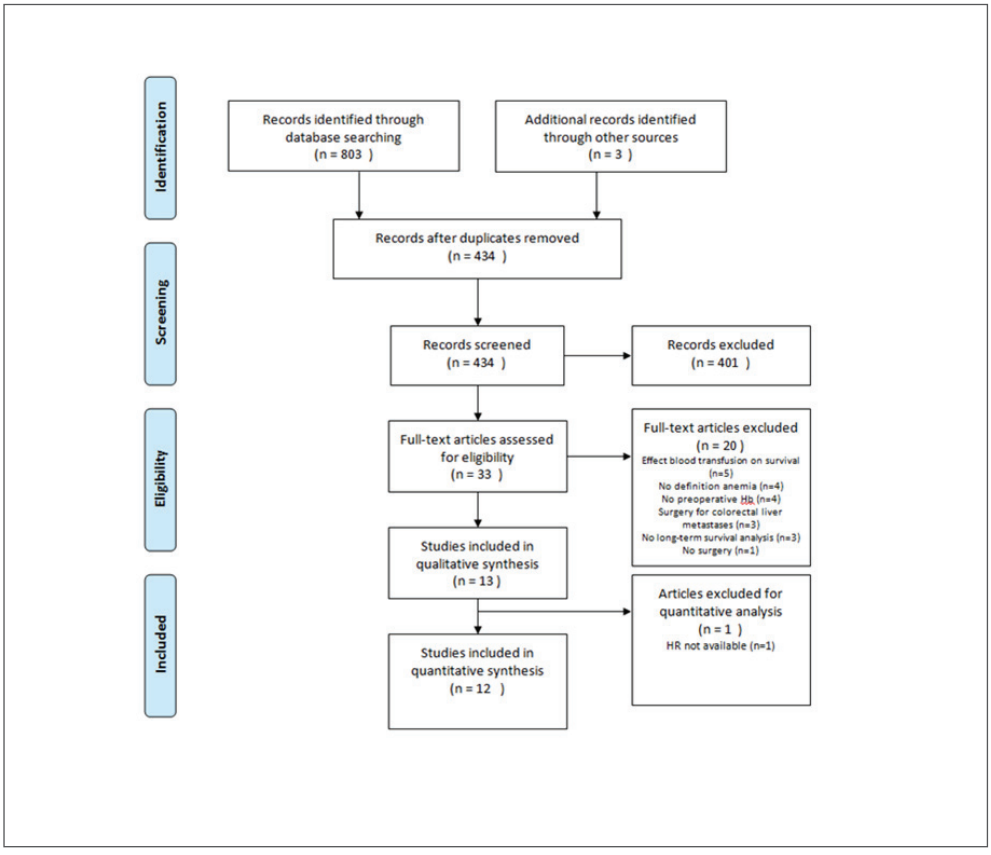


Figure 1. PRISMA flowchart

Table 1: Study characteristics

Study	NOS	Study type	Year	Sample size	Tumor type	Definition anemia (g/dL)	Prevalence anemia	Therapy anemia	Follow-up (months)	Survival analysis	HR
An	8	Retro	2015	196	colon	male<14, female<12	59%	NA	mean 61	OS, DFS	multivariate OS + DFS (R)
Berardi	5	Pros	2006	317	rectum	<12	NA	1 blood transfusion, 1 ESA	NA	DFS	multivariate DFS (R)
Buunen*	8	Pros	2009	1076	colon	male<13, female<12	53%	NA	median 53	OS, DFS	multivariate OS + DFS (E)
Box	5	Retro	2005	100	rectum	male<12, female<11.5	25%	NA	median 39	OS, LR, DR	univariate OS (E)
Cengiz	6	Retro	2006	99	colon + rectum	male<14.5, female <12.5	61%	NA	mean 30	OS, DFS	univariate OS (E)
Fjortoft	6	Pros	2013	234	colon	male<13, female<12	54%	blood transfusion 42%	at least 24	OS	multivariate OS (R)
Giessen	8	Retro	2014	256	rectum	<14.2 (median)	50%	NA	median 101	DFS, CSS	univariate DFS (E)
Giessen-Jung	8	Retro	2015	472	colon	<13.4 (median)	50%	NA	median 71	DFS, CSS	univariate DFS (E)
Khan**	3	Pros	2012	463	rectum	<12	NA	NA	median 24	OS, LR, DR	NA
Lee*	7	Retro	2012	247	rectum	male<13.5, female<12	36%	blood transfusion 5%	median 44	OS, DFS, LR, DR	multivariate OS + DFS (E)
Peng	8	Retro	2012	84	colon	<11	38%	NA	median 45	DFS	multivariate DFS (R)
Qiu	7	Retro	2010	363	colon + rectum	<11	21%	blood transfusion excluded	median 26	OS	multivariate OS (R)
van Halteren	5	Retro	2004	144	rectum	<12	18%	NA	at least 24	OS	multivariate OS (R)

*** = with availability individual patient data**
**** = excluded for meta-analyses**
Abbreviatons: NOS = Newcastle-Ottawa Score; HR = hazard ratio NA = not available; Pros = prospective; Retro = retrospective; RCT = randomized controlled trial; ESA = erythropoiesis-stimulating agent;
DFS = disease-free survival; OS = overall survivall; LR = local recurrence; DR = distant recurrence; CSS = cancer-specific survival; R = reported; E = estimated

For quantitative analysis, 12 studies were included.[10-13, 16-21, 23, 24] In two studies both colon and rectal cancer patients were included, while five studies reported exclusively on colon cancer, and five studies reported exclusively on rectal cancer. In two studies [16, 23], hazard ratio (HR) could not be estimated from reported data, but patient-level survival data were provided by study authors, making it possible to include the studies in the quantitative analysis. One study was excluded because the HR could not be estimated from reported data and while patient-level data were not

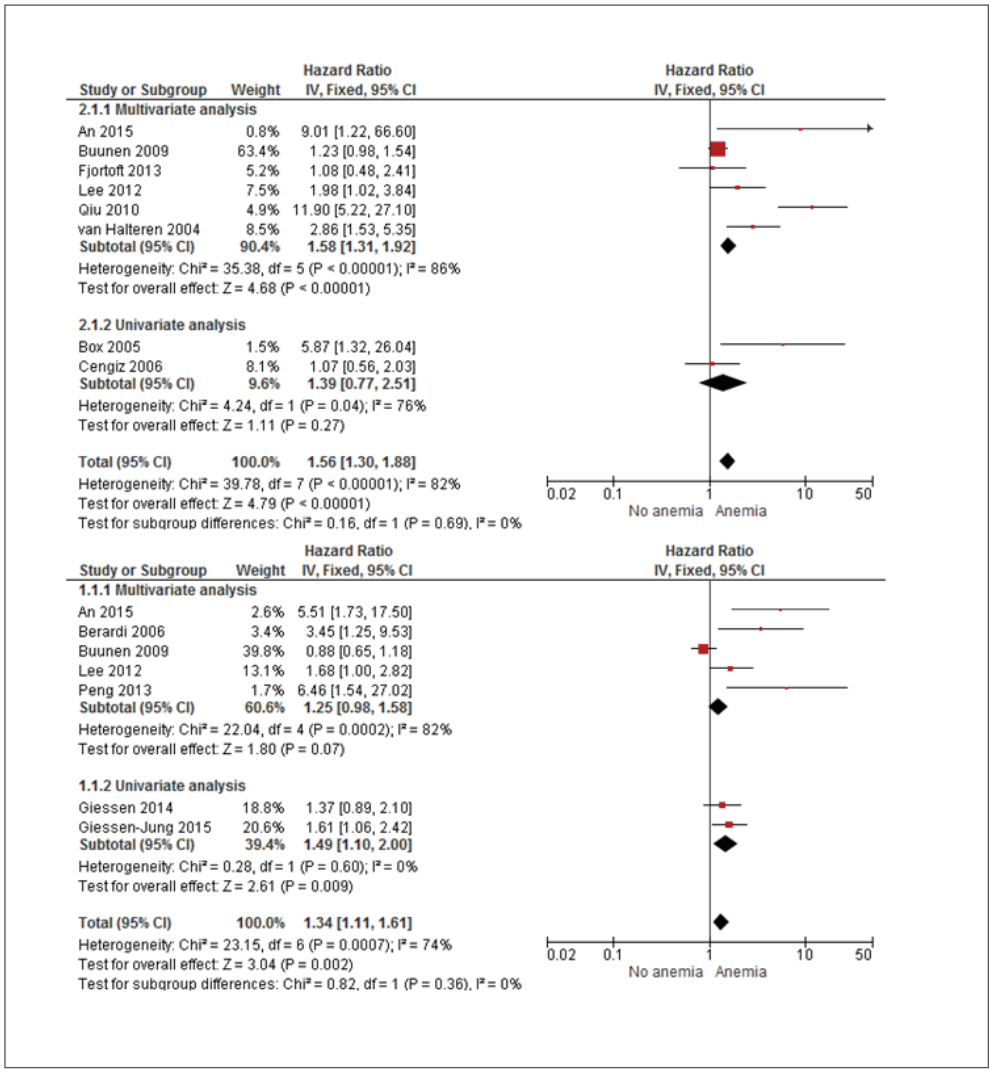


Figure 2. Forest plot of 8 evaluable studies assessing OS in colorectal cancer according to methods of analysis by a fixed-effects model (above) and forest plot of 7 evaluable studies assessing DFS in colorectal cancer according to methods of analysis by a fixed-effects model (under). HR = hazard ratio, CI = confidence interval, IV = inverse variance (statistical method RevMan)

shared by the author.[22] In the included studies, the prevalence of anemia varied between 18 and 61%.

In figure 2, meta-analysis of eight studies, including colon and rectal cancer patients, demonstrated that preoperative anemia was significantly associated with poor OS (HR 1.56; 95% CI 1.30 to 1.88; p < 0.001; I2 = 82%). Among studies reporting HRs based on multivariate analysis, preoperative anemia was significantly associated with poor OS as well (HR 1.58; 95% CI 1.31 to 1.92; p < 0.001; I2 = 86%). Two studies reporting HRs based on univariate analysis did not show significance for preoperative anemia (HR 1.39; 95% CI 0.77 to 2.51; p = 0.27; I2 = 76%) (figure 2). Preoperative anemia was also significantly associated with poor DFS (HR 1.34; 95% CI 1.11 to 1.61; p = 0.002; I2 = 74%). When restricted to studies reporting multivariate HR, pooled HR was 1.25 (95% CI 0.98 to 1.58; p = 0.07; I2 = 82%). Pooled HR for studies reporting univariate analysis was 1.49 (95% CI 1.10 to 2.00; p = 0.009; I2 = 0%).

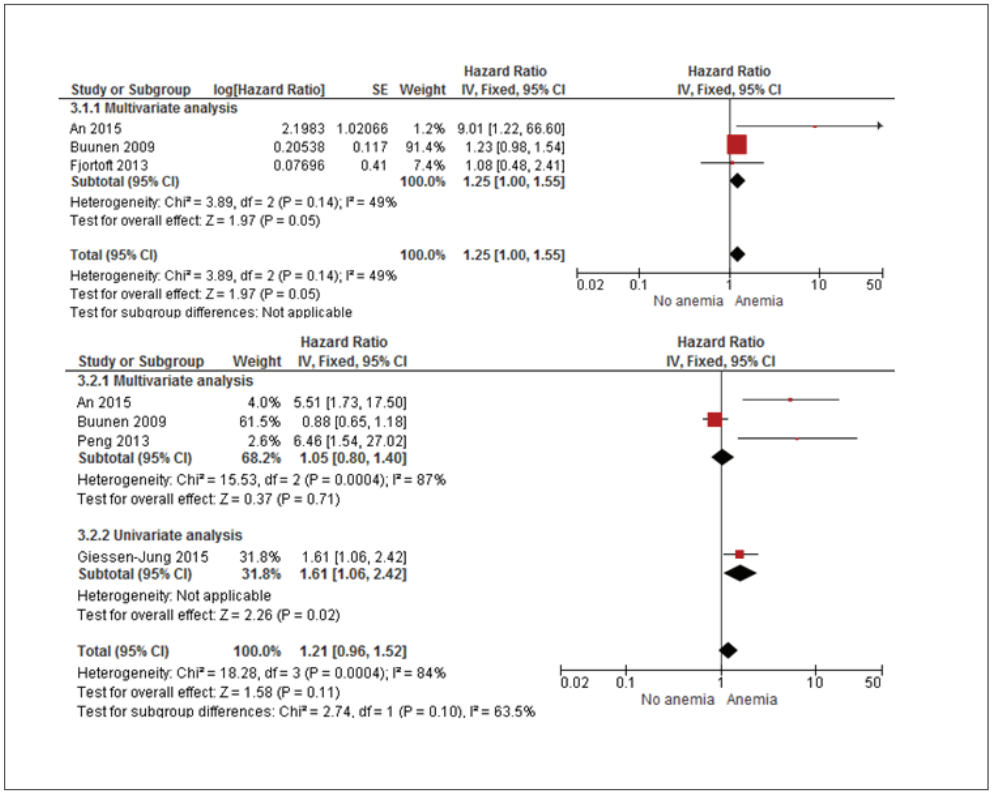


Figure 3. Forest plot of 3 evaluable studies assessing OS in colon cancer according to methods of analysis by a fixed-effects model (above) and forest plot of 4 evaluable studies assessing DFS in colon cancer according to methods of analysis by a fixed-effects model (under). HR = hazard ratio, CI = confidence interval, IV = inverse variance (statistical method Revman)

In meta-analysis of three studies including only colon cancer patients, preoperative anemia showed a near significant association with poor OS (HR 1.25; 95% CI 1.00 to 1.55; p = 0.05; I2 = 49%) (figure 3), while in meta-analysis of four studies addressing DFS, significance was clearly lacking (HR 1.21; 95% CI 0.96 to 1.52; p = 0.11; I2 = 84%) (figure 3).

As shown in figure 4, in meta-analysis of four studies including only rectal cancer patients, preoperative anemia was significantly associated with poor OS (HR 2.59; 95% CI 1.68 to 4.01; p <0.001; I2 = 0%). In three studies addressing DFS, pooled HR for preoperative anemia was 1.61 (95% CI 1.18 to 2.21; p = 0.003; I2 = 27%) (figure 4).

As shown in table 2, in subgroup analyses, including both colon and rectal cancer patients and when restricted to studies adjusting for age and tumor stage, pooled HRs for preoperative anemia

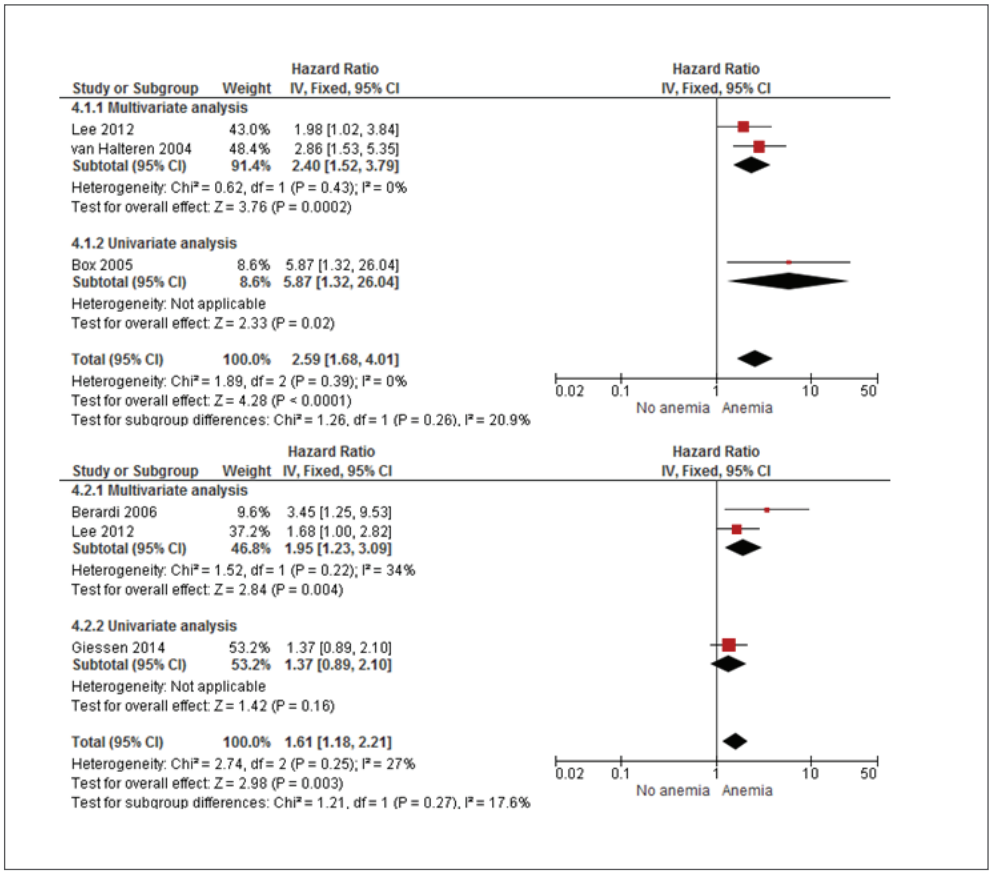


Figure 4. Forest plot of 3 evaluable studies assessing OS in rectal cancer according to methods of analysis by a fixed-effects model (above) and forest plot of 3 studies assessing DFS in rectal cancer according to methods of analysis by a fixed-effects model (under). HR = hazard ratio, CI = confidence interval, IV = inverse variance (statistical method Revman)

in OS were 1.28 (95% CI 1.04 to 1.57; I2 = 0%) and 1.25 (95% CI 1.00 to 1.55; I2 = 49%). In DFS, pooled HRs for studies adjusting for age and tumor stage were 1.11 (95% CI 0.86 to 1.42; I2 = 79%) and 1.98 (95% CI 0.82 to 1.42; I2 = 86), respectively. In subgroup analyses based on the various definitions of anemia used by included studies, pooled HRs in OS and DFS were 1.56 (95% CI 1.28 to 1.91; I2 = 89%) and 1.05 (95% CI 0.79 to 1.38; I2 = 84%) respectively, for studies using a restricted cut off defining anemia (<12 g/dL in female and <13 g/dL in male), as compared to pooled HRs of 1.58 (95% CI 1.01 to 2.47; I2 = 58%) and 1.63 (95% CI 1.27 to 2.10; I2 = 39%) respectively, for studies using a more liberal cut off (>12 g/dL in female or >13 in male).

In the quantitative analysis of specifically rectal cancer patients, two studies did report data on hemoglobin levels prior to or during neoadjuvant therapy, in contrast to 3 studies reporting data on directly preoperative hemoglobin level. On average, neoadjuvant therapy was 5 weeks prior to surgery. In a sensitivity analysis in which the pooled HR was calculated for all studies, excluding the two studies reporting data on hemoglobin level prior to or during neoadjuvant therapy, pooled HR for preoperative anemia remained significant for OS (p = 0.0002; HR 2.40; 95% CI 1.52 to 3.79) and DFS (p = 0.02; HR 1.49; 95% CI 1.07 to 2.07) and was almost equal in both OS and DFS compared to HR in main meta-analysis (OS; HR 2.59, DFS; HR 1.61).

Analysis	Number of studies	Fixed effects HR (95% CI)	I2 statistics (%)
Colorectal cancer			
Overall survival			
Adjustment			
Age	3 (Buunen Fjortoft, Lee)	1.28 (1.04-1.57)	0
Stage	3 (An, Buunen, Fjortoft)	1.25 (1.00-1.55)	49
Definition anemia (g/dL)			
<12 female and <13 male	5 (Box, Buunen, Fjortoft, Qiu, Halteren)	1.56 (1.28-1.91)	89
>12 female or >13 male	3 (An, Cengiz, Lee)	1.58 (1.01-2.47)	58
Disease-free survival			
Adjustment			
Age	3 (Berardi, Buunen, Lee)	1.11 (0.86-1.42)	79
Stage	3 (An, Berardi, Buunen)	1.98 (0.82-1.42)	86
Definition anemia (g/dL)			
<12 female and <13 male	3 (Berardi, Buunen, Peng)	1.05 (0.79-1.38)	84
>12 female or >13 male	4 (An, Lee, Giessen, Giessen-Jung)	1.63 (1.27-2.10)	39

Table 2: Main meta-analysis results

DISCUSSION

This meta-analysis shows that preoperative anemia is significantly associated with decreased long-term OS and DFS in patients with colorectal cancer. For colorectal cancer patients, separate subgroup analyses of studies with adjustment for important prognostic factors, such as age and tumor stage, showed that preoperative anemia is particularly associated with decreased long-term OS. However, since the effect of all confounding factors could not be assessed, a causal relationship cannot definitely be claimed.

A difference was found in the prognostic value of preoperative anemia between colon and rectal cancer patients. Namely after subdividing colorectal cancer patients into colon and rectal cancer patients, our findings only apply to rectal cancer patients. In colon cancer patients, statistical significance is no longer present, however, a similar clear trend for preoperative anemia as a negative long-term prognostic factor is observed. As a whole, survival rates are known to be different for colon and rectal cancer patients, and different treatment strategies are required. In rectal cancer, particularly in stage 2 and 3, neoadjuvant chemoradiation therapy plays a pivotal role in treatment, whilst in colon cancer this is not the case. Furthermore, despite the lack of good quality comparative studies, in general, rectal cancer surgery is associated with longer operation time and more blood loss as compared to surgery for colon cancer.[16, 25] Hence, in rectal cancer, more extensive blood loss in these patients likely aggravates anemia even further. This condition therefore will increase the chance on hypoxia and hypoxia driven survival of remnant tumor mass and might explain a stronger association of preoperative anemia with long-term OS and DFS in rectal cancer patients than in colon cancer patients. In these respects, a separate analysis for colon and rectal cancer patients seems to be justified.

In studying the prognostic value of preoperative anemia, gender should be preferably considered. In defining anemia distinction is made between male and female, and moreover, in colorectal cancer patients, sex differences in long-term survival are demonstrated. This is best known for the survival advantage of young and middle-aged female colorectal cancer patients with localized disease.[26] Unfortunately, despite this known variation, in defining anemia only half of included studies used gender based anemia criteria, and the vast majority of studies failed to include gender in the analyses. As a result, gender could not be included in our subgroup analyses.

Meta-analysis in patients with other cancer types similarly showed that anemia, at any point during course of the disease, is associated with shorter survival. This was the case for patients with lung cancer, cervicouterine cancer, head and neck cancer, prostate cancer, lymphoma and multiple myeloma.[27] Anemia in this respect may be a common cause for treatment resistance, progression or even recurrence of cancer by several mechanisms, of which tumor hypoxia leading to an imbalance between oxygen supply and consumption receives most attention. Experimental studies indeed showed that the oxygen supply to tumors is greatly reduced and hypoxia is

intensified at hemoglobin levels below 10–12 g/dl. Tumor hypoxia in its turn is known to reduce the effectiveness of both chemotherapy and radiotherapy, and can also negatively impact therapeutic outcome by causing a broad variety of proteomic and genetic changes, leading to increased metastatic potential.[28] Moreover, under hypoxic conditions, the concentration of transcription factor hypoxia-inducible factor 1 is increased and may stimulate hypoxia-inducible gene transcription resulting in metabolic, invasive and apoptotic changes; up regulation of vascular endothelial growth factor; and tumor angiogenesis.[6, 29]

Results from experimental studies, showing that tumor hypoxia is intensified below hemoglobin levels 10-12 g/dl, may suggest that not every anemic condition will result in tumor hypoxia. However, when anemia is abruptly intensified by surgery, hypoxia driven survival of remnant tumor mass is likely important for eventual outcome. Our results from subgroup analyses based on the definition of anemia do not support the finding from experimental studies showing that tumor hypoxia, suggested to be the cause of inferior outcome, is intensified at decreasing hemoglobin levels. No trend was observed suggesting that lower hemoglobin levels are associated with worse long-term prognosis, however, high statistical heterogeneity was found in the various analyses.

The reported association between anemia and survival might suggest that correcting the preoperative anemia might positively influence long-term survival of colorectal cancer patients. However, treatment modalities for correcting anemia may also negatively influence outcome. Three principal options for treatment of anemia are to be considered, namely red blood cell transfusions, erythropoiesis-stimulating agents (ESAs) and iron, but so far there is no solid evidence that correction of anemia would improve long-term tumor prognosis.

Blood transfusions are implicated to have immunomodulatory effects that could compromise wound-healing and pathogen control, and also the immune-surveillance against cancer.30 Especially in patients with colorectal cancer, blood transfusions have been reported to be associated with worse prognosis.[31, 32] Interestingly, and refuting the immunomodulation of allogeneic blood transfusion, autologous blood transfusion showed no benefit as compared to standard allogeneic blood transfusion. From these studies, it was concluded that blood transfusion was not likely to modulate prognosis.[33, 34] Hence, a restrictive transfusion policy was implemented in favour for iron and erythropoiesis-stimulating agents (ESAs) therapy as transfusion sparing alternative. However, both these alternatives might not be indifferent for the prognosis of colorectal cancer either.

Indeed, ESAs reduce anemia and transfusion requirements in cancer patients. However, ESAs have also been reported to worsen cancer prognosis.[35-37] Possible mechanisms by which ESAs enhance tumor growth in general, and tumor recurrence in particular, is by increasing the production of pro-angiogenic factors, such as VEGF and by anti-apoptotic action.38 Increased serum VEGF is

associated with decreased disease-free and overall survival in patients with advanced colorectal cancer. In current clinical practice, treatment with ESAs should only be considered in patients with symptomatic chemotherapy-induced anemia and hemoglobin levels <10 g/dL. Moreover in patients treated with curative intent, ESAs should be used with caution. However, available analyses of data from RCTs have not stratified results on the basis of treatment intent (i.e. palliative versus curative), and therefore, future research on this topic is warranted [39, 40]

Similarly, iron therapy is reported to increase hemoglobin levels and with it reduction in allogeneic blood transfusion in patients with colorectal cancer.[41, 42] However, iron is also known to be an essential nutrient for proliferating tumor cells, and anemia of chronic disease, characterized by adequate iron stage but insufficient iron supply for erythroblasts and other iron dependent tissues, is believed to be a potentially effective defense strategy of the human body to inhibit growth of tumor cells.[4] Numerous studies support the hypothesis that both dietary iron and elevated iron levels increase the risk of colorectal cancer,[43-45] and a relationship between levels of iron stores and cancer risk is suggested by studies showing that blood donation, which reduces body iron stores, is associated with lower cancer risk.[46] This notion that iron therapy and high iron levels could pose a risk, is further enforced by the reversed, namely that systemic iron reduction by phlebotomy decreased the incidence of visceral malignancies and mortality in patients with peripheral arterial disease.[47] Finally, several animal experiment studies show iron as a risk factor for developing colorectal cancer and tumor growth.[48, 49] Clearly, while preoperative anemia is a risk factor for OS and DFS, to our knowledge, no study has addressed the long-term hazards of iron therapy in patients with colorectal cancer.

LIMITATIONS

In this meta-analysis, next to 12 observational studies, one RCT was included. This RCT was not designed primarily to examine the effect of anemia, but HR of preoperative anemia could be computed from shared individual patient data. However, this was limited by the lack of information on anemia related factors, for example blood transfusion rates.

Anemia is associated with important prognostic factors as disease severity, and with treatment strategies, and thus with outcome itself. In this meta-analysis, to adjust for important prognostic factors, subgroup analyses of studies adjusting for age and tumor stage were performed. However, since the vast majority of the included studies were observational and of retrospective nature, many factors significantly associated with DFS and OS could not be corrected for. For example, blood management strategies themselves, like preoperative blood transfusion, ESAs and iron therapy, all reduce anemia and should ideally be known and corrected for. Only in one study, the patients receiving blood transfusion were excluded, while in two studies, blood transfusion was adjusted for in the multivariate analysis. In these studies, the HRs for preoperative anemia differed greatly.

Additionally, a principal limitation to this study was the high level of statistical and clinical heterogeneity in the findings, likely due to the variety of populations studied and the different definitions of anemia used by included studies. Before including studies in the meta-analysis, a quality assessment was performed, which showed that the evidence of each individual study varied from high to very low. However, the study with the lowest quality assessment score did not provide sufficient data to include in the quantitative analysis. As the larger studies tended to be those conducted with more methodological rigour, a fixed-effects analysis was used. In this fixed-effects meta-analysis relatively more weight is rewarded to larger studies. This seems to be justified as results of the study with the most patients, addressing colon cancer, differed considerably from results of the other studies, particularly in the DFS. In general, the studies addressing rectal cancer were of less quality.

CONCLUSION

The present systematic review and meta-analysis reveals the long-term prognostic value of preoperative anemia in colorectal cancer patients This finding is particularly the case for rectal cancer patients and is supported by subgroup analyses of studies adjusting for important prognostic factors, such as age and tumor stage. However, since the effect of all confounding factors could not be assessed, a causal relationship can still not be claimed. The results should be interpreted with care given the retrospective observational nature of the vast majority of included studies, with high levels of heterogeneity. This meta-analysis does not answer the intriguing question if, and to what extent, correction of anemia by blood transfusion, ESAs or iron, will also modulate the outcome. Instead of improving survival, circumstantial evidence seems to indicate that these treatment modalities may even negatively influence long-term outcome. Future well designed RCTs therefore have to prove the observed associations of our meta-analysis and have to provide evidence if the present preoperative blood management strategy for colorectal cancer patients is optimal and safe as regards to long-term outcome.

CONTRIBUTORS

Study concept and design: MW, MvH. JH, JJ, JJZ, MS. Literature search and figures: MW, MvH. Data acquisition: MW, JB, HCP. Data analysis and interpretation: MW, JH, JJ, JJZ, MS. Writing manuscript: MW, JH, JJZ, MS. Reviewing manuscript: MvH, JH, JB, HCP JJ, JJZ, MS.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136(5):E359-86.
2. Edge S, Byrd, DR, Compton, CC, et al. AJCC (American Joint Committee on Cancer) Cancer Staging Manual, 7th edition. Vol. 7th edtion. *New York: Springer*, 2010.
3. Knight K, Wade S, Balducci L. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. *Am J Med* 2004; 116 Suppl 7A:11S-26S.
4. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
5. Steensma DP. Is anemia of cancer different from chemotherapy-induced anemia? *J Clin Oncol* 2008; 26(7):1022-4.
6. Graeber TG, Osmanian C, Jacks T, et al. Hypoxia-mediated selection of cells with diminished apoptotic potential in solid tumours. *Nature* 1996; 379(6560):88-91.
7. Harrison L, Blackwell K. Hypoxia and anemia: factors in decreased sensitivity to radiation therapy and chemotherapy? *Oncologist* 2004; 9 Suppl 5:31-40.
8. Prosnitz RG, Yao B, Farrell CL, et al. Pretreatment anemia is correlated with the reduced effectiveness of radiation and concurrent chemotherapy in advanced head and neck cancer. *Int J Radiat Oncol Biol Phys* 2005; 61(4):1087-95.
9. Vaupel P, Briest S, Hockel M. Hypoxia in breast cancer: pathogenesis, characterization and biological/therapeutic implications. *Wien Med Wochenschr* 2002; 152(13-14):334-42.
10. An MS, Yoo JH, Kim KH, et al. T4 stage and preoperative anemia as prognostic factors for the patients with colon cancer treated with adjuvant FOLFOX chemotherapy. *World J Surg Oncol* 2015; 13:64.
11. Berardi R, Braconi C, Mantello G, et al. Anemia may influence the outcome of patients undergoing neo-adjuvant treatment of rectal cancer. *Ann Oncol* 2006; 17(11):1661-4.
12. Qiu MZ, Yuan ZY, Luo HY, et al. Impact of pretreatment hematologic profile on survival of colorectal cancer patients. *Tumor Biol* 2010; 31(4):255-60.
13. van Halteren HK, Houterman S, Verheij CD, et al. Anemia prior to operation is related with poorer long-term survival in patients with operable rectal cancer. *Eur J Surg Oncol* 2004; 30(6):628-32.
14. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Int J Surg* 2010; 8(5):336-41.
15. Tierney JF, Stewart LA, Ghersi D, et al. Practical methods for incorporating summary time-to-event data into meta-analysis. *Trials* 2007; 8:16.
16. Colon Cancer Laparoscopic or Open Resection Study G, Buunen M, Veldkamp R, et al. Survival after laparoscopic surgery versus open surgery for colon cancer: long-term outcome of a randomised clinical trial. *Lancet Oncol* 2009; 10(1):44-52.
17. Box B, Lindsey I, Wheeler JM, et al. Neoadjuvant therapy for rectal cancer: improved tumor response, local recurrence, and overall survival in nonanemic patients. *Dis Colon Rectum* 2005; 48(6):1153-60.
18. Cengiz O, Kocer B, Surmeli S, et al. Are pretreatment serum albumin and cholesterol levels prognostic tools in patients with colorectal carcinoma? *Med Sci Monit* 2006; 12(6):CR240-7.
19. Fjortoft I, Furnes B, Hausken T, et al. Pre-operative anemia in colon cancer patients became normal after more than a year post-operatively but did not influence oncological outcome in the final analysis. *Scand J Gastroenterol* 2013; 48(6):663-71.
20. Giessen C, Nagel D, Glas M, et al. Evaluation of preoperative serum markers for individual patient prognosis in stage I-III rectal cancer. *Tumor Biol* 2014; 35(10):10237-48.
21. Giessen-Jung C, Nagel D, Glas M, et al. Preoperative serum markers for individual patient prognosis in stage I-III colon cancer. *Tumor Biol* 2015; 36(10):7897-906.
22. Khan AA, Klonizakis M, Shabaan A, et al. Association between pretreatment hemoglobin levels and morphometric characteristics of the tumour, response to neoadjuvant treatment and long-term outcomes in patients with locally advanced rectal cancers. *Colorectal Dis* 2013; 15(10):1232-7.

23. Lee H, Park HC, Park W, et al. Negative impact of pretreatment anemia on local control after neoadjuvant chemoradiotherapy and surgery for rectal cancer. *Radiat Oncol J* 2012; 30(3):117-23.
24. Peng Y, Wang L, Gu J. Elevated preoperative carcinoembryonic antigen (CEA) and Ki67 is predictor of decreased survival in IIA stage colon cancer. *World J Surg* 2013; 37(1):208-13.
25. van der Pas MH, Haglind E, Cuesta MA, et al. Laparoscopic versus open surgery for rectal cancer (COLOR II): short-term outcomes of a randomised, phase 3 trial. *Lancet Oncol* 2013; 14(3):210-8.
26. Majek O, Gondos A, Jansen L, et al. Sex differences in colorectal cancer survival: population-based analysis of 164,996 colorectal cancer patients in Germany. *PLoS One* 2013; 8(7):e68077.
27. Caro JJ, Salas M, Ward A, et al. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* 2001; 91(12):2214-21.
28. Rey*nolds TY, Rockwell S, Glazer PM. Genetic instability induced by the tumor microenvironment. Cancer Res 1996; 56(24):5754-7.*
29. Leo C, Giaccia AJ, Denko NC. The hypoxic tumor microenvironment and gene expression. *Semin Radiat Oncol* 2004; 14(3):207-14.
30. Vamvakas EC, Blajchman MA. Deleterious clinical effects of transfusion-associated immunomodulation: fact or fiction? *Blood* 2001; 97(5):1180-95.
31. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
32. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
33. Busch OR, Hop WC, Hoynck van Papendrecht MA, et al. Blood transfusions and prognosis in colorectal cancer. *N Engl J Med* 1993; 328(19):1372-6.
34. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.
35. Adamson JW, Spivak JL. Physiologic basis for the pharmacologic use of recombinant human erythropoietin in surgery and cancer treatment. *Surgery* 1994; 115(1):7-15.
36. Alghamdi AA, Albanna MJ, Guru V, et al. Does the use of erythropoietin reduce the risk of exposure to allogeneic blood transfusion in cardiac surgery? A systematic review and meta-analysis. *J Card Surg* 2006; 21(3):320-6.
37. Bohlius J, Schmidlin K, Brillant C, et al. Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. *Lancet* 2009; 373(9674):1532-42.
38. Pascual M, Bohle B, Alonso S, et al. 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* 2013; 183(1):270-7.
39. Schrijvers D, De Samblanx H, Roila F, et al. Erythropoiesis-stimulating agents in the treatment of anemia in cancer patients: ESMO Clinical Practice Guidelines for use. *Ann Oncol* 2010; 21 Suppl 5:v244-7.
40. Rizzo JD, Brouwers M, Hurley P, et al. American Society of Clinical Oncology/American Society of Hematology clinical practice guideline update on the use of epoetin and darbepoetin in adult patients with cancer. *J Clin Oncol* 2010; 28(33):4996-5010.
41. Keeler BD, Simpson JA, Ng S, et al. The feasibility and clinical efficacy of intravenous iron administration for preoperative anemia in patients with colorectal cancer. *Colorectal Dis* 2014; 16(10):794-800.
42. Borstlap W, Stellingwerf ME, Moolla Z, et al. Iron therapy for the treatment of preoperative anemia in patients with colorectal carcinoma: a systematic review. *Colorectal Dis* 2015.
43. Chua AC, Klopcic B, Lawrance IC, et al. Iron: an emerging factor in colorectal carcinogenesis. *World J Gastroenterol* 2010; 16(6):663-72.
44. Nelson RL. Iron and colorectal cancer risk: human studies. *Nutr Rev* 2001; 59(5):140-8.
45. Knekt P, Reunanen A, Takkunen H, et al. Body iron stores and risk of cancer. *Int J Cancer* 1994; 56(3):379-82.
46. Edgren G, Reilly M, Hjalgrim H, et al. Donation frequency, iron loss, and risk of cancer among blood donors. *J Natl Cancer Inst* 2008; 100(8):572-9.

47. Zacharski LR, Chow BK, Howes PS, et al. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. *J Natl Cancer Inst* 2008; 100(14):996-1002.

48. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.

49. Radulescu S, Brookes MJ, Salgueiro P, et al. Luminal iron levels govern intestinal tumorigenesis after Apc loss in vivo. *Cell Rep* 2012; 2(2):270-82.

50. Weiland DE, Bay RC, Del Sordi S. Choosing the best abdominal closure by meta-analysis. *Am J Surg* 1998; 176(6):666-70.

51. Berretta R, Rolla M, Patrelli TS, et al. Randomised prospective study of abdominal wall closure in patients with gynaecological cancer. *Aust N Z J Obstet Gynaecol* 2010; 50(4):391-6.

52. DesCoteaux JG, Temple WJ, Huchcroft SA, et al. Linea alba closure: determination of ideal distance between sutures. *J Invest Surg* 1993; 6(2):201-9.

53. Harlaar JJ, van Ramshorst GH, Nieuwenhuizen J, et al. Small stitches with small suture distances increase laparotomy closure strength. *Am J Surg* 2009; 198(3):392-5.

54. Deerenberg EB, Harlaar JJ, Steyerberg EW, et al. Small bites versus large bites for closure of abdominal midline incisions (STITCH): a double-blind, multicentre, randomised controlled trial. *Lancet* 2015; 386(10000):1254-60.

55. Sajid MS, Parampalli U, Baig MK, et al. A systematic review on the effectiveness of slowly-absorbable versus non-absorbable sutures for abdominal fascial closure following laparotomy. *Int J Surg* 2011; 9(8):615-25.

56. Israelsson LA, Millbourn D. Prevention of incisional hernias: how to close a midline incision. *Surg Clin North Am* 2013; 93(5):1027-40.

57. Burger JW, Luijendijk RW, Hop WC, et al. Long-term follow-up of a randomized controlled trial of suture versus mesh repair of incisional hernia. *Ann Surg* 2004; 240(4):578-83; discussion 583-5.

58. Luijendijk RW, Hop WC, van den Tol MP, et al. A comparison of suture repair with mesh repair for incisional hernia. *N Engl J Med* 2000; 343(6):392-8.

59. Pans A, Desaive C. Use of an absorbable polyglactin mesh for the prevention of incisional hernias. *Acta Chir Belg* 1995; 95(6):265-8.

60. Timmermans L, de Goede B, Eker HH, et al. Meta-analysis of primary mesh augmentation as prophylactic measure to prevent incisional hernia. *Dig Surg* 2013; 30(4-6):401-9.

61. Bhangu A, Fitzgerald JE, Singh P, et al. Systematic review and meta-analysis of prophylactic mesh placement for prevention of incisional hernia following midline laparotomy. *Hernia* 2013; 17(4):445-55.

62. Timmermans L, Eker HH, Steyerberg EW, et al. Short-term results of a randomized controlled trial comparing primary suture with primary glued mesh augmentation to prevent incisional hernia. *Ann Surg* 2015; 261(2):276-81.

63. Caro-Tarrago A, Olona Casas C, Jimenez Salido A, et al. Prevention of incisional hernia in midline laparotomy with an onlay mesh: a randomized clinical trial. *World J Surg* 2014; 38(9):2223-30.

64. Muysoms FE, Detry O, Vierendeels T, et al. Prevention of Incisional Hernias by Prophylactic Mesh-augmented Reinforcement of Midline Laparotomies for Abdominal Aortic Aneurysm Treatment: A Randomized Controlled Trial. *Ann Surg* 2016; 263(4):638-645.

Chapter 3 // **The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment**

M.J. Wilson, J.W.T. Dekker, J.J. Harlaar, J. Jeekel, M. Schipperus, J.J. Zwaginga

ABSTRACT

Background: In preoperative blood management of colorectal cancer patients, intravenous iron therapy is increasingly used to treat anemia and prevent red blood cell transfusions. However, while iron deficiency is the most common cause of anemia, little is known about the prevalence and namely type of iron deficiency in this population, whereas both types of iron deficiency (i.e. absolute and functional iron deficiency) are recommended to be treated differently by international cancer guidelines.

Objective: To investigate the prevalence and namely type of iron deficiency in colorectal cancer patients, and to assess its clinical relevance.

Methods: Preoperative iron status, clinical parameters (i.e. age, ASA classification, tumor location, tumor stage) and postoperative complications were retrospectively collected for all newly diagnosed colorectal cancer patients in our institution over a 3-year period.

Results: Iron deficiency was observed in 163 (48.1%) of 339 patients. Of these iron deficient patients, 3.7% had an isolated absolute iron deficiency (AID) and 15.3% a functional iron deficiency (FID), while the rest had a combination of AID and FID. Anemia was present in 66.1% of iron deficient patients. Iron deficiency was significantly associated with increased postoperative complication rate (univariable OR 1.94, p=0.03, multivariable OR 1.84, p=0.07), with right-sided tumors (p<0.001), high ASA classification (p=0.002), advanced tumor stage (p=0.01), and advanced age (p=0.04). In comparing clinical parameters between patients with AID and FID, advanced age was significantly associated with FID (p=0.03), and the presence of anemia with AID (p=0.02).

Conclusion: In preoperative colorectal cancer patients, there is a high prevalence of iron deficiency, including a high percentage of patients with - a component of - functional iron deficiency, associated with increased postoperative complication rate. As both types of iron deficiency require a different treatment strategy, our results illustrate the therapeutic potential of especially intravenous iron supplementation in patients with severe iron deficiency, and stress the urgency of routinely monitoring preoperative iron status and differentiation between types of iron deficiency. As iron therapy may also be potentially harmful in respect to stimulation of tumor growth, future clinical trials assessing the long-term effect of iron therapy are necessary.

INTRODUCTION

Preoperative anemia is frequently observed in colorectal cancer patients, with reported case incidences of >30 %.[1] Preoperative anemia generally is associated with increased postoperative morbidity and mortality,[2] and is also reported to be a cause of inferior long-term outcome, possibly by worsening of tumor hypoxia.[3,4] Furthermore, preoperative anemia is associated with increased utilization of allogeneic red blood cell transfusion (RBC), which, for its part, is also associated with deleterious effects on the short- and long-term outcome in colorectal cancer patients.[5,6]

Iron deficiency (ID) is the most common cause of preoperative anemia in colorectal cancer patients.[7] Contributing mechanisms to the development iron deficiency anemia include chronic tumor-induced blood loss and impaired iron homeostasis associated with chronic disease. While chronic blood loss will deplete iron stores and cause absolute iron deficiency (AID), functional iron deficiency (FID) is characterized by both reduced iron uptake in the gut and sequestration in the reticulo-endothelial system of absorbed iron, resulting in a reduction of biologically available iron.[8] Next to AID, FID is the second most prevalent cause of anemia. FID is especially known from patients with immune activation and therefore termed as anemia of inflammation of anemia of chronic disease.

The importance of this differentiation lies in the fact that the indication for initiation and the administration route of iron therapy differs between AID and FID.[9] In patients with AID, iron therapy is recommended to be started independently of the actual hemoglobin(Hb) level, while in patients with FID, iron therapy is advised only if patients are symptomatic because of iron deficiency and/or anemia and should be withheld in patients with high ferritin levels (i.e. >1000 ng/ml). In addition, in patients with FID, oral iron is poorly absorbed in the duodenum, while intravenous iron is more effective. On the other hand, restrictive iron therapy might be advisable for cancer patients in general, as iron is reported to stimulate tumor growth. The latter could be even more important for cancer patients with FID. This cancer-induced immune response namely might well protect against proliferation of tumor cells.[8,10]

Notwithstanding possible detrimental effects, iron in preoperative blood management to reverse the anemia associated prognosis has gained more attention.[11] In particular, this has led to the increased use of preoperative intravenous iron supplementation. Whereas preoperative anemia is a well-known and frequent complication in colorectal cancer patients, little is known about the prevalence of iron deficiency.[7,12] Whilst research is being carried out on the efficacy of preoperative oral and intravenous iron therapy in patients with iron deficiency anemia, no trials differentiate between AID and FID and often only the Hb increase and reduction in RBC transfusions are studied.[13,14]

Despite the recommendations by international oncological guidelines,[15,16] routinely monitoring

preoperative iron status is often not standard of care, and is, for example, not incorporated into the Dutch guideline on the treatment of anemia in oncological patients. The aim of present study is to identify the prevalence and type of iron deficiency, and to assess the clinical relevance of iron deficiency.

METHODS

All patients undergoing resection for colorectal cancer between 1 July 2013 and 1 July 2016 at the Department of Surgery, Reinier de Graaf Hospital, were eligible for inclusion. In these patients, the inclusion criterion was the availability of iron status (i.e. iron, transferrin, transferrin saturation, ferritin), measured directly after colonoscopy and suspicion of colorectal cancer. Clinical and pathological data, including age, gender, ASA classification, tumor type, pathological tumor stage, neoadjuvant treatment and 30-day overall postoperative complications (i.e. pulmonic, cardiologic, thrombotic, infectious, neurologic) were collected by the Dutch Surgical Colorectal Audit (DSCA), a disease-specific national audit. This audit collects information on patient, tumor, treatment, and 30-day and in-hospital outcome characteristics of all patients undergoing a resection for primary colorectal carcinoma in the Netherlands. The data set is based on evidence-based guidelines and is cross-checked on a yearly basis with data from the Netherlands Cancer Registry. In addition, hemoglobinvalues (i.e. at diagnosis, preoperative and postoperative), and iron status at diagnosis, were retrospectively collected.

According to the World Health Organisation (WHO), anemia was defined as Hb <8 mmol/L in men and <7.5 mmol/L in women. Iron deficiency was defined as transferrin saturation (TSAT) <20% and was further classified as absolute iron deficiency (AID), functional iron deficiency (FID), or a combination of both conditions. AID was defined as TSAT <20% and increased transferrin (>3.6 g/L); FID as TSAT <20%, reduced to normal transferrin and increased ferritin (>200 µg/L).

Tumor locations were classified as right colon (i.e. cecum, colon ascendens, hepatic flexure), transverse colon, left colon (i.e splenic flexure, colon descendens, sigmoid) and rectum. Tumor staging and tumor grading was determined according to the AJCC recommendations in colorectal cancer, and was given by pathologic examination. The ASA physical status classification system was used for assessing the fitness of patients before surgery.

The results are mainly illustrated by descriptive statistics. $\chi 2$, Fisher's exact and Student's t test were used to compare the frequencies of both categorical and continuous variables with iron status (i.e. iron deficiency versus non-iron deficiency, and absolute versus functional iron deficiency) and tumor location (i.e. colon versus rectum). Binary logistic regression analysis was performed to identify the relationship between iron deficiency at diagnosis and postoperative complication. All variables in the univariable analysis were included in the multivariable analysis. A significance level of 0.05 was considered to be statistical significant.

Approval by the Local Medical Ethics Committee was obtained. Our institution, a teaching hospital, is making use of opt-out consent. Each included patients had given consent by not declining to give consent.

RESULTS

Incidence of iron deficiencies

In total, 429 patients underwent surgery for colorectal cancer, and iron status was available in 339 patients (all measured at diagnosis). Table 1 shows the baseline characteristics of included patients. The mean age at presentation was 69.6 (range 28-95); 185 males and 154 females were included. Most patients (58.1%) were classified as ASA 2 and the most frequent site of tumor occurrence was the left colon (36.6%), followed by the rectum (29.5%), right colon (25.4%) and transverse colon (8.6%). The majority of patients were classified as pTNM stage 2 (33.6%), followed by stage 1 (29.8%), stage 3 (28.0%), stage 4 (8.6%). Of 339 patients, preoperatively, 35 patients (10.3%) received radiotherapy alone, 19 patients (5.6%) received concomitant chemo-radiotherapy, and 8 patients (2.4%) received chemotherapy alone. In total, 256 patients (79.0%) were symptomatic at presentation; most patients presented with blood loss (n=108), followed by change in stool (n=72), other (n=43)(i.e. abdominal pain, weight loss, fatigue), and anemia (n=33). Iron deficiency was observed in 163 patients (48.1%), and anemia in 115 patients (33.9%). Among these iron deficient patients, 6 (3.7%) and 25 (15.3%) patients were absolute and functional iron deficient, respectively. In the majority of patients (n=132; 81.0%), iron deficiency was caused by a combination of AID and FID. In total, 80% of anemic patients had some form of iron deficiency (5.2% AID, 9.6% FID, 65.2% combination AID and FID). Of non-anemic patients, 14 (6.3%) were functional iron deficient, and 57 (25.4%) had a combination of AID and FID; no patients were absolute iron deficient (figure 1).

Associations between iron deficiency and patient and tumor characteristics

In table 2, the proportion of patients with and without iron deficiency are given in relation to gender, age, ASA classification, tumor location, pTNM stage, and the presence of anemia. Iron deficiency was significantly more likely to occur in the right colon ($p < 0.001$), in patients with a more advanced pTNM stage ($p=0.01$), and in patients with a higher ASA classification ($p=0.002$), and in patients with more advanced age ($p=0.043$). Moreover, anemia was significantly more observed in iron deficient patients ($p < 0.001$). Gender did not show a significant association with the presence of iron deficiency. Iron deficient patients presented more often in the workup of anemia, as compared to non-iron deficient patients (16.2% versus 4.7%), while non-iron deficient patients more often were diagnosed due to the screening program.

In table 3, the mentioned variables (i.e. gender, age, tumor location, ASA classification, pTNM stage and anemia) were compared between patients with AID and those with FID. Results showed

Table 1. Patient baseline characteristics (n=339)

	n	%
Gender		
male	185	54.6
female	154	45.4
Age (years)		
mean (range)	69.63 (28-95)	
ASA classification		
I	76	22.4
II	197	58.1
III	65	19.2
IV	1	0.3
Tumor location		
right colon	86	25.4
transverse colon	29	8.6
left colon	124	36.6
rectum	100	29.5
Neoadjuvant treatment		
chemotherapy	8	2.4
radiotherapy	35	10.3
concomitant chemoradiotherapy	19	5.6
none	277	81.7
pTNM stage*		
I	101	29.8
II	114	33.6
III	95	28.0
IV	29	8.6
Presenting symptoms		
asymptomatic (population screening)	68	21.0
symptomatic	256	
blood loss	108	33.3
change in stool	72	22.2
workup of anemia	33	10.2
other	43	13.3
unknown	15	
Iron deficiency		
no	176	51.9
yes	163	48.1
absolute iron deficiency	6	3.7
functional iron deficiency	25	15.3
both conditions	132	81.0
Anemia at presentation		
no	224	66.1
yes	115	33.9
absolute iron deficiency	6	5.2
functional iron deficiency	11	9.6
both conditions	75	65.2

* after chemo and/or radiotherapy in 62 patients

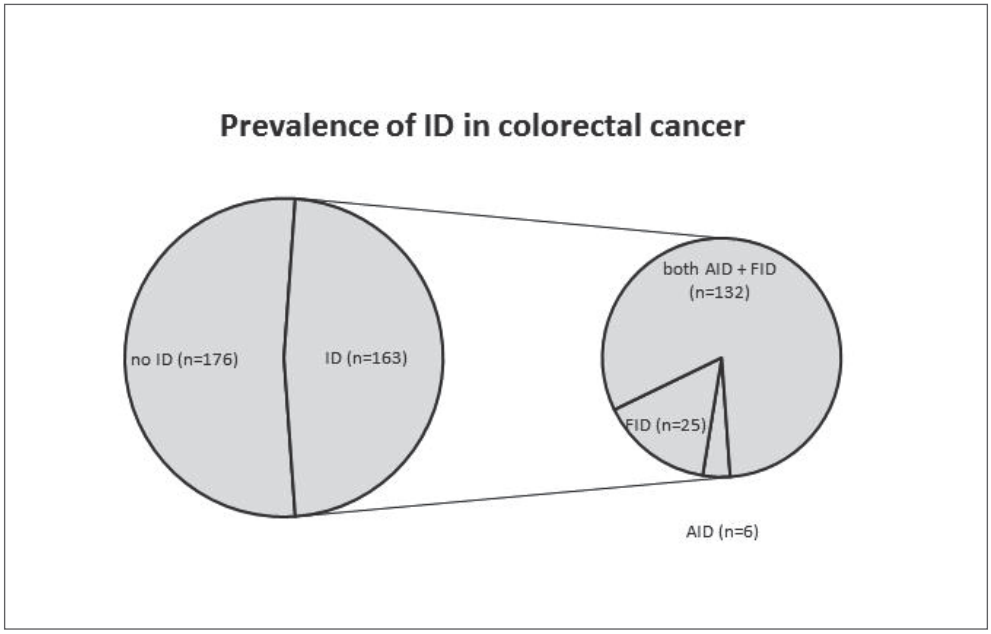


Figure 1. Prevalence of iron deficiency

that advanced age was significantly associated with FID (p=0.03), while the presence of anemia was significantly associated with AID (p=0.02). Gender, tumor location, ASA classification and pTNM stage were not found to have any significant relationship with AID or FID.

Association between iron deficiency and postoperative complication

In table 4, the association between iron deficiency and postoperative complications is assessed by uni- and multivariable logistic regression analysis. In total, postoperative complications were observed in 75 of 339 patients. Initially, in univariable analysis, the categorical variable of severity of iron deficiency was included (i.e. no iron deficiency versus mild iron deficiency (TSAT <20%) versus severe iron deficiency (TSAT <10%)). As merely severe iron deficiency appeared to be significantly associated with postoperative complications (OR 1.92, p=0.045, versus mild iron deficiency OR 0.97, p=0.92), severe iron deficiency was included in uni- and multivariable logistic regression analyses, as shown in table 4. In univariable analysis, severe iron deficiency was significantly associated with postoperative complications (OR 1.94, p=0.030). No significant result was found in multivariable analysis (OR 1.84, p=0.074).

Distinction between colon and rectum tumors

In table 5, the different variables between colon and rectum tumors are shown. Anemia, both

at diagnosis, preoperative and postoperative, was more prevalent in colon tumors (p <0.001, p <0.001, p=0.04, respectively). Reduced Hb levels at diagnosis, preoperative and postoperative were found to be significantly associated with colon tumors (all p <0.001), while a reduction in the Hb level due to surgery was more pronounced in patients with rectum tumors as compared to those with colon tumors (1.09 mmol/L versus 0.96 mmol/L, p=0.05). Mean duration from diagnosis to surgery was 7.4 weeks for all colorectal tumors, but was significantly different for colon cancer patients (5.1 weeks) as compared to patients with rectum cancer (12.2 weeks).

Table 2: Characteristics non-iron deficiency versus iron deficiency

	Non-iron deficiency	Iron deficiency	p-value
Number, n (%)	176	163	
Gender, %			0.13
male	58.5	50.3	
female	41.5	49.7	
Age (years)			0.043
mean ±SD	68.5 ±10.86	70.8 ±10.56	
ASA, %			0.002
I + II	86.9	76.3	
III + IV	13.1	26.4	
Tumor location, %			<0.001
right colon	13.6	38	
transverse colon	8.5	8.6	
left colon	39.8	33.1	
rectum	38.1	20.2	
pTNM stage, %			0.01
I	36.9	22.1	
II	27.3	40.5	
III	28.4	27.6	
IV	7.4	9.8	
Anemia at diagnosis, n (%)	23 (13.1)	92 (56.4)	<0.001
Presenting symptoms, n (%)			<0.001
asymptomatic	47 (27.6)	21 (13.6)	
symptomatic	123	133	
blood loss	60 (35.3)	48 (31.2)	
change in stool	41 (24.1)	31 (20.1)	
workup of anemia	8 (4.7)	25 (16.2)	
other	14 (8.2)	29 (18.8)	

Table 3. Characteristics in absolute versus functional iron deficiency

	Absolute iron deficiency	Functional iron deficiency	p-value
Number, n	6	25	
Gender, %			0.79
male	66.7	72	
female	33.3	28	
Age (years)			0.03
mean ±SD	68.5 ±4.23	74.2 ±7.40	
Tumor location, %			0.64
colon	83.3	68.0	
rectum	16.7	32.0	
ASA, %			0.60
I + II	66.7	80.0	
III + IV	33.3	20.0	
pTNM stage, %			0.66
I + II	66.7	52.0	
III + IV	33.3	48.0	
Anemia, %			0.02
no	0	56.0	
yes	100	44.0	

Table 4. Univariable and multivariable logistic regression analysis for risk factors of postoperative complications

		univariable			multivariable	
	OR	95% CI	p-value	OR	95% CI	p-value
Age (years)	1.02	0.99 - 1.05	0.074	1.01	0.99 - 1.04	0.336
Gender						
female versus male	0.38	0.22 - 0.67	0.001	0.38	0.21 - 0.68	0.001
ASA-classification						
III-IV vs. I-II	2.08	1.15 - 3.76	0.016	1.71	0.87 - 3.36	0.118
Surgery						
laparoscopic versus open	0.34	0.17 - 0.68	0.002	0.29	0.13 - 0.62	0.002
Tumor localisation						
rectum vs. colon	1.47	0.86 - 2.53	0.16	2.07	1.12 - 3.82	0.021
Severe iron deficiency at diagnosis	1.94	1.07 - 3.54	0.030	1.84	0.94 - 3.60	0.074

Table 5. Characteristics in colon versus rectum cancer patients

	colon	rectum	p-value
Number, n	239	100	
Age (years)			0.15
mean ±SD	70.2 ±10.4	68.3 ±11.6	
Anaemia at diagnosis, %	41.8	15.0	<0.001
Preoperative anaemia, %	45.1	20.6	<0.001
Postoperative anaemia, %	76.2	65.0	0.04
Hb at diagnosis*			
mean ±SD	7.78 ±1.4	8.54 ±1.0	<0.001
Preoperative Hb			
mean ±SD	7.87 ±1.2	8.45 ±0.9	<0.001
Postoperative Hb			
mean ±SD	6.91 ±1.1	7.34 ±0.9	<0.001
Reduction in Hb level due to surgery			
mean ±SD	0.96 ±0.6	1.09 ±0.5	0.05
* in mmol/L			

DISCUSSION

The present study firstly shows a high prevalence of preoperative ID in colorectal cancer patients. Almost half of the patients with newly diagnosed colorectal cancer are iron deficient at presentation. Interestingly, most patients have isolated FID (15%) or a combination of FID and AID (81%), compared to only 4% with isolated AID. From these results, we may conclude that the high percentage of patients with FID or a component of FID suggests that inflammation plays an important role in the development of iron deficiency in colorectal cancer patients. Secondly, patients with an advanced tumor, advanced age, a tumor in the right colon, and a high ASA classification, are more prone to develop iron deficiency. Thirdly, iron deficiency clearly plays a role in 80% of anemic patients (5.2% AID, 9.6% FID, 65.2% combined AID and FID), however, iron deficiency is also encountered in 32% of non-anemic patients (6.3% FID, 25.4% combined AID and FID).

In addition to the high prevalence of iron deficiency, the clinical relevance of iron deficiency is studied in the present study. Particularly, in univariable analysis, severe iron deficiency is significantly associated with increased postoperative complication rate. Despite the fact that in present cohort loss of significance is observed in multivariable analysis, most likely due to the relative small sample size, iron deficiency still seems to be independently associated with postoperative complications. Previous published studies namely have demonstrated the efficacy of preoperative iron supplementation with regard to reduction of the need for blood transfusion and reduction of hospital length of stay [17, 18]. In addition, lower total numbers of postoperative complications were found. These results implicate iron deficiency as an attractive treatment target to at least ameliorate short-term outcomes.

Preoperative anemia is emerging as an important health problem in colorectal cancer patients. Importantly, preoperative anemia has already been associated with increased short-term postoperative morbidity and mortality (<30 days)[2, 19], and worse colorectal tumor prognosis[3, 4, 20]. Whereas preoperative anemia is often associated with iron deficiency, up to now, guidelines for the management of cancer or chemotherapy-induced anemia make only a few remarks on the management of iron deficiency.

The ASCO (American Society of Clinical Oncology) guideline[21] on *the use of epoetin and darbepoietin in adult patients with cancer* recommends to only start iron supplementation in order to improve the efficacy of erythropoietin-stimulating agents (ESAs), and to monitor iron status during the course of ESA therapy. The ESMO (European Society for Medical Oncology) guideline16 states that intravenous iron therapy is more effective in terms of Hb optimisation as compared to oral iron therapy, and that iron therapy seems to reduce the total number of patients receiving blood transfusions. Most elaborate is the NCCN (National Comprehensive Cancer Network) guideline[15] on *cancer- and chemotherapy-induced anemia* that recommends to start iron monotherapy in absolute iron deficiency patients, independently of the presence of anemia, to start iron therapy in patients receiving ESA, and to withheld iron therapy in patients with active infections. The NCCN guideline additionally briefly addresses treatment of merely iron deficiency in non-anemic patients. This seems to be clinically relevant as iron deficiency itself, in the absence of anemia, can cause symptoms as impaired physical function and fatigue.[22, 23] The observed high prevalence of iron deficiency in colorectal cancer patients causes the authors to advise routinely monitoring of preoperative iron status.

In general, guidelines and literature stress the high therapeutic potential of iron therapy in patients with iron deficiency anemia to increase preoperative hemoglobinlevel, to lower the need for blood transfusions and to improve short-term postoperative outcomes. An important caveat raised by ESMO is that oral - as opposed to intravenous - iron administration is quite ineffective in, as our study shows, the major part of patients that have some form of FID. Inflammation-

related IL-6 increased hepcidin production namely hampers iron absorption from the duodenum.[8, 24] Furthermore, there is an increased uptake and retention of iron in macrophages, resulting in limitation of availability of iron for iron-restricted erythropoiesis.

Notwithstanding its increased efficacy, timing and dosing is crucial for intravenous iron therapy. Maximal Hb response namely usually takes four to six weeks,[25] while often more than one dose, maximum of 1 gram weekly (i.e. Ferinject or Monofer), is required. As highlighted in our study, such an approach is well feasible for patients with rectum tumors, however, for patients with colon tumors with only on average a 5-week period between diagnosis and surgery, this would be quite a challenge. Furthermore, preoperatively, anemia was found in almost half of all colon cancer patients, compared to only 20% of rectum cancer patients. However, surgery mediated blood loss and decrease in Hb level was substantially higher in rectum cancer patients, with an increase in postoperative anemia to 66%, compared to 77% in colon cancer patients. This finding suggests that an even more proactive approach to correct preoperative anemia in all rectal cancer patients seems to be warranted.

An additional comment, however, should be made. Despite the increased use and success of preoperative - often intravenous - iron therapy to correct anemia, there are no clinical studies addressing long-term effects of iron therapy in colorectal cancer patients. The importance of this is highlighted by the fact that iron is an important growth factor for rapidly proliferating cells including bacteria and tumor cells. FID in this regard is believed to be a potentially effective defense strategy of the human body to inhibit the growth of pathogens. Several experimental animal studies have shown that exposure to iron can be a risk factor for developing colorectal cancer and tumor growth.[10, 26, 27] While oral iron might induce intraluminal tumor growth, intravenous iron could in this respect additionally be a potential risk for stimulating growth of metastases.
Ultimately, in preoperative blood management, the potential risks of blood transfusion and iron supplementation have to be cautiously weighed up against the risks of anemia and iron deficiency. Importantly, concerning oncological patients, not only short-term, but also long-term oncological effects have to be included in this risk assessment. Preoperative anemia and blood transfusion have already been strongly associated with a worse oncological outcome [5, 28]. The oncological effects of iron supplementation, however, have not been studied yet. Therefore, clinical studies comparing the long-term effects of anemia and iron deficiency with the long-term effects of iron supplementation and blood transfusion are required to establish the optimal blood management strategy in oncological patients.

Strengths and limitations

One of the strengths of the present study is the timing of measuring iron status of patients. Iron status was measured directly after colonoscopy, where a lesion suspicious of colorectal cancer

was noticed. As a result, in the vast majority, the iron status we used was not yet affected by any iron supplementation and therefore a reliable representation of condition around diagnosis. The major limitation of this study was the sample size. Therefore, in comparing characteristics of AID and FID, and in assessing the association between iron deficiency and postoperative complication, the small sample size did not allow us to draw firm conclusion on associations. In addition, Nonetheless, up till now, this is the largest group of colorectal cancer patients in which the prevalence and type of iron deficiency is described.

CONCLUSION

This study shows a high prevalence of preoperative iron deficiency in colorectal cancer patients, including a high percentage of patients with - a component of - functional iron deficiency, and frequently associated increased postoperative complication rate, anemia, right-sided colon tumors, advanced age and tumor stage, and poor physical status. As both types of iron deficiency require a different treatment strategy, our results illustrate the therapeutic potential of especially intravenous iron supplementation in patients with severe iron deficiency, and stress the urgency of routinely monitoring preoperative iron status and differentiation between types of iron deficiency. As iron therapy may also be potentially harmful in respect to stimulation of tumor growth, future clinical trials assessing the long-term effect of iron therapy are necessary.

REFERENCES

1. Bastide NM, Chenni F, Audebert M, et al. A central role for heme iron in colon carcinogenesis associated with red meat intake. *Cancer Res* 2015; 75(5):870-9.
2. Fowler AJ, Ahmad T, Phull MK, et al. Meta-analysis of the association between preoperative anemia and mortality after surgery. *Br J Surg* 2015; 102(11):1314-24.
3. van Halteren HK, Houterman S, Verheij CD, et al. Anemia prior to operation is related with poorer long-term survival in patients with operable rectal cancer. *Eur J Surg Oncol* 2004; 30(6):628-32.
4. An MS, Yoo JH, Kim KH, et al. T4 stage and preoperative anemia as prognostic factors for the patients with colon cancer treated with adjuvant FOLFOX chemotherapy. *World J Surg Oncol* 2015; 13:64.
5. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
6. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
7. Ludwig H, Muldur E, Endler G, et al. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol* 2013; 24(7):1886-92.
8. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
9. Ludwig H, Evstatiev R, Kornek G, et al. Iron metabolism and iron supplementation in cancer patients. *Wien Klin Wochenschr* 2015.
10. Brookes MJ, Hughes S, Turner FE, et al. Modulation of iron transport proteins in human colorectal carcinogenesis. *Gut* 2006; 55(10):1449-60.
11. Borstlap W, Stellingwerf ME, Moolla Z, et al. Iron therapy for the treatment of preoperative anemia in patients with colorectal carcinoma: a systematic review. *Colorectal Dis* 2015.
12. Beale AL, Penney MD, Allison MC. The prevalence of iron deficiency among patients presenting with colorectal cancer. *Colorectal Dis* 2005; 7(4):398-402.
13. Richards T, Clevenger B, Keidan J, et al. PREVENTT: preoperative intravenous iron to treat anemia in major surgery: study protocol for a randomised controlled trial. *Trials* 2015; 16:254.
14. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemia in colorectal cancer patients. *BMC Surg* 2015; 15:78.
15. NCCN. *Cancer- and chemotherapy-induced anemia*. 2014.
16. Schrijvers D, De Samblanx H, Roila F, et al. Erythropoiesis-stimulating agents in the treatment of anemia in cancer patients: ESMO Clinical Practice Guidelines for use. *Ann Oncol* 2010; 21 Suppl 5:v244-7.
17. Calleja JL, Delgado S, del Val A, et al. Ferric carboxymaltose reduces transfusions and hospital stay in patients with colon cancer and anemia. *Int J Colorectal Dis* 2016; 31(3):543-51.
18. Froessler B, Palm P, Weber I, et al. The Important Role for Intravenous Iron in Perioperative Patient Blood Management in Major Abdominal Surgery: A Randomized Controlled Trial. *Ann Surg* 2016.
19. Leichtle SW, Mouawad NJ, Lampman R, et al. Does preoperative anemia adversely affect colon and rectal surgery outcomes? *J Am Coll Surg* 2011; 212(2):187-94.
20. Zhen L, Zhe S, Zhenning W, et al. Iron-deficiency anemia: a predictor of diminished disease-free survival of T3N0M0 stage colon cancer. *J Surg Oncol* 2012; 105(4):371-5.
21. Rizzo JD, Brouwers M, Hurley P, et al. American Society of Hematology/American Society of Clinical Oncology clinical practice guideline update on the use of epoetin and darbepoetin in adult patients with cancer. *Blood* 2010; 116(20):4045-59.
22. Brownlie Tt, Utermohlen V, Hinton PS, et al. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. *Am J Clin Nutr* 2004; 79(3):437-43.
23. Verdon F, Burnand B, Stubi CL, et al. Iron supplementation for unexplained fatigue in non-anemicwomen: double blind

randomised placebo controlled trial. *Bmj* 2003; 326(7399):1124.

24. Ludwig H, Evstatiev R, Kornek G, et al. Iron metabolism and iron supplementation in cancer patients. *Wien Klin Wochenschr* 2015; 127(23-24):907-19.

25. Keeler BD, Simpson JA, Ng S, et al. The feasibility and clinical efficacy of intravenous iron administration for preoperative anemia in patients with colorectal cancer. *Colorectal Dis* 2014; 16(10):794-800.

26. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.

27. Radulescu S, Brookes MJ, Salgueiro P, et al. Luminal iron levels govern intestinal tumorigenesis after Apc loss in vivo. *Cell Rep* 2012; 2(2):270-82.

28. Wilson MJ, van Haaren M, Harlaar JJ, et al. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis. *Surg Oncol* 2017; 26(1):96-104

Chapter 4 // **Short-term effect of preoperative intravenous iron therapy in anemic colorectal cancer patients: results of a cohort study**

M.J. Wilson, J.W.T. Dekker, E.R.J. Bruns, W.A. Borstlap, J. Jeekel, J.J. Zwaginga, M. Schipperus

ABSTRACT

Background: Preoperative anemia is associated with increased postoperative morbidity and delayed recovery in oncological patients. In the treatment of such anemia , iron suppletion can replace blood transfusion and erythropoiesis-stimulating agents, which both have been associated with substantial side effects and increased risk of cancer recurrence. The aim of this study was to assess the efficacy of preoperative intravenous iron infusion in optimising hemoglobin (Hb) level in anemic colorectal cancer patients and to identify patient characteristics that are associated with an increase in Hb level after iron infusion.

Methods: A retrospective cohort study was performed on patients who underwent surgery for colorectal cancer between 2010-2016 in a single teaching hospital. The primary outcome measure, the change in hemoglobin level, was assessed by comparing anemic patients receiving usual care (UC)(i.e. no iron therapy and no blood transfusion) with anemic patients receiving intravenous iron (IV) therapy (no blood transfusion). In addition, in assessing the association between intravenous iron therapy and postoperative blood transfusions and complications, all anemic patients were included in logistic regression analyses.

Results: 758 patients with colorectal cancer were eligible, of which 318 (41.9%) were anemic. The IV and the UC group included 52 and 153 patients with mean Hb levels at diagnosis of 6.3 and 6.9 mmol/L, respectively. In the IV group, preoperative Hb level was significantly increased as compared to UC group (0.65 mmol/L vs 0.10 mmol/L, $p<0.001$). High increase in Hb level after iron infusion was associated with initial higher transferrin and lower ferritin levels (high versus poor responders: median transferrin 2.9 vs 2.7 g/L, median ferritin 12 vs 27 µg/L). Multivariable logistic regression analyses on all anemic patients (n=318) showed that administration of intravenous iron therapy did not affect postoperative blood transfusion and complication rate (OR 0.54, p=0.14 and OR=0.91, p=0.77, respectively).

Discussion: Based on this cohort study, implementation of intravenous iron therapy in anemic colorectal cancer patients leads to a distinct increase of preoperative hemoglobin level. Intravenous iron therapy is most effective in patients presenting with more severe anemia, and with higher transferrin and lower ferritin levels, markers for an absolute iron deficiency, as compared to functional iron deficiency. Our finding, that the distinct Hb increase did not coincide with an expected decrease in the percentage of patients with a postoperative blood transfusion or complication, should be viewed with caution due to the retrospective nature of this study. Future randomised trials are thus required to establish the short-term benefits.

INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in men and second in women worldwide[1], and patients present with anemia in up to a third of the cases.[2] Anemia in this respect is emerging as an important health problem. It is not only associated with fatigue[3], impaired physical performance and cognitive function, but most importantly also with increased morbidity and mortality.[4-6]

Iron deficiency (ID) is the most common cause of preoperative anemia in colorectal cancer patients.[7] Contributing mechanisms to the development of iron deficiency anemia (IDA) include chronic tumour-induced blood loss and also impaired iron homeostasis associated with chronic disease. While chronic blood loss will cause absolute iron deficiency (AID), characterized by depleted iron stores, impaired iron homeostasis will cause functional iron deficiency (FID), characterized by reduced iron uptake and iron mobilisation from the reticulo-endothelial system, both leading to a reduction of biologically available iron for erythropoiesis.[8]

Enhancement of a patient's condition prior to surgery has been gaining attention ever since the beneficial outcomes of such protocols were shown.[9, 10] More specifically, normalization of preoperative hemoglobin (Hb) level by blood management strategy is an important element in this spectrum of preoperative care.[11-13]

The high prevalence of IDA in colorectal cancer patients provides an opportunity to optimise preoperative hemoglobin level by preoperative iron supplementation with the purpose of reducing the use of blood transfusions and erythropoiesis-stimulating agents (ESAs).[14] Avoiding blood transfusions and ESAs in oncological patients seems important because of its association with an increased risk of cancer recurrence and increased mortality[15-17]. Oral iron has been shown to correct anemia , but is also known to be slow in terms of absorption rate, to cause constipation, and to be ineffective in patients with FID as oral iron is poorly absorbed in the duodenum in these patients, due to increased production of hepcidin.

Therefore as compared to oral iron, intravenous iron therapy is likely to be more effective in treating anemia , as shown in patients undergoing orthopedic[18] or general abdominal surgery[19]. Based on these advantages, over the course of the last five years administration of intravenous iron has also been introduced in our institution. In this study, we retrospectively compare preoperative intravenous iron with usual care (i.e. no iron therapy) in anemic colorectal cancer patients, with regard to increasing preoperative hemoglobin level, and reducing postoperative complications and blood transfusions. In addition, predictive factors of good response to intravenous iron therapy will be studied.

METHODS

Patient selection

All patients undergoing resection for colorectal cancer between 1 January 2010 and 1 July 2016 at the Department of Surgery, Reinier de Graaf Hospital, the Netherlands, were identified. Patients who had surgery in the emergency setting, and those with missing data with respect to baseline Hb levels and blood transfusions were excluded.

Outcome Measures

Primary outcome was the change in hemoglobin level (i.e. Hb at diagnosis – Hb preoperative), secondary outcomes included the percentage of patients with a blood transfusion and complication <30 days postoperatively.

Defining Patient Groups

Consecutive patients diagnosed with anemia (men Hb <8.0 mmol/L, 12.9 g/dL; women Hb <7.5 mmol/L, 12.0 g/dL) were eligible for inclusion. Initially, to provide a clear overview, the total anemic cohort was divided in two main groups (IV versus UC).

The UC group consisted of patients receiving usual care, defined by no intravenous iron therapy <6 weeks prior to surgery. In general and following the disadvantages of oral iron suppletion, none of the patients awaiting surgery in our center did receive preoperative oral iron therapy. According to the criteria of the Dutch Blood Transfusion Guideline, during the entire study period, a blood transfusion was given according to the 4-5-6 rule, depending on the severity of the anemia and the condition of the patient.[20]

The IV group consisted of patients receiving intravenous iron therapy <6 weeks prior to surgery, defined by a dose of 1000-2000mg iron(III)carboxymaltose (Ferinject) or iron(III)isomaltoside (Monofer). In our institution, a patient blood management protocol (PBM) was implemented in July 2013. Prior to implementation of this protocol, treatment of preoperative anemia was heavily depending on the interest in, and knowledge of PBM of each physician. As a result, there was heterogeneity in the cohort of anemic patients treated with intravenous iron therapy before July 2013. As part of the implemented PBM protocol, iron status was measured in all consecutive patients diagnosed with colorectal cancer and treatment with intravenous iron therapy was considered for patients with anemia . However, each physician did have the possibility to deviate from the PBM protocol, depending on their clinical assessment. As a result, there was also heterogeneity in the cohort of anemic patients treated with intravenous iron therapy after July 2013. Due to this heterogeneity, comparing a before- and after July 2013 cohort would not yield relevant results.

In addition, two subgroups (IV vs UC) were formed, in which all factors possibly directly affecting

Hb level (i.e. preoperative blood transfusion and neoadjuvant chemotherapy) were excluded. Patients receiving their first intravenous iron infusion <7 days prior to surgery (IV group), and patients receiving intravenous iron infusion between 6 and 12 weeks prior to surgery (UC group) were additionally excluded.

Statistical Analyses

To assess the primary outcome, the difference between Hb level at diagnosis and preoperative Hb level were calculated and analysed in the two subgroups. In addition, predictive factors of good response to intravenous iron were identified. For comparison, $\chi 2$ and Mann Whitney U tests were performed. To assess the association between intravenous iron therapy and postoperative blood transfusion and complication, all anemic (i.e. UC + IV group) patients were included in uni- and multivariable logistic regression analyses. Amongst the variables included in the logistic regression analyses is timeframe surgery (2014-2016 vs. 2010-2013), because in the course of time new surgical techniques or procedures could potentially contribute to a decrease in the postoperative blood transfusion and complication rate. A significance level of 0.05 was considered to be statistical significant.

Data Collection

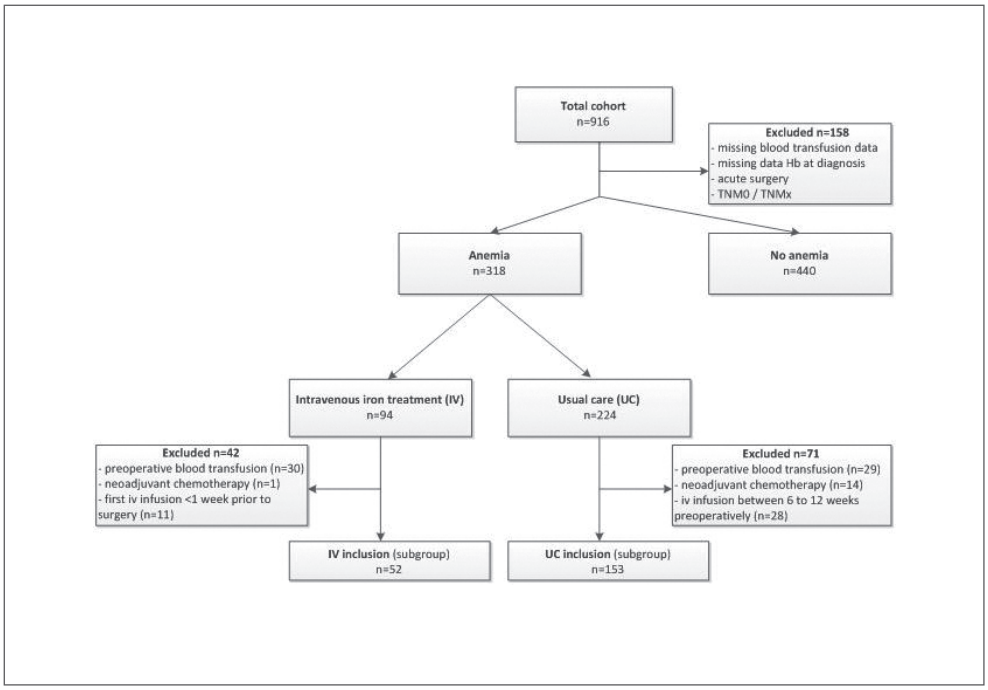
The use of preoperative intravenous iron therapy and pre-, peri-, and postoperative blood transfusion was retrospectively collected. In this respect, preoperative period was defined as <6 weeks before surgery, and postoperative period as <30 days after surgery. In addition, Hb values at diagnosis of colorectal cancer, preoperative (i.e. one day before surgery) and postoperative (i.e. one day after surgery) were manually obtained from medical records. Clinical and pathological data, including age, gender, ASA-classification (i.e. American Society of Anesthesiologists physical status classification), overall comorbidities (i.e. cardiologic, vascular, diabetes, pulmonic, neurologic, thrombotic, urologic, musculoskeletal, infectious, malignancy, endocrine) tumor type, pathological tumor stage, neoadjuvant treatment, and postoperative overall complications (i.e. pulmonic, cardiologic, thrombotic, infectious, neurologic) were collected by the Dutch Surgical Colorectal Audit (DSCA), a disease-specific national audit.[21] This audit collects information on patient, tumour, treatment, and 30-day and in-hospital outcome characteristics of all patients undergoing a resection for primary colorectal carcinoma in the Netherlands. The data set is based on evidence-based guidelines and is cross-checked on a yearly basis with data from the Netherlands Cancer Registry.

Ethical approval for this study was provided by the Ethical Committee METC Zuidwest Holland (METC-nr 16-012, approved by secretary mw. drs. E. Roep, date of approval 03/02/2016). Our institution, a teaching hospital, is making use of opt-out consent. Each included patients had given consent by not declining to give consent.

RESULTS

In total, 916 patients underwent surgery for colorectal cancer. A total of 158 patients were excluded because of missing data on blood transfusion or Hb level at diagnosis, or surgery in the emergency setting. A total of 318 patients (41.9%) were anemic at diagnosis, of which 94 patients received intravenous iron treatment and 224 patients received usual care. After excluding all factors possibly directly affecting Hb level, 52 and 153 patients remained in the IV and UC subgroup (figure 1).

Figure 1. Flow diagram



IV versus UC, total anemic cohort

An overview of the baseline characteristics is presented in table 1. Both groups had a mean age above 70 years (IV=71.8 ±11.1, UC=73.7 ±9.9, p=0.15). In the UC group, the majority was male as compared to the IV group (58.5% vs 44.7%, p=0.02) and there were more patients with comorbidity (87.1% vs 79.8%, p=0.01) and with a rectum tumor (20.5% vs 5.3%, p=0.001). Regarding physical condition, surgical procedure and tumor stage, no significant differences were found. In the IV group, Hb level at diagnosis was significantly lower (6.12 mmol/L vs 6.61 mmol/L, p<0.001) and more patients received a preoperative blood transfusion (31.9% vs 12.9%, p<0.001). Out of 30 IV patients additionally receiving a preoperative blood transfusion, 13 patients (mean Hb level at diagnosis of 5.7 mmol/L) received blood transfusion prior to iron infusion, while in 17 patients

Table 1. Patient baseline characteristics of all anemic patients at diagnosis, IV versus UC group

	IV group (n=94)	UC group (n=224)	p-value
Age (years mean, SD)	71.8 ± 11.1	73.7 ±9.9	0.15
Gender (male) (%)	42 (44.7)	131 (58.5)	0.02
ASA-classification			0.06
I-II	71 (75.5)	145 (64.7)	
III-IV	23 (24.5)	79 (35.3)	
Comorbidity (overall) (%)	75 (79.8)	195 (87.1)	0.01
Tumor localisation (%)			0.001
colon	89 (94.7)	178 (79.5)	
rectum	5 (5.3)	46 (20.5)	
TNM stage (%)			0.68
I-II	59 (62.8)	135 (60.3)	
III-IV	35 (37.2)	89 (39.7)	
Surgery			
timeframe			0.06
2010-2013	53 (56.4)	151 (67.4)	
2014-2016	41 (43.6)	73 (32.6)	
laparoscopic (%)	72 (76.6)	153 (68.3)	0.14
Hemoglobin (mmol/L)			
at diagnosis (mean, SD)	6.12 ±0.89	6.61 ±0.87	<0.001
Number patients with preop. BT (%) | Hb at diagnosis			<0.001
yes	30 (31.9) | 5.67 mmol/L	29 (12.9) | 5.56 mmol/L	
prior to iron infusion	13 | 5.68 mmol/L	NA	
after iron infusion	17 | 5.67 mmol/L	NA	
no	64 (68.1) | 6.32 mmol/L	195 (87.1) | 6.77 mmol/L	
Number patients with postop. BT (%) | number of units transfused			
yes	10 (10.6) | 28	45 (20.1) | 91	
no	84 (89.4)	179 (79.9)	
Number patients with postop. complication (%)			
yes	24 (25.5)	77 (34.4)	
no	70 (74.5)	147 (65.6)	

Abbreviations: IV = intravenous iron group, UC = usual care group, BT = blood transfusion, preop. = preoperative, postop. = postoperative

Table 2. Patient baseline characteristics and outcome, IV versus UC subgroup

	IV (n=52)	UC (n=153)	p-value
Characteristics			
Age (years mean, SD)	71.3 ± 11.6	74.3 ± 9.5	0.09
Gender (male) (%)	23 (44.2)	93 (60.8)	0.04
ASA-classification			0.045
I-II	42 (80.8)	101 (66.0)	
III-IV	10 (19.2)	52 (34.0)	
Comorbidity (overall) (%)	11 (21.2)	21 (13.7)	0.20
Tumor localisation (%)			0.08
colon	48 (92.3)	126 (82.4)	
rectum	4 (7.7)	27 (17.6)	
TNM stage (%)			0.36
I-II	34 (65.4)	89 (58.2)	
III-IV	18 (34.6)	64 (41.8)	
Surgery			
timeframe			0.31
2010-2013	31 (59.6)	103 (67.3)	
2014-2016	21 (40.4)	50 (32.7)	
laparoscopic (%)	43 (82.7)	99 (64.7)	0.02
Hemoglobin (mmol/L)			
at diagnosis (mean, SD)	6.3 ± 0.8	6.9 ±0.7	<0.001
Outcome			
Hemoglobin (mmol/L)			
increase diagnosis-preop. (mean, SD)	0.65 ±0.74	0.10 ±0.74	<0.001

Abbreviations: IV = intravenous iron group, UC = usual care group, preop. = preoperative

(mean Hb level at diagnosis of 5.7 mmol/L) blood infusion was administered after iron transfusion. Mean Hb level at diagnosis was considerably higher in IV patients who did not receive preoperative blood transfusion (6.3 mmol/L).

IV versus UC, subgroup

An overview of the baseline characteristics is presented in table 2. In total, 105 patients were included (IV=52, UC=153). In the IV group, 32 and 20 patients received a 1000-2000mg dose of iron(III)isomaltoside and iron(III)carboxymaltose, respectively. Both groups had a mean age above 70 years (IV=71.3 ±11.6, UC=74.3 ±9.5, p=0.09). In the UC group, more males were included as compared to the IV group (60.8% vs 44.2%, p=0.04) and there were more patients with a high ASA score (34% versus 19.2%, p=0.04). In the IV group, significantly more patients were operated

Table 3. Patient baseline characteristics high responder (=>0.6 mmol/L Hb increase) versus poor responder (<0.6 mmol/L Hb increase), receiving 1 dose iron infusion (1000mg)

	IV high responder (n=17)	IV poor responder (n=16)	p-value
Age (years mean, SD)	69.3 ±13.1	73.6 ±9.0	0.28
Gender (male) (%)	5 (29.4)	5 (31.2)	0.91
ASA-classification			1.0
I-II	13 (76.5)	13 (81.2)	
III-IV	4 (23.5)	3 (18.8)	
Comorbidity (overall) (%)	14 (82.4)	12 (75.0)	0.69
Tumor localisation (%)			0.60
colon	16 (94.1)	14 (87.5)	
rectum	1 (5.9)	2 (12.5)	
TNM stage (%)			0.62
I-II	12 (70.6)	10 (62.5)	
III-IV	5 (29.4)	6 (37.5)	
Iron status at diagnosis			
(median; IQR - mean ± SD)			
Hb (mmol/L)	6.0; 1.5 – 6.2 ±0.8	6.8; 1.1 – 6.6 ±0.7	
TSAT (%)	5.3; 4.6 – 7.3 ± 4.6	11; 15 – 16.3 ±14.3	
transferrin (g/L)	2.9; 0.4 – 3.1 ±0.5	2.7; 0.2 – 2.7 ±0.4	
ferritin (µg/L)	12; 27 – 36 ±52	27; 67 – 142 ±360	

Abbreviations: IV = intravenous iron group, TSAT = transferrin saturation

laparoscopically (82.7% vs 64.7%, p=0.02). Regarding comorbidity, tumor localisation and tumor stage, no significant differences were found. In the IV group, Hb level at diagnosis was significantly lower (6.3 mmol/L vs 6.9 mmol/L, p<0.001).

Patients with intravenous iron treatment showed a significant higher increase of Hb level as compared to patients with UC (IV=0.65 mmol/L vs UC=0.10 mmol/L, p<0.001). In identifying characteristics associated with Hb level response after iron infusion, patients receiving one dose of iron infusion (1000mg) were classified into high and poor responders. A cut-off value of 0.6 mmol/L (i.e. median Hb level increase) was used (table 3). In total, 33 patients were included (high responder=17, poor responder=16). No significant differences were found for age, gender, ASA score, comorbidity, tumor localisation and tumor stage. Regarding iron status at diagnosis,

Table 4. Regression analysis on relationship between preoperative intravenous iron and postoperative blood transfusion in anemic patients (n=318)

	univariable			multivariable		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Age (years)	1.02	0.99 - 1.05	0.23	1.02	0.99 - 1.06	0.26
Gender						
female vs. male	0.69	0.38 - 1.26	0.23	0.52	0.27 1.04	0.06
Comorbidity (overall)	1.27	0.54 - 2.99	0.59	1.04	0.39 2.74	0.94
ASA-classification						
III-IV vs. I-II	1.84	1.01 - 3.33	0.045	1.77	0.89 - 3.53	0.11
TNM stage						
III-IV vs. I-II	0.72	0.39 - 1.33	0.30	0.66	0.34 - 1.28	0.22
Surgery						
laparoscopic versus open	0.51	0.28 - 0.92	0.026	0.55	0.28 - 1.06	0.08
Tumor localisation						
rectum vs. colon	1.03	0.47 - 2.26	0.94	1.10	0.98 - 1.24	0.12
Timeframe surgery						
2014-2016 vs 2010-2013	0.69	0.37 - 1.30	0.25	0.65	0.32 - 1.32	0.24
Preoperative Hb (0.1 mmol/L increase)	0.48	0.33 - 0.69	<0.001	0.40	0.26 - 0.60	<0.001
Preoperative intravenous iron	**0.47**	**0.23 - 0.99**	**0.046**	**0.54**	**0.24 - 1.21**	**0.14**

high responders showed more distinct signs of anemia and iron deficiency as compared to poor responders (high versus poor responder, median values: Hb 6.0 mmol/L vs 6.8 mmol/L, transferrin saturation (TSAT) 5.3% vs 11%). In addition, increased transferrin (median 2.9 g/L vs 2.7 g/L), and decreased ferritin (median 12 µg/L vs 27 µg/L) levels were found in the high responder group.

Association between intravenous iron therapy and postoperative complications and blood transfusions

All anemic patients, as presented in table 1, were included in logistic regression analyses. In univariable analysis, preoperative intravenous iron administration (OR=0.47, 95%CI 0.23 to 0.99, p=0.04) was observed to prevent the administration of postoperative blood transfusion. No significant result was found in multivariable analysis (OR=0.54, 95%CI 0.24 to 1.21, p=0.14)(table

Table 5. Regression analysis on relationship between preoperative intravenous iron and postoperative complications in anemic patients (n=318)

	univariable			multivariable		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Age (years)	1.01	0.99 - 1.03	0.51	1.02	0.99 - 1.04	0.30
Gender						
female vs. male	0.43	0.26 - 0.70	0.001	0.36	0.20 - 0.63	<0.001
Comorbidity (overall)	0.67	0.35 - 1.26	0.21	0.48	0.23 - 0.99	0.049
ASA-classification						
III-IV vs. I-II	1.54	0.94 - 2.53	0.09	1.62	0.90 - 2.90	0.11
TNM stage						
III-IV vs. I-II	0.76	0.47 - 1.25	0.28	0.58	0.34 - 1.00	0.050
Surgery						
laparoscopic versus open	0.33	0.20 - 0.55	<0.001	0.32	0.18 - 0.55	<0.001
Tumor localisation						
rectum vs. colon	1.09	0.58 - 2.06	0.79	1.03	0.94 - 1.13	0.54
Timeframe surgery						
2014-2016 vs 2010-2013	0.99	0.60 - 1.62	0.96	0.94	0.54 - 1.63	0.81
Preoperative Hb (0.1 mmol/L increase)	1.12	0.85 - 1.47	0.44	1.08	0.79 - 1.48	0.65
Preoperative intravenous iron	**0.66**	**0.38 - 1.12**	**0.12**	**0.91**	**0.50 - 1.68**	**0.77**

4). In both uni- and multivariable analysis, no advantageous effect was found on postoperative complications (OR=0.66, 95% CI 0.28 to 1.12, p=0.12 and OR=0.91, 95%CI 0.50 to 1.68, p=0.77, respectively)(table 5).

DISCUSSION

The present study illustrates the efficacy of intravenous iron therapy in the optimisation of preoperative hemoglobin level in anemic colorectal cancer patients, as compared to usual care. We found that intravenous iron therapy is most effective in patients presenting with more severe anemia , and with higher transferrin and lower ferritin levels, markers for an absolute iron deficiency, as compared to functional iron deficiency. In present study, the distinct Hb increase after iron infusion did not translate into an expected decrease in the percentage of patients with

a postoperative blood transfusion. This is most likely due to the confounding effect of preoperative blood transfusions, which could not be adequately corrected for in this retrospective cohort. Our observed perioperative blood transfusion rates are fairly comparable with the perioperative blood transfusion rates presented in other large cohort studies[22, 23], and our results, therefore, could legitimately be generalised.

Our results add to a growing body of evidence in the literature demonstrating the efficacy of preoperative intravenous iron therapy in colorectal cancer patients, and contribute to the ongoing debate whether preoperative intravenous iron therapy is improving postoperative outcome. Our results are consistent with the results of a prospective randomised trial by Keeler et al., comparing the effect of preoperative oral versus intravenous iron in anemic colorectal cancer patients.24 No overall benefit was seen with intravenous iron in reducing blood transfusions and postoperative complications, despite the fact that in the study by Keeler et al. oral iron administration represented usual care. However, in addition to the study by Keeler et al., we also identified patients characteristics associated with hemoglobin level response after iron infusion. Evidently, higher transferrin and lower ferritin levels, markers for absolute iron deficiency, were associated with a higher hemoglobin level response after iron infusion. Increased ferritin level, a marker for functional iron deficiency, could be the cause of poor hemoglobin level response after iron infusion. In this respect, increased uptake and retention of the administered intravenous iron within cells of the reticuloendothelial system may lead to a poor availability of administered iron for erythropoiesis.[8] Therefore, these results stress the importance of distinguishing between the two types of iron deficiency and emphasize the efficacy of intravenous iron namely in patients with absolute iron deficiency. It is noteworthy that in present international guidelines on the treatment of anemia in oncological patients a distinction between type of iron deficiency is already made: intravenous iron should be withheld in patients with an active infection and/or if serum ferritin exceeds 1000 µg/L [25, 26]. Despite this, in current clinical practice, no distinction is made between type of iron deficiency. Ongoing and future randomised clinical trials have to establish whether the optimisation of preoperative hemoglobin level by preoperative intravenous iron therapy is resulting in improved postoperative outcome.[11, 13]

STRENGTH AND LIMITATIONS

A key strength of our study is the identification of patient characteristics associated with hemoglobin level response after iron infusion in colorectal cancer patients. To our knowledge, this is the first study identifying the potential clinical relevance of identifying the type of iron deficiency in the treatment of preoperative anemia not only with oral iron but even with intravenous iron.

The main limitations of our study are three-fold, leading to key recommendations for future research.

First, this study represents a retrospective cohort of consecutive patients, involving several limitations. The significant differences between the intravenous iron and usual care group (e.g. baseline hemoglobin levels and timeframe surgery) could, despite correction in the multivariable regressions analyses, potentially indicate selection bias and have significant impact on the outcome. Moreover, iron status was not consistently monitored in each patient. The past years, great efforts have been made to optimise the results of colorectal cancer surgery. In addition to surgical techniques and procedures[9, 10, 27], also blood transfusion strategy, as part of patient blood management (PBM), has changed in the course of time. In this regard, the optimal transfusion threshold, dosing, and age of red blood cell (RBC) units have been studied. Presently, a restrictive transfusion threshold is recommended for hospitalised adult patients and seems to be safe in the oncological setting.[28, 29] Moreover, standard-issue RBC units rather than fresh RBC units (storage length: <10 days), and, to initiate, one rather than two RBC units are advised.[29] Although we corrected our results for the year of treatment, the combined efforts to optimise colorectal cancer care (e.g. centralisation, protocols, laparoscopy) might have contributed differently to the results. This emphasises the importance of performing a randomised controlled trial comparing usual care (i.e. no therapy or oral iron) with intravenous iron supplementation in colorectal cancer patients, in which, importantly, intravenous iron has to be administered as early as possibly, preferably at least three weeks prior to surgery for its optimal effect[11].

Second, this study focused specifically on preoperative treatment of anemia . However, investigation and treatment of merely hemoglobin levels appears to be a suboptimal way to indicate overall performance and therefore, presently, various multimodal programs are being introduced.[30, 31] The use of such various modalities could be valuable in preoperative prehabilitation, specifically in elderly patients (>75 years), in which an increased 1-year mortality of up to 25 percent is observed.[32, 33] In line with the previous limitation, in present study, various multimodal programs may similarly introduce confounding of our results that are not easily corrected for. A randomised trial could correct for both continuing pre- as well as postoperative care optimisation.

The third limitation was that only short-term effects of intravenous iron therapy were studied. In this respect, iron is an important growth factor for rapidly proliferating cells, including bacteria and tumor cells.[8, 34] Several animal experiment studies have shown that exposure to iron to be a risk factor for developing colorectal cancer and tumor growth.[35, 36] In this regard, intraluminal colorectal tumours might be more affected by oral iron administration, while intravenous iron with a higher risk of non-transferrin bound serum iron and reactive oxygen species presence might also influence systemic tumor growth. Randomised trials on the short-term benefits versus the potential long-term hazards of iron therapy in colorectal cancer patients should therefore acknowledge the type of anemia and the associated choice of iron therapy.

CONCLUSION

We were able to show that implementation of intravenous iron therapy leads to optimisation of preoperative hemoglobin level. Furthermore, we showed the importance of assessing the type of iron deficiency. Iron infusion is most effective in patients with more severe anemia and with higher transferrin and lower ferritin levels, markers for absolute iron deficiency, as compared to functional iron deficiency. Following the optimisation of preoperative hemoglobin level, strikingly, no significant decrease in the percentage of patients with a postoperative blood transfusion and postoperative complication were observed. However, from present cohort study, due to its retrospective nature, we cannot entirely conclude that intravenous iron and the associated Hb increase does decrease the postoperative blood transfusion and complication rate. Future randomised trials are thus required to not only establish the short-term benefits, but also the potential long-term hazards of preoperative intravenous iron therapy in colorectal cancer patients.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136(5):E359-86.
2. Knight K, Wade S, Balducci L. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. *Am J Med* 2004; 116 Suppl 7A:11S-26S.
3. Anker SD, Comin Colet J, Filippatos G, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med* 2009; 361(25):2436-48.
4. Fowler AJ, Ahmad T, Phull MK, et al. Meta-analysis of the association between preoperative anemia and mortality after surgery. *Br J Surg* 2015; 102(11):1314-24.
5. Caro JJ, Salas M, Ward A, et al. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* 2001; 91(12):2214-21.
6. Wilson MJ, van Haaren M, Harlaar JJ, et al. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis. *Surg Oncol* 2017; 26(1):96-104.
7. Ludwig H, Muldur E, Endler G, et al. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol* 2013; 24(7):1886-92.
8. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
9. Veenhof AA, Vlug MS, van der Pas MH, et al. Surgical stress response and postoperative immune function after laparoscopy or open surgery with fast track or standard perioperative care: a randomized trial. *Ann Surg* 2012; 255(2):216-21.
10. van Bree SH, Vlug MS, Bemelman WA, et al. Faster recovery of gastrointestinal transit after laparoscopy and fast-track care in patients undergoing colonic surgery. *Gastroenterology* 2011; 141(3):872-880 e1-4.
11. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemia in colorectal cancer patients. *BMC Surg* 2015; 15:78.
12. Munoz M, Acheson AG, Auerbach M, et al. International consensus statement on the peri-operative management of anemia and iron deficiency. *Anaesthesia* 2017; 72(2):233-247.
13. Richards T, Clevenger B, Keidan J, et al. PREVENTT: preoperative intravenous iron to treat anemia in major surgery: study protocol for a randomised controlled trial. *Trials* 2015; 16:254.
14. Beale AL, Penney MD, Allison MC. The prevalence of iron deficiency among patients presenting with colorectal cancer. *Colorectal Dis* 2005; 7(4):398-402.
15. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
16. Pascual M, Bohle B, Alonso S, et al. 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* 2013; 183(1):270-7.
17. Bohlius J, Schmidlin K, Brillant C, et al. Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. *Lancet* 2009; 373(9674):1532-42.
18. Cuenca J, Garcia-Erce JA, Martinez F, et al. Perioperative intravenous iron, with or without erythropoietin, plus restrictive transfusion protocol reduce the need for allogeneic blood after knee replacement surgery. *Transfusion* 2006; 46(7):1112-9.
19. Froessler B, Palm P, Weber I, et al. The Important Role for Intravenous Iron in Perioperative Patient Blood Management in Major Abdominal Surgery: A Randomized Controlled Trial. *Ann Surg* 2016.
20. Sanquin. *Blood Transfusion Guideline* https://www.sanquin.nl/repository/documenten/en/prod-en-dienst/287294/blood-transfusion-guideline.pdf. 2014.
21. Van Leersum NJ, Snijders HS, Henneman D, et al. The Dutch surgical colorectal audit. Eur J Surg Oncol 2013; 39(10):1063-70.
22. Aquina CT, Blumberg N, Becerra AZ, et al. Association Among Blood Transfusion, Sepsis, and Decreased Long-term Survival After Colon Cancer Resection. *Ann Surg* 2017; 266(2):311-317.

23. Halabi WJ, Jafari MD, Nguyen VQ, et al. Blood transfusions in colorectal cancer surgery: incidence, outcomes, and predictive factors: an American College of Surgeons National Surgical Quality Improvement Program analysis. *Am J Surg* 2013; 206(6):1024-32; discussion 1032-3.
24. Keeler BD, Simpson JA, Ng O, et al. Randomized clinical trial of preoperative oral versus intravenous iron in anemic patients with colorectal cancer. *Br J Surg* 2017.
25. NCCN. Cancer- and chemotherapy-induced anemia. 2014.
26. Schrijvers D, De Samblanx H, Roila F, et al. Erythropoiesis-stimulating agents in the treatment of anemia in cancer patients: ESMO Clinical Practice Guidelines for use. *Ann Oncol* 2010; 21 Suppl 5:v244-7.
27. de Vries EN, Eikens-Jansen MP, Hamersma AM, et al. Prevention of surgical malpractice claims by use of a surgical safety checklist. *Ann Surg* 2011; 253(3):624-8.
28. Prescott LS, Taylor JS, Lopez-Olivo MA, et al. How low should we go: A systematic review and meta-analysis of the impact of restrictive red blood cell transfusion strategies in oncology. *Cancer Treat Rev* 2016; 46:1-8.
29. Carson JL, Guyatt G, Heddle NM, et al. Clinical Practice Guidelines From the AABB: Red Blood Cell Transfusion Thresholds and Storage. *Jama* 2016; 316(19):2025-2035.
30. Chia CL, Mantoo SK, Tan KY. 'Start to finish trans-institutional transdisciplinary care': a novel approach improves colorectal surgical results in frail elderly patients. *Colorectal Dis* 2016; 18(1):O43-50.
31. Gillis C, Li C, Lee L, et al. Prehabilitation versus rehabilitation: a randomized control trial in patients undergoing colorectal resection for cancer. *Anesthesiology* 2014; 121(5):937-47.
32. Dekker JW, van den Broek CB, Bastiaannet E, et al. Importance of the first postoperative year in the prognosis of elderly colorectal cancer patients. *Ann Surg Oncol* 2011; 18(6):1533-9.
33. Dekker JW, Gooiker GA, Bastiaannet E, et al. Cause of death the first year after curative colorectal cancer surgery; a prolonged impact of the surgery in elderly colorectal cancer patients. *Eur J Surg Oncol* 2014; 40(11):1481-7.
34. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.
35. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.
36. Radulescu S, Brookes MJ, Salgueiro P, et al. Luminal iron levels govern intestinal tumorigenesis after Apc loss in vivo. *Cell Rep* 2012; 2(2):270-82.

Chapter 5 // **Patient blood management in colorectal cancer patients: a survey among Dutch gastroenterologists, surgeons and anesthesiologists**

M.J. Wilson, A.W.M.M. Koopman-van Gemert, J.J. Harlaar, J. Jeekel, J.J. Zwaginga, M. Schipperus

ABSTRACT

Introduction: There is an increasing awareness to integrate patient blood management (PBM) within routine surgical care in order to improve patient outcome. Although often standard in orthopedic and cardiac surgery, limited information about the use and implementation of PBM in colorectal cancer surgery is available. This is curious, as preoperative anemia, associated with increased morbidity and mortality, is highly prevalent in colorectal cancer patients. The present study therefore aimed to assess the current preoperative blood management strategies in the Netherlands, and to identify preferences of different physicians in the treatment of preoperative anemia in this particular patient group.

Methods: An online electronic survey was developed and sent to all surgeons of the Dutch Taskforce Coloproctology (177 in total). In addition, for each hospital in which surgery for colorectal cancer surgery is performed (75 in total), the survey was sent to one gastroenterologist and one anesthesiologist. Analyses of survey data were performed using descriptive statistics

Results: A total of 192 physicians responded to the survey (overall response rate 58.7%). In 73 hospitals (97.3%) the survey was conducted by at least one physician, and in more detail, in 21 hospitals (28.0%) the survey was conducted by a surgeon, an anesthesiologist and a gastroenterologist. Regarding the management of a mild to moderate preoperative anemia, no clear policy was reported in half of the hospitals (49.3%). Treatment of a mild to moderate preoperative anemia was initiated by the gastroenterologist, 14.7%; surgeon, 20.0%; colon care nurse, 5.3%; hematologist, 2.7%; anesthesiologist, 2.7%. In 38.7% of the hospitals, iron parameters were measured during screening for colorectal cancer. In addition, in only 13.3% of the hospitals, iron parameters were measured by the anesthesiologist during preoperative assessment. The most important objective for the treatment of anemia was 'the prevention of blood transfusions because of their association with impaired long-term tumor prognosis'. Furthermore, the severity of anemia was considered as the most important factor to treat anemia (98% of all respondents).

Conclusion: The present study shows a distinct variability in preoperative blood management practices in colorectal cancer care. Strikingly, this variability which was not only seen among, but also within Dutch hospitals, was demonstrated by variable responses from gastroenterologists, surgeons and anesthesiologists from the same institution. As a result, the present study clearly demonstrates the lack of consensus on PBM among gastroenterologists, surgeons and anesthesiologists, resulting in a suboptimal preoperative blood management strategy.

INTRODUCTION

Preoperative anemia in colorectal cancer patients is associated with an increased risk of short-term mortality and morbidity, and a decrease in long-term tumor survival.[1, 2] Iron deficiency is the principal cause of preoperative anemia and is reported in almost 50% of preoperative colorectal cancer patients.[3, 4] Transfusion, in earlier days the default therapy to correct this anemia, however, is also known to be associated with increased morbidity and mortality, as already demonstrated by Busch et al. in 1993.[5-9] This has resulted in alternative approaches to treat preoperative anemia, which are collectively known as patient blood management (PBM).

PBM refers to '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'. It has been developed to promote strategies to reduce or avoid the need of blood transfusion, and therefore questions blood transfusion as the primary treatment strategy of anemia. PBM is a continuous process, initiated early in the preoperative period and continued intra- and postoperatively. Importantly, and by definition, PBM requires a multimodal and multidisciplinary strategy, and should at least involve surgeons, anesthesiologists, gastroenterologists, hematologists and nurses.[10]

The increasing awareness to integrate PBM within routine surgical care resulted in numerous ongoing trials studying the optimal blood management strategy in all types of surgery.[11-13] To date, studies on the use and implementation of these PBM strategies are mostly limited to orthopedic and cardiac surgery.[10, 14] Despite the high prevalence of preoperative anemia, associated with increased morbidity and mortality, limited information about the use and implementation of PBM strategies in colorectal cancer care is available. A review by Munoz et al. represents a clear exception to this.[15] In this review, the prevalence and consequences of anemia are discussed and a pragmatic approach to the treatment of perioperative anemia in colorectal cancer patients is presented.

With the present study, we focused on the preoperative assessment and treatment of anemia in colorectal cancer patients and aimed to 1) assess the current preoperative blood management strategies in the Netherlands, 2) identify preferences of different physicians (surgeons, anesthesiologists and gastroenterologists) in the treatment of preoperative anemia, and 3) evaluate physicians' general knowledge of blood management issues.

METHODS

Study design

To accomplish our objectives, an online electronic survey was developed. The survey included three topics: 1) questions on the current preoperative blood management practice in colorectal cancer patients (measurement of iron parameters and treatment of preoperative anemia), 2)

questions on physicians' preferences in treatment of preoperative anemia (best treatment of preoperative anemia and the goal of treatment) and 3) questions to test physicians' knowledge of blood management issues. The survey questions were made by the research fellow (MJW) and two hematologists (MS and JJZ), and were subsequently tested at two sites (Department of Surgery Reinier de Graaf Hospital, and Department of Anesthesiology Albert Schweitzer Hospital). The eventual revised questionnaire was sent by e-mail to the eligible participants. The online survey tool SurveyMonkey was used to conduct the survey.

Study population

After obtaining the mailing list, the survey was first sent to all surgeons of the Dutch Taskforce ColoProctology (Werkgroep Coloprotocologie) in May 2017. Subsequent e-mail reminders were sent out in July and September 2017. In addition, for each hospital in which surgery for colorectal cancer is performed (75 in total), the survey was sent to one gastroenterologist and one anesthesiologist, all involved in colorectal cancer care and usually the head of department. For this purpose, the survey was slightly modified to suit the clinical situation of the gastroenterologist and anesthesiologist. The first invitation was sent in June 2017 and subsequent e-mail reminders were sent out in July and September 2017. The participation period closed in October 2017.
Removal of undeliverable e-mails from the mailing list, as well as retired or relocated surgeons resulted in an adjusted study population of 177 surgeons. In addition, the study population included 75 gastroenterologists and 75 anesthesiologists. As a result, the total targeted study population was 327 physicians.

Statistics

Survey data were extracted into an Excel database. Statistical analyses were performed using SPSS (Version 21) and GraphPad Prism (Version 5). Analyses were performed using descriptive statistics.

RESULTS

Participation

As shown in table 1, a total of 192 physicians responded to the survey (response rate 58.7%), including 95 surgeons, 48 anesthesiologists and 49 gastroenterologists. Of 192 respondents, 158 (82.2%) completed the survey, including 79 surgeons, 38 anesthesiologists and 41 gastroenterologists. In total, in 73 of 75 hospitals (97.3%) one or more physicians responded to the survey. In 21 of 75 hospitals (28.0%) the survey was conducted by a surgeon, an anesthesiologist, and a gastroenterologist.

Table 1. Characteristics/distribution respondents

	n	%
Responses per specialism		
Surgeons (177 invited in total)	95	53.7
Gastroenterologists (75 invited in total)	49	65.3
Anesthesiologists (75 invited in total)	48	64.0
Responses per hospital (75 in total)		
Surgeon(s)	8	10.7
Gastroenterologist	4	5.3
Anesthesiologist	9	12.0
Surgeon(s) + gastroenterologist	14	18.7
Surgeon(s) + anesthesiologist	12	16.0
Gastroenterologist + anesthesiologist	5	6.7
Surgeon(s) + gastroenterologist + anesthesiologist	21	28.0
No response	2	2.7

Preoperative blood management practice

Regarding the use of red blood cell transfusions and the treatment of severe anemia, respondents from all hospitals indicated the perioperative use of a restrictive blood transfusion policy. According to the adapted 4-5-6 mmol/L hemoglobin transfusion trigger rule (Dutch transfusion guideline), the severity of anemia and the patient-specific cardiopulmonary compensation capacity was acknowledged.[16]

To determine the current preoperative blood management practice per hospital, all respondents were first asked to indicate the primarily responsible specialist (gastroenterologist, surgeon, hematologist, anesthesiologist, unknown or none) for the management of mild to moderate preoperative anemia in colorectal cancer patients. Strikingly, in 33 of 44 hospitals with multiple respondents (minimum of two physicians per hospital), these responses differed and were contradictory, and needed reclassification:

1. when per hospital multiple and different responses were given to the question who is primarily responsible for the treatment of mild to moderate anemia, the current preoperative blood management practice in the hospital was categorized as unclear/ambiguous.

2. when per hospital multiple and different answers were given to the question whether iron parameters are measured during screening for colorectal cancer, the answer of the gastroenterologist was determinant.

In twelve hospitals, an ongoing randomized clinical trial (FIT trial) during the survey period studied the efficacy of preoperative intravenous iron supplementation in comparison with preoperative oral supplementation in anemic patients with colorectal cancer.[11] For these twelve hospitals, the content of the study protocol of the randomized trial was reflected in all answers regarding pre-operative blood management practice.

As shown in table 2, iron parameters (iron, ferritin, transferrin, or transferrin saturation) were indicated to be measured during screening for colorectal cancer in 38.7% of all hospitals. Of these 29 hospitals, complete iron status (iron, ferritin, transferrin, and transferrin saturation) was

Table 2. Current practices for preoperative blood management in all centers (n=75), according to respondents

	n	%
Iron status measured during screening colorectal cancer		
Answered by: gastroenterologists and surgeons		
Yes	29	38.7
No	35	46.7
Unknown/missing	11	14.7
Treatment of anemia first started by		
Answered by: gastroenterologists, surgeons and anesthesiologists		
Gastroentrologist	11	14.7
Surgeon	15	20.0
Colon care nurse	4	5.3
Hematologist/internist	2	2.7
Anesthesiologist	2	2.7
Unclear/ambiguous policy	37	49.3
No treatment	1	1.3
Unknown/missing	3	4.0
Iron status measured at preoperative assessment anesthesiology		
Answered by: anesthesiologists		
Yes	8	10.7
No	37	49.3
Unknown/missing	30	40.0
Treatment of anemia by anesthesiologists, regardless of previous treatment		
Answered by: anesthesiologists		
Yes	10	13.3
No	34	45.3
Unknown/missing	31	41.3

indicated to be measured in four hospitals. In a total of 35 hospitals (46.7%), iron parameters were not measured during screening. In addition to the measurement of iron parameters during screening for colorectal cancer, in ten hospitals (13.3%) iron parameters were measured by the anesthesiologist at preoperative assessment, as compared to 37 hospitals (49.3%) in which iron parameters were not measured at preoperative assessment. In ten hospitals (13.3%), it was indicated that anemia observed at preoperative assessment, regardless of possible previous treatment by gastroenterologist or surgeon, was treated by the anesthesiologist, as compared to 34 hospitals (45.3%) in which this was not the case.

Regarding the treatment of preoperative anemia, respondents from nineteen hospitals (25.3%), including the twelve hospitals participating in the ongoing FIT trial, indicated that the surgeons or colon care nurses were primarily responsible (table 2). In eleven hospitals (14.7%) the gastroenterologist was the first responsible to treat a mild to moderate preoperative anemia. In two hospitals each (2.7%), hematologists and anesthesiologists were indicated as primarily responsible for treatment, while in one hospital (1.3%) it was indicated that a mild to moderate preoperative anemia was not treated. In only four hospitals, the treatment of preoperative anemia was reported to be part of a protocol, with the aim of optimizing the preoperative condition of a patient. In half of the hospitals (49.3%), no clear policy regarding the treatment of preoperative anemia was reported. In 44 hospitals multiple responses (minimum of two physicians per hospital) to the question regarding the treatment of preoperative anemia were given, and in 33 hospitals (75.0%) these responses differed and were contradictory.

Objectives for treatment of preoperative anemia

All respondents were asked to prioritize their objectives for treatment of preoperative anemia. Results are shown in figure 1. Pooled responses demonstrated that, 'prevention of blood transfusion, because of its association with impaired long-term tumor prognosis' was ranked first, followed by 'prevention of blood transfusion, because of its short-term side effects', 'prevention of preoperative anemia, because of its association with impaired long-term tumor prognosis', 'prevention of blood transfusion, because of its high expenses', and 'optimization of preoperative hemoglobin level for enhanced hemostasis'. This order of preference was similar for the different specialisms.

Decision-making in treatment preoperative anemia

Table 3 provides percentages of respondents considering different variables in their decision to treat preoperative anemia. Only respondents who had indicated to treat preoperative anemia themselves were asked this question. Overall, 'age of patient' was considered by 63.3% of all respondents, 'presence iron deficiency' by 75.5%, 'presence clinical symptoms of anemia' by 85.7%, 'presence of comorbidities' by 83.7%, and 'severity of anemia' by 98.0%.

Figure 1. Objective treatment preoperative anemia

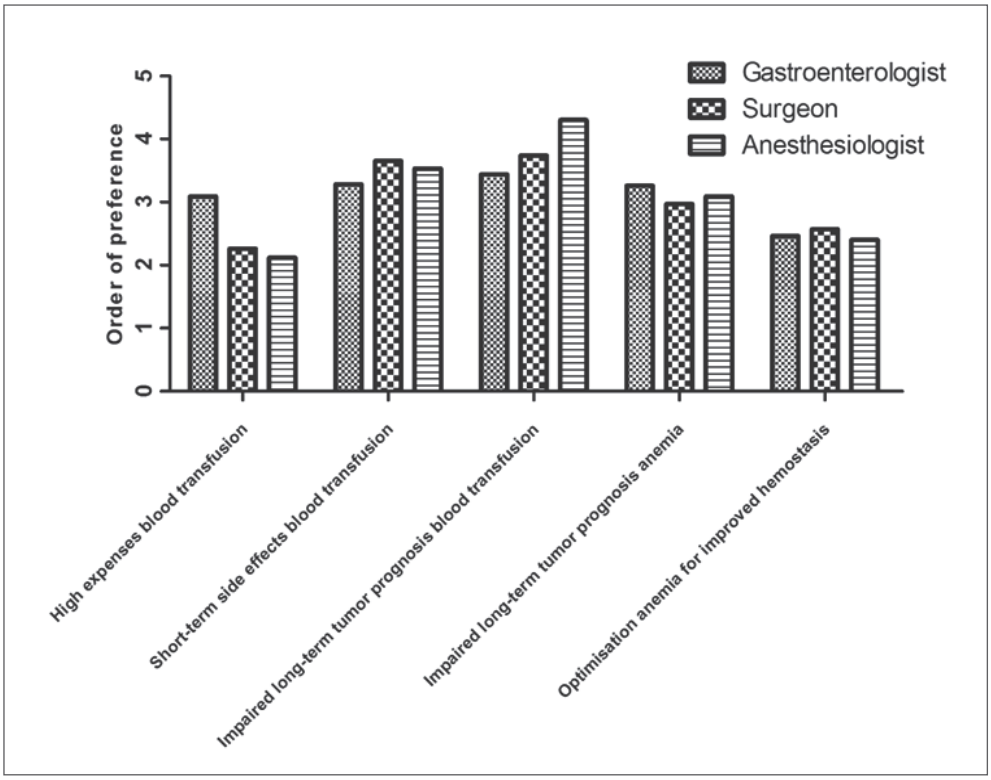
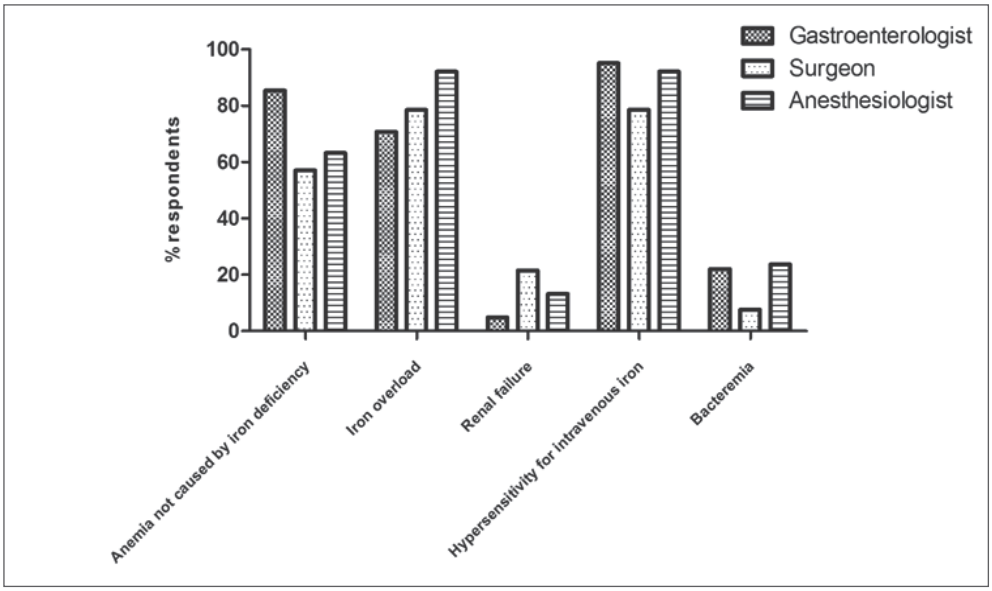


Figure 2. Respondents opinions on contraindications for intravenous iron



In case respondents indicated that their decision to treat anemia is dependent on the presence of iron deficiency, they were asked if the iron formulation (oral or intravenous) would depend on the type of iron deficiency (absolute versus functional iron deficiency), and if so, what treatment was chosen for an absolute and a functional iron deficiency anemia. Absolute iron deficiency is characterized by depleted iron stores (defined by decreased transferrin saturation and increased transferrin), while functional iron deficiency is caused by impaired iron homeostasis and is, due to increased hepcidin production, characterized by reduced iron uptake and iron mobilization from the reticulo-endothelial system (defined by decreased transferrin saturation and increased ferritin). For a small minority of respondents (44.4%) the type of iron deficiency made a difference for their treatment. In case of an absolute iron deficiency anemia, intravenous iron was the first choice of treatment for 71.4% of these respondents (versus 28.6% oral iron). In case of a functional iron deficiency, the choice of treatment was equally divided (50% oral iron versus 50% intravenous iron).

Contraindications to intravenous iron therapy

Figure 2 provides the percentages of respondents identifying different variables as an absolute contraindication to intravenous iron therapy. Overall, contraindications to intravenous iron

Table 3. Decision making in treatment preoperative anemia

	Gastroenterologists, n (%)	Surgeons, n (%)	Anesthesiologists, n (%)	Total, n (%)
A. Variables considered in decision making treatment preoperative anemia				
Age of patient	16 (76.2)	12 (66.7)	3 (30)	31 (63.3)
Presence iron deficiency	19 (90.5)	13 (72.2)	5 (50)	37 (75.5)
Presence clinical symptoms anemia	20 (95.2)	14 (77.8)	8 (80)	42 (85.7)
Presence comorbidities	18 (85.7)	15 (83.3)	8 (80)	41 (83.7)
Severity of anemia	21 (100)	17 (94.4)	10 (100)	48 (98)
B. Type of treatment (oral or intravenous iron) is depending on type of iron deficiency (absolute versus functional iron deficiency)				
Yes	3 (30)	4 (66.7)	1 (50)	8 (44.4)
No	7 (70)	2 (33.3)	1 (50)	10 (55.6)
C. First choice of treatment in case of an absolute iron deficiency anemia				
Oral iron	0 (0)	2 (50)	0 (0)	2 (28.6)
Intravenous iron	3 (100)	2 (50)	0 (0)	5 (71.4)
D. First choice of treatment in case of a functional iron deficiency anemia				
Oral iron	1 (33.3)	2 (66.7)	0 (0)	3 (50)
Intravenous iron	2 (66.7)	1 (33.3)	0 (0)	3 (50)

therapy included 'anemia not caused by iron deficiency' for 65.8% of all respondents, 'iron overload' for 79.7%, 'hypersensitivity for intravenous iron' for 86.1%, and 'bacteremia' for 15.2%. 'Renal failure' was indicated as an absolute contraindication by 15.2% of all respondents. Surgeons, gastroenterologists and anesthesiologists (78.5%, 92.1% and 95.1%, respectively) most frequently identified hypersensitivity for intravenous iron as absolute contraindication. Gastroenterologists and anesthesiologists (4.9% and 13.2%, respectively) least frequently indicated renal failure as absolute contraindication, while surgeons least frequently indicated bacteremia in this regard (7.6%).

International guidelines on the long-term effects of iron therapy

Overall, 8.9% of the respondents indicated to believe that the long-term oncological effects of intravenous iron therapy are known and already incorporated in the international guidelines on the treatment of anemia in cancer patients. 5.7% of the respondents indicated to regard intravenous iron as safe, while in contrast 3.2% of respondents believed intravenous iron therapy to be associated with impaired long-term tumor prognosis. 22.2% of the respondents indicated that the long-term oncological effects of intravenous iron therapy are not studied and therefore not included in the international guidelines. A vast majority (69%) indicated to be ignorant on this subject.

DISCUSSION

The results of our national survey show a distinct variability in preoperative blood management practices in colorectal cancer patients. Strikingly, this variability is not only found among hospitals, but also within hospitals, demonstrated by variable responses from gastroenterologists, surgeons and anesthesiologists from the same institution. As a result, the present study clearly demonstrates the lack of consensus on PBM among gastroenterologists, surgeons and anesthesiologists, resulting in a suboptimal preoperative blood management strategy.

Extensive research on barriers limiting the translation of PBM into clinical practice has led to simplified international recommendations for the implementation of PBM[17-21]. One of these recommendations is that each hospital should appoint a key leader for the PBM project management, who should have a central role in charge of communication, education, and documentation. This should contribute to a more clear division in responsibilities among treating physicians. Our study clearly demonstrates that this is not the case for the vast majority of Dutch hospitals. Most gastroenterologists, surgeons and anesthesiologists referred to different persons they held primarily responsible for the treatment of a mild to moderate preoperative anemia in colorectal cancer patients. In the few hospitals practicing preoperative blood management according to protocol, the primarily responsible persons were clear for all respondents.

A second simplified recommendation is derived from the fact that effective correction of anemia will depend on the underlying disorder, and states that optimal PBM should involve screening for the underlying cause, preferably at the earliest opportunity to allow optimal correction. With respect to this recommendation, our study again showed a high variation to which extent anemia and underlying causes were investigated and identified. In only 38.7% of hospitals, iron parameters, essential for identifying type of anemia, were indicated to be measured during screening for colorectal cancer (by gastroenterologist or surgeon). In addition, in only 13.3% of hospitals, iron parameters were measured by the anesthesiologist during preoperative assessment. Most strikingly, anemia is, regardless of previous treatment by surgeon or gastroenterologists, treated by the anesthesiologist in only a quarter of the hospitals. These results clearly indicate that the majority of the Dutch hospitals are failing in the assessment and treatment of preoperative anemia.

A third recommendation is that both physicians and nurses need to be trained in PBM clinical protocols and transfusion algorithms. According to our results, much progress could be made by improving the knowledge of physicians' on these subjects. For example, in case of a functional iron deficiency, the choice of treatment was equally divided between oral and intravenous iron. This is a striking and counter-intuitive result, as oral iron is known to be nearly inefficacious in patients with a functional iron deficiency. In addition, the results of the acknowledgement of contraindications to intravenous iron emphasize the knowledge gap of the responding physicians. Renal failure, the commonest indication for intravenous iron therapy, was considered as an absolute contraindication by up to 15.2% of all respondents. Anemia not caused by iron deficiency and iron overload, which are clear contraindications to intravenous iron therapy, were indicated as such by only 66% and 80% of all respondents respectively. Hypersensitivity for intravenous iron is the most dangerous contraindication and acute hypersensitivity reactions during infusion are very rare but can be life-threatening. A review by Rampton et al. provides recommendations about their management and prevention.[29] Importantly, if intravenous iron is to be given to individuals with any of the risk factors for acute hypersensitivity reactions (previous reaction to an iron infusion, a fast iron infusion rate, multiple drug allergies, severe atopy, systemic inflammatory diseases), an extremely slow infusion rate and meticulous observation is recommended. Finally, and notwithstanding the observed knowledge gap on PBM issues, possible long-term and potential hazardous effects of iron therapy in colorectal cancer patients are still unclear. Therefore, the long-term effects of iron therapy are not discussed in regard to the most optimal preoperative blood management strategy. Uncertainty on the potential role of iron in tumor progression arises from epidemiological and non-clinical studies, showing iron's role in all aspects of cancer development and cancer growth.[22-26] Despite the fact that the conditions in these epidemiological and non-clinical studies often not reflect the clinical situation in anemic patients and often use excessive iron doses iron-replete animals, we believe well-designed clinical studies are required to exclude the potential long-term hazardous effect of iron therapy in cancer patients.

The increasing awareness to integrate PBM within routine surgical care, has resulted in numerous completed and ongoing trials studying the optimal blood management strategy in all types of surgery.[11, 13, 27, 28] With regard to colorectal cancer, a pragmatic approach to the management of perioperative anemia is presented by Munoz et al.[15] In this review, the use of PBM is strongly advocated to minimize or eliminate the use of allogeneic blood transfusion. Regarding the treatment of perioperative anemia, the use of oral iron is clearly dissuaded as it is poorly tolerated with low adherence based on published evidence, while the use of intravenous iron is strongly advised as it is safe and effective, but also frequently avoided due to misinformation and misinterpretation concerning the incidence and clinical nature of minor infusion reactions. In addition to this review and regarding the efficacy of intravenous iron therapy, a study by Keeler et al.[12] showed that intravenous iron is more effective in increasing hemoglobin level compared to oral iron, but did not observe a relevant difference in the administration of red blood cell transfusions. However, in this trial the sample size was small and only primary outcomes in terms of increasing hemoglobin level and the use of red blood cell transfusions were reported, stressing the need for larger trials with a focus on functional performance and quality of life.[11] The results of such trials should provide more evidence surrounding the effectiveness of the management of preoperative anemia, and should contribute to successful implementation of PBM protocols, specifically in colorectal cancer patients.

STRENGTHS AND LIMITATIONS

The key strength of our study is the availability of responses from gastroenterologists, surgeons and anesthesiologists. This enables comparison between different medical disciplines within and between hospitals. Our data sets allows assessing the knowledge of the different types of physicians and assessing the consensus in the management of preoperative anemia. In addition, in all hospitals except two, at least one physician responded to the survey. While the availability of responses from gastroenterologists, surgeons and anesthesiologists is a key strength of our study, it also appeared to be a limitation. Due to the high variety in responses, it was extremely difficult to determine the actual preoperative blood management strategy per hospital. An additional limitation of our study is that it is a national survey, hampering generalization of our results to an international setting. However, the Netherlands are known to be a pioneer in the implementation of PBM, using PBM strategies for more than two decades, especially for major orthopedic surgery.[10] Therefore in other countries, physicians' knowledge of blood management issues and implementation of PBM in colorectal cancer care will presumably not be superior to the Dutch setting.

CONCLUSION

The present study shows a distinct variability in preoperative blood management practices in colorectal cancer care. Strikingly, this variability which was not only seen among, but also within

Dutch hospitals, was demonstrated by variable responses from gastroenterologists, surgeons and anesthesiologists from the same institution. As a result, the present study clearly demonstrates the lack of consensus on PBM among gastroenterologists, surgeons and anesthesiologists, resulting in a suboptimal preoperative blood management strategy. For a more effective and uniform implementation of PBM, much progress could be made on education as the present study clearly demonstrates a significant information deficit among physicians dealing with PBM issues. In addition, results of clinical trials providing evidence surrounding the effectiveness of treatment of preoperative anemia should contribute to more evidence-based guidelines. Finally, appointing key leaders for PBM project management should contribute to improved communication and cooperation, resulting in a more clear division in responsibilities among treating physicians.

REFERENCES

1. Fowler AJ, Ahmad T, Phull MK, et al. Meta-analysis of the association between preoperative anemia and mortality after surgery. *Br J Surg* 2015; 102(11):1314-24.
2. Wilson MJ, van Haaren M, Harlaar JJ, et al. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis. *Surg Oncol* 2017; 26(1):96-104.
3. Ludwig H, Muldur E, Endler G, et al. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol* 2013; 24(7):1886-92.
4. Wilson MJ, Dekker JWT, Harlaar JJ, et al. The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment. *Int J Colorectal Dis* 2017.
5. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
6. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
7. Busch OR, Hop WC, Hoynck van Papendrecht MA, et al. Blood transfusions and prognosis in colorectal cancer. *N Engl J Med* 1993; 328(19):1372-6.
8. Halabi WJ, Jafari MD, Nguyen VQ, et al. Blood transfusions in colorectal cancer surgery: incidence, outcomes, and predictive factors: an American College of Surgeons National Surgical Quality Improvement Program analysis. *Am J Surg* 2013; 206(6):1024-32; discussion 1032-3.
9. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.
10. Shander A, Van Aken H, Colomina MJ, et al. Patient blood management in Europe. *Br J Anaesth* 2012; 109(1):55-68.
11. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemia in colorectal cancer patients. *BMC Surg* 2015; 15:78.
12. Keeler BD, Simpson JA, Ng O, et al. Randomized clinical trial of preoperative oral versus intravenous iron in anemic patients with colorectal cancer. *Br J Surg* 2017.
13. Richards T, Clevenger B, Keidan J, et al. PREVENTT: preoperative intravenous iron to treat anemia in major surgery: study protocol for a randomised controlled trial. *Trials* 2015; 16:254.
14. Van der Linden P, Hardy JF. Implementation of patient blood management remains extremely variable in Europe and Canada: the NATA benchmark project: An observational study. *Eur J Anaesthesiol* 2016; 33(12):913-921.
15. Munoz M, Gomez-Ramirez S, Martin-Montanez E, et al. Perioperative anemia management in colorectal cancer patients: a pragmatic approach. World J Gastroenterol. 2014 Feb 28;20(8):1972-1985. *PubMed PMID*: 24587673.
16. Sanquin. *Blood Transfusion Guideline* https://www.sanquin.nl/repository/documenten/en/prod-en-dienst/287294/blood-transfusion-guideline.pdf. 2014.
17. Fischer DP, Zacharowski KD, Muller MM, et al. Patient blood management implementation strategies and their effect on physicians' risk perception, clinical knowledge and perioperative practice - the frankfurt experience. *Transfus Med Hemother* 2015; 42(2):91-7.
18. Mbanya D. Barriers and enablers to introducing comprehensive patient blood management in the hospital. *Biologicals* 2012; 40(3):205-8.
19. Meybohm P, Froessler B, Goodnough LT, et al. "Simplified International Recommendations for the Implementation of Patient Blood Management" (SIR4PBM). *Perioper Med* (Lond) 2017; 6:5.
20. Munoz M, Gomez-Ramirez S, Kozek-Langeneker S, et al. 'Fit to fly': overcoming barriers to preoperative hemoglobin optimization in surgical patients. *Br J Anaesth* 2015; 115(1):15-24.
21. Vamvakas EC. Reasons for moving toward a patient-centric paradigm of clinical transfusion medicine practice. *Transfusion* 2013; 53(4):888-901.

22. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1):12-31.

23. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.

24. Stevens RG, Jones DY, Micozzi MS, et al. Body iron stores and the risk of cancer. *N Engl J Med* 1988; 319(16):1047-52.

25. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.

26. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. Protein Cell 2015; 6(2):88-100.

27. Froessler B, Palm P, Weber I, et al. The Important Role for Intravenous Iron in Perioperative Patient Blood Management in Major Abdominal Surgery: A Randomized Controlled Trial. *Ann Surg* 2016.

28. Investigators I, Litton E, Baker S, et al. Intravenous iron or placebo for anemia in intensive care: the IRONMAN multicentre randomized blinded trial : A randomized trial of IV iron in critical illness. *Intensive Care Med* 2016; 42(11):1715-1722.

29. Rampton D, Folkersen J, Fishbane S, et al. Hypersensitivity reactions to intravenous iron: guidance for risk minimization and management. Haematologica. 2014 Nov;99(11):1671-1676. *PubMed PMID*: 25420283.

Chapter 6 // **Iron therapy as treatment of anemia: a potentially detrimental and hazardous strategy in colorectal cancer patients**

M.J. Wilson, J.J. Harlaar, J. Jeekel, M. Schipperus, J.J. Zwaginga

ABSTRACT

In colorectal cancer patients, iron therapy, and especially intravenous iron therapy, is increasingly used to treat anemia and reduce the use of blood transfusions. However, iron has also been shown to be an essential nutrient for rapidly proliferating tissues and cells. In this respect, anemia of inflammation, characterized by limited duodenal iron uptake and sequestration of iron into the reticuloendothelial system, might be regarded as a potentially effective defense strategy of the human body against tumor growth. We therefore hypothesize that iron therapy, by supporting colorectal tumor growth and increasing the metastatic potential, may worsen tumor prognosis in colorectal cancer patients. This hypothesis is particularly supported for colorectal cancer by laboratory, epidemiological and animal studies, demonstrating the role of iron in all aspects of tumor development growth. Compared to non-malignant colon cells, tumor cells differ in the levels and activity of many iron import and export proteins, resulting in an increase in intracellular iron level and enhanced proliferation. In addition, it is demonstrated that iron is able to amplify Wnt signaling in tumors with Apc mutation, a critical mutation in the development of colorectal cancer. If our hypothesis is to be confirmed, current practice of iron administration, as treatment for anemia and as replacement of blood transfusions, can be hazardous and should be completely reconsidered.

INTRODUCTION

Colorectal cancer is the third most commonly diagnosed cancer in men and the second in women worldwide, accounting for more than 1.4 million new cases and 694 000 associated deaths worldwide.[1] Anemia (hemoglobin <12.0 g/dL) is the most frequent hematological manifestation in patients with cancer, occurring in >40% of the cases. In colorectal cancer, anemia is even reported in around 60% of the cases.[2] This anemia is most often associated with iron deficiency,[3] but more importantly also with impaired disease-free and overall survival in cancer patients.[4, 5]

As both blood transfusions and erythropoiesis-stimulating agents (ESAs) are, similar to anemia, independently associated with an increased risk of colorectal cancer recurrence and increased mortality,[6-9] the use of iron to reverse anemia has gained more attention. In this regard, while oral iron does correct anemia, it also causes constipation, and is largely ineffective in patients with anemia of inflammation, characterized by reduced duodenal iron uptake and iron mobilization from the reticulo-endothelial system. In comparison with oral iron, intravenous iron does not have these disadvantages and is therefore more and more preferred.[10] In colorectal cancer patients, several cohort studies have shown that intravenous iron therapy indeed optimizes preoperative hemoglobin level. A net reduction of blood transfusions by intravenous iron, however, is not conclusively shown as of yet. [11-14]

In contrast to the short-term effect of iron therapy to increase the hemoglobin level, strikingly, possible long-term effects in colorectal cancer patients, such as survival, are so far hardly studied. These long-term effects are of special interest since anemia of inflammation is believed to be a potentially defense strategy of the human body to limit the growth of tumor cells.[15] In this respect, the results of laboratory, epidemiological and animal studies indeed have shown iron's role in all aspects of cancer development and cancer growth.[11, 16-21] Finally, corroborating evidence implicates that especially gastrointestinal cancer cells, likely by their original iron-absorbing nature, have an altered iron homeostasis.[22]

THE HYPOTHESIS

We hypothesize that iron therapy may worsen colorectal tumor prognosis by supporting colorectal tumor growth and increasing the metastatic potential. Although no direct evidence is available to date, accumulative data from experimental studies are in favor of this hypothesis. In this respect, importantly, iron therapy is increasingly used with the aim of optimizing hemoglobin level and reducing the need for blood transfusions. Therefore, this hypothesis is in striking contrast with current practice in patient blood management. We evaluated the current evidence supporting this hypothesis in the following part.

EVALUATION OF THE HYPOTHESIS

Iron is an essential nutrient participating in numerous biological and cellular processes such as hemoglobin-mediated oxygen transport, DNA synthesis and cell proliferation and growth. As mammals do not possess any regulated mechanisms for iron excretion from the body, iron metabolism is maintained by the tight control of dietary iron absorption in the duodenum. Intracellular iron transport is mainly controlled by three iron transport proteins: 1. divalent metal transporter 1 (DMT1), facilitating the transport of dietary iron across the apical membrane of enterocytes 2. ferroportin, facilitating the export across the basolateral membrane into the bloodstream, and 3. transferrin receptor 1 (TfR1), facilitating the import across the basolateral membrane into the cell. Systemically, iron homeostasis is regulated by hepcidin, which is produced by hepatocytes and inhibits the release of iron from enterocytes and macrophages into the circulation by inducing the internalization and subsequent degradation of ferroportin.[23, 24] The level of hepcidin is controlled by many factors including iron stores, hypoxia, anemia and erythropoiesis.[25, 26] Whereas iron deficiency, enhanced red blood cell production, and hypoxia decrease hepcidin expression to accelerate iron absorption, iron overload and inflammatory stimuli like IL-6 induce increased hepcidin expression. The latter is the cause of a hepcidin-mediated decrease in iron uptake and utilization, so called anemia of chronic disease or anemia of inflammation.

Abovementioned background information on the normal regulation of iron metabolism is essential to put the modifications in intracellular iron regulation in colorectal cancer cells in perspective. In distinct favor of our hypothesis, many transport proteins that were originally studied for their roles in normal iron metabolism have now been shown to contribute to malignant tumor growth. Compared to non-malignant colon cells, iron import proteins, such as DMT1 and TfR1, are upregulated, while ferroportin, the only known iron export protein, is downregulated in colon tumor cells, subverting the normal homeostatic control into a chronic iron acquisition state enabling enhanced proliferation.[27-29] More in detail, the presence of the key intestinal tumor suppressor Apc seems to play a pivotal role. The Apc gene is the most commonly mutated tumor suppressor gene in sporadic colorectal cancer,[30] and it is shown that especially Apc-deficient (i.e. mutant Apc) cells appear critically dependent iron for efficient tumor growth. In Apc-deficient cells, raising the levels of iron induces the expression of TfR1 and DMT1, resulting in increased iron content in the cells and increased proliferation, while removal of iron drives apoptosis of Apc-deficient cells. This is the exact opposite to what is observed in colorectal cancer cells with wildtype Apc.[17, 31] In addition, it is demonstrated that, in mouse models, the growth rate of tumor xenografts is increased by high levels of dietary iron.[32, 33]

Finally, in studying the proliferative effect of iron in colorectal tumors, a clear link between iron and Wnt signaling was found[30, 34, 35]. Wnt signaling plays a critical role in regulating homeostasis and self-renewal of tissues, and in the intestinal epithelium it promotes proliferation and differentiation of stem cells in the intestinal crypts. Aberrant Wnt signaling is closely related to a mutation

in Apc, and is an important hallmark for colorectal cancer development. Importantly, and supporting our hypothesis, it is demonstrated that iron, in the background of an Apc mutation, is able to amplify Wnt signaling, and with it induction of cell growth.[36] Therefore, in the presence of an Apc mutation, iron will affect Wnt signaling and with it an increase in the tumorigenic and metastatic potential.[37]

CONSEQUENCES OF THE HYPOTHESIS AND DISCUSSION

Substantial evidence suggests that iron promotes colorectal tumor growth and potentially increases the metastatic potential of colorectal tumor cells. Therefore, the legitimate question arises as to whether the use of iron therapy, either orally or intravenously, is a safe and optimal treatment strategy in anemic colorectal cancer patients.

We hypothesize that iron in general (i.e. both oral and intravenous) may worsen colorectal tumor prognosis, but the different routes of administration should be considered. In colorectal cancer patients, oral iron often is poorly absorbed. As a consequence, only a fraction of the dietary (i.e. orally administered) iron will reach the sites of erythropoiesis and a significant part will reach the site of the primary tumor. At the site of the primary tumor, the oral iron will be able to affect Wnt signaling and contribute to enhanced tumor growth and increased metastatic potential. However, and in contrast with intravenous iron, the effect of oral iron will be limited to the primary tumor. In this respect, intravenous iron might have more influence if metastases are present.

In addition to the different routes of administration, we hypothesize that the assessment of type of iron deficiency anemia might be important in studying the long-term effect of iron therapy. Chronic blood loss, as can be envisioned by bleeding from gastrointestinal tumors, in this respect causes absolute iron deficiency (AID), characterized by depleted iron stores. Functional iron deficiency (FID), in contrast, is caused by impaired iron homeostasis and is, due to increased hepcidin production, characterized by reduced iron uptake from the duodenum and iron mobilization from the reticulo-endothelial system. FID resulting in anemia is also known as anemia of inflammation or anemia of chronic disease. Despite definite evidence, FID could be regarded as a potentially effective defense strategy to inhibit growth of pathogens and tumor cells.[15] As, in the event of FID, a large fraction of oral iron will, due to poor absorption in the duodenum, reach the site of the primary tumor, we hypothesize that iron supplementation will be more hazardous in patients with FID, as compared to patients with AID.

In assessing the effect of iron therapy on tumor growth and tumor prognosis, the dose-response relationship will be particularly important. A single dose of intravenous iron normally contains 1000 mg, which is, as compared to a daily iron uptake of 1-2 mg, an extremely high amount of extra iron. Total body iron is 3-4 g, and this quantity is tightly regulated. However, the body has no mechanism to excrete excess iron and only less than 0.1% of total iron is lost on average daily,

mostly through urine, sweat and feces. Therefore, and despite the relatively large amount of 3-4 g of total body iron, we hypothesize that a single dose of intravenous iron or continuous supplementation of oral iron (i.e. normally 400-500 mg daily) could already affect tumor growth and therefore be harmful, especially in iron-dependent tumors.

Testing our hypothesis will be challenging. In a retrospective cohort study significant differences between an iron-treatment and non-iron treatment group (e.g. baseline hemoglobin levels and use of blood transfusions) could, despite possible correction by multivariable regressions analyses, potentially indicate selection bias and have significant impact on the outcome. Therefore, to show the validity of our hypothesis, the clinical long-term effect of iron therapy should be studied in a prospective trial. In such a trial, anemic patients should be randomized into an iron-treatment and a non-treatment group. In addition, clinical outcome should be correlated with both tumor (e.g. expression levels of iron transporters, Apc status, tumor stage and molecular subtype[38]) and patient characteristics. Only such a set up will allow identification of iron-dependent tumor growth and potential high-risk patients. Apart from studying the clinical long-term consequences of iron therapy, assessing the optimal management of anemia in colorectal cancer patients is more challenging. For this purpose, the detrimental long-term effects of iron treatment must be compared with those of not only anemia, but also alternatives to treat anemia like ESAs and more important blood transfusions. The problem here is that a head-to-head comparison of blood transfusion and iron therapy seems almost impossible. The indications for both therapies are namely clearly different. Iron therapy is indicated in patients with a mild to moderate anemia, while blood transfusion are only administered in case of severe anemia. As a useful alternative for clinically assessing the optimal management of anemia, animal experiments could be considered. In an ortho- or heterotopic rodent model, the effect on tumor growth of both anemia, blood transfusion and iron therapy (both oral and intravenous) could be accurately assessed and compared.

In conducting a prospective trial randomizing anemic patients in an iron-treatment and a non-treatment group, ethical issues should be considered. Anemia itself is considered as a major risk factor for impaired disease-free and overall survival in cancer patients,4, 5 and therefore the inclusion of a non-treatment (i.e. anemic) group could be considered unethical. To challenge this ethical issue, the proposed and abovementioned animal experiments studying the effect on tumor growth of both anemia, blood transfusion and iron therapy will play a pivotal role. These animal experiments should precede a clinical trial studying the long-term effects of iron therapy. If anemia is proven to be significantly associated with highest tumor growth, the clinical study design should be reconsidered. This could, for example, include the administration of iron therapy in all anemic patients, subdivided into patients with iron-dependent and non-iron dependent colorectal tumor phenotypes. Assessment of the iron-dependency of the tumor could be done by identification of gene and protein expression levels of iron transporters. If iron therapy is proven

to be significantly associated with impaired tumor prognosis in the iron-dependent group, this probably indicates the hazardous effect of iron therapy.

Interestingly, corroborating data about the possible detrimental effects of iron can be deduced from a report by Harlaar et al,[39] comparing the long-term effects of autologous and allogeneic blood transfusions in colorectal cancer patients. They unexpectedly showed that patients with autologous blood transfusion, with an a priori lower probability to dysregulate the immune system of the recipient, showed an inferior long-term survival as compared to patients transfused with allogeneic blood. Interestingly, the patients with autologous blood transfusion additionally received preoperative oral iron therapy to maintain normal hemoglobin levels after blood donation.

In conclusion, if iron therapy indeed can be shown to worsen colorectal tumor prognosis, this should change current management of anemia in colorectal cancer patients. Focus will be shifted, and minimization of the use of blood transfusions will no longer be the main objective. Paradigms will be shifted in patient blood management, which will bear major changes in oncological care as a whole.

REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015; 136(5):E359-86.
2. Ludwig H, Van Belle S, Barrett-Lee P, et al. The European Cancer AnemiaSurvey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anemiain cancer patients. *Eur J Cancer* 2004; 40(15):2293-306.
3. Wilson MJ, Dekker JWT, Harlaar JJ, et al. The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment. *Int J Colorectal Dis* 2017.
4. Caro JJ, Salas M, Ward A, et al. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* 2001; 91(12):2214-21.
5. Wilson MJ, van Haaren M, Harlaar JJ, et al. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis. *Surg Oncol* 2017; 26(1):96-104.
6. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
7. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
8. Bohlius J, Schmidlin K, Brillant C, et al. Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. *Lancet* 2009; 373(9674):1532-42.
9. Pascual M, Bohle B, Alonso S, et al. 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* 2013; 183(1):270-7.
10. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemiain colorectal cancer patients. *BMC Surg* 2015; 15:78.
11. Borstlap W, Stellingwerf ME, Moolla Z, et al. Iron therapy for the treatment of preoperative anemiain patients with colorectal carcinoma: a systematic review. *Colorectal Dis* 2015.
12. Calleja JL, Delgado S, del Val A, et al. Ferric carboxymaltose reduces transfusions and hospital stay in patients with colon cancer and anemia. *Int J Colorectal Dis* 2016; 31(3):543-51.
13. Keeler BD, Simpson JA, Ng O, et al. Randomized clinical trial of preoperative oral versus intravenous iron in anemic patients with colorectal cancer. *Br J Surg* 2017.
14. Laso-Morales M, Jerico C, Gomez-Ramirez S, et al. Preoperative management of colorectal cancer-induced iron deficiency anemia in clinical practice: data from a large observational cohort. *Transfusion* 2017.
15. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
16. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1):12-31.
17. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.
18. Stevens RG, Jones DY, Micozzi MS, et al. Body iron stores and the risk of cancer. *N Engl J Med* 1988; 319(16):1047-52.
19. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.
20. Xue X, Shah YM. Intestinal iron homeostasis and colon tumorigenesis. *Nutrients* 2013; 5(7):2333-51.
21. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. *Protein Cell* 2015; 6(2):88-100.
22. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5):646-74.
23. Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; 306(5704):2090-3.

24. Wu XN, Su D, Wang L, et al. Roles of the hepcidin-ferroportin axis and iron in cancer. *Eur J Cancer Prev* 2014; 23(2):122-33.
25. Ganz T, Olbina G, Girelli D, et al. Immunoassay for human serum hepcidin. *Blood* 2008; 112(10):4292-7.
26. Nicolas G, Chauvet C, Viatte L, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. *J Clin Invest* 2002; 110(7):1037-44.
27. Brookes MJ, Hughes S, Turner FE, et al. Modulation of iron transport proteins in human colorectal carcinogenesis. *Gut* 2006; 55(10):1449-60.
28. Hamara K, Bielecka-Kowalska A, Przybylowska-Sygut K, et al. Alterations in expression profile of iron-related genes in colorectal cancer. *Mol Biol Rep* 2013; 40(10):5573-85.
29. Ward DG, Roberts K, Brookes MJ, et al. Increased hepcidin expression in colorectal carcinogenesis. *World J Gastroenterol* 2008; 14(9):1339-45.
30. Bienz M, Clevers H. Linking colorectal cancer to Wnt signaling. *Cell* 2000; 103(2):311-20.
31. Radulescu S, Brookes MJ, Salgueiro P, et al. Luminal iron levels govern intestinal tumorigenesis after Apc loss in vivo. *Cell Rep* 2012; 2(2):270-82.
32. Hann HW, Stahlhut MW, Blumberg BS. Iron nutrition and tumor growth: decreased tumor growth in iron-deficient mice. *Cancer Re*s 1988; 48(15):4168-70.
33. Hann HW, Stahlhut MW, Menduke H. Iron enhances tumor growth. Observation on spontaneous mammary tumors in mice. *Cancer 1991; 68(11):2407-10.*
34. Klaus A, Birchmeier W. Wnt signalling and its impact on development and cancer. Nat Rev Cancer 2008; 8(5):387-98.
35. Reya T, Clevers H. Wnt signalling in stem cells and cancer. *Nature* 2005; 434(7035):843-50.
36. Brookes MJ, Boult J, Roberts K, et al. A role for iron in Wnt signalling. *Oncogene* 2008; 27(7):966-75.
37. Vermeulen L, De Sousa EMF, van der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; 12(5):468-76.
38. Guinney J, Dienstmann R, Wang X, et al. The consensus molecular subtypes of colorectal cancer. Nat Med 2015.
39. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.

Chapter 7 // **The effect of intravenous iron therapy on long-term survival in anemic colorectal cancer patients: results from a matched cohort study**

M.J. Wilson, J.W.T. Dekker, S. Buettner, J.J. Harlaar, J. Jeekel, M. Schipperus, J.J. Zwaginga

ABSTRACT

Introduction: Intravenous iron therapy has been shown to be advantageous in treating anemia and reducing the need for blood transfusions. Iron treatment, however, may also be hazardous by supporting cancer growth. Present clinical study explores, for the first time, the effect of preoperative intravenous iron therapy on tumor prognosis in anemiccolorectal cancer patients.

Methods: A retrospective cohort study was performed on consecutive patients who underwent surgery for colorectal cancer between 2010-2016 in a single teaching hospital. The primary outcomes were 5-year overall survival (OS) and disease-free survival (DFS). Survival estimates were calculated using the Kaplan-Meier method and patients were matched based on propensity score.

Results: 320 (41.0%) of all eligible patients were anaemic, of whom 102 patients received preoperative intravenous iron treatment (31.9%). After propensity score matching 83 patients were included in both intravenous and non-intravenous iron group. The estimated 1-, 3-, and 5-year OS (91.6%, 73.1%, 64.3%, respectively) and DFS (94.5%, 86.7%, 83.4%, respectively) in the intravenous iron group were comparable with the non-intravenous iron group (p=0.456 and p=0.240, respectively). In comparing patients with an event (death or recurrence) and no event in the intravenous iron group, a distinct trend was found for decreased transferrin in the event group (median 2.53g/L vs 2.83g/L, p=0.052).

Conclusion: The present study illustrates that a dose of 1000-2000mg preoperative intravenous iron therapy does not have a profound effect on long-term overall and disease-free survival in anemiccolorectal cancer patients. Future randomised trials with sufficient power are required to draw definite conclusions on the safety of intravenous iron therapy.

INTRODUCTION

Anemia is a frequent complication in malignancies and is present in up to 30% of all colorectal cancer patients.[1, 2] Preoperative anemia is reported to be an independent prognostic factor for impaired short- and long-term outcome.[3-5] Although a causal relationship has not yet been demonstrated, the reported association has led clinicians to aim for correcting preoperative anemia with the aim of improving survival of colorectal cancer patients. Treatment options for anemia include the use of erythropoiesis-stimulating agents (ESAs), blood transfusion and iron therapy. As both blood transfusions and ESAs are, similar to preoperative anemia, independently associated with an increased risk of cancer recurrence and increased mortality[6-10], the use of iron therapy is gaining more attention.[11]

Iron deficiency (ID) is the most common cause of preoperative anemia in colorectal cancer patients.[2, 12] This implicates that optimising preoperative hemoglobinlevel often can be accomplished by preoperative iron supplementation. While oral iron has been shown to correct anemia, it is also known to be absorbed slowly, to cause constipation, and to be largely ineffective in patients with anemia of chronic disease.[13] Therefore, the use of intravenous iron has received more consideration. In this regard, several cohort studies have shown that intravenous iron therapy in colorectal cancer patients indeed optimises preoperative hemoglobinlevel and reduces the use of red blood cell transfusions.[14-16]

The effect of intravenous iron, however, on immediate postoperative complication rate has not been elucidated. Also possible long-term effects in colorectal cancer patients are unknown. These long-term effects are of special interest while laboratory, epidemiological and animal studies have shown iron's role in all aspects of cancer development and cancer growth.[17-23] Corroborating evidence moreover implicates changes in the uptake and management of iron as crucial feature of growth of gastrointestinal cancer cells, and suggests that altered iron metabolism is a key metabolic hallmark of gastrointestinal cancer. [24]

Considering this established hazardous role of iron in cancer development and especially cancer growth, the important question arises as to whether intravenous iron therapy is negatively affecting tumor prognosis in colorectal cancer patients, independently of the presence of anemia and the use of blood transfusions. The present clinical study explores, for the first time, this long-term effect of intravenous iron therapy on tumor prognosis in colorectal cancer patients.

METHODS

Patient Selection

In this retrospective analysis, all patients undergoing resection for colorectal cancer between 1 January 2010 and 1 July 2016 at the Department of Surgery of Reinier de Graaf, a teaching hospital

in the Netherlands, were identified. Consecutive patients diagnosed with anemia (men Hb <8.0 mmol/L, 12.9 g/dL; women Hb <7.5 mmol/L, 12.0 g/dL) were eligible for inclusion. Patients who had surgery in an emergency setting were excluded. In our institution, over the course of the last 5 years, preoperative intravenous iron therapy was administered more frequently in anemicpatients. As treatment of anemia was mostly depending on the clinical assessment, and on the knowledge of patient blood management of each physician, not all anemicpatiens received intravenous iron therapy. None of the patients awaiting surgery in our center did receive preoperative oral iron therapy or erythropoiese-stimulating agents (ESAs).

Data Collection

All data concerning preoperative blood management and long-term survival, including the use of preoperative iron therapy and blood transfusion, Hb values and iron status (i.e. ferritin, transferrin, transferrin saturation) at diagnosis of colorectal cancer, and overall survival (OS) and disease-free survival (DFS) were manually obtained from medical records. In this respect, the preoperative period was defined as the time from diagnosis to surgery. Administration of intravenous iron therapy was defined by a dose of 1000-2000mg iron(III)carboxymaltose (Ferinject) or iron(III) isomaltoside (Monofer). None of the patients awaiting surgery in our center did receive preoperative oral iron therapy or erythropoiese-stimulating agents (ESAs). Clinical and pathological data, including age, gender, ASA-classification, overall comorbidities (i.e. cardiologic, vascular, diabetes, pulmonic, neurologic, thrombotic, urologic, musculoskeletal, infectious, malignancy, endocrine) tumor type, pathological tumor stage and neoadjuvant treatment were collected by the Dutch Surgical Colorectal Audit (DSCA), a disease-specific national audit. This audit collects information on patient, tumor, treatment, and 30-day and in-hospital outcome characteristics of all patients undergoing a resection for primary colorectal carcinoma in the Netherlands. The data set is cross-checked on a yearly basis with data from the Netherlands Cancer Registry.[25]

Statistical Analysis

Categorical variables were described as whole numbers and percentages while continuous variables were reported as medians with interquartile (IQR) range. Percentages for each variable were calculated based on available data, excluding missing values. Univariable comparison, comparing patients with preoperative intravenous iron therapy with patients with no intravenous iron therapy, was performed using the Pearson chi-square test for categorical variables and using the Mann-Whitney U-test for continuous variables. The primary outcomes of the study were 5-year DFS and OS. DFS was calculated from the date of surgery to the first date of radiological or pathological evidence of recurrence or metastases or the date of last follow-up, as applicable. OS was calculated as the time from the date of surgery to the date of death or date of last available follow-up. Survival estimates were calculated using the Kaplan-Meier method. Patients diagnosed with metastatic disease (i.e. AJCC TNM stage 4) and with non-curative intent treatment

were excluded in calculating DFS estimate. In addition, in patients with recurrent disease time to recurrence was compared between the intravenous iron and non-intravenous iron group using the Mann-Whitney U-test. In order to correct for the baseline differences between the treatment groups, patients were matched based on propensity score, with a caliper of 0.10. Variables matched for were tumor location, Hb level at diagnosis, treatment approach and resection type (all $p<0.1$). Finally, in all patients in the intravenous iron group and with a minimum follow up of 2.5 years, iron status was studied as a predictive factor for long-term survival using the Mann-Whitney U-test. All analyses were performed using SPSS 22.0 (IBM, New York) and the MatchIt package for R 3.0.3 (https://cran.r-project.org/). All tests were 2-sided and $p<0.05$ defined statistical significance.

Ethical approval for this study was provided by the Ethical Committee METC Zuidwest Holland. Our institution, a teaching hospital, is making use of opt-out consent. Each included patients had given consent by not declining to give consent.

RESULTS

In total, 863 patients underwent surgery for colorectal cancer, of whom 82 patients were excluded because of surgery in an emergency setting. A total of 320 patients (41.0%) were anemicat diagnosis, of whom 102 patients received preoperative intravenous iron treatment (31.9%). No patient received oral iron or ESAs in the preoperative period. Baseline characteristics are shown in table 1. Median age at presentation was 74 years (66-80) and the majority of patients was male (54.4%). Most patients were operated laparoscopically (70.9%) and the most frequently performed resection was right colectomy (56.6%), followed by sigmoid resection/low anterior resection/abdominoperineal resection (31.9%), left colectomy (9.7%) and other (i.e. panproctolectomy, subtotal colectomy) (1.9%). 125 (39.1%) patients were classified as TNM stage 3 or 4, and 24 patients (7.5%) presented with distant metastases (i.e. TNM 4).

Baseline characteristics of the intravenous iron (IV) versus the non-intravenous iron (no IV) group are presented in table 2. Both groups had a median age above 70 years (IV=75.0 (67-80), no IV=73.5 (66-80); p=0.44). In the IV iron group, the majority of patients presented with a tumor in the colon (91.2% vs 80.3%, p=0.01) and was more often operated laparoscopically (77.5% vs 67.9%, p=0.08). In addition, right colectomy was most performed in IV iron patients (67.6% vs 51.4%, p=0.004). Hb level (mmol/L) at diagnosis was significantly lower in the IV iron group (6.0 (5.5-7.0) vs 6.7 (6.1-7.3), $p<0.001$). Regarding gender, ASA classification, TNM stage and administration of preoperative blood transfusion, no significant differences were observed. Median follow-up was 47 months.

To correct for the baseline differences between the treatments groups, propensity score matching was executed for tumor location, Hb level at diagnosis, treatment approach and resection type, and led to 83 patients in each group. With this, no significant differences were observed in the

baseline characteristics of both groups (tumor location p=0.48, Hb level p=0.89, treatment approach p=0.72, resection type p=0.76)(table 3).

Table 1. Baseline characteristics of all anemic patients (n = 320)

Characteristic	n (%) / median (IQR)
Male gender	174 (54.4)
Age, years	74 (66-80)
Tumor location	
Colon	268 (83.8)
Rectum	52 (16.3)
Comorbidities	21 (84.7)
ASA	
1-2	217 (67.8)
3-4	103 (32.2)
Neoadjuvant treatment	
Chemotherapy	8 (2.5)
Radiotherapy	39 (12.2)
Treatment approach	
Open	93 (29.1)
Laparoscopic	227 (70.9)
Resection	
(Extended) right colectomy/transversum	181 (56.6)
(Extended) left colectomy	31 (9.7)
Sigmoid resection/low anterior resection/abdominoperineal resection	102 (31.9)
Other	6 (1.9)
AJCC TNM stage	
1-2	195 (60.9)
3-4	125 (39.1)
Distant metastases	24 (7.5)
Preoperative blood transfusion	50 (15.6)
Preoperative intravenous iron	102 (31.9)

Table 2. Baseline characteristics, IV versus no IV

Characteristic, n (%) / median (IQR)	No IV Iron (n = 218)	IV Iron (n = 102)	*p* value
Male gender	120 (55.0)	54 (52.9)	0.73
Age, years	73.5 (66.0-80.0)	75.0 (67.0-80.0)	0.44
Tumor location			0.01
Colon	175 (80.3)	93 (91.2)	
Rectum	43 (19.7)	9 (8.8)	
ASA			0.96
1-2	148 (67.9)	69 (67.6)	
3-4	70 (32.1)	33 (32.4)	
Treatment approach			0.08
Open	70 (32.1)	23 (22.5)	
Laparoscopic	148 (67.9)	79 (77.5)	
Resection			0.004
(Extended) right colectomy/transversum	112 (51.4)	69 (67.6)	
(Extended) left colectomy	23 (10.6)	8 (7.8)	
Sigmoid resection/low anterior resection/abdominoperineal resection	81 (37.2)	21 (20.6)	
Other	2 (0.9)	4 (3.9)	
AJCC TNM stage			
1-2	132 (60.6)	63 (61.8)	
3-4	86 (39.4)	39 (38.2)	0.78
Distant metastases (TNM 4)	18 (8.3)	6 (5.9)	0.45
Dose intravenous iron			
1000mg	-	68 (66.7)	
2000mg	-	34 (33.3)	
Preoperative blood transfusion	34 (15.7)	16 (15.7)	0.99
Hb at diagnosis (mmol/L)	6.7 (6.1-7.3)	6.0 (5.5-7.0)	<0.001
Recurrent disease (excluding M+ patients)	38 (18.9)	11 (11.3)	
Local	0 (0)	1 (1.0)	
Distant	33 (16.4)	10 (10.3)	
Local and distant	5 (2.5)	0 (0)	

Table 3. Propensity score matched baseline characteristics, IV iron versus no IV iron

Characteristic, n (%) / median (IQR)	No IV Iron (n = 83)	IV Iron (n = 83)	*p* value
Male gender	38 (45.8)	45 (54.2)	0.28
Age, years	72 (66-79)	75 (67-80)	0.38
Tumor location			0.48
Colon	71 (85.5)	74 (89.2)	
Rectum	12 (14.5)	9 (10.8)	
ASA			0.87
1-2	54 (65.1)	55 (66.3)	
3-4	29 (34.9)	28 (33.7)	
Treatment approach			0.72
Open	19 (22.9)	21 (25.3)	
Laparoscopic	64 (77.1)	62 (74.7)	
Resection			0.76
(Extended) right colectomy/transversum	50 (60.2)	51 (61.4)	
(Extended) left colectomy	9 (10.8)	8 (9.6)	
Sigmoid resection/low anterior resection /abdominoperineal resection	23 (27.7)	21 (25.3)	
Other	1 (1.2)	3 (3.6)	
AJCC TNM stage			0.42
1-2	54 (65.1)	49 (59.0)	
3-4	29 (34.9)	34 (41.0)	
Distant metastases	5 (6.0)	6 (7.2)	0.76
Dose intravenous iron			
1000mg	-	55 (66.3)	
2000mg	-	28 (33.7)	
Blood transfusion	16 (19.5)	12 (14.5)	0.39
Hb at diagnosis (mmol/L)	6.4 (5.7-7.1)	6.3 (5.7-7.1)	0.89
Recurrent disease (excluding M+ patients)	16 (20.5)	9 (11.4)	
Distant	15 (19.1)	8 (10.1)	
Local and distant	1 (1.3)	1 (1.3)	

Table 4. Baseline characteristics of IV iron patients, event (death of recurrence) versus no event at minimum follow up of 2.5 years

Characteristic, n (%) / median (IQR)	No Event (n = 49)	Event (n = 28)	*p* value
Male gender	25 (51.0)	15 (53.6)	0.83
Age, years	72 (67-79)	79 (73-82)	0.009
Tumor location			0.65
Colon	44 (89.8)	26 (92.9)	
Rectum	5 (10.2)	2 (7.1)	
ASA			0.25
1-2	36 (73.5)	17 (60.7)	
3-4	13 (26.5)	11 (39.3)	
Treatment approach			0.053
Open	8 (16.3)	10 (35.7)	
Laparoscopic	41 (83.7)	18 (64.3)	
Resection			0.39
(Extended) right colectomy/transversum	35 (71.4)	17 (60.7)	
(Extended) left colectomy	4 (8.2)	3 (10.7)	
Sigmoid resection/low anterior resection /abdominoperineal resection	9 (18.4)	5 (17.9)	
Other	1 (2.0)	3 (10.7)	
AJCC TNM stage			0.65
1-2	34 (68.0)	18 (56.2)	
3-4	15 (30.6)	10 (35.7)	
Distant metastases	1 (2.0)	1 (3.6)	0.69
Preoperative blood transfusion	6 (12.2)	6 (21.4)	0.29
Iron status at diagnosis			
Hb (mmol/L)	6.0 (5.5-7.1)	5.9 (5.3-6.9)	0.35
Ferritin (µg/L)	23.0 (9.5-81)	47.5 (13.0-131.0)	0.31
Transferrin (g/L)	2.83 (2.57-3.23)	2.53 (1.99-3.07)	0.052
TSAT (%)	8.93 (4.11-16.27)	6.57 (4.49-15.27)	1.00

In the propensity matched intravenous iron group, the mean OS was 58 months and the estimated 1-, 3- and 5-year OS was 91.6%, 73.1%, and 64.3%, respectively, which was comparable with the matched non-intravenous iron group (p=0.456)(fig. 1). Mean DFS was 58 months in the matched intravenous iron group, with estimated 1-, 3- and 5-year DFS of 94.5%, 86.7%, and 83.4%, which did not differ from the matched non-intravenous iron group (p=0.240; fig 2). In addition to the survival curves, no significant difference was observed in the time to recurrence between the intravenous iron (13.8 months; 10.3-21) and the non-intravenous iron group (13.3 months; 8.0-19.8, p=0275) (fig. 3).

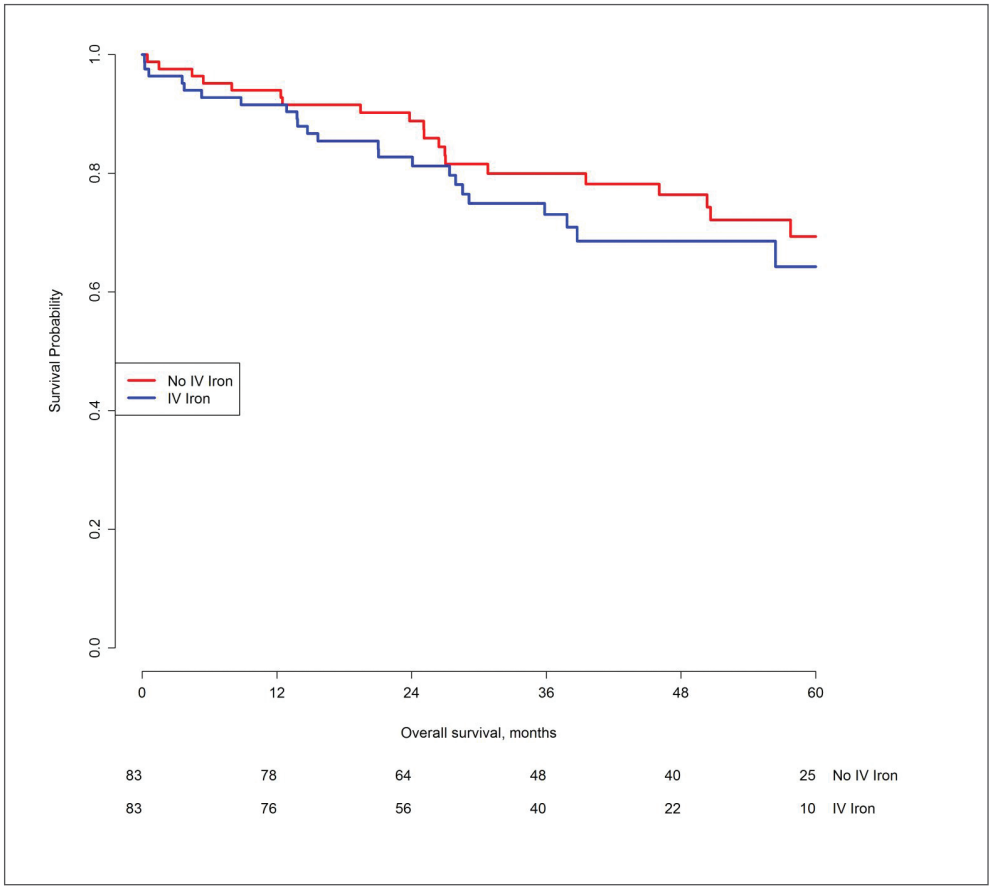
For more detail on the iron status as potential predictive factor for long-term survival in patients receiving preoperative intravenous iron therapy, all patients in the intravenous iron group and with a minimum follow up of 2.5 years were divided in an event (i.e. death of recurrence) and a non-event group (table 4). In baseline characteristics, only age was significantly different between both groups (event 79 (73-82) vs no event 72 (67-79), p=0.009). Regarding gender, tumor location, ASA classification, TNM stage, treatment approach and resection type, no significant differences were observed. With regard to iron status, TSAT was equal in both groups (event 6.6 (4.5-15.3) vs no event 8.9 (4.1-16.3), p=1.00), while a trend was found for decreased transferrin in the event group (2.53 (1.99-3.07) vs 2.83 (2.57-2.23), p=0.052). Ferritin was increased in the event group, but statistical significance was not reached (47.5 (13.0-131.0) vs 23.0 (9.5-81.0), p=0.3).

DISCUSSION

The present study shows that a dose of 1000-200mg preoperative intravenous iron therapy does not have a distinct effect on long-term overall and disease-free survival in anemiccolorectal cancer patients. In addition, in all patients with recurrent disease, intravenous iron therapy did not negatively affect the time to recurrence. On the other hand, long-term benefits, as were hoped for, were neither observed. Interestingly, in assessing the predictive value of iron status in patients receiving intravenous iron therapy, decreased transferrin, and to lesser extent increased ferritin, emerge to be potentially associated with impaired long-term survival.

Elemental iron is involved in numerous biological and cellular processes such as DNA synthesis, oxygen transport and cellular growth, and therefore is an essential nutrient for mammalian life.[21, 26] Clinically, the presence of preoperative anemia and the often-associated iron deficiency results in fatigue and impaired physical performance. Most important, however, anemia was found to be associated with increased postoperative morbidity and mortality.[3-5] The use of blood transfusions to treat the anemia, paradoxically and possibly due to transfusion-related immunomodulation (TRIM), however, was reported to be independently associated with impaired postoperative outcome in colorectal cancer patients.[6, 7] These data, combined with the high prevalence of iron deficiency anemia in these patients[12], explains why at present iron therapy is regarded as the most logical, and intravenous iron therapy as the most efficient way to optimise preoperative Hb level.

Figure 1. Overall survival stratified for intravenous iron treatment group

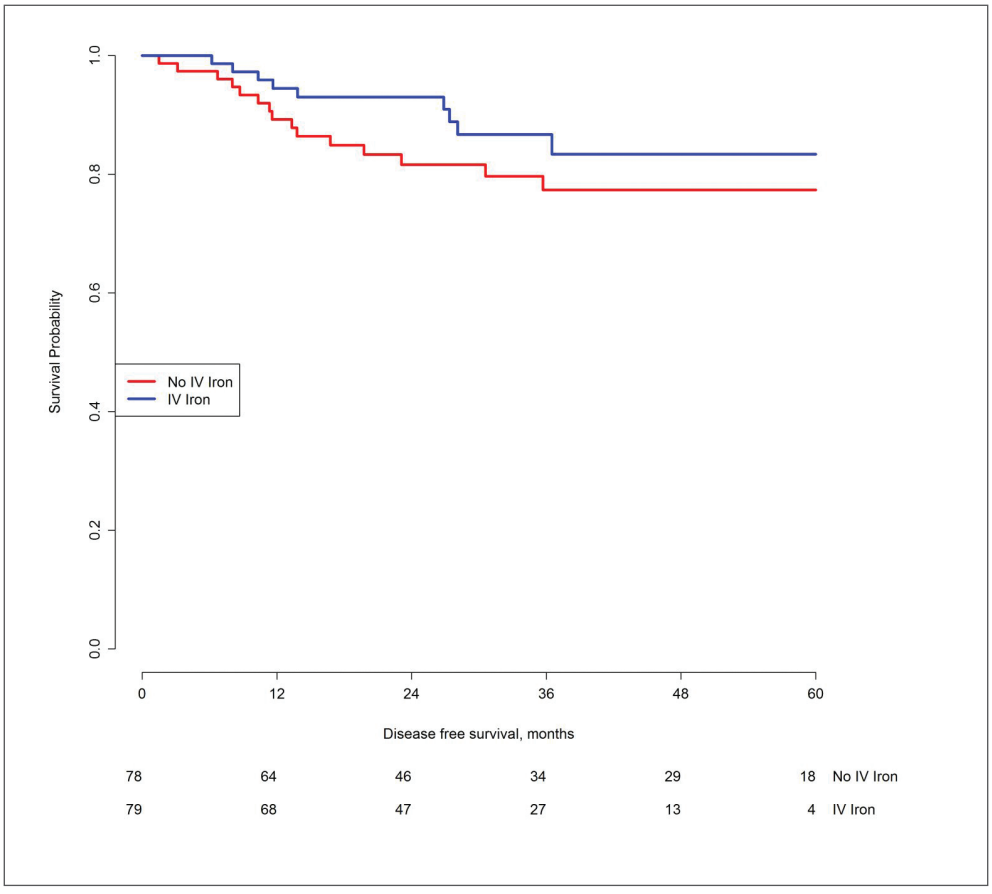


The greater efficacy of intravenous iron is to a great extent explained by the high prevalence of anemia of chronic disease (or functional iron deficiency anemia) in colorectal cancer patients.[2] While several cohort studies have indeed already demonstrated that intravenous iron therapy is positively influencing short-term outcomes with optimisation of Hb level and reduction of blood transfusion use[14-16], our study is the first to focus on possible effects of iron on long-term outcomes.

In this regard, it is important to realise that iron could be potentially harmful. As indicated, iron mediates free radical formation and it regulates crucial cell proliferation signaling pathways, including the Wnt pathway, in tumors.[27] Therefore, excess iron can contribute to both tumor initiation and tumor growth, demonstrated by several experimental animal studies [18, 28, 29], and supported by epidemiological studies showing the association between high body iron stores and increased cancer risk.[19, 20]

In addition to the role of iron in tumor initiation and tumor growth, another potential argument against the use of intravenous iron therapy in anemiccolorectal patients is the indistinctness of the causal relationship between anemia and blood transfusion on the one hand, and impaired postoperative outcome (i.e. morbidity and mortality) on the other. A paucity of evidence exists on this causality, and this is stressed by recent meta-analyses investigating the prognostic factor of preoperative anemia and blood transfusions[4-6]. Moreover, a randomised controlled trial failed to demonstrate that at long-term follow up colorectal cancer patients did benefit from autologous transfusion (i.e. no transfusion-related immunomodulation) as compared with standard allogeneic transfusion.[30] Question this causality between anemia and survival, the legitimate question arises as to whether correction of preoperative anemia, independent of therapy, will ever positively influence outcome or that anemia simply is a marker of the severity of the underlying disease. Causing harm by correction of the anemia should by all means be avoided.

Figure 2. Disease-free survival stratified for intravenous iron treatment group

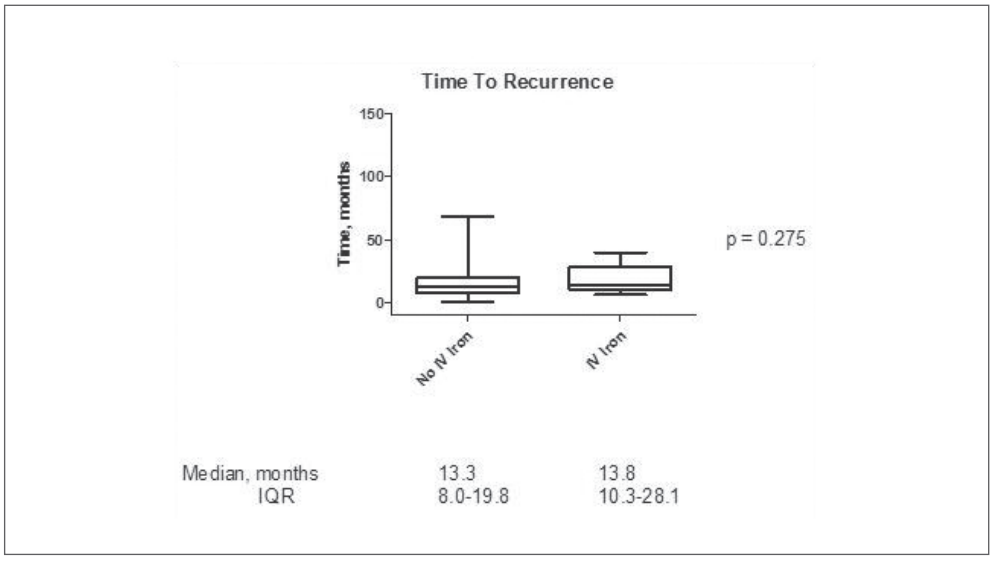


Assessing the type of iron deficiency anemia might be an additional important variable in studying the long-term effect of intravenous iron therapy. Chronic blood loss, as can be envisioned by bleeding from gastrointestinal tumors, in this respect causes absolute iron deficiency (AID), characterised by depleted iron stores. Functional iron deficiency (FID), in contrast, is caused by impaired iron homeostasis and is, due to increased hepcidin production, characterised by reduced iron uptake and iron mobilisation from the reticulo-endothelial system. Despite definite evidence, FID in this respect could be regarded as a potentially effective defence strategy to inhibit growth of pathogens and tumor cells.[13] Interestingly, some support for this hypothesis could be found in our results, showing that lower transferrin and higher ferritin levels, markers for functional iron deficiency, were associated with decreased long-term survival after iron therapy. However, an important note of caution is due here since the event and non-event group were not completely comparable (i.e. significant difference for age) and since ferritin and transferrin values were not significantly different between both groups.

STRENGTH AND LIMITATIONS

The key strength of present study is that it is the first to focus on the effect of intravenous iron therapy on long-term survival in colorectal cancer patients. Despite a growing body of epidemiological and animal studies stressing the eminent role of iron in cancer development and cancer growth, till now no studies addressed the safety of intravenous iron therapy in a clinical cohort. Although of retrospective nature, the baseline differences between the intravenous and non-intravenous iron group were corrected for by propensity score matching. This enabled us to properly calculate survival estimates using the Kaplan-Meier method. Finally, due to the propensity score

Figure 3. Time to recurrence (months), intravenous iron versus no intravenous iron



matching, the most important confounding factors of intravenous iron therapy, Hb level at diagnosis and blood transfusions, were equal in both groups. Median follow up duration of 47 months was sufficiently long.

The major limitations of this study derive from its retrospective nature and its small sample size. Over the course of the study period, preoperative intravenous iron therapy was administered more frequently in anemicpatients. This treatment was mostly depending on the clinical assessment, and on the knowledge of patient blood management of each individual physician. Increased knowledge of patient blood management probably have caused the increased administration of intravenous iron therapy. In this respect, selection bias, inherent to a retrospective cohort study, might have affected the outcome. We attempted to minimize this selection bias by propensity score matching for several important prognostic factors, however, possible confounders may be hidden and therefore selection bias cannot be completely ruled out. In assessing the predictive value of iron status in patients receiving intravenous iron therapy (table 4), the small sample size did not allow us to correct for the variable of age, which was significantly different between the event and no event group. In addition, we were not able to study the dose-response relationship. Finally, we focused on the surgical patient and by doing so, the legitimate question could arise as to whether surgery and tumor eradication might neutralise the possible detrimental effects of intravenous iron on tumor growth and long-term prognosis. Future studies should therefore also address non-surgical patients.

CONCLUSION

Despite a growing body of epidemiological and experimental studies stressing the important role of iron in cancer development and cancer growth, the present study shows that preoperative intravenous iron therapy does not have a profound effect on long-term overall and disease-free survival in anemiccolorectal cancer patients. Future randomised trials with sufficient power are required to draw definite conclusions on the safety of intravenous iron therapy, and to further investigate the predictive value of type of iron deficiency, to establish the dose-response relationship, and to study the safety of intravenous iron therapy in non-surgical patients.

CONTRIBUTORS

Conception and design: MJW, JWTD, SB, JJH, JJ, MS, JJZ. Acquisition of data: MJW, JWTD. Analysis and interpretation of data: MW, JWTD, SB, MS, JJZ. Drafting the article or revising: MW, JWTD, SB, JJH, JJ, MS, JJZ. Final approval: MW, JWTD, SB, JJH, JJ, MS, JJZ.

REFERENCES

1. Knight K, Wade S, Balducci L. Prevalence and outcomes of anemia in cancer: a systematic review of the literature. *Am J Med* 2004; 116 Suppl 7A:11S-26S.
2. Wilson MJ, Dekker JWT, Harlaar JJ, et al. The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment. *Int J Colorectal Dis* 2017.
3. Caro JJ, Salas M, Ward A, et al. Anemia as an independent prognostic factor for survival in patients with cancer: a systemic, quantitative review. *Cancer* 2001; 91(12):2214-21.
4. Fowler AJ, Ahmad T, Phull MK, et al. Meta-analysis of the association between preoperative anemia and mortality after surgery. *Br J Surg* 2015; 102(11):1314-24.
5. Wilson MJ, van Haaren M, Harlaar JJ, et al. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis. *Surg Oncol* 2017; 26(1):96-104.
6. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
7. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
8. Bohlius J, Schmidlin K, Brillant C, et al. Recombinant human erythropoiesis-stimulating agents and mortality in patients with cancer: a meta-analysis of randomised trials. *Lancet* 2009; 373(9674):1532-42.
9. Busch OR, Hop WC, Hoynck van Papendrecht MA, et al. Blood transfusions and prognosis in colorectal cancer. *N Engl J Med* 1993; 328(19):1372-6.
10. Pascual M, Bohle B, Alonso S, et al. 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* 2013; 183(1):270-7.
11. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anemia in colorectal cancer patients. *BMC Surg* 2015; 15:78.
12. Ludwig H, Muldur E, Endler G, et al. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol* 2013; 24(7):1886-92.
13. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
14. Calleja JL, Delgado S, del Val A, et al. Ferric carboxymaltose reduces transfusions and hospital stay in patients with colon cancer and anemia. *Int J Colorectal Dis* 2016; 31(3):543-51.
15. Froessler B, Palm P, Weber I, et al. The Important Role for Intravenous Iron in Perioperative Patient Blood Management in Major Abdominal Surgery: A Randomized Controlled Trial. *Ann Surg* 2016.
16. Keeler BD, Simpson JA, Ng S, et al. The feasibility and clinical efficacy of intravenous iron administration for preoperative anemia in patients with colorectal cancer. *Colorectal Dis* 2014; 16(10):794-800.
17. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1):12-31.
18. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.
19. Nelson RL, Davis FG, Sutter E, et al. Body iron stores and risk of colonic neoplasia. *J Natl Cancer Inst* 1994; 86(6):455-60.
20. Stevens RG, Jones DY, Micozzi MS, et al. Body iron stores and the risk of cancer. *N Engl J Med* 1988; 319(16):1047-52.
21. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.
22. Xue X, Shah YM. Intestinal iron homeostasis and colon tumorigenesis. *Nutrients* 2013; 5(7):2333-51.
23. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. *Protein Cell* 2015; 6(2):88-100.
24. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell* 2011; 144(5):646-74.

25. Van Leersum NJ, Snijders HS, Henneman D, et al. The Dutch surgical colorectal audit. *Eur J Surg Oncol* 2013; 39(10):1063-70.

26. Padmanabhan H, Brookes MJ, Iqbal T. Iron and colorectal cancer: evidence from in vitro and animal studies. *Nutr Rev* 2015; 73(5):308-17.

27. Brookes MJ, Boult J, Roberts K, et al. A role for iron in Wnt signalling. *Oncogene* 2008; 27(7):966-75.

28. Siegers CP, Bumann D, Trepkau HD, et al. Influence of dietary iron overload on cell proliferation and intestinal tumorigenesis in mice. *Cancer Lett* 1992; 65(3):245-9.

29. Siegers CP, Bumann D, Baretton G, et al. Dietary iron enhances the tumor rate in dimethylhydrazine-induced colon carcinogenesis in mice. *Cancer Lett* 1988; 41(3):251-6.

30. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.

Chapter 8 // **General discussion and future perspectives**

GENERAL DISCUSSION

Anemia is a common finding in cancer patients and is observed in up to 40 percent of the patients diagnosed with colorectal cancer.[1] Whilst preoperative anemia has been established as an independent risk factor for adverse short-term outcome following colorectal cancer surgery,[2] such as an increased number of complications and a longer hospital stay, studies on the association between preoperative anemia and long-term cancer outcome show inconsistent results. It has been hypothesized that anemia impairs long-term prognosis via worsening of tumor hypoxia, which is linked to radiotherapy and chemotherapy resistance.[3-5] In addition, by inducing proteomic and genomic changes, hypoxia may also increase the proliferative and metastatic potential.[3] Due to the inconsistencies demonstrated by various studies, we conducted a systematic review and meta-analysis to assess the long-term prognostic value of preoperative anemia in colorectal cancer patients (chapter 2). In meta-analyses including both colon and rectal cancer patients, preoperative anemia was significantly associated with decreased overall survival (HR 1.56, 95% CI 1.30 to 1.88) and disease-free survival (HR 1.34, 95% CI 1.11 to 1.61). However, when restricted to studies exclusively on colon or rectal cancer patients, analyses demonstrated that preoperative anemia is only significantly associated with decreased long-term overall and disease-free survival in rectal cancer patients, and not in colon cancer patients. Following the results of this meta-analysis, raised awareness about the impact of preoperative anemia on long-term survival is justified, but it remains uncertain whether anemia is an independent risk factor for impaired long-term survival or just a marker of the severity of co-morbid disease.

Causes of anemia in patients with colorectal cancer are often multifactorial. Anemia may be attributed to the underlying disease or to therapy-related factors. At diagnosis, however, anemia most often results from iron deficiency (i.e. absolute or functional). The prevalence of iron deficiency is reported to be approximately 50 percent in colorectal cancer patients.[6] The malignancy itself can lead to this iron deficiency in two major ways. Firstly, cancer cells can produce cytokines that lead to increased hepcidin production by the liver, inducing reduced duodenal iron uptake as well as iron mobilization from the reticulo-endothelial system. These effects lead to a functional iron deficiency, often referred as anemia of chronic disease or anemia of inflammation.[7] In this condition, the amount of stored iron is sufficient, but the bioavailable iron necessary for erythroblast production is deficient. Secondly, chronic blood loss at tumor site can deplete iron stores and cause an absolute iron deficiency anemia. Identification of the type of iron deficiency is of major importance because both types of iron deficiency are recommended to be treated differently.[8, 9] Whereas iron is recommended for patients who develop absolute iron deficiency, it is not recommended for patients who develop functional iron deficiency as a result of disease-related factors (i.e. infection or inflammation).9 Oral iron in these patients namely is ineffective, as hepcidin blocks the duodenal iron uptake and thus the subsequent iron transport to the bone marrow.

Our retrospective study in chapter 3 demonstrated that the prevalence of iron deficiency is approximately 50 percent in all colorectal cancer patients, and 80 percent in anemic colorectal cancer patients. This result is in accordance with the prevalence observed in a previous study.6 In regard of the type of iron deficiency, the vast majority was a combination of absolute and functional iron deficiency (81.0%); only 3.7% and 15.3% was an isolated absolute and isolated functional iron deficiency, respectively. The clinical relevance of iron deficiency in our patient cohort, however, was disputable, as only severe iron deficiency was significantly associated with increased postoperative complication rate in a univariate analysis.

Elaborating on the observed prevalence numbers of (type of) iron deficiency, we decided to perform a national survey to assess the current preoperative blood management strategies in the Netherlands, and to determine whether the recommendations formulated in international oncological guidelines are being followed. The concept of patient blood management (PBM) has been developed to promote 'the appropriate provision and use of blood, its components and derivatives, and strategies to reduce or avoid the need for a blood transfusion.[10] Special focus in PBM has been the early identification and treatment of preoperative anemia, the strongest indicator for perioperative blood transfusion. Moreover, there is an increasing awareness of the need and also a debate on how to integrate patient blood management within routine surgical care, resulting in numerous ongoing trials studying the optimal blood management strategy in all types of surgery, including colorectal cancer surgery.[11-13] Results of our survey among surgeons, gastroenterologists and anesthesiologists, as shown in chapter 4, demonstrated a distinct variability in preoperative blood management practices. Strikingly, this variability was not only seen between, but also within Dutch hospitals, as indicated by the varying responses from surgeons, gastroenterologists and anesthesiologists. In general, poor compliance with the recommendations in international guidelines on the management of anemia in cancer patients was observed. This was for example illustrated by the low number of hospitals in which iron status was measured during screening for colorectal cancer (i.e. less than 40 percent), crucial to identify the type of anemia and to determine the optimal treatment.

As anemia is most frequently iron-deficient in etiology, iron supplementation is regarded as a feasible technique to optimize hemoglobin level and minimize the use of blood transfusion, which itself has been independently associated with worse patient outcome, as demonstrated in multiple randomized trials.[14-18] In this regard, the effect of intravenous iron, in opposition to oral iron, is increasingly being explored. Compared to the side effects present in the majority of people taking oral iron preparations, the side effects with intravenous iron are minor, infrequent and short-lasting.[19] Early intravenous iron preparations were associated with a high rate of serious adverse events, most notably anaphylactic shock. Newer formulations, which bind the elemental iron more tightly resulting in a much slower release, are found to be much safer.[20] With this improved short-term safety of intravenous iron preparations, the efficacy of intravenous iron,

in terms of optimizing hemoglobin level and reducing the need for blood transfusions, received new interest.[11, 12] Although all previous studies consistently demonstrated that intravenous iron, as compared to oral iron or placebo, is more effective in treating preoperative anemia, inconsistent results are observed in reducing the blood transfusion requirement.[12, 18, 21, 22] While none of the previous studies identified characteristics/biomarkers associated with the magnitude of response in raising hemoglobin level, our results demonstrated that intravenous iron therapy is most effective in patients presenting with more severe anemia, and with higher transferrin and lower ferritin levels (chapter 5). Our results failed to demonstrate that the distinct hemoglobin increase after iron infusion leads to a decreased proportion of patients with a postoperative complication and/or blood transfusion. Following our results, we believe future studies on the short-term efficacy of intravenous iron should take the type of anemia/iron deficiency (i.e. absolute or functional iron deficiency) into account when studying the potential blood transfusion reducing effect.

Supported by data on the short-term safety and efficacy, many studies advocate a more prominent role for intravenous iron therapy in preoperative/patient blood management.[20, 23] However, before intravenous iron should be considered as the default therapy to treat a mild to moderate preoperative anemia in cancer patients, well-designed trials are required to evaluate the long-term effects and safety. By introducing intravenous iron as a therapy to reduce the blood transfusion requirement, it is essential to extensively investigate all safety aspects of intravenous iron, including the long-term effects, and to compare these results to those of blood transfusion. These long-term oncological effects of iron therapy are of special interest, as the results of laboratory, epidemiological and animal studies have shown iron's role in all aspects of cancer development and cancer growth.[24-29] Iron is an essential nutrient participating in numerous biological and cellular processes, facilitating normal cell proliferation and growth. Results of experimental studies moreover demonstrated that iron therapy is also able to enhance colorectal tumor growth and might contribute to an increased metastatic potential.[25, 26, 30] In addition, many transport proteins that were originally studied for their roles in normal iron metabolism have now been shown to also contribute to malignant tumor growth. Compared to non-malignant colon cells, iron import proteins, such as DMT1 and TfR1, are upregulated, while ferroportin, the only known iron export protein, is downregulated in colon tumor cells, seemingly subverting the normal homeostatic control into a chronic iron acquisition state enabling enhanced proliferation.[31-33] Hypothetically, as extension of these data, anemia of inflammation could be regarded as a potentially effective defense strategy of the human body to limit the growth of tumor cells.7 In this respect, due to increased hepcidin production, iron is sequestered from tumor cells into the reticuloendothelial system, resulting in a limitation of the availability of iron for the growth of tumor cells. In agreement, the avidity of cancer cells for iron has also led to the question of whether iron chelators could be used as anticancer therapy. Iron chelators, such as desferrioxamine (DFO), were initially designed to prevent iron-mediated toxicity in patient with hemoglo-

binopathy. The potential of iron chelators as anticancer therapy first came to light in studies assessing the anticancer effect of iron chelators in experimental studies,[34-37] and ever since, there is a growing interest in iron chelators as anticancer therapy. To date, promising clinical results are demonstrated in patients with hepatocellular cancer,38 prompting future research to iron chelators as a new iron-directed anticancer therapeutic.

Supported by all abovementioned circumstantial evidence, we hence decided to explore the effect of preoperative intravenous iron therapy on colorectal tumor prognosis in a matched cohort study (chapter 7). Our study failed to demonstrate that preoperative intravenous iron therapy has a profound effect on long-term overall and disease-free survival in anemic colorectal cancer patients. However, it should be stressed that the results were derived from a small-sized retrospective study, and therefore should be interpreted with caution.

To conclude, the high prevalence of iron deficiency and associated anemia in our colorectal cancer patient group often involves a functional hepcidin-mediated iron deficiency. This stresses the particular potential of preoperative intravenous iron therapy to 1) optimize hemoglobin level, namely in patients with more severe anemia and with higher transferrin and lower ferritin levels, and 2) reduce the blood transfusion requirement. In this respect, intravenous iron might benefit the patient by reducing the blood transfusion and anemia-related adverse effects. However, to date, only our small-sized retrospective study has addressed the long-term effects of preoperative intravenous iron therapy in colorectal cancer patients, showing no profound effect. Therefore, new well-designed trials studying the possible long-term effects of intravenous iron are required to answer the question whether the use of intravenous iron as first-line/default therapy to treat a mild to moderate preoperative anemia is a safe strategy for oncological patients.

FUTURE PERSPECTIVES: NEW IRON-DIRECTED DIAGNOSTICS AND THERAPEUTICS

Assessment of the long-term effects of iron therapy is needed to identify the optimal blood management strategy in colorectal cancer patients, and therefore, we believe future research should put more focus on this subject. Proper assessment of such long-term effects can be achieved by different means.

Firstly, the long-term effects can be monitored in an observational cohort study, as demonstrated in this thesis. However, this study design involves significant limitations. In a cohort study, significant differences between an iron-treatment and non-iron treatment group (e.g. hemoglobin levels and administration of blood transfusion) would, despite possible matching, likely introduce selection bias or confounding by indication, and significantly affect outcome. Therefore, to eventually demonstrate a potential causal relationship between iron therapy and long-term survival, randomized controlled trials should be considered. Randomized controlled

trials avoid selection bias and are the gold standard for establishing causal conclusions. However, in studying the effect of iron therapy, we believe that it is of importance to not only look at clinical endpoints, such as long-term overall and disease-free survival, but also, more specific and in line with many in vitro and animal studies, to look at what extent iron might enhance tumor growth, and, importantly, which colorectal tumor phenotypes are 'iron-hungry' for their growth. We strongly believe a more patient and tumor specific approach is required, and therefore, a comprehensive picture of exactly how iron metabolism is altered in malignant cells is needed. This namely will determine how iron therapy, or even iron chelation therapy, might influence the tumor itself. For this purpose, new and feasible methods to assess the iron content in colorectal tumors should be explored and be implemented.

To date, multiple experimental studies have already shown the possibility of quantifying iron and assessing the gene and protein expression levels of iron transporters in colorectal cancer specimens/samples. Evidently, the main disadvantage of these experiments is that surgery or biopsy is required in order to enable direct iron assessment in tumor samples. As a non-invasive alternative, novel diagnostic methods to visualize and quantify iron-rich biochemical compounds are being explored.[39, 40] As an example, a novel magnetic resonance protocol might allow reliable preoperative quantification of iron in colorectal tumors and is presently studied. This novel and non-invasive proton magnetic resonance spectroscopy (H-MRS) should of course first be validated against the actual iron content in tumor samples. If the novel scanning method provides high diagnostic accuracy for the assessment of iron load in colorectal tumors, it is the first demonstration of a non-invasive iron-directed test with the possibility to 1) quantity the iron load in colorectal tumors, 2) assess the effect of iron therapy on iron load in the tumor, and 3) to identify iron-dependent and non-iron dependent colorectal tumors, and 4) to investigate whether different treatments in different phenotypes could change the prognosis.

Apart from studying the effects of iron therapy by H-MRS, assessing the optimal management of anemia in colorectal cancer patients may be even more challenging. For this purpose, the detrimental long-term effects of iron treatment must be compared with those of not only anemia, but also with alternatives to treat anemia like ESAs and more important also blood transfusions. The problem here is that a head-to-head comparison of blood transfusion and iron therapy might be impossible in the clinical setting. The indications for both therapies are namely clearly different. While iron therapy is indicated and used in patients with a mild to moderate anemia, blood transfusions are only administered in case of severe anemia. To still assess the optimal management of anemia, animal experiments could be considered. In a colorectal cancer rodent model, the effect on tumor growth of both anemia, blood transfusion and iron therapy (both oral and intravenous) could be accurately assessed and compared.

The abovementioned animal and H-MRS studies will provide a more detailed understanding of

the oncological effect of iron therapy, enabling the identification of high- and low-risk patients for iron therapy. Based on these studies, the execution of a prospective randomized trial should be considered to test the effect of iron therapy on long-term survival. In such a trial, preoperative anemic patients should be randomized into an iron-treatment and a non-treatment group. In designing a study protocol, multiple important issues should be taken into consideration.

Firstly, in conducting a prospective trial randomizing anemic patients in an iron-treatment and a non-treatment group, it could be held unethical to have a non-treatment group because anemia itself is considered as a major risk factor for impaired disease-free and overall survival in cancer patients. As a first alternative, iron therapy could be considered in all anemic patients, subdivided into patients with iron-dependent and non-iron dependent colorectal tumor phenotypes. Assessment of the iron-dependency of the tumor could be, potentially, done by the novel scanning method and by identification of protein expression levels of iron transporters and iron itself in tumor samples. We hypothesize that iron therapy will show to be especially hazardous in patients with an iron-dependent tumor. As a second alternative, all patients with a non-anemic iron deficiency could be randomized into an iron-treatment and a non-treatment group.

Secondly, the dose-response relationship and the administration route (oral versus intravenous) should be considered. Often, the conditions in experimental studies, demonstrating the tumor-growing effect of iron, do not properly reflect the situation in anemic patients using excessive iron doses in iron-replete animals. In addition and of special interest, colorectal cancer animal models studying the effect of intravenous iron administration have so far not been published. In humans, particularly the effect of intravenous iron is of special interest as intravenous iron is presently more frequently being used as compared to oral iron.

Thirdly, as cancer types are shown to be iron-dependent, one might not only refrain from iron administration, but, apart from the presence of anemia or complete surgical eradication, even add iron chelation. In addition to iron chelation, two other iron-directed therapeutics can be explored. First, antibodies targeted towards the transferrin receptor 1 (TFR1) that effectively deplete intracellular iron are being studied. These antibodies have shown to effectively antagonize the growth of leukemia in mice.[41] Second, hepcidin-targeted treatment approaches, aiming at increasing ferroportin or decreasing local hepcidin levels, are being under investigation. The hepcidin depletion could be realized by neutralizing antibodies or hepciding small interfering RNAs.[42-44]

Additionally to all above, also the study of non-surgical patients should be deliberated. By focusing on only preoperative patients, the legitimate question could arise as to whether surgery and resection of the tumor might neutralize the possible detrimental effect of iron therapy on tumor growth and long-term prognosis. Therefore, future research studying the effect of iron therapy should also consider patients with premalignant colorectal adenomas.

REFERENCES

1. Ludwig H, Van Belle S, Barrett-Lee P, et al. The European Cancer Anaemia Survey (ECAS): a large, multinational, prospective survey defining the prevalence, incidence, and treatment of anaemia in cancer patients. *Eur J Cancer* 2004; 40(15):2293-306.
2. Leichtle SW, Mouawad NJ, Lampman R, et al. Does preoperative anemia adversely affect colon and rectal surgery outcomes? *J Am Coll Surg* 2011; 212(2):187-94.
3. Harrison L, Blackwell K. Hypoxia and anemia: factors in decreased sensitivity to radiation therapy and chemotherapy? *Oncologist* 2004; 9 Suppl 5:31-40.
4. Prosnitz RG, Yao B, Farrell CL, et al. Pretreatment anemia is correlated with the reduced effectiveness of radiation and concurrent chemotherapy in advanced head and neck cancer. *Int J Radiat Oncol Biol Phys* 2005; 61(4):1087-95.
5. Vaupel P, Briest S, Hockel M. Hypoxia in breast cancer: pathogenesis, characterization and biological/therapeutic implications. *Wien Med Wochenschr* 2002; 152(13-14):334-42.
6. Ludwig H, Muldur E, Endler G, et al. Prevalence of iron deficiency across different tumors and its association with poor performance status, disease status and anemia. *Ann Oncol* 2013; 24(7):1886-92.
7. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005; 352(10):1011-23.
8. Schrijvers D, De Samblanx H, Roila F, et al. Erythropoiesis-stimulating agents in the treatment of anaemia in cancer patients: ESMO Clinical Practice Guidelines for use. *Ann Oncol* 2010; 21 Suppl 5:v244-7.
9. NCCN. Cancer- and chemotherapy-induced anemia. 2014.
10. (SABM). SABM administrative and clinical standards for patient blood management programs. https://www.sabm.org/publications - adminstandards 2014 Accessed 09 Sept 2016.
11. Borstlap WA, Buskens CJ, Tytgat KM, et al. Multicentre randomized controlled trial comparing ferric(III)carboxymaltose infusion with oral iron supplementation in the treatment of preoperative anaemia in colorectal cancer patients. *BMC Surg* 2015; 15:78.
12. Keeler BD, Simpson JA, Ng O, et al. Randomized clinical trial of preoperative oral versus intravenous iron in anaemic patients with colorectal cancer. *Br J Surg* 2017.
13. Richards T, Clevenger B, Keidan J, et al. PREVENTT: preoperative intravenous iron to treat anaemia in major surgery: study protocol for a randomised controlled trial. *Trials* 2015; 16:254.
14. Acheson AG, Brookes MJ, Spahn DR. Effects of allogeneic red blood cell transfusions on clinical outcomes in patients undergoing colorectal cancer surgery: a systematic review and meta-analysis. *Ann Surg* 2012; 256(2):235-44.
15. Amato A, Pescatori M. Perioperative blood transfusions for the recurrence of colorectal cancer. *Cochrane Database Syst Rev* 2006(1):CD005033.
16. Busch OR, Hop WC, Hoynck van Papendrecht MA, et al. Blood transfusions and prognosis in colorectal cancer. *N Engl J Med* 1993; 328(19):1372-6.
17. Harlaar JJ, Gosselink MP, Hop WC, et al. Blood transfusions and prognosis in colorectal cancer: long-term results of a randomized controlled trial. *Ann Surg* 2012; 256(5):681-6; discussion 686-7.
18. Keeler BD, Simpson JA, Ng S, et al. The feasibility and clinical efficacy of intravenous iron administration for preoperative anaemia in patients with colorectal cancer. *Colorectal Dis* 2014; 16(10):794-800.
19. Auerbach M, Macdougall IC. Safety of intravenous iron formulations: facts and folklore. *Blood Transfus* 2014; 12(3):296-300.
20. Munoz M, Acheson AG, Auerbach M, et al. International consensus statement on the peri-operative management of anaemia and iron deficiency. *Anaesthesia* 2017; 72(2):233-247.
21. Calleja JL, Delgado S, del Val A, et al. Ferric carboxymaltose reduces transfusions and hospital stay in patients with colon cancer and anemia. *Int J Colorectal Dis* 2016; 31(3):543-51.
22. Edwards TJ, Noble EJ, Durran A, et al. Randomized clinical trial of preoperative intravenous iron sucrose to reduce blood transfusion in anaemic patients after colorectal cancer surgery. *Br J Surg* 2009; 96(10):1122-8.
23. Munoz M, Gomez-Ramirez S, Martin-Montanez E, et al. Perioperative anemia management in colorectal cancer patients: a pragmatic approach. *World J Gastroenterol* 2014; 20(8):1972-85.

24. Fonseca-Nunes A, Jakszyn P, Agudo A. Iron and cancer risk--a systematic review and meta-analysis of the epidemiological evidence. *Cancer Epidemiol Biomarkers Prev* 2014; 23(1):12-31.
25. Ilsley JN, Belinsky GS, Guda K, et al. Dietary iron promotes azoxymethane-induced colon tumors in mice. *Nutr Cancer* 2004; 49(2):162-9.
26. Stevens RG, Jones DY, Micozzi MS, et al. Body iron stores and the risk of cancer. *N Engl J Med* 1988; 319(16):1047-52.
27. Torti SV, Torti FM. Iron and cancer: more ore to be mined. *Nat Rev Cancer* 2013; 13(5):342-55.
28. Xue X, Shah YM. Intestinal iron homeostasis and colon tumorigenesis. Nutrients 2013; 5(7):2333-51.
29. Zhang C, Zhang F. Iron homeostasis and tumorigenesis: molecular mechanisms and therapeutic opportunities. *Protein Cell* 2015; 6(2):88-100.
30. Vermeulen L, De Sousa EMF, van der Heijden M, et al. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat Cell Biol* 2010; 12(5):468-76.
31. Brookes MJ, Hughes S, Turner FE, et al. Modulation of iron transport proteins in human colorectal carcinogenesis. *Gut* 2006; 55(10):1449-60.
32. Hamara K, Bielecka-Kowalska A, Przybylowska-Sygut K, et al. Alterations in expression profile of iron-related genes in colorectal cancer. *Mol Biol Rep* 2013; 40(10):5573-85.
33. Ward DG, Roberts K, Brookes MJ, et al. Increased hepcidin expression in colorectal carcinogenesis. *World J Gastroenterol* 2008; 14(9):1339-45.
34. Blatt J, Stitely S. Antineuroblastoma activity of desferoxamine in human cell lines. *Cancer Res* 1987; 47(7):1749-50.
35. Estrov Z, Tawa A, Wang XH, et al. In vitro and in vivo effects of deferoxamine in neonatal acute leukemia. Blood 1987; 69(3):757-61.
36. Kontoghiorghes GJ, Piga A, Hoffbrand AV. Cytotoxic and DNA-inhibitory effects of iron chelators on human leukaemic cell lines. *Hematol Oncol* 1986; 4(3):195-204.
37. Sakaida I, Kayano K, Wasaki S, et al. Protection against acetaminophen-induced liver injury in vivo by an iron chelator, deferoxamine. *Scand J Gastroenterol* 1995; 30(1):61-7.
38. Yamasaki T, Terai S, Sakaida I. Deferoxamine for advanced hepatocellular carcinoma. *N Engl J Med* 2011; 365(6):576-8.
39. Dekkers IA, de Heer P, Bizino MB, et al. (1) H-MRS for the assessment of renal triglyceride content in humans at 3T: A primer and reproducibility study. *J Magn Reson Imaging* 2018.
40. de Vries AP, Ruggenenti P, Ruan XZ, et al. Fatty kidney: emerging role of ectopic lipid in obesity-related renal disease. *Lancet Diabetes Endocrinol* 2014; 2(5):417-26.
41. Crepin R, Goenaga AL, Jullienne B, et al. Development of human single-chain antibodies to the transferrin receptor that effectively antagonize the growth of leukemias and lymphomas. *Cancer Res* 2010; 70(13):5497-506.
42. Courselaud B, Pigeon C, Inoue Y, et al. C/EBPalpha regulates hepatic transcription of hepcidin, an antimicrobial peptide and regulator of iron metabolism. Cross-talk between C/EBP pathway and iron metabolism. *J Biol Chem* 2002; 277(43):41163-70.
43. Sow FB, Alvarez GR, Gross RP, et al. Role of STAT1, NF-kappaB, and C/EBPbeta in the macrophage transcriptional regulation of hepcidin by mycobacterial infection and IFN-gamma. *J Leukoc Biol* 2009; 86(5):1247-58.
44. Wu XN, Su D, Wang L, et al. Roles of the hepcidin-ferroportin axis and iron in cancer. *Eur J Cancer Prev* 2014; 23(2):122-33.

Chapter 9 // **Summary and conclusions**

In **chapter 1** a general outline of this thesis is given and the topic of perioperative blood management is introduced with a special focus on the detrimental effects of anti-anemic therapies and the role of iron in colorectal cancer patients.

Anemia is a common finding in cancer patients and is observed in up to 40 percent of the patients diagnosed with colorectal cancer. It has been hypothesized that anemia impairs long-term prognosis via worsening of tumor hypoxia, which is linked to radiotherapy and chemotherapy resistance. In **chapter 2** the results of a systematic review and meta-analysis assessing the long-term prognostic value of preoperative anemia in colorectal cancer patients are presented. In meta-analyses including both colon and rectal cancer patients, preoperative anemia was significantly associated with decreased overall survival (HR 1.56, 95% CI 1.30 to 1.88) and disease-free survival (HR 1.34, 95% CI 1.11 to 1.61). However, when restricted to studies exclusively on colon or rectal cancer patients, analyses demonstrated that preoperative anemia is only significantly associated with decreased long-term overall and disease-free survival in rectal cancer patients, and not in colon cancer patients.

In **chapter 3** an overview of the prevalence and treatment of preoperative iron deficiency in colorectal cancer patients is provided. In approximately 50 percent of all colorectal cancer patients, and 80 percent of anemic colorectal cancer patients, iron deficiency is observed. In regard of the type of iron deficiency, the vast majority was a combination of absolute and functional iron deficiency (81.0%); only 3.7% and 15.3% was an isolated absolute and isolated functional iron deficiency, respectively. The clinical relevance of iron deficiency in our patient cohort, however, was disputable, as only severe iron deficiency was significantly associated with increased postoperative complication rate in a univariate analysis.

Results of a survey among surgeons, gastroenterologists and anesthesiologists to assess the preoperative blood management strategy in colorectal cancer patients in Dutch hospitals are presented in **chapter 4**. A distinct variability in preoperative blood management practices was demonstrated. Strikingly, this variability was not only seen between, but also within Dutch hospitals, as indicated by the varying responses from surgeons, gastroenterologists and anesthesiologists. In general, poor compliance with the recommendations in international guidelines on the management of anemia in cancer patients was observed. This was for example illustrated by the low number of hospitals in which iron status was measured during screening for colorectal cancer (i.e. less than 40 percent), crucial to identify the type of anemia and to determine the optimal treatment.

In **chapter 5** the short-term effect of preoperative intravenous iron therapy is studied in a cohort study. Preoperative intravenous iron therapy is most effective in patients presenting with more severe anemia, and with higher transferrin and lower ferritin levels. The results failed

to demonstrate that the distinct hemoglobin increase after iron infusion leads to a decreased proportion of patients with a postoperative complication and/or blood transfusion.

In **chapter 6** the long-term safety of preoperative intravenous iron therapy is being questioned. The hypothesis that iron therapy as treatment of anemia could be a potentially detrimental and hazardous strategy in colorectal cancer patients is elaborated.

Supported by the hypothesis in chapter 6, the effect of preoperative intravenous iron therapy on long-term survival in anemic colorectal cancer patients is studied in a matched cohort study in **chapter 7.** The study failed to demonstrate that preoperative intravenous iron therapy has a profound effect on long-term overall and disease-free survival in anemic colorectal cancer patients. However, it should be stressed that the results were derived from a small-sized retrospective study, and therefore should be interpreted with caution.

Chapter 10 // **Dutch summary**

IIn **hoofdstuk 1** wordt een algemene schets van dit proefschrift gegeven en wordt het onderwerp peri-operatief bloedbeleid geïntroduceerd met speciale aandacht voor de schadelijke effecten van anti-bloedarmoede therapieën en de rol van ijzer bij patiënten met dikkedarmkanker.

Bloedarmoede is een veel voorkomende bevinding bij kankerpatiënten en wordt waargenomen bij maximaal 40 procent van de patiënten met dikkedarmkanker. Er werd verondersteld dat bloedarmoede de langetermijnprognose schaadt door verergering van het zuurstoftekort van de tumor, wat verband houdt met resistentie tegen bestraling en chemotherapie. In **hoofdstuk 2** worden de resultaten gepresenteerd van een systematische review en meta-analyse van de langetermijn prognostische waarde van pre-operatieve bloedarmoede bij patiënten met dikkedarmkanker. In meta-analyses met zowel colon- als rectumkankerpatiënten, was preoperatieve bloedarmoede significant geassocieerd met een afname van de algehele overleving (HR 1,56, 95% CI 1,30 tot 1,88) en ziektevrije overleving (HR 1,34, 95% betrouwbaarheidsinterval 1,11 tot 1,61). Echter, wanneer beperkt tot studies met uitsluitend colon- of rectumkankerpatiënten, toonden analyses aan dat preoperatieve bloedarmoede alleen significant geassocieerd is met een verminderde algemene en ziektevrije overleving op de lange termijn bij patiënten met rectumkanker, en niet bij patiënten met colonkanker.

In **hoofdstuk 3** wordt een overzicht gegeven van de prevalentie en behandeling van pre-operatieve ijzerdeficiëntie bij patiënten met dikkedarmkanker. In ongeveer 50 procent van alle patiënten met dikkedarmkanker werd ijzerdeficientie geobserveerd. Dit percentage lag op 80% bij patiënten met dikkedarmkanker en bloedarmoede. Met betrekking tot het type ijzerdeficiëntie was de overgrote meerderheid een combinatie van absolute en functionele ijzerdeficiëntie (81,0%); slechts 3,7% en 15,3% was een geïsoleerde absolute en geïsoleerde functionele ijzertekort, respectievelijk. De klinische relevantie van ijzerdeficiëntie in ons patiëntencohort was echter discutabel, omdat alleen ernstige ijzerdeficiëntie in een univariate analyse significant geassocieerd was met een verhoogd postoperatief complicatiepercentage.

Resultaten van een onderzoek onder chirurgen, gastro-enterologen en anesthesiologen om de preoperatieve strategieën voor bloedbeleid bij patiënten met dikkedarmkanker in Nederlandse ziekenhuizen te achterhalen, worden gepresenteerd in **hoofdstuk 4**. Een duidelijke variabiliteit in pre-operatief bloedbeleid werd aangetoond. Opvallend was dat deze variabiliteit niet alleen tussen, maar ook binnen Nederlandse ziekenhuizen werd waargenomen, zoals blijkt uit de uiteenlopende reacties van chirurgen, gastro-enterologen en anesthesiologen. Over het algemeen werd een slechte naleving van de aanbevelingen in internationale richtlijnen over de behandeling van bloedarmoede bij kankerpatiënten waargenomen. Dit werd bijvoorbeeld geïllustreerd door het lage aantal ziekenhuizen waarin de ijzerstatus werd gemeten tijdens screening op colorectale kanker (d.w.z. minder dan 40%), cruciaal om het type bloedarmoede te identificeren en om de optimale behandeling te bepalen.

In **hoofdstuk 5** wordt het kortetermijneffect van pre-operatieve intraveneuze ijzertherapie bestudeerd in een cohortonderzoek. Pre-operatieve intraveneuze ijzertherapie is het meest effectief bij patiënten met ernstigere bloedarmoede en met hogere transferrine- en lagere ferritinespiegels. De resultaten lieten niet zien dat de duidelijke hemoglobinestijging na ijzerinfusie leidt tot een verminderd aantal patiënten met een postoperatieve complicatie en / of bloedtransfusie.

In **hoofdstuk 6** wordt de veiligheid op lange termijn van pre-operatieve intraveneuze ijzertherapie in twijfel getrokken. De hypothese dat ijzertherapie als behandeling van bloedarmoede een potentieel schadelijke en gevaarlijke strategie zou kunnen zijn bij patiënten met dikkedarmkanker, is uitgewerkt.

Ondersteund door de hypothese in hoofdstuk 6, wordt het effect van pre-operatieve intraveneuze ijzertherapie op de langetermijnoverleving bij patiënten met dikkedarmkanker en bloedarmoede bestudeerd in een gematchte cohortstudie in **hoofdstuk 7**. Het onderzoek kon niet aantonen dat pre-operatieve intraveneuze ijzertherapie een diepgaand effect heeft op lange termijn algehele en ziektevrije overleving bij patiënten met dikkedarmkanker en bloedarmoede. Er dient echter te worden benadrukt dat de resultaten zijn afgeleid van een retrospectief onderzoek van kleine omvang en daarom met de nodige voorzichtigheid geïnterpreteerd moeten worden.

ABOUT THE AUTHOR

Michael Wilson was born on December 23rd 1989 in Delft, the Netherlands. After graduation from 'Stanislas College Westplantsoen' in Delft, he started medical school at the Erasmus University Medical Center in 2008. During his study period he was working at the student team of Skillsplaza, educating medical students in basic suturing techniques. He followed his elective research project the Department of Surgery at the Red Cross Children's Hospital in Cape Town. Following his clinical rotations, he obtained his medical degree in 2015, after which he started as a PhD-candidate at TRIP (Transfusie Reacties in Patienten) and at the Department of Surgery at Erasmus Medical Center under supervision of prof. dr. J.J. Zwaginga, prof. dr. J. Jeekel and dr. M. Schipperus. During his time as PhD-candidate he simultaneously participated in the board of Promeras, the representing body of all PhD students at Erasmus MC, and Young Medical Delta. Since May 2018, he is working as a resident not in training at the Department of Surgery at the Maasstad Hospital (Rotterdam) under supervision of drs. R.A. Klaassen.

LIST OF PUBLICATIONS

Wilson MJ, van Haaren M, Harlaar JJ, Park HC, Bonjer HJ, Jeekel J, Zwaginga JJ, Schipperus *M. Long-term prognostic value of preoperative anemia in patients with colorectal cancer: A systematic review and meta-analysis.*
Surg Oncol. 2017 Mar;26(1):96-104

Wilson MJ, Dekker JWT, Harlaar JJ, Jeekel J, Schipperus M, Zwaginga JJ.
The role of preoperative iron deficiency in colorectal cancer patients: prevalence and treatment.
Int J Colorectal Dis. 2017 Nov;32(11):1617-1624

Wilson MJ, Dekker JWT, Bruns E, Borstlap W, Jeekel J, Zwaginga JJ, Schipperus M.
Short-term effect of preoperative intravenous iron therapy in colorectal cancer patients with anemia: results of a cohort study.
Transfusion. 2018 Mar;58(3):795-803

Wilson MJ, Koopman-van Gemert AWMM, Harlaar JJ, Jeekel J, Zwaginga JJ, Schipperus M. *Patient blood management in colorectal cancer patients: a survey among Dutch gastroenterologists, surgeons and anesthesiologists.*
Transfusion. 2018 Oct;58(10):2345-2351

Wilson MJ, Harlaar JJ, Jeekel J, Schipperus M, Zwaginga JJ.
Iron therapy as treatment of anemia: A potentially detrimental and hazardous strategy in colorectal cancer patients.
Med Hypotheses. 2018 Jan;110:110-113

Wilson MJ, Dekker JWT, Buettner S, Harlaar JJ, Jeekel J, Schipperus M, Zwaginga JJ.
The effect of intravenous iron therapy on long-term survival in anemiccolorectal cancer patients: results from a matched cohort study
Surg Oncol. 2018 Jun;27(2):192-199

Jairam AP, **Wilson MJ**, Steyerberg EW, Jeekel J, Lange JF.
Patient reported outcome measurements in the diagnosis of incisional hernia: PROMIS questionnaire, a pilot study.
J Surg Res. 2016 Jun 15;203(2):378-82

Jairam AP, **Wilson MJ**, Steyerberg EW, Jeekel J, Lange JF.
Corrigendum to Patient reported outcome measurements in the diagnosis of incisional hernia: PROMIS questionnaire, a pilot study.
J Surg Res. 2018 Feb 13. pii: S0022-4804(17)30730-8

DANKWOORD

Beste professor Zwaginga, beste Jaap Jan, zeer veel dank voor de afgelopen jaren. Ondanks de alledaagse drukte was je altijd op een bewonderenswaardige manier zeer betrokken en bevlogen. Daarnaast opmerkelijk: als je niet binnen 1 dag mijn stuk had nagekeken en van commentaar had voorzien moest ik je vooral snel een reminder sturen, omdat je de mail dan waarschijnlijk over het hoofd had gezien. Dit alles maakte het voor mij een heel groot plezier om jou als promotor te hebben, nogmaals veel dank hiervoor.

Beste dr. Schipperus, beste Martin, al snel bleek het onderzoek een geheel andere wending te krijgen dan vooraf eigenlijke voor ogen was. Van de klinische hemovigilantie weken we soms uit naar het fundamentele ijzermetabolisme, wat eigenlijk alleen maar mogelijk was omdat jij mij ook die vrijheid gaf. Dit alles heeft het voor mij denk ik vooral heel leerzaam en ook leuk gemaakt. Veel dank hiervoor, het was een plezier samen te werken!

Beste professor Jeekel, dank voor het vertrouwen dat u mij heeft gegeven, waardoor ik in de eerste plaats aan dit mooie project kon beginnen. Ik heb zeer veel respect voor uw eindeloze passie voor wetenschap, uw interesse in de wereld, en vooral uw eeuwige positivisme. Ik ben er trots op dat ik met u als copromotor mijn onderzoek heb kunnen voltooien.

Beste Joris, zeer veel dank voor jouw bewonderenswaardige en tomeloze enthousiasme. Je hielp me op weg en je was altijd bereid op en neer te reizen richting Rotterdam of Leiden voor een onderzoeksafspraak. Dat heb ik zeer gewaardeerd, dank.

Beste professor Lange, beste professor Kleinrensink, beste dr. Menon, binnen de REPAIR was ik met mijn hematologische onderzoek een beetje de vreemd eend in de bijt. Toch heb ik me altijd zeer welkom gevoeld en heb ik genoten van de passende humor tijdens de vergaderingen. Ik heb dit zeer gewaardeerd, dank hiervoor.

Beste collega's van TRIP, ondanks dat ik zelden op het kantoor in Leiden te vinden was heb ik me altijd zeer welkom gevoeld. Dit heb ik zeer gewaardeerd, waarvoor veel dank! Het idee van de 'roestige' kaft is ontstaan tijdens het TRIP-uitje in museum Voorlinden! Succes met alle hemovigilantie werkzaamheden.

Beste labvrienden, het was met jullie altijd een waar feest in de kelder, wat denk toch wel de meest deprimerende werkplek in het Erasmus is. Mijn experimenten waren al vroeg gesneuveld, maar toch bleef ik er dankzij jullie extreem slappe grappen nog maandenlang rondhangen. Laten we vooral de jaarlijkse theaterbezoekjes erin houden, wel altijd met een vrij plekje naast dr. Buettner.

Lieve Z-flatters, jullie gezellige aanwezigheid zorgden ervoor dat mijn werkdagen altijd stipt om 8 uur in de vroege ochtend startten! Dank hiervoor, en dank voor alle goedbedoelde dieetadviezen tijdens de lunch, zonder jullie was ik nooit zo fit aan de start in de woestijn verschenen.

Beste co-auteurs, allen dank voor jullie bijdrage aan dit proefschrift.

Lieve papa en mama, bij mijn diploma-uitreiking liet ik de gelegenheid aan mij voorbij gaan, maar nu zeker niet: dankjewel! Dank voor jullie onvoorwaardelijke steun en liefde!