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Original Article

Associations between non-anaemic iron deficiency and outcomes following elective surgery for colorectal cancer: a prospective cohort study*

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Summary

Background Iron deficiency is present in up to 75% of patients presenting for colorectal cancer surgery. It is unclear whether iron deficiency without anaemia is associated with worse postoperative outcomes. We hypothesised that, in adults without anaemia undergoing surgery for colorectal cancer, iron deficiency would be associated with worse postoperative outcomes relative to an iron-replete state.

Methods We performed a prospective, observational study, recruiting adults (aged ≥ 18 y) without anaemia who were undergoing surgery for colorectal cancer in 16 hospitals across Australia and Aotearoa/New Zealand. Anaemia was defined as a haemoglobin concentration < 130 $g.l^{-1}$ for men and < 120 $g.l^{-1}$ for women. Iron deficiency was defined primarily as transferrin saturation < 20%. The primary endpoint was days alive and at home on postoperative day 90. The primary endpoint analysis was adjusted for surgical risk based on recruiting institution; sex; Charlson comorbidity index; CR-POSSUM score; surgical approach; and requirement for neoadjuvant therapy.

Results Of 420 patients, 170 were iron deficient and 250 were iron replete. The median (IQR [range]) days alive and at home in the iron-deficient group was 84.0 (80.7–85.9 [0–88.2]) days and in the iron-replete group was 83.1 (78.7–85.1 [0–88.9]) days. The unadjusted difference in medians between groups was 0.9 (95%CI 0–1.8, p = 0.047) days and the adjusted difference was 0.9 (95%CI 0–1.80, p = 0.042) days, favouring the iron-deficient group.

Conclusions In adult patients without anaemia undergoing surgery for colorectal cancer, iron deficiency defined by transferrin saturation < 20% was not associated with worse patient outcomes and appeared to be associated with more days alive and at home on postoperative day 90.

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Introduction

Iron deficiency is present in 66–75% of patients presenting for colorectal cancer surgery, depending on the definition used. Iron deficiency anaemia has been associated with a variety of poor cancer outcomes in operative and non-operative patients [1–3]. These associations underpin best practice guidelines that recommend clinicians should identify and treat iron deficiency anaemia before major surgery [4].

Based on these associations, it has been postulated that early or 'non-anaemic' iron deficiency might also be associated with worse outcomes after major surgery [5]. Despite non-anaemic iron deficiency being twice as common as iron deficiency anaemia on a population scale [6], data examining associations between non-anaemic iron deficiency and postoperative outcomes are sparse [7]. Data from cardiac surgery patients suggest no association between non-anaemic iron deficiency and worse patient-centred postoperative outcomes [8]. However, there are no prospective studies in any abdominal surgical population that examine a similar research question, and it is unclear whether patients undergoing major surgery for colorectal cancer should be screened routinely for non-anaemic iron deficiency.

To address this gap in the evidence, we aimed to determine if pre-operative non-anaemic iron deficiency is associated with worse outcomes in patients undergoing major or complex major surgery for colorectal cancer, and if so, for which definition(s) this association was strongest.

Methods

We performed a prospective, observational study in adult patients who underwent isolated, elective surgery for colorectal cancer in 16 hospitals across Australia and Aotearoa/New Zealand (online Supporting Information Appendix S1). The study was approved by the Austin Health Human Research Ethics Committee and the study protocol has been published elsewhere [9].

All patients were screened for inclusion using the results of routine pre-operative blood tests [10]. Patients who met the World Health Organization diagnostic criteria for anaemia (haemoglobin concentration < 130 $g.l^{-1}$ for men and < 120 $g.l^{-1}$ for women) were not studied [11]. We also did not study patients meeting any of the following criteria: emergency or inpatient surgery; pregnancy or breastfeeding; age < 18 y; treatment with exogenous iron (oral or intravenous) or erythropoiesis stimulating drugs between pre-operative determination of iron status and surgery; haemoglobinopathy; primary or secondary bone marrow disease; and end-stage renal failure requiring dialysis. The study is reported in accordance with the STROBE statement [12].

All patients received routine pre-operative laboratory studies recommended by the Australian National Blood Authority, including full blood count; serum ferritin; and transferrin saturation (TSAT) [10]. The study protocol required pre-operative iron status be established no more than 45 days before surgery wherever possible. Where a patient did not meet criteria for anaemia based on a pre-operative full blood count, but iron studies had not been performed, these were performed on the day of surgery before surgical incision. Patients with a TSAT < 20% were analysed in the iron-deficient group. Patients with a TSAT ≥ 20% were analysed in the iron-replete group. This definition has been used in observational studies [13] and

interventional trials [14] to stratify iron status in patients undergoing surgery for colorectal cancer.

Baseline patient characteristic data collection included: age; sex; weight; BMI; ethnicity; medications; smoking history; and requirement for neoadjuvant chemotherapy or radiotherapy. Predicted 10-year and 30-day mortality were determined by the Charlson comorbidity index [15] and the Colorectal Physiological and Operative Severity Score for the Enumeration of Mortality and Morbidity (CR-POSSUM) respectively [16]. Pre-operative investigation variables included haemoglobin concentration; white cell count; platelet count; serum creatinine; serum albumin; serum ferritin; TSAT; and C-reactive protein. Heart rhythm was determined by a pre-operative 12-lead electrocardiogram. Intra-operative data collected included: surgical approach (open vs. minimally invasive); operation type (refers to the extent of the surgery as determined by CR-POSSUM score); planned procedure; duration of surgery (time between surgical incision and wound closure); and requirement for and type of regional anaesthesia. Postoperative data (additional to outcomes) collected included: requirement for high dependency or intensive care; intra-abdominal drain loss measured on postoperative day 3; and requirement for and time to return to intended oncological therapy (RIOT) (i.e. adjuvant chemotherapy or radiotherapy).

Patients could withdraw from the study on request. Where patients were not able to be contacted following hospital discharge, their general practitioner was contacted to determine the duration of any subacute stay and/or readmission to ensure primary outcome data were complete.

The primary outcome was days alive and at home at postoperative day 90 (DAOH90), a validated patient-centred endpoint in peri-operative clinical trials and cohort studies. It combines several key indicators for measuring effectiveness of interventions [17]. It is calculated from the following:

Days alive and at home = time available for followup
- − duration of initial length of stay
- − duration of rehabilitation or subacute care
- − duration of any readmission to hospital

Time spent in subacute care is included in the calculation only if it is in a location different to the patient's usual home environment. If a patient dies during the follow-up period, days alive and at home is recorded as zero, irrespective of when death occurred. The minimum difference that has been reported to be meaningful to patients is 3 days [18].

Secondary outcomes were: days alive and at home on postoperative day 30 (DAOH30); duration of inpatient stay; requirement for allogeneic red blood cell transfusion measured up to postoperative day 9; requirement for readmission to acute care measured up to postoperative day 90; haemoglobin concentration measured at postoperative day 3 and day of acute care discharge; recovery from surgery measured by the 15-item Quality of Recovery questionnaire (QoR-15) at postoperative day 3 [19]; change in functional status between baseline and postoperative day 30 measured by the five-level European Quality of Life Score (EQ-5D-5L) [20]; and change in disability status between baseline and postoperative day 90 measured by the WHO Disability Assessment Schedule (WHODAS 2.0) [21].

Safety endpoints included postoperative complications (including all cause infection and mortality). These were assessed at three time-points: on discharge from acute care; and via telephone interviews on postoperative days 30 and 90. Complications were stratified by body system using predetermined clinical and laboratory criteria (online Supporting Information Appendix S2).

The sample size was derived from a re-analysis of the observed difference of 2 days in DAOH90 in a similar cohort of patients who were iron deficient and iron replete [22]. These data were collected retrospectively from one of the participating hospitals [9]. Using 20,000 simulations in R (version 3.4.0; R Project for Statistical Computing, Vienna, Austria), we calibrated a lognormal distribution truncated at 90 days to generate days alive and at home with a median of 82 days and 5% zeros in the control group; a sample size of 422 patients would have 80.5% power to detect a between-group difference of medians of 2 days in the primary outcome, allowing for 5% loss of follow-up and using a two-sided significance level of 5%. The protocol allowed for unequal recruitment to one or the other group up to an enrolment ratio of 60:40 or 40:60. While we recruited consecutive patients irrespective of iron status, we planned to cease recruitment to one group if the number of patients in one group exceeded 253 (60% of the planned study cohort), with only patients with the converse iron status recruited from that point forward.

Simultaneous quantile regression modelled DAOH90, with 5000 bootstrap replications to obtain the difference in medians between the iron-deficient and iron-replete groups. The models included group membership and recruiting institution alone (hereafter referred to as

'unadjusted'), and sex, Charlson comorbidity index, CR-POSSUM, surgical approach (minimally invasive vs. open) and requirement for neoadjuvant chemotherapy or radiotherapy added subsequently (hereafter referred to as 'adjusted'). These covariates, derived from our pilot study and expert consensus within the multidisciplinary steering committee, incorporating by extension the component variables of the included risk prediction scores, account for a range of patient and surgery-specific risk factors that alter postoperative outcome. In addition, we obtained the between-group differences in the 25th and 75th quantiles.

We analysed binary outcomes using logistic regression to estimate odds ratios (OR). If the number of events in either group was < 10, we used exact logistic or Firth's logistic regression if the model failed to converge. We analysed recovery from surgery (QoR-15) and haemoglobin concentration using linear regression and adjusted for baseline value where applicable. The change in functional (EQ-5D-5L) and disability (WHODAS 2.0) status were analysed similarly to the primary outcome and adjusted for baseline value. We analysed time-to-event outcomes such as duration of inpatient stay using a generalised γ accelerated failure time model with censoring at 30 days, and time to first readmission using a Cox regression with censoring at 90 days. For DAOH90, predefined subgroup analyses were performed for sex (male or female), C-reactive protein (≤ 5 mg.l^{-1} or > 5 mg.l^{-1}) and estimated glomerular filtration rate (eGFR) (30–59 ml.min^{-1} per 1.73 m^2, 60–89 ml.min^{-1} per 1.73 m^2 and ≥ 90 ml.min^{-1} per 1.73 m^2) by adding a term for subgroup and interaction between subgroup and group in the models. We also explored heterogeneity in between-group effects for pre-operative haemoglobin concentration within males and females separately. Additional, prespecified analyses using alternative definitions for iron deficiency were performed for DAOH90 and DAOH30, all cause postoperative complication and requirement for allogeneic red blood cell transfusion. The different definitions were suboptimal iron stores (serum ferritin < 100 µg.l^{-1}) [10]; iron-restricted erythropoiesis (serum ferritin < 50 µg.l^{-1}) [23]; and absolute iron deficiency (serum ferritin < 30 µg.l^{-1}) [24]. A post hoc analysis for a common definition of functional iron deficiency used in the peri-operative literature (serum ferritin < 100 µg.l^{-1} where TSAT $< 20\%$) [25, 26] and specific inpatient complications were also performed.

All analyses were performed unadjusted and adjusted using the covariates specified above, with the iron-replete group as the reference category. Provided the proportion of missing data did not exceed 5% we performed a complete case analysis with all eligible patients including all non-missing data. A hierarchical testing procedure was applied to the primary and key secondary outcome, whereby the p value for DAOH30 was not presented if the null hypothesis was not rejected for DAOH90 at the two-sided 5% level of significance. All 95%CIs and p values are two-sided and unadjusted for multiple testing. Analyses were performed using Stata (version 16.1, StataCorp LP, College Station, TX, USA).

Results

Overall, 1761 patients were assessed for eligibility between 7 May 2019 and 18 April 2023 (online Supporting Information Figures S1 and S2) and we enrolled 423 patients. The last patient in the iron-replete group was recruited on 17 April 2023, and the last patient with iron deficiency was recruited on 18 April 2023. The last follow-up was completed on 24 July 2023 (Fig. 1). Three patients were excluded after enrolment: two were found to have non-malignant disease and one had malignant disease not affecting the colon or rectum (terminal ileum). Four (all iron replete) were lost to follow-up between days 30 and 90. Hence, 416 (98%) enrolled patients contributed data to the primary endpoint analysis. Included in the analysis were eight patients who received exogenous iron therapy in the postoperative period and one male patient who had a haemoglobin concentration of 129 g.l^{-1} on the day of surgery having not had evidence of anaemia on pre-operative screening.

The between-group baseline characteristics were well balanced (Table 1; online Supporting Information Table S1); 170 (40%) patients were analysed in the iron-deficient group and 250 (60%) in the iron-replete group. Additionally, 14 patients received neoadjuvant chemotherapy alone, eight received neoadjuvant radiotherapy alone and 63 received both, with 48 patients diagnosed with metastatic disease before surgery.

Pre-operative iron status established a median (IQR [range]) of 9 (1–21 [0–131]) days before surgery (Table 1). Iron status was assessed within 45 days of surgery in 406 (97%) patients. The baseline haemoglobin concentration was slightly lower in the iron-deficient group relative to the iron-replete group. Apart from serum ferritin and TSAT, clinically significant between-group differences were not noted. Intra-operative patient characteristics were similarly well balanced (online Supporting Information Table S2). The median (IQR [range]) duration of surgery was 215 (163–267 [30–539]) min, and nearly one-fifth of patients (78, 19%) required an open approach. Overall, 201 (48%) patients underwent major surgery (isolated left- or right-sided colectomy), 219 (52%) underwent complex major

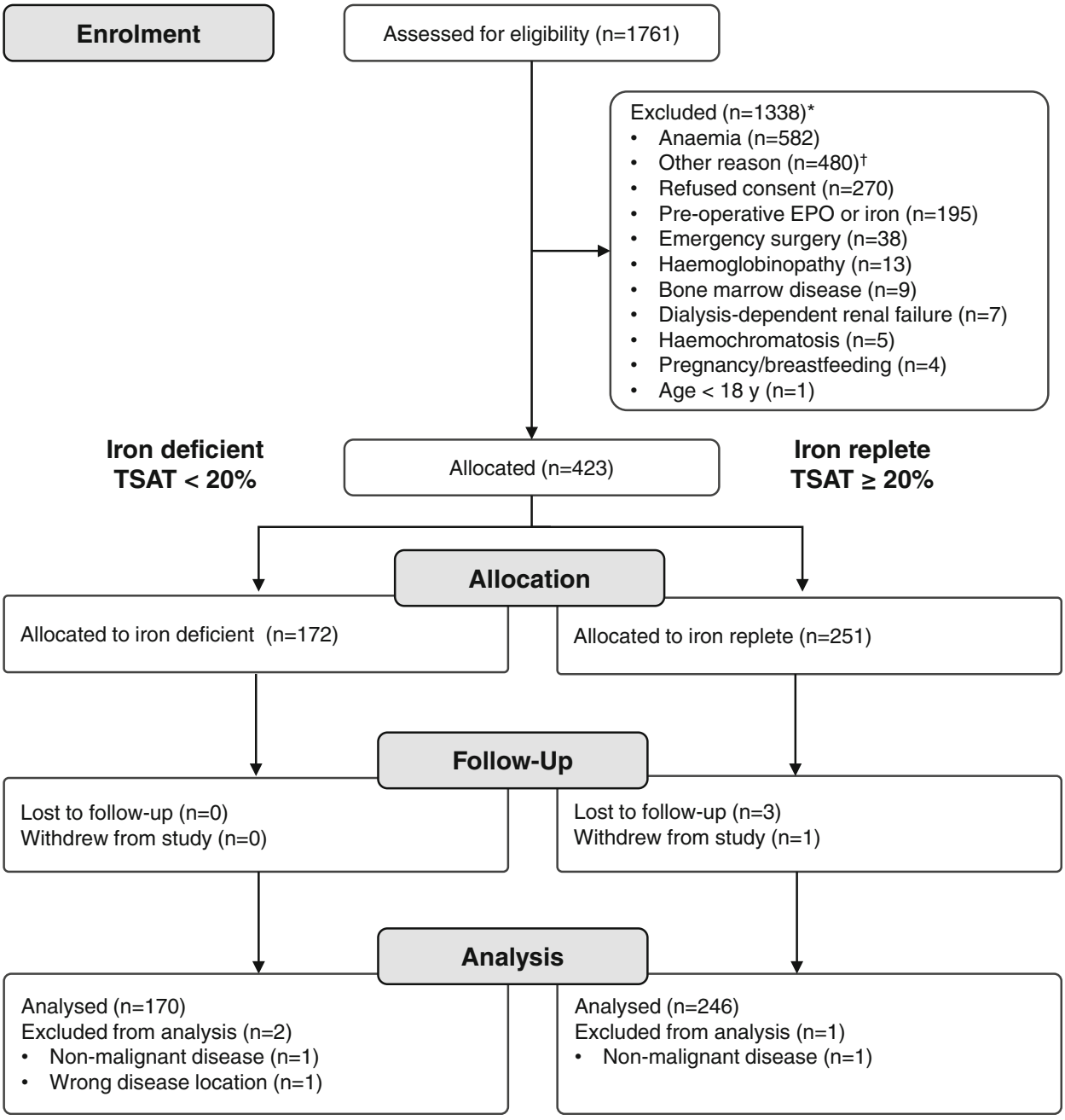

Figure 1 Study flow diagram. EPO, erythropoietin; TSAT, transferrin saturation. *Some patients assessed for eligibility and thereafter excluded from the study met > 1 exclusion criteria. †'Other reason': patients who were not fluent in the same language(s) as research staff; suspension of face-to-face enrolment during the COVID-19 pandemic; research staff not available or missed by research staff; co-enrolment in an unsuitable clinical trial; or other, non-specified reason.

surgery (anterior resection or abdominoperineal resection) and 134 (32%) had a stoma formed as part of their index surgery. Notably, a lower proportion of patients in the iron-deficient group had a stoma formed as part of their surgery (47, 28% vs. 87, 35%). However, this difference was not statistically significant (p = 0.14).

The median (IQR [range]) DAOH90 in the iron-deficient group was 84.0 (80.7–85.9 [0–88.2]) days compared with a DAOH90 of 83.1 (IQR 78.7–85.1 [0–88.9]) days in the iron-replete group (Table 2; Fig. 2). When the difference in medians was assessed, this reflected 0.91 (95%CI 0.01–1.81, p = 0.047) more days spent at home in the iron-

Table 1 Pre-operative patient and clinical characteristics. Values are median (IQR [range]), mean (SD) or number (proportion).

	Iron deficient n = 170	**Iron replete n = 250**
Age at enrolment; y	63 (54–70 [29–98])	64 (54–70 [22–91])
Sex; male	95 (56%)	177 (71%)
BMI; $kg.m^{-2}$	30.1 (25.3–34.4 [16.9–55.5])	28.0 (24.9–31.9 [16.7–48.6])
Ethnicity		
White	166 (98%)	230 (92%)
Asian	2 (1%)	12 (5%)
Black (African or Caribbean)	1 (1%)	0
Australian Aboriginal or Torres Strait Islander	0	2 (1%)
Māori, Polynesian or Pacific Islander	1 (1%)	6 (2%)
Charlson Comorbidity Index 10-y mortality; %	53.4 (2.2–77.5 [0–90.1])	53.4 (2.2–77.5 [0–90.1])
Smoking status		
Current smoker	27 (16%), n = 169	38 (15%)
Former smoker	74 (44%), n = 169	115 (46%)
Alcohol consumption per day; units	0 (0–1 [0–10]), n = 169	1 (0–2 [0–30])
CR-POSSUM score 30-d mortality; %	2.6 (1.3–4.4 [0.7–32.3])	2.3 (1.3–5.6 [0.7–48.4])
Neoadjuvant therapy before surgery		
None	137 (81%)	198 (79%)
Chemotherapy alone	4 (2%)	10 (4%)
Radiotherapy alone	3 (2%)	5 (2%)
Both chemotherapy and radiotherapy	26 (15%)	37 (15%)
Pre-existing medical conditions		
Heart failure	6 (4%)	8 (3%)
Previous myocardial infarction	8 (5%)	9 (4%)
Peripheral vascular disease	6 (4%)	10 (4%)
Chronic obstructive pulmonary disease	20 (12%)	17 (7%)
Diabetes mellitus	29 (17%)	25 (10%)
Metastatic solid tumour	20 (12%)	28 (11%)
Pre-operative laboratory parameters		
Haemoglobin; $g.l^{-1}$	140 (9.9)	146 (10.9)
White blood cell count; $\times 10^9.l^{-1}$	7.6 (2.3)	6.8 (2.0)
Platelet count; $\times 10^9.l^{-1}$	260 (224–295)	243 (205–284)
Creatinine; $\mu mol.l^{-1}$	74 (65–87 [39–166])	78 (66–89 [41–162])
Albumin; $g.l^{-1}$	38 (37–41 [26–48]), n = 163	40 (38–42 [32–48]), n = 243
Ferritin; $\mu g.l^{-1}$	62 (31–128 [3–995])	124 (73–225 [2–1049]), n = 249
Transferrin saturation; %	15 (12–18 [3–19])	27 (23–34 [20–57])
C-reactive protein; $mg.l^{-1}$	3.3 (1.8–7.0 [0.2–123.5]), n = 142	2.0 (1.0–4.0 [0–29.0]), n = 201
Estimated GFR; $ml.min^{-1}$ per 1.73 m^2	86 (74–97 [33–125])	87 (75–98 [38–130])
Duration between pre-operative iron studies and date of surgery; d	11 (2–23 [0–130])	7 (0–20 [0–104])
Atrial fibrillation or flutter	5 (3%)	10 (4%)
Baseline EQ-5D-5L	0.934 (0.885–0.968 [0.411–1.000])	0.959 (0.919–1.000 [-0. 105–1.000])
Baseline WHODAS 2.0	4 (2–13 [0–52])	4 (2–10 [0–77])

CR-POSSUM, Colorectal Physiological and Operative Severity Score for the Enumeration of Mortality and Morbidity; GFR, glomerular filtration rate; WHODAS, World Health Organisation Disability Assessment Schedule.

Table 2 Primary and key secondary outcomes. Values are presented separately for the median and the 25th and 75th centiles, and as difference in quantiles for each of the median and the 25th and 75th centiles (95%CI).

	Iron deficient	Iron replete	Unadjusted difference in quantiles (95%CI)	p value	Adjusted difference in quantiles (95%CI)	p value
Primary outcome						
Days alive and at home at POD 90	n = 170	n = 246*				
25th percentile	80.73	78.70	2.19 (-0.02–4.41)	0.053	1.65 (-0.03–3.33)	0.054
Median	83.96	83.06	0.91 (0.01–1.81)	0.047	0.92 (0.03–1.80)	0.042
75th percentile	85.86	85.09	0.88 (0.17–1.59)	0.015	0.69 (0.08–1.29)	0.027
Key secondary outcome						
Days alive and at home at POD 30	n = 170	n = 250				
25th percentile	22.18	19.92	1.74 (0–3.48)	0.050	1.14 (-0.33–2.61)	0.130
Median	24.09	23.79	0.71 (-0.13–1.55)	0.096	0.70 (-0.05–1.46)	0.068
75th percentile	25.95	25.19	0.87 (0.24–1.50)	0.007	0.64 (0.05–1.23)	0.032

POD, postoperative day.
*1 patient withdrew from the study after POD 30 and 3 were lost to follow-up.

deficient group in the unadjusted analysis, and 0.92 (95%CI 0.03–1.80, p = 0.042) more days in the adjusted analysis. The upper limit of the 95%CI did not exceed 3 days (the minimum difference identified by patients to be meaningful to them) in either analysis [18]. No detectable differences were seen between the groups for the key secondary outcome, DAOH30 (adjusted difference in medians (95%) 0.70 (-0.05–1.46) days).

No significant between-group differences were observed for any secondary outcomes (Table 3). The duration of index hospital stay was similar between the groups, as was requirement for allogeneic red blood cell transfusion and readmission to acute care. Twelve patients required an allogeneic red blood cell transfusion, with a range of 1–16 units being administered over the course of the study depending on the clinical situation. The mean haemoglobin concentration was lower in the iron-deficient group relative to the iron-replete group on both postoperative day 3 (adjusted mean difference (95%CI) -0.67 (-3.32–1.98) $g.l^{-1}$) and at discharge from hospital after the index admission (adjusted mean difference (95%CI) -0.28 (-3.08–2.51) $g.l^{-1}$), but this difference was not clinically significant and not statistically significant after adjustment for covariates. The adjusted incidence of postoperative complications was not significantly different between the groups (online Supporting Information Table S3), nor was the incidence of organ-specific complications including infection (adjusted OR (95%CI) 1.51 (0.87–2.63), p = 0.15; online Supporting Information Table S4).

Pre-specified unadjusted and adjusted subgroup analyses for the primary outcome stratified by sex, C-reactive protein concentration and eGFR rate did not show any meaningful between-group differences (online Supporting Information Table S5). Similarly, an exploratory analysis of the interactions between iron status, sex and haemoglobin concentration did not yield any significant result (online Supporting Information Table S6).

Unadjusted and adjusted sensitivity analyses for DAOH90, all cause postoperative complications and requirement for allogeneic red blood cell transfusion based on alternative definitions of iron deficiency or suboptimal iron stores (ferritin $< 30\ \mu g.l^{-1}$ vs. $\geq 30\ \mu g.l^{-1}$; ferritin $< 50\ \mu g.l^{-1}$ vs. $\geq 50\ \mu g.l^{-1}$; ferritin $< 100\ \mu g.l^{-1}$ vs. $\geq 100\ \mu g.l^{-1}$; and ferritin $< 100\ \mu g.l^{-1}$ and TSAT < 20% vs. ferritin $\geq 100\ \mu g.l^{-1}$ and/or TSAT ≥ 20%) also showed no differences between iron-deficient and iron-replete groups, with the exception of a higher rate of allogeneic red blood cell transfusion in the iron-deficient group when iron deficiency was defined as ferritin $< 30\ \mu g.l^{-1}$ (adjusted OR (95%CI) 4.96 (1.43–17.14), p = 0.011) (online Supporting Information Tables S7–S10).

Discussion

In this prospective, observational, multicentre study in patients without anaemia undergoing elective colorectal surgery we found that iron deficiency (TSAT < 20%) was associated with more DAOH90 than an iron-replete state (TSAT ≥ 20%). We further explored different definitions of iron deficiency and their associations with patient outcomes. Overall, we found no differences in patient complications, duration of stay, quality of recovery or quality of life when assessed at 30 days and 90 days after surgery for any other definition of iron deficiency.

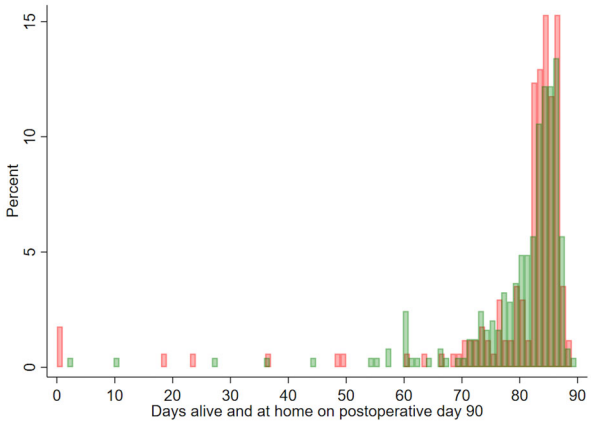

Figure 2 Frequency histogram for days alive and at home at postoperative day 90. Red bars, iron-deficient group (transferrin saturation < 20%); green bars, iron-replete group (transferrin saturation ≥ 20%).

However, patients with a pre-operative ferritin concentration < 30 μg.l^{-1} did have a higher rate of allogeneic red blood cell transfusion.

Over the last decade, non-anaemic iron deficiency has been proposed as an independent disease entity that exists on a continuum with iron deficiency anaemia [27]. In the peri-operative setting, definitions of iron deficiency are heterogeneous, encompassing absolute iron deficiency (ferritin < 30 μg.l^{-1}), functional iron deficiency (ferritin < 100 μg.l^{-1} where TSAT < 20% or TSAT < 20% in isolation) and suboptimal iron stores (ferritin < 100 μg.l^{-1} irrespective of TSAT) [28]. Data from non-anaemic patients undergoing cardiac surgery suggest that associations between functional iron deficiency and suboptimal iron stores and postoperative outcomes are tenuous, with no significant between-group differences detected for any outcome other than transfusion [8]; however, patients with ferritin < 30 μ.l^{-1} may be underrepresented in this cohort [29]. Data examining postoperative outcomes in iron-deficient patients undergoing major abdominal surgery are limited to retrospective studies, most of which do not exclude patients with anaemia [7, 13, 22]. Recently, data from large, randomised trials of intravenous iron therapy to treat anaemia before major abdominal surgery have shown rapid increases in haemoglobin in response to therapy are limited to patients with ferritin < 30 μg.l^{-1}, with any haemoglobin increment in patients with functional iron deficiency taking weeks to become apparent [26]. These findings have prompted the British Society of Haematology to redefine pre-operative iron deficiency anaemia, stating that while patients with ferritin < 30 μg.l^{-1} are likely to benefit from pre-operative treatment (grade 1B recommendation), patients with ferritin 30–100 μg.l^{-1} and TSAT < 20% only 'may' benefit (grade 2B recommendation) [24].

Our results provide high-quality evidence that could allow similar recommendations to be made for the non-anaemic population. First, we have shown that TSAT < 20% is not associated with worse outcomes after colorectal cancer surgery. While our results suggest that patients with TSAT < 20% have a higher DAOH90, a causal interpretation of this result is not tenable, and the possibility of unmeasured (i.e. stoma formation) or residual confounding cannot be excluded [30]. We are, however, confident that our original hypothesis has not been proven. Second, we have not shown any association between functional iron deficiency or suboptimal iron stores and postoperative outcomes. Accordingly, subsequent iterations of best practice guidelines should consider whether these definitions remain acceptable thresholds for treatment [10, 25]. Finally, we have shown that ferritin < 30 μg.l^{-1} is associated with a higher rate of allogeneic red blood cell transfusion. While future interventional trials could foreseeably aim to treat ferritin < 30 μg.l^{-1} in patients without anaemia awaiting surgery for colorectal cancer, using transfusion as the primary outcome and thereby proving causality, such trials are probably not feasible for several reasons, not least of which being that the incidence of allogeneic red blood cell transfusion is generally low when other patient blood management measures are implemented [26]. A ferritin concentration < 30 μg.l^{-1} was found in only 12% of patients in our study, and while the observed effect size was relatively large, the CIs were wide. Red blood cell transfusion in isolation is not a patient-centred outcome metric.

Our study has several strengths. We recruited prospectively, allowing accurate assessment of several recommended patient-centred outcomes and health-related quality of life scores [17]. Our study design was robust, with a pre-published study protocol [9] and low attrition rates and the study was conducted in several urban and regional centres in two countries. Accordingly, our results are generalisable to high-income settings. While all observational studies are at some risk of bias, the covariates in our statistical model were identified by a pilot study, and included additional metrics suggested by a broad, interdisciplinary steering committee that included anaesthetists, colorectal surgeons, haematologists and patient representatives.

Our study also has limitations. Patients and clinicians were not masked to the patient's iron status. We selected TSAT < 20% as our 'primary' definition of iron deficiency as it has seen the greatest use in clinical trials of pre-operative

Table 3 Other secondary outcomes. Values are median (IQR [range]), number (proportion) or mean (SD).

	Iron deficient n = 170	Iron replete n = 250	Unadjusted estimate (95%CI)	p value	Adjusted estimate (95%CI)	p value
Duration of inpatient stay; d	6 (4–7 [2–60])	6 (4–9 [1–45])	0.92 (0.83–1.02)	0.10	0.92 (0.84–1.01)	0.09
Odds ratio						
Allogeneic red blood cell transfusion	6 (4%)	6 (2%)	1.22 (0.40–3.71)	0.73	0.97 (0.32–2.98)	0.96
Readmission to hospital (n = 167)	34 (20%)	61 (24%)	0.77 (0.47–1.26)	0.30	0.80 (0.48–1.33)	0.39
Successful RIOT within 90 d if eligible	63 (89%), n = 71	75 (95%), n = 79	0.35 (0.09–1.35)	0.13	0.22 (0.04–1.22)	0.08
Mean difference						
POD 3 QoR-15	100.3 (25.7), n = 163	100.0 (24.0), n = 249	1.80 (-3.11–6.72)	0.47	1.26 (-3.61–6.13)	0.61
Clinical laboratory evaluations*						
POD 3 haemoglobin; $g.l^{-1}$	120.5 (14.7), n = 167	125.7 (15.6), n = 246	-0.43 (-3.17–2.30)	0.75	-0.67 (-3.32–1.98)	0.62
Hospital discharge haemoglobin; $g.l^{-1}$† (n = 168)	122.0 (15.3)	126.5 (15.5)	-0.11 (-2.98–2.75)	0.94	-0.28 (-3.08–2.51)	0.84
Difference in medians						
EQ-5D-5L; change from baseline to POD 30‡	0 (-0.08–0.03 [-0.69–0.47]),	-0.01 (-0.08–0.03 [-1.12–0.96]),	0.01 (-0.01–0.03)	0.52	0.01 (-0.01–0.03)	0.51
WHODAS 2.0; change from baseline to POD 90§	0 (-4–10 [-27–81]), n = 139	0 (-2–4 [-73–96]), n = 221	0.87 (-1.12–2.86)	0.39	0.57 (-1.44–2.58)	0.58

RIOT, return to intended oncological therapy; POD, postoperative day; QoR-15, Quality of Recovery (QoR) 15; WHODAS, World Health Organisation Disability Assessment Schedule.
*The model was adjusted for pre-operative values.
†Median hospital discharge was POD 6 and ranged between POD 1 (minimum) and POD 57 (maximum).
‡Values (35/414, 8%) outside of a ±14 day visit window were excluded to reflect quality of life at POD 30 accurately.
§Values (47/407, 12%) outside of a ±14 day visit window were excluded to reflect quality of life at POD 90 accurately.

iron therapy [28], was supported by consensus recommendations [25, 31] and was considered most likely to encompass patients with both absolute and functional iron deficiency. While we prespecified and performed several sensitivity analyses based on different definitions of iron deficiency, our results are only applicable to the definitions we tested and the laboratory tests we used. Other guidelines may use alternate tests of iron status such as soluble transferrin receptor [32], proportion of hypochromic red cells [33] and reticulocyte haemoglobin concentration to determine iron status [34]. However, these tests are not used routinely in most countries, and their inclusion in the study design would have diminished the feasibility, acceptability and 'real-world' applicability of the study considerably. We included only patients undergoing elective surgery; hence the results should be applied cautiously to individuals undergoing inpatient or emergency surgery for colorectal cancer. Eight patients received postoperative iron in contravention of the study protocol and 14 patients had their iron status assessed more than 45 days before surgery; but these values were too small to justify separate sensitivity analyses and are unlikely to confound our results. The study was conducted in two high income countries with a study cohort that was mostly of White ethnicity; generalisability to low- and middle-income countries and other ethnicities populations may therefore be limited. Finally, the observational design and lack of an intervention means we are not able to determine whether administering exogenous iron to the iron-deficient group would have changed the study outcome. Given the possible signal for harm when TSAT ≥ 20%, we consider this possibility unlikely. However, the increased rate of transfusion in our study was seen when ferritin was defined as $< 30\ \mu g.l^{-1}$ and the results of a recent sub-study of the PREVENTT trial, suggesting haemoglobin concentration increments rapidly only when ferritin is $< 30\ \mu g.l^{-1}$ [26],

support the extension of the recent British Society of Haematology guidelines to patients without anaemia [24].

In summary, we have found that iron deficiency in patients without anaemia (defined as TSAT $< 20\%$) is associated with more DAOH90 after elective colorectal surgery when compared with patients who are iron replete. However, we are reluctant to attribute causality to this finding. Instead, we conclude that iron deficiency and suboptimal iron stores do not appear associated with worse outcomes in this population, except for those patients with ferritin $< 30\,\mu g.l^{-1}$, who have a higher incidence of transfusion. We suggest iron deficiency be redefined as ferritin $< 30\,\mu g.l^{-1}$ in adult patients without anaemia undergoing surgery for colorectal cancer. Validation of our findings in different socio-economic and ethnic groups should be considered.

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Supporting Information

Additional supporting information may be found online via the journal website.

Appendix S1. Participating sites and investigators.
Appendix S2. Study recruitment.
Figure S1. Cumulative recruitment.
Figure S2. Recruitment by month.
Table S1. All collected pre-operative patient demographic and clinical characteristics.
Table S2. Intra-operative patient characteristics.
Table S3. Specific inpatient complications and subsequent hospital admissions.
Table S4. Postoperative complications.
Table S5. Difference in median days alive and at home on postoperative day 90 in prespecified subgroups.
Table S6. Exploratory analyses for effects of different strata of haemoglobin concentration on days alive and at home on postoperative day 90.
Table S7. Sensitivity analyses for ferritin $< 100\ \mu g.l^{-1}$ and $\geq 100\ \mu g.l^{-1}$.
Table S8. Sensitivity analyses for ferritin $< 50\ \mu g.l^{-1}$ and $\geq 50\ \mu g.l^{-1}$.
Table S9. Sensitivity analyses for ferritin $< 30\ \mu g.l^{-1}$ and $\geq 30\ \mu g.l^{-1}$.
Table S10. Sensitivity analyses for ferritin $< 100\ \mu g.l^{-1}$ and TSAT $< 20\%$ vs. ferritin $\geq 100\ \mu g.l^{-1}$ or TSAT $\geq 20\%$.