



Preoperative iron status is a prognosis factor for stage II and III colorectal cancer

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Abstract

Background Iron deficiency anemia is represented in colorectal cancer (CRC) patients. Iron surplus lead to increase non-transferrin bound iron (NTBI), and NTBI promotes cancer progression and influences microbiota. This study investigated whether preoperative serum iron status was associated with prognosis after CRC resection.

Methods We evaluated preoperative iron and transferrin saturation (TSAT), which was calculated as iron divided by total iron-binding capacity, in 327 patients who underwent surgery for Stage II–III CRC. Fe < 60 µg/dl and TSAT > 40% were defined as low and high iron, respectively. The associations between iron status and overall survival (OS) were evaluated in univariate and multivariate Cox proportional hazards analysis.

Results Of the 327 patients, 179 (54.7%), 124 (37.9%) and 24 (7.3%) had low, normal and high iron, respectively. In univariate analysis, low iron was associated with shorter OS (hazard ratio [HR] 2.821, 95% confidence interval [CI] 1.451–5.485, $P=0.002$). High iron was also associated with shorter OS (HR 3.396, 95% CI 1.359–8.489, $P=0.009$). In multivariate analysis, high age ($P=0.002$), depth of invasion pT4 ($P=0.012$), lymph-node metastasis presence ($P=0.035$), low albumin ($P=0.011$), low iron (HR 2.282, 95% CI 1.163–4.478, $P=0.016$) and high iron (HR 3.757, 95% CI 1.486–9.494 $P=0.005$) were independently associated with shorter OS. High iron was associated with the amount of intratumoral *Fusobacterium nucleatum* compared with normal iron.

Conclusion Both low and high preoperative iron in Stage II–III CRC patients were associated with unfavorable OS in univariate and multivariate analyses.

Keywords Colorectal cancer · Prognosis · Iron · Transferrin saturation · *Fusobacterium nucleatum*

Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignant disease in men and the second in women worldwide [1]. CRC patients are likely to suffer from iron

deficiency anemia. The most effective treatment for resectable CRC is surgical resection with lymph-node (LN) dissection [2], but CRC patients with anemia often receive perioperative blood transfusion, which has been associated with unfavorable short- and long-term prognosis in many studies [3, 4]. Therefore, iron supplementation has been performed to improve anemia and thus to avoid perioperative blood transfusion. In a few studies, preoperative iron supplementation has been reported to decrease perioperative blood transfusion and improve short-term outcome [5]. However, the association between preoperative iron status and long-term outcome has remained unclear.

Iron deficiency anemia has been observed in CRC patients; however, iron surplus can contribute to both tumor initiation and tumor growth. Iron also plays a role in the tumor microenvironment and in metastasis. Reprogramming

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of iron metabolism is a crucial aspect of tumor cell survival. Hypoxia-inducible factor and WNT pathways may contribute to altered iron metabolism in cancer [6]. Iron surplus leads to increased non-transferrin bound iron (NTBI), and NTBI promotes cancer progression [7] and influences microbiota. High transferrin saturation (TSAT) results in elevated NTBI, and elevating NTBI leads to unfavorable side effects at the highly interactive host–microbe interface of the human gastrointestinal tract. Microbial populations change in the gut in response to increased luminal iron concentration [8]. Microbial change has been associated with prognosis in many gastrointestinal cancers [9–11].

Fusobacterium nucleatum (*F. nucleatum*) is associated with unfavorable prognosis after curative resection in colorectal cancer. To our knowledge, no association between *F. nucleatum* and iron status has been reported. However, the Western dietary pattern has been strongly associated with *F. nucleatum*-positive colorectal cancer [12], and Western dietary patterns are characterized by red and processed meats, which contain large amounts of iron [13, 14].

The aim of this study is to evaluate the relationship between preoperative iron status and long-term outcome in Stage II and III CRC patients who underwent colorectal resection. This study may lead to novel insights into the association between preoperative iron status and prognosis of the CRC, and iron and transferrin saturation should be measured to ‘to inform decisions about whether to perform iron supplementation.

Materials and methods

Patients and evaluation for iron status

Four hundred thirty-six Stage II and III CRC patients underwent surgery at the Department of Gastroenterological Surgery, Kumamoto University between January 2005 and March 2018. Blood iron and unsaturated iron-binding capacity were measured in 327 of these 436 patients. TSAT was calculated as iron divided by total iron-binding capacity. The lower limit of the normal range of iron was 60 µg/dl, as specified by the recommendations of the measuring kit our institute adopted. TSAT > 40% was defined as high iron in accordance with a previous report [7]. Clinical data, including age, gender, body mass index, depth of invasion (pT), presence of metastatic lymph node (LN), pathological type, lymphatic invasion, and vascular invasion, were retrospectively available for all 327 patients. The laboratory data were collected less than 2 months before surgery. Laboratory measurements included carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), white blood cells (WBC), serum total protein, albumin, CRP, hemoglobin, platelets, peripheral neutrophils, and lymphocytes.

Each cut-off value was defined according to the recommendations of the measuring kits we used. The cut-off values of hemoglobin in males and females were 13.0 and 12.0 g/dl, respectively, as defined by the World Health Organization. Neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), as inflammatory markers, were evaluated, because the association between these markers and metastatic CRC patient prognosis has been reported. Cut-off values of NLR and PLR were determined as 5 and 150, respectively, in accordance with a previous study [15]. Complication after surgery was evaluated in accordance with the Clavien–Dindo classification [16]. Written informed consent was obtained from all patients for the treatments and the study was approved by the Institute Review Board of the Graduate School of Medical Science, Kumamoto University.

Treatment strategy and follow-up evaluation

The treatment strategy followed the Japanese colorectal cancer guidelines, which recommend surgery with LN dissection for Stage II and Stage III CRC. Patients were followed up at 3-month intervals. Recurrence was confirmed by clinical examinations, including computed tomography (CT). Tumor marker levels were measured every 3 months for 5 years after surgery. CT scanning studies that included the neck to the pelvis were performed at least twice a year for 3 years after surgery.

Quantitative polymerase chain reaction (PCR) for intratumor *Fusobacterium nucleatum*

Genomic DNA was extracted from CRC tissue using a QIAamp DNA Mini Kit (Qiagen). Quantitative PCR assays were performed to measure the amount of tissue DNA of *F. nucleatum*. Custom TaqMan primer/probe sets (Applied Biosystems) for the nusG gene of *F. nucleatum* and the reference human gene SLCO2A1 were used as previously described [17]. Each 10-µl reaction contained 12.5 ng of genomic DNA, 1 × final concentration LightCycler 480 Probes Master (Roche). Amplification and detection of DNA were performed with a LightCycler 480 Instrument II (Roche). The primer and probe sequences for each TaqMan Gene Expression Assay were as follows: *F. nucleatum* forward primer, 5'-TGGTGCATTCTCCAAAAATATCA-3'; *F. nucleatum* reverse primer, 5'-AGATCAAGAAGGACAAGTTGCTGAA-3'; *F. nucleatum* FAM probe, 5'-ACTTTA ACTTACCATGTTCA-3'. Each specimen was analyzed in duplicate in a single batch, and we used the average of the two cycle threshold (Ct) values. The amount of *F. nucleatum* in each specimen was calculated as a relative unitless value normalized with SLCO2A1 using the 2-ΔCt method as previously described [17].

Statistical analysis

The association of transferrin with recorded clinical and pathological characteristics was determined by chi-squared tests. All P values were two-sided; $P < 0.05$ was considered significant. Analysis of risk factors for survival included age, gender, depth of invasion, LN metastasis, albumin, CRP, hemoglobin, NLR, PLR and iron. Mortality was estimated from cancer-specific survival (CSS) and overall survival (OS). The log-rank test and Wilcoxon test were used in the survival analysis; the Kaplan–Meier method was used to assess cumulative survival. Cox proportional hazards regression models were utilized to calculate hazard ratios (HR) and 95% confidence interval (CI). We performed multivariate Cox proportional hazards regression analysis to compute an HR according to age ≥ 70 , male sex, depth of invasion (pT4), the presence of LN metastasis, low albumin (< 4.1 g/dl), CPR (≥ 0.14 mg/dl), low hemoglobin, NLR (≥ 5), PLR (≥ 150) and iron status. Backward stepwise elimination (likelihood method) with a threshold of $P = 0.20$ was used to select variables for the final model. Prognostic analysis was performed following the REMARK Guidelines [18]. Probability values < 0.05 were considered significant. All data were processed and analyzed using SPSS and JMP 11 software programs.

Results

Association between iron and clinicopathological findings

Of the 327 patients, 179 (54.7%), 124 (37.9%) and 24 (7.3%) had low, normal and high iron, respectively. For these patients, age ($P = 0.016$), gender ($P = 0.016$), depth of invasion ($P < 0.001$), CEA ($P = 0.024$), CA19-9 ($P = 0.028$), WBC ($P = 0.001$), albumin ($P = 0.001$), CRP ($P < 0.001$), hemoglobin ($P < 0.001$), NLR ($P = 0.001$) and PLR ($P = 0.001$) were significantly different between the three groups (Table 1). For these patients, low iron was significantly associated with high age ($P = 0.0087$), right-sided tumor location ($P = 0.0241$), depth of invasion T4 ($P < 0.0001$), high CEA ($P = 0.0064$), high CA19-9 ($P = 0.0121$), high WBC ($P = 0.0021$), low albumin ($P = 0.0013$), high CRP ($P < 0.0001$), low hemoglobin ($P < 0.0001$), high NLR ($P = 0.0001$), and high PLR ($P = 0.0003$) compared with normal iron using Bonferroni-adjusted p values in univariate analysis (Supplementary Table S1). High age (risk ratio (RR) 1.896, 95%CI 1.087–3.307, $P = 0.024$), depth of invasion T4 (RR 2.293, 95%CI 1.123–4.682 $P = 0.023$), high CRP (RR 2.646, 95%CI 1.514–4.625, $P < 0.001$) and low hemoglobin (RR 4.509, 95%CI 2.600–7.820, $P < 0.001$) were independently associated with low iron in multivariate binomial logistic analysis. High

iron was not significantly associated with any clinicopathological factors compared with normal iron (Supplementary Table S2).

Association of iron with blood transfusion and postoperative complication

The association of the prognosis of CRC patients after surgery with blood transfusion and postoperative complication was previously reported [19, 20]. Of the 327 patients, 19 received blood transfusion 7 days before surgery, and 57 received intraoperative blood transfusion. Preoperative and intraoperative blood transfusion was more frequently performed for patients with low iron than for those with normal iron ($P = 0.0303$ and $P < 0.0001$, respectively) (Table 2). Postoperative complication was evaluated with the Clavien–Dindo classification. Low iron was not associated with anastomotic leakage ($P = 0.5297$) or postoperative complication with Clavien–Dindo classification ≥ 3 ($P = 0.3167$) compared with normal iron. Preoperative high iron was associated with preoperative blood transfusion ($P = 0.0066$), but not with intraoperative blood transfusion or with any postoperative complication.

Association between iron status and survival

The 327 patients, of whom 61 (18.7%) were dead and 35 (10.7%) were cancer-specific deaths, were monitored over a median follow-up of 54.7 months. Five-year CSS and OS rates were 89.1% and 81.3%. We evaluated the association between survival and iron status with the Kaplan–Meier method. Low iron was significantly associated with shorter OS (log-rank test $P = 0.002$, Wilcoxon test $P < 0.001$), and tended to result in shorter CSS (log-rank test $P = 0.095$, Wilcoxon test $P = 0.001$) compared with normal iron. Five-year OS rates of CRC patients with low and normal iron were 75.1% and 93.7%, respectively. Five-year CSS rates of CRC patients with low and normal iron were 84.9% and 95.9%, respectively (Fig. 1).

High iron was significantly associated with shorter OS (log-rank test $P < 0.001$, Wilcoxon test $P < 0.001$) but not with CSS (log-rank test $P = 0.541$, Wilcoxon test $P = 0.103$) compared with normal iron. Five-year OS rates of CRC patients with low and normal iron were 66.2% and 93.7%, respectively. Five-year CSS rates of CRC patients with high and normal iron were 85.0% and 95.9%, respectively (Fig. 1C, D).

Association between clinicopathological factors and survival

Many clinicopathological factors are associated with OS after colorectal resection. Both low and high iron were also

Table 1 Association between iron status and clinicopathological factors

Factor	<i>N</i> = 327	Low iron <i>N</i> = 179 (%)	Normal iron <i>N</i> = 124 (%)	High iron <i>N</i> = 24 (%)	<i>P</i> value
Age (years old)					0.016*
< 70	160	78 (43.6)	73 (58.9)	9 (37.5)	
≥ 70	167	101 (56.4)	51 (41.1)	15 (62.5)	
Gender					0.016*
Male	154	95 (53.1)	53 (42.7)	6 (25.0)	
Female	173	84 (46.9)	71 (57.3)	18 (75.0)	
Body mass index (kg/m ²)					0.486
< 18.5	38	25 (14.0)	11 (8.9)	2 (8.3)	
18.5–25	220	121 (67.6)	82 (66.1)	17 (70.8)	
25 <	69	33 (18.4)	31 (25.0)	5 (20.8)	
Tumor location					0.069
Right-sided	104	65 (36.3)	30 (24.2)	9 (37.5)	
Left-sided	223	114 (63.7)	94 (75.8)	15 (62.5)	
Depth of invasion					< 0.001*
pT1–3	252	121 (67.6)	108 (87.1)	23 (95.8)	
pT4	75	58 (32.4)	16 (12.9)	1 (4.2)	
LN metastasis					0.194
Absent	179	102 (57.0)	61 (49.2)	16 (66.7)	
Present	148	77 (43.0)	63 (50.8)	8 (33.3)	
Pathological type					0.176
Tub, pap	299	160 (89.4)	115 (92.7)	24 (100.0)	
Por, sig, muc	43	19 (10.6)	24 (19.4)	0 (0.0)	
Lymphatic invasion					0.399
Absent	230	121 (67.6)	90 (72.6)	19 (79.2)	
Present	97	58 (32.4)	34 (27.4)	5 (20.8)	
Vascular invasion					0.371
Absent	135	74 (41.3)	48 (38.7)	13 (54.2)	
Present	192	105 (58.7)	76 (61.3)	11 (45.8)	
CEA (ng/ml)					0.024*
< 3.4	144	67 (37.4)	66 (53.2)	11 (45.8)	
≥ 3.4	183	112 (62.6)	58 (46.8)	13 (54.2)	
CA19-9 (U/ml)					0.028*
< 37	264	135 (75.4)	108 (87.1)	21 (87.5)	
≥ 37	68	44 (24.6)	21 (16.9)	3 (12.5)	
WBC (/μl)					0.001*
< 8600	277	140 (78.2)	113 (91.1)	24 (100.0)	
≥ 8600	50	39 (21.8)	11 (8.9)	0 (0.0)	
Total protein (g/dl)					0.77
< 6.6	135	77 (43.0)	49 (39.5)	9 (37.5)	
≥ 6.6	192	102 (57.0)	75 (60.5)	15 (62.5)	
Albumin (g/dl)					0.001*
< 4.1	227	139 (77.7)	75 (60.5)	13 (54.2)	
≥ 4.1	100	40 (22.3)	49 (39.5)	11 (45.8)	
CRP (mg/dl)					< 0.001*
< 0.14	130	44 (24.6)	74 (59.7)	12 (50.0)	
≥ 0.14	197	135 (75.4)	50 (40.3)	12 (50.0)	
Hemoglobin (g/dl)					< 0.001*
Male: < 13, female: < 12	189	130 (72.6)	46 (37.1)	13 (54.2)	
Male: ≥ 13, female: ≥ 12	133	44 (24.6)	78 (62.9)	11 (45.8)	
Neutrophil/lymphocyte					0.001*

Table 1 (continued)

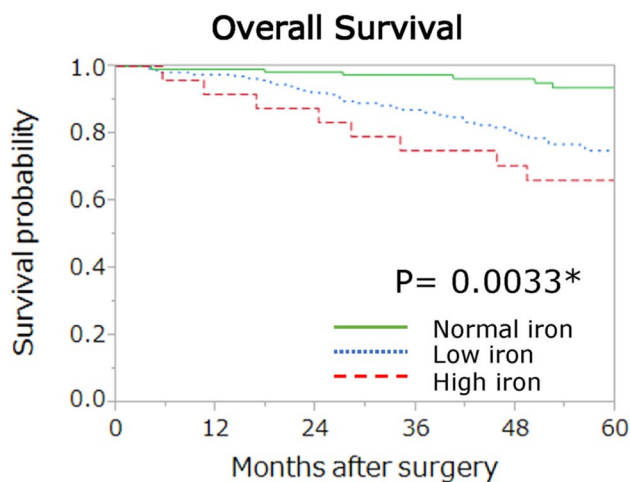
Factor	<i>N</i> = 327	Low iron <i>N</i> = 179 (%)	Normal iron <i>N</i> = 124 (%)	High iron <i>N</i> = 24 (%)	<i>P</i> value
< 5	295	152 (84.9)	121 (97.6)	22 (91.7)	0.001*
≥ 5	32	27 (15.1)	3 (2.4)	2 (8.3)	
Platelets/lymphocyte					0.001*
< 150	156	68 (38.0)	73 (58.9)	15 (62.5)	
≥ 150	171	111 (62.0)	51 (41.1)	9 (37.5)	

LN lymph node, *CEA* carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19–9, *WBC* white blood cell, *CRP* C-reactive protein

*Significant difference

Table 2 Association between iron status and blood transfusion

Factors	<i>n</i> = 327	Low iron <i>n</i> = 179 (%)	Normal iron <i>n</i> = 124 (%)	High iron <i>n</i> = 24 (%)	<i>P</i> value
Preoperative blood transfusion					0.009*
Done	19	13 (7.3)	2 (1.6)	4 (16.7)	
Not done	308	166 (92.7)	122 (98.4)	20 (83.3)	
Intraoperative blood transfusion					0.0002*
Done	57	45 (25.1)	9 (7.3)	3 (12.5)	
Not done	270	134 (74.9)	115 (92.7)	21 (87.5)	

**Fig. 1** Kaplan–Meier curve of overall survival (OS) of colorectal cancer patients who underwent colorectal resection

significantly associated with shorter OS (HR 2.821, 95%CI 1.451–5.485, $P=0.002$ and HR 3.396, 95%CI 1.359–8.489, $P=0.009$, respectively) compared with normal iron, in univariate Cox proportional hazards regression analysis. Preoperative or intraoperative blood transfusion was not associated with prognosis after colorectal resection (Supplementary Figure S1). High age ($P=0.002$), depth of invasion pT4 ($P=0.012$), LN metastasis presence ($P=0.035$), low albumin ($P=0.011$), low iron (HR 2.282, 95%CI 1.163–4.478, $P=0.016$) and high iron (HR 3.757, 95%CI 1.486–9.494 $P=0.005$) were

independently associated with shorter OS in multivariate Cox proportional hazards regression analysis (Table 3).

Association between iron status and *Fusobacterium nucleatum*

Recent work has demonstrated that TSAT is associated with microbiota in the gut [8]. Of the 327 CRC patients, frozen samples were available for 142. The quantity of *F. nucleatum* within the primary tumor of 142 patients was measured. These patients consisted of 80 with low iron, 48 with normal iron and 14 with high iron. *F. nucleatum* was more frequently detected in high-iron patients compared with normal-iron patients ($P=0.0138$) (Fig. 2A). An association between *F. nucleatum* and prognosis of CRC patients has been reported. In accordance with that previous study, we divided the patients into three groups, *F. nucleatum*-negative, -low and -high [9]. The proportions of *F. nucleatum*-negative, -low and -high were 43.8%, 31.3% and 25.0%, respectively, in the primary tumor of normal-iron patients, whereas they were 21.4%, 28.6% and 50.0%, respectively, in the tumor of high-iron patients (Fig. 2B).

Discussion

Preoperative low iron levels tended to correlate with unfavorable CSS and were significantly associated with shorter OS of Stage II and III CRC patients. Iron supplementation

Table 3 Overall survival of CRC patients in univariate and multivariate Cox proportional hazards analysis

Factors	Ref.	Univariate analysis			Multivariate analysis		
		HR	95% CI	P value	HR	95% CI	P value
Age: ≥ 70	< 70	2.410	(1.399–4.153)	0.001*	1.558	(1.178–9.688)	0.002*
Gender: male	Female	1.050	(0.631–1.749)	0.850			
Depth of invasion: pT4	pT1-3	1.989	(1.176–3.364)	0.010*	1.417	(1.078–1.863)	0.012*
LN metastasis: present	Absent	1.470	(0.889–2.433)	0.133	1.319	(1.020–1.704)	0.035*
Albumin (g/dl): < 4.1	≥ 4.1	2.753	(1.430–5.302)	0.002*	1.544	(1.104–2.161)	0.011*
CRP (mg/dl): ≥ 0.14	< 0.14	1.814	(1.034–3.182)	0.038*			
Hemoglobin (g/dl): low	Normal	1.654	(0.969–2.825)	0.065			
NLR: ≥ 5	< 5	1.267	(0.545–1.267)	0.583			
PLR: ≥ 150	< 150	0.937	(0.567–1.550)	0.800			
Preoperative blood transfusion	Not done	1.156	(0.459–2.915)	0.759			
Intraoperative blood transfusion	Not done	1.494	(0.831–2.684)	0.180			
Low iron	Normal	2.821	(1.451–5.485)	0.002*	2.282	(1.163–4.478)	0.016*
High iron	Normal	3.396	(1.359–8.489)	0.009*	3.757	(1.486–9.494)	0.005*

Low male: < 13 g/dl; female: < 12 g/dl, Normal male: ≥ 13 g/dl; female: ≥ 12 g/dl

HR hazard ratio, CI confidence interval, LN lymph node, CRP C-reactive protein

*Significant difference

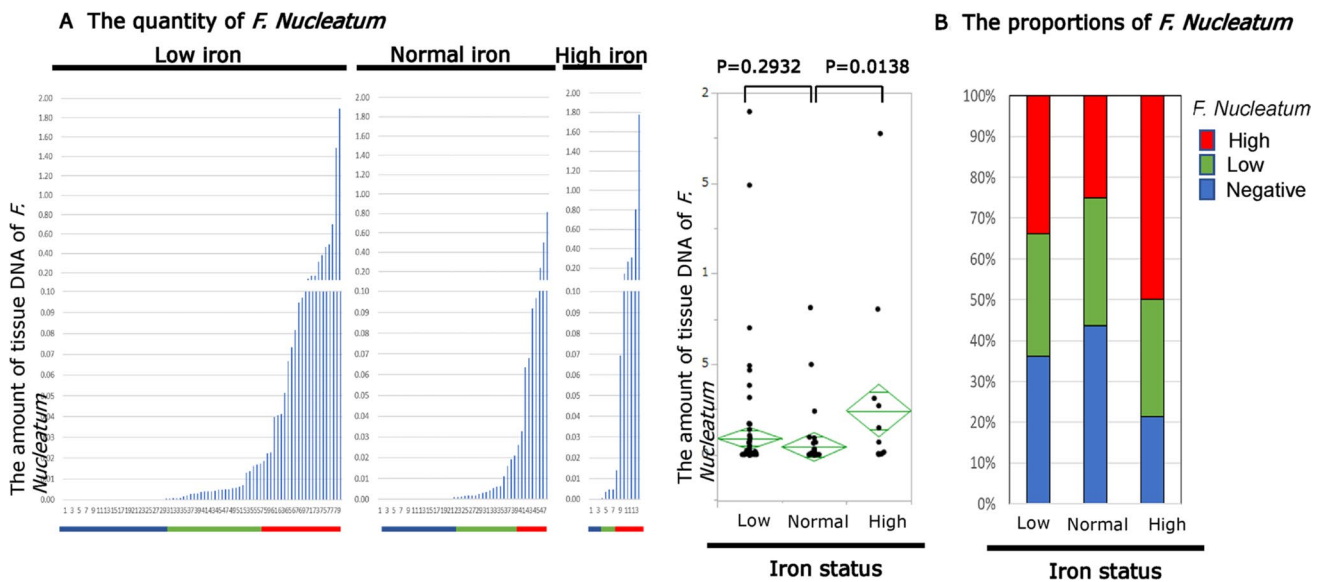


Fig. 2 Association of intratumor *Fusobacterium nucleatum* with iron status. The quantity of *F. nucleatum* within the primary tumor of the 142 patients was measured by real-time PCR (A). The 142 patients

were divided into three groups, *Fusobacterium nucleatum*-negative, -low and -high. Association between *F. nucleatum* and iron status is shown (B)

may improve prognosis of CRC patients with iron deficiency anemia. However, preoperative high iron was also associated with shorter OS. These data indicated that Stage II and III CRC patients who displayed both a lack and a surplus of iron had unfavorable prognoses, and further research on this finding will be required.

Preoperative anemia was significantly associated with shorter OS and disease-free survival in rectal cancer in a

meta-analysis [21]. In our study, anemia was associated with shorter CSS and OS in univariate analysis. The treatment of anemia encompasses three strategies: erythropoietin-stimulating agents, blood transfusion and iron supplementation. Recombinant human erythropoiesis-stimulating agents improve anemia in many studies [22–24]; however, a total of 13,933 patients with cancer in 53 trials were surveyed in a meta-analysis of randomized trials, and

erythropoiesis-stimulating agents increased mortality (HR 1.17, 95%CI 1.06–1.30) [25]. Erythropoietin-stimulating agents and blood transfusion are effective in increasing hemoglobin levels, but both modalities should be given with caution to cancer patients [26]. However, perioperative blood transfusion was also associated with unfavorable long-term prognosis and increased short-term complications after CRC surgery in another meta-analysis. A total of 174,036 patients in 36 trials were analyzed and perioperative blood transfusion was associated with shorter OS (HR 0.33, 95%CI 0.24–0.41, $P < 0.0001$) and shorter CSS (HR 0.34, 95%CI 0.21–0.47, $P < 0.0001$). Perioperative blood transfusion was associated with postoperative infectious complications, pulmonary complications, cardiac complications, anastomotic complications, reoperation and general complications [4].

Iron supplementation is another strategy for treatment of anemia. Clinical trials have revealed that iron supplementation improved short-term outcome in CRC patients who underwent surgery. Oral ferrous sulfate (200 mg twice daily for 2 weeks) or no iron therapy before surgery was compared in a randomized-controlled trial (RCT). The iron-supplemented group had higher hemoglobin than the non-iron-treated group (13.1 g/dl, range 9.6–17 g/dl versus 11.8 g/dl) at the time of admission to hospital [27]. Intravenous iron infusion, as a single 1000-mg dose of ferric carboxymaltose, was preoperatively administered in the outpatient clinic to CRC patients with anemia. Iron infusion was associated with increased hemoglobin levels and reduced allogeneic red blood cell transfusions use [28]. In other studies, intravenous iron could also increase hemoglobin level and reduce red blood cell transfusion [27, 29, 30]. Postoperative intravenous iron administration also improved the recovery of hemoglobin level at postoperative day 30 without increasing postoperative complication [31]. Iron supplementation has been found to reduce blood transfusion and improve short-term outcome in many studies, but the association between iron supplementation and long-term outcome remains unclear; 1000–2000 mg preoperative intravenous iron therapy had little effect on long-term OS or disease-free survival in CRC patients with anemia [32]. In our study, low iron was not associated with postoperative complication, although preoperative low iron was significantly associated with anemia and perioperative blood transfusion. Iron supplementation may therefore reduce perioperative blood transfusion.

Low iron levels were associated with shorter OS than were normal iron levels in the present study, as well as with both nutritional and inflammatory status. In turn, nutritional and inflammatory status were correlated with NLR and PLR, which were associated with prognosis in many types of cancers. Therefore, we performed survival analysis with these factors to test for confounding. However, low iron levels were independently associated with an unfavorable prognosis.

Iron status has been associated with cancer initiation and progression. Iron homeostasis is regulated via several mechanisms and may promote tumor growth or cell death. Mutations in APC are present in more than 80% of sporadic colon cancers, while mutations in β -catenin are present in approximately 10% of colon cancers [33]. Wnt signaling is activated by iron in APC-mutant malignant cell lines [34]. Cancer cells exhibit an increased dependence on iron compared with normal cells [35]. The mean concentration of iron in CRC (46.1 $\mu\text{g/g}$) was higher than in polyps (43.2 $\mu\text{g/g}$), as determined by the total-reflection X-ray fluorescence method [36]. In a case–control study within a prostate, lung, colorectal and ovarian cancer screening trial, patients with lower iron had a reduced risk of developing colorectal adenoma [37]. Higher TSAT or serum iron concentrations were associated with increased nonskin cancer risk and increased risk of cancer death in 1597 men and 1795 women [38]. The relative risk of CRC was 1.18 (95%CI 1.06–1.32) for patients in the highest category of heme iron intake compared with those in the lowest category [39]. Qualitative requirements for iron of normal and neoplastic cells are similar. Anemia can develop in states of iron deficiency, whereas iron excess increases oxidative stress in body tissues [33]. High TSAT results in elevated NTBI, which has unfavorable side effects at the highly interactive host–microbe interface of the human gastrointestinal tract. Microbial composition changes in the gut in response to increased luminal iron concentration [8]. Boyer E. et al. reported that significant correlations were found between TSAT and the proportions of microbiota when TSAT exceeded 45% [40]. NTBI was disappeared when TSAT was less than 35% [41]. We determined cut-off value as 40% in this study in accordance with these studies. In this study, intratumor *F. nucleatum* in CRC patients was associated with high iron. This result may indicate that microbial change is induced by NTBI. Lactoferrin is a secreted, iron-binding glycoprotein originally discovered as a component of milk, and daily intake of 3 g of lactoferrin has been suggested as a clinically beneficial adjunct to colorectal polyp extraction [42]. Morita et al. reported that *F. nucleatum* on the tongue was significantly less frequent in patients receiving lactoferrin and lactoperoxidase-containing tablets for 8 weeks than in those receiving placebo tablets [43].

The aim of iron supplementation in perioperative CRC patients is to improve anemia and decrease blood transfusion. However, the association between perioperative iron supplementation and long-term prognosis remained unclear. This study revealed that preoperative low iron was associated with shorter OS compared with normal iron in univariate and multivariate analysis. However, high iron was also associated with an unfavorable prognosis and surplus iron supplementation may lead to an unfavorable prognosis.

Therefore, iron supplementation should be performed in conjunction with monitoring TSAT.

This study had limitations. It was a retrospective study and 109 of the 436 CRC patients (24.9%) were not evaluated for their iron level before surgery. This potentially leads to selection bias. The supplied iron agents depended on the attending doctor, because the doctors had not participated in any trials involving iron support. The administration of iron supplementation could not be accurately monitored before and after surgery.

In conclusion, both low and high preoperative iron in patients who underwent CRC resection was identified as being associated with an unfavorable prognosis by univariate and multivariate analyses. Suppling iron to alleviate iron deficiency anemia, together with monitoring TSAT to avoid surplus iron, may improve the prognosis of patients who receive CRC resection.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10147-021-01995-9>.

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Declarations

Conflict of interest No author has any conflict of interest.

Ethical standards All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1964 and later versions. All patients gave informed consent for being included in the study.

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