High preoperative serum ferritin predicted poor prognosis in non-metastatic colorectal cancer

Hong Tingting, MD, Shen Di, MD, Chen Xiaoping, MD, Wu Xiaohong, MD, Hua Dong, MD, PhD.

ABSTRACT

الأهداف: للتحقق من صحة الدلالات المنذرة من متغيرات استقلاب الحديد في مصل الدم قبل الجراحة في مرضى سرطان القولون والمستقيم غير المنتشر الذين تلقوا العلاج الجراحي.

الطريقة: أجرينا دراسة استباقية في قسم جراحة الأورام، مستشفى ويكسي الرابع للناس، ويكسي، الصين خلال الفترة من مارس 2010م وسبتمبر 2013م. تم فحص علاقات متغيرات استقلاب الحديد في الدم مع المتغيرات الأخرى. تم تقييم أهمية النذير باستخدام منحنى كابلان ماير ونموذج انحدار مخاطر كوكس النسبية.

النتائج: اشتملت الدراسة على ٥١٤ مريض مؤهلين لتحليلها. وكانت مستويات المعلمات الثلاث لاستقلاب الحديد مترابطة. ارتبط مستوى الهيموجلوبين بشكل إيجابي مع الحديد في الدم وترانسفيرين، وارتبط سلبياً مع الفيريتين. مقارنة مع الغزو شبه العصبية (PNI) المرضى السلبية، كان مستوى الحديد للمرضى PNI إيجابي في مصل الدم (p=0.03) ومستويات الفيريتين (p=0.01) بالمقارنة مع المرضى الذين يعانون من أدنى من مستوى الربع من الفيريتين، بينما المرضى الذين يعانون أعلى من مستوى الربع من الفيريتين 2.21 (95% CI: 1.18-4.14) زيادة خطر الوفاة لديهم أضعاف في نموذج الانحدار المنفرد و2.56 (95% CI: 1.10-5.96) في نموذج انحدار مخاطر كوكس المتعدد النسبي. عندما مستويات مختلفة حسب مراحل TNM، إلا أنه في المرحلة الثالثة للمرضى كان مستوى الفيريتين في المصل إنذار مهم إحصائي.

الخاتمة: أظهرت الدراسة أن مستوى فيريتين المصل قبل الجراحة يعد عامل خطر مستقل سلبي في سرطان القولون والمستقيم غير المنتشر.

Objectives: To validate the prognostic significance of preoperative serum iron metabolism parameters in non-metastatic colorectal cancer patients treated with curative resection.

Methods: We conducted a prospective cohort study in the Department of Surgical Oncology, WuXi 4th People's Hospital, WuxiChina, between March 2010 and September 2013. The relationships of serum iron metabolism parameters with other variables were examined. The prognostic significance was evaluated using the Kaplan Meier curve and Cox proportional hazards regression model.

Results: Five hundred and fourteen patients were eligible for analysis. The levels of the 3 iron metabolism parameters were interdependent. Hemoglobin level was positively correlated with serum iron and transferrin, and was negatively correlated with ferritin. Compared with peri-neural invasion (PNI)-negative patients, PNI-positive patients had higher serum iron (p=0.03) and ferritin levels (p=0.01). Compared with patients with the lowest quartile level of ferritin, patients with the highest quartile level of ferritin had a 2.21 (95% CI: 1.18-4.14) fold increased mortality risk in the univariate and 2.56 (95% CI: 1.10-5.96) in the multivariate Cox proportional hazards models. When stratified by TNM stages, it was only in stage III patients that serum ferritin remained statistically prognostically significant.

Conclusions: Preoperative serum ferritin appeared as an independent adverse risk factor in non-metastatic colorectal cancer.

Saudi Med J 2017; Vol. 38 (3): 268-275
doi: 10.15537/smj.2017.3.16110

From the Department of Medical Oncology, The Second Affiliated Hospital of Soochow University, Suzhou, People Republic of China.

Received 23rd August 2016. Accepted 21st November 2016.

Address correspondence and reprint request to: Dr. Hua Dong, Department of Medical Oncology, The Second Affiliated Hospital of Soochow University, Suzhou, People Republic of China. E-mail: xxlsmile00@gmail.com

Iron is an essential nutrient for life. Cancer cells have altered cellular iron metabolism, exhibit enhanced dependence on cellular iron for proliferation, dramatically more susceptible to iron depletion than their normal counterparts, a phenomenon termed iron addiction.[1] Iron chelators thus appeared as an attractive anti-neoplastic treatment.[2,3] Ferritin is the primary iron-binding protein that exists both intracellularly and extracellularly. Recent evidence indicated that ferritin might play a role in cancer proliferation, angiogenesis, immunosuppression,[4] and therapeutic resistance.[5] The associations of serum iron metabolism parameters and cancers have long been studied. As early as 1976, it was observed that breast cancer patients with higher serum ferritin levels appeared to have higher tumor recurrence rates. In metastatic breast cancer, elevated serum ferritin was associated with reduced progression-free survival and overall survival.[6] A higher level of serum ferritin has been demonstrated as a poor prognostic factor in a variety of other malignancies, including lung cancer,[7-9] pancreatic cancer,[10,11] multiple myeloma,[12] hepatocellular carcinoma,[13] stage IV neuroblastoma,[14] and colorectal cancer.[15] Nevertheless, the evidence was not always consistent. Serum ferritin was not prognostic in melanoma patients treated with adjuvant interferon-alfa,[16] nor in operable breast cancer.[17] Population based epidemiological research also did not support the association of serum ferritin with cancer mortality either.[18,19] It seems that the prognostic significance of serum iron metabolism parameters is dependent on both tumor-type and clinical context.

In colorectal cancer, high baseline ferritin was shown as an independent poor prognostic factor in metastatic colorectal cancer,[15] but was not prognostic in stage I-III colorectal cancer.[20,21] For further clarification, we conducted a prospective cohort study to validate the significance of pre-operative serum iron metabolism parameters on the outcome of non- metastatic colorectal cancer treated with curative surgery.

Methods. Between March 2010 and September 2013, all patients with newly diagnosed colorectal cancer scheduled to have radical surgery at the Department of Surgical Oncology of Wuxi 4th People's Hospital, Jiangsu, China were invited to participate in this study. The study was approved by the Hospital's Ethics Review Board. Patients were first informed on the purpose and process of the study. Individuals who agreed to participate, signed the written informed consents form and enrolled. The study was performed according to the principles of the Helsinki Declaration.

Patients with the following conditions were excluded: 1) patients who had undergone neo-adjuvant treatments, 2) who had metastatic diseases at diagnosis, 3) who had previously been diagnosed with other malignancy including colorectal cancer at different sites, and 4) who had multiple synchronous primary colorectal cancers. Before surgery, enrolled patients had their serum iron, ferritin and transferrin tested as required. After surgery, patients who had metastatic diseases found intraoperatively or who died within one month after surgery were further excluded.

The following information was individually collected from medical charts and recorded: patient's name, age, gender, hemoglobin (Hb) level, pathological characteristics including site of disease, histology, depth of primary tumor invasion, number of lymph nodes with metastases (LNM), histological grade, number of total lymph nodes sampled, number of tumor deposits (TDs), status of peri-neural invasion (PNI) and lympho-vascular invasion (LVI), tumor necrosis and adjuvant treatment. Patients were staged according to the 7th edition of American Joint Committee on Cancer (AJCC) TNM staging manual.

All patients were routinely followed up every 3 months by either telephone interviews or scheduled physician visits until their death. The final follow up was conducted on June 2016.

Laboratory measurement. Serum iron hemostasis parameters, including iron, ferritin, and transferrin, were tested preoperatively after patients signed the informed consent forms. Briefly, subjects fasted at least for 8 hours before sampling, and 4-5 ml blood was collected in an ethylenediaminetetraacetic acid tube. Ferritin was measured by a chemiluminescence immunoassay (Roche Module E170 Chemiluminescence Immunoassay System, Rotkreuz, Switzerland). Transferrin and iron were measured by turbidimetric immunoassay and colorimetric method, respectively (Roche Modular P800 Automatic Biochemistry Analyzer, Rotkreuz, Switzerland).

Statistical analysis. The Spearman rank correlation test was used to analyze the associations among continuous variables (age, Hb, CRP, iron, ferritin and transferrin). Since the distribution of serum iron, ferritin, and transferrin were not normal, the relationships of the 3 parameters with other categorical variables were examined with Spearman rank correlation tests. Levels

Disclosure. Authors have no conflict of interests, and the work was not supported or funded by any drug company.

of iron metabolism parameters were modeled as 4 equal-sized quartiles based on the distribution among the whole cohort. The Kaplan Meier curve was used to estimate survival probability, and the log-rank test was used to detect the differences. The assumption of proportionality was checked by including time dependent covariates in the model. Univariate and multivariate Cox proportional hazards models were used to test the independent prognostic significance of each variable. To achieve better prediction and to keep the variables continuous, the multivariate fractional polynominals (MFP) approach was used to model any non-linearity effects and avoid categorization. Subgroup analysis and tests of interaction were used to detect potential confounding effects.

All hypothesis tests were 2-sided with $p<0.05$ as statistically significant. All statistical analysis was performed using STATA (STATACorp. LP, College Station, TX, USA) version 10.

Results. A total of 576 patients were initially screened for eligibility for this study. A total of 54 patients had neo-adjuvant treatment, 53 of whom had rectal cancer and one had colon cancer. Fifty-four patients had neo-adjuvant chemotherapy, and 26 patients had neo-adjuvant radiation. One rectal cancer patient had intra-artery neo-adjuvant chemotherapy. Twelve patients had a previous history of other malignancy, including 6 patients with colorectal cancer at different sites, 3 patients with breast cancer, one patient with cervical cancer, one patient with endometrial cancer, and one patient with prostate cancer. Five patients were found intraoperatively to have intra-abdominal metastasis, and 4 patients had metastatic diseases found preoperatively. Two patients died within 30 days postoperatively. Twelve patients were lost to follow up. The final follow up was conducted on June 2016. A final total of 514 patients were eligible for analysis. With a median follow up period of 52 months, a total of 150 cancer related deaths (26.1%) occurred.

The demographic and biochemical characteristics for the cohort are listed in **Table 1**. Most patients had normal serum iron, ferritin, and transferrin. Seventy-three percent of patients had normal iron levels, 25% of patients had abnormally low iron levels, and only 2% of patients had abnormally high iron levels. Seventy-nine percent of patients had normal ferritin levels, 18% of patients had abnormally low ferritin, and only 3% had abnormally high ferritin. Seventy-five percent of patients had normal transferrin levels and 24% of patients had abnormally low transferrin levels. The pathological characteristics are listed in **Table 2**. Most patients had stage IIIB disease, followed by stage IIA disease. Approximately 90% of patients had 12 or more lymph nodes sampled. Relationships of serum iron, ferritin, and transferrin with other variables. The levels of the 3 iron metabolism parameters were interdependent. Serum iron was inversely correlated with serum transferrin (rho=-0.14, $p=0.02$). Serum transferrin was inversely correlated with ferritin (rho=-0.56, $p<0.001$). Serum ferritin was positively correlated with serum iron (rho=0.57, $p<0.001$). The data reported in **Tables 3 & 4** describe the relationships of the iron metabolism parameters with other variables. Age was inversely correlated with transferrin level and had a borderline positive correlation with ferritin level. Compared with male patients, female patients had higher serum iron levels, higher ferritin levels, and lower transferrin levels. Serum CRP level was inversely correlated with serum iron level. Hemoglobin showed positive correlation with serum iron and ferrintin, and a negative correlation with transferrin. Serum iron level was negatively correlated with T stage. Compared to PNI negative patients, PNI positive patients had both higher serum iron and ferritin levels.

Table 1 - Demographic and biochemical characteristics of 576 patients initially screened for eligibility.

Characteristics	n	(%)
Age, years (mean± SD)	63.08 ± 11.82	
Gender		
Male	269	(52.3)
Female	245	(47.7)
Iron (umol/L)		
High	11	(2.1)
Normal	368	(73.2)
Low	124	(24.7)
Ferritin (ng/mL)		
High	13	(3.2)
Normal	321	(78.7)
Low	74	(18.1)
Transferrin(G/L)		
High	2	(0.6)
Normal	231	(75.0)
Low	75	(24.4)
*Severity of anemia**		
0	327	(63.6)
1	91	(17.7)
2	55	(10.7)
3	29	(5.7)
4	12	(2.3)
CRP (mmol/L)		
High	114	(22.7)
Normal	388	(77.3)

*Severity of anemia was graded according to the National Cancer Institute (NCI) criteria: grade 0: normal values (for men: >12 g/L, for women: >11 g/L); grade 1: 10 g/L-normal values; grade 2: 8-10 g/L; grade 3: 6.5-7.9 g/L; grade 4: <6.5 g/L. CRP - C-reactive protein

Table 2 - Pathological characteristics of the cohort.

Characteristics	n	(%)
T		
T1	5	(1.0)
T2	137	(26.7)
T3	222	(43.1)
T4a	144	(28.0)
T4b	6	(1.2)
N		
N0	286	(55.6)
N1a	59	(11.5)
N1b	82	(16.0)
N1c	24	(4.7)
N2a	48	(9.3)
N2b	15	(2.9)
Staging		
I	103	(20.0)
IIA	126	(24.5)
IIB	53	(10.3)
IIC	4	(0.8)
IIIA	33	(6.4)
IIIB	161	(31.3)
IIIC	34	(6.6)
Grade		
I	104	(21.8)
II	265	(55.7)
III	107	(22.5)
Tumor deposits		
Positive	52	(10.1)
Negative	462	(89.9)
Number of lymph nodes sampled		
<12	51	(9.9)
≥12	463	(90.1)
LVI		
Positive	48	(9.4)
Negative	456	(88.9)
indeterminate	9	(1.7)
PNI		
Positive	29	(5.6)
Negative	474	(92.2)
Indeterminate	11	(2.1)
Necrosis		
Positive	95	(18.5)
Negative	419	(81.5)
Adjuvant treatment		
Yes	192	(37.4)
No	322	(62.6)

LVI - lympho-vascular invasion, PNI - peri-neural invasion

Table 3 - Relationships of serum iron, ferritin, and transferrin with patients' demographic and biochemical characteristics.

Characteristics	Iron Rho (*p*-value)	Ferritin Rho (*p*-value)	Transferrin Rho (*p*-value)
Age[a]	-0.06 (0.177)	0.10 (0.04)	-0.27 (<0.001)
Gender			
Male/Female	-0.21 (<0.001)	-0.28 (<0.001)	0.17 (<0.001)
Hemoglobin	0.70 (<0.001)	0.61 (<0.001)	-0.16 (<0.01)
CRP	-0.29 (<0.001)	-0.07 (0.19)	0.04 (0.51)

CRP - C-reactive protein

Prognostic associations. The effects of the predictor variables were constant over time, and there was a linear relationship between overall survival and predictor variables (data not shown). Univariate analysis identified T stage (p=0.003), N stage (p<0.001), TNM stage (p<0.001), histological grade (p=0.01), status of TDs (HR=2.79, 95% CI:1.79-4.36, p<0.001), status of PNI (HR=2.92, 95% CI:1.62-5.25, p=0.002), status of LVI (HR:2.09, 95% CI:1.25-3.51, p=0.01), and grade 4 anemia (HR=2.85, 95% CI: 1.24-6.59) as significant prognostic factors. Necrosis (p=0.67), adjuvant treatment (p=0.21) and number of total lymph nodes sampled (p=0.40), CRP levels (p=0.11) were not predictive of patient survival. Multivariate analysis showed T stage, N stage, TNM stage, status of TDs (p=0.001), PNI (p=0.01), and grade 4 anemia (p=0.01) to be independent prognostic factors for this cohort.

Fitted as categorical variables, neither serum iron nor transferrin was predictive of overall survival by either univariate or multivariate Cox proportional hazards regression analysis (**Table 5**). The lack of separation of the Kaplan-Meier curves and log-rank test confirmed the lack of such an association (**Figure 1**). Multivariate fractional polynominals analysis also did not detect any significant associations of iron metabolism parameters with overall survival either (iron: p=0.29, transferrin: p=0.35, ferritin: p=0.22).

Compared to patients with the lowest quartile level of ferritin, patients with the highest quartile level of ferritin had 2.21 (95% CI: 1.18-4.14) fold increased mortality risk in the univariate and 2.56 (95% CI: 1.10-5.96), in the multivariate model. Because serum ferritin was significantly associated with PNI status and anemic status, tests of interaction were performed to detect possible confounding effects. Tests of interaction were significant for ferritin with PNI status (p=0.01), anemic status (p=0.01) and TNM stages (p<0.001). When stratified by TNM stages, serum ferritin remained statistically prognostic significant (p=0.02) only in stage III patients (**Table 6**). Ferritin levels were not prognostic of PNI negative patients (data not shown), and the sample of PNI positive patients was too small to perform analysis.

Discussion. The study is the first to prospectively investigate the prognostic significance of serum iron metabolism parameters exclusively in non-metastatic colorectal cancer treated with curative surgery. It was

Table 4 - Relationships of serum iron, ferritin, and transferrin of 576 patients screened for eligibility.

Characteristics	Iron Rho (p value)	Ferritin Rho (p value)	Transferrin Rho (p value)
T			
T1/T2/T3/T4a/T4b	-0.14 (<0.001)	-0.07 (0.18)	0.02 (0.69)
N			
N0/N1a/N1b/N1c/N2a/N2b	0.02 (0.58)	0.06 (0.20)	-0.01 (0.86)
Staging			
I/IIA/IIB/IIC/IIIA/IIIB/IIIC	-0.03 (0.54)	0.02 (0.62)	-0.00 (0.99)
Grade			
I/II/III	-0.02 (0.66)	0.002 (0.96)	0.11 (0.06)
Tumor deposits			
Negative/positive	-0.07 (0.10)	0.03 (0.49)	0.11 (0.05)
LVI			
Negative/positive	0.07 (0.10)	0.08 (0.10)	-0.05 (0.40)
PNI			
Negative/positive	0.10 (0.03)	0.12 (0.01)	-0.10 (0.09)

Kruskal-wallis test and Mann-whitney U test were used to detect differences in the iron metabolism parameters between subgroups of categorical variables. If significant, the Spearman rank correlation coefficient was further calculated. LVI - lympho-vascular invasion, PNI - peri-neural invasion

observed that higher serum ferritin was an independent poor prognostic factor. The underlying biological mechanism of the prognostic significance of serum ferritin is quite intriguing. The intracellular functions of ferritin are for the most part well-characterized and believed to be protective of cancer cells,[22,23] but the significance of serum (extracellular) ferritin in the host is poorly understood.[4] Neoplasm was initially believed to be the source of increased serum ferritin. Previous research demonstrated that nude mice bearing human neuroblastoma xenografts had human ferritin in the mice sera. Serum ferritin levels became normal as tumors entered remission. Cancer patients with metastasis had higher serum ferritin than patients

Table 5 - Univariate and multivariate prognostic analysis of iron, ferritin, and transferrin of 576 patients screened for eligibility.

Iron metabolism parameters	N	Univariate HR[a] (95%CI)	Multivariate HR[b] (95%CI)
Iron (umol/L)			
Q1 (0.6-6.8)	126	1.00	1.00
Q2 (6.9-11.6)	129	0.83(0.50-1.39)	0.88(0.50-1.55)
Q3 (11.7-16.3)	123	0.89(0.53-1.47)	0.85(0.48-1.52)
Q4 (16.4-44.2)	125	0.72(0.42-1.23)	0.67(0.35-1.27)
		$P_{trend =}$ 0.68	
Transferrin (g/L)			
Q1 (1.64-2.52)	76	1.00	1.00
Q2 (2.53-2.86)	81	1.30(0.69-2.42)	1.09(0.53-2.26)
Q3 (2.87- 3.19)	74	0.56(0.26-1.20)	0.68(0.29-1.60)
Q4 (3.2-4.88)	77	0.79(0.40-1.56)	0.64(0.27-1.50)
		$P_{trend =}$ 0.10	
Ferritin (ng/mL)			
Q1 (1.32 - 21.99)	102	1.00	1.00
Q2 (22.61 - 73.11)	102	1.5(0.81-3.00)	1.93(0.91-4.12)
Q3 (73.64 - 149)	103	1.20(0.61-2.39)	1.40(0.64-3.08)
Q4 (150 - 641)	101	2.21(1.18-4.14)	2.56(1.10-5.96)
		$P_{trend =}$ 0.06	

HR - hazard ratio, 95%CI - 95% confidence interval, [a]univariate cox proportional hazards regression analysis. [b]multivariate cox proportional hazards regression analysis adjusted by age (continuous), gender (female / male), T (T1 / T2 / T3 / T4a / T4b), N (N0 / N1a / N1b / N1c / N2a / N2b), stage (I / IIA / IIB / IIC / IIIA / IIIB / IIIC), histological grade (I / II / III), tumor deposits (continuous), LVI (positive / negative), PNI (positive / negative) , adjuvant treatment (yes/no) and anemic status.

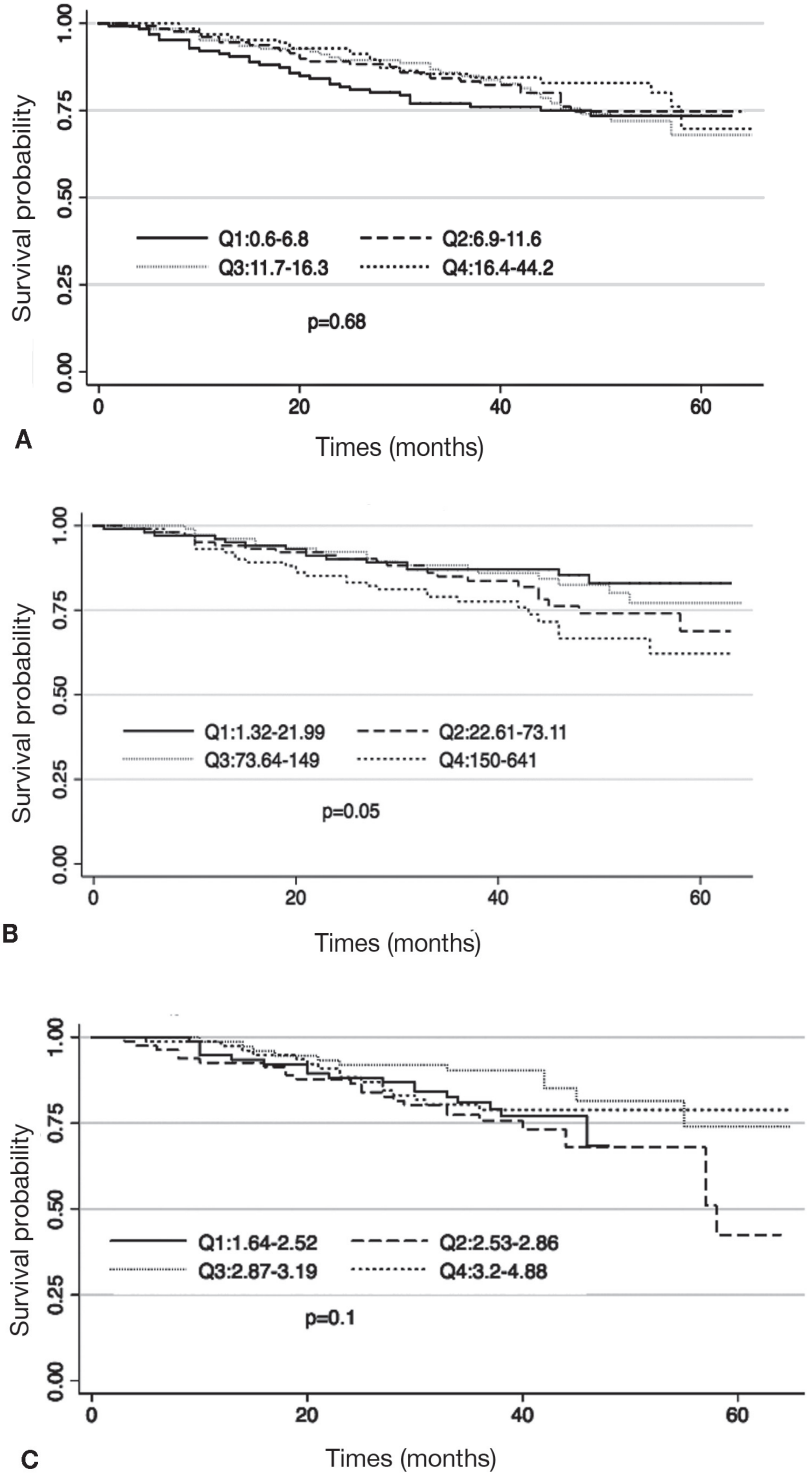

Figure 1 - Kaplan-Meier curve of colorectal cancer survival by quartiles of A) iron, B) transferrin, and C) ferritin

Table 6 - Univariate prognostic analysis of ferritin stratified by TNM stages and status of anemia status among 411 patients.

Ferritin (ng/mL)	Hazard ratio (95%CI)		
	Stage I	Stage II	Stage III
Q1 (1.32 - 21.99)	1.00	1.00	1.00
Q2 (22.61 - 73.11)	1.03(0.14-7.36)	0.50(0.13-2.00)	2.61(1.08-6.32)
Q3 (73.64 - 149)	0.63(0.09-4.46)	0.91(0.26-3.21)	1.63(0.64-4.16)
Q4 (150 - 641)	1.24(0.20-7.54)	0.98(0.27-3.46)	3.21(1.35-7.56)
	$P_{trend =}$ 0.89	$P_{trend =}$ 0.74	$P_{trend =}$ 0.02
	Anemic		Non-anemic
Q1 (1.32 - 21.99)	1.00		1.00
Q2 (22.61 - 73.11)	2.32(1.01-5.37)		2.53(0.33-19.37)
Q3 (73.64 - 149)	2.00(0.77-5.21)		2.04(0.26-15.67)
Q4 (150 - 641)	2.74(0.99-7.65)		4.34(0.59-21.18)
	$P_{trend =}$ 0.11		$P_{trend =}$ 0.08

TNM - classification of malignant tumors

without metastasis and had lower serum ferritin after surgery.[24] Nevertheless, the level of colon tissue ferritin was not related to the systemic parameters of iron metabolism.[25] The expression levels of ferritin heavy chain (FHC) were gradually decreased from normal colon tissues and non-LNM colorectal cancer to LNM colorectal cancer.[26] If it is not directly from tumor cells other sources of elevated serum ferritin must be considered. Indeed, studies in breast cancer have indicated that the rise in serum ferritin might be attributable to stromal reaction rather than to tumor synthesis. It was validated that ferritin light chain (FLT) was stored in tumor-associated macrophages (TAMs) with M2-like phenotype and an independent prognostic marker.[27] In cell culture, macrophages, but not breast cancer cells, were capable of ferritin secretion. The secreted ferritin stimulated the proliferation of MCF7 and T47D.[28] Additionally, ferritin has been shown to antagonize the anti-angiogenic effect of HKa and enhance the migration, assembly, and survival of HKa-treated endothelial cells.[29] We postulate that elevated serum ferritin mainly originates from TAMs. Tumor-associated macrophages-derived ferritin then promots tumorigenesis and angiogenesis.

Study limitations. The data for this study were not robust enough to clarify the confounding effects of PNI status, TNM stages, and anemic status. In fact, the results of the non-prespecified subgroup analysis were error prone, either for false-positivity due to multiplicity, or for false-negativity due to chance and lack of power. Even with significant interaction tests, readers are advised to base their interpretation of the findings on biological plausibility, the pre-specification of analysis, and the statistical strength. In this study, the sample was too small to perform subgroup analysis on PNI-positive patients. The statistics were not very strong for their interaction tests of PNI status and anemic status. Meanwhile, biological plausibility was absent for such interactions. Thus, caution is indicated when interpreting these results. Second, there are always difficulties in modeling continuous predictors. In fact, we believe the discrepancy of the results between our study and the other studies[20,21] can be partly attributed to the different modeling of continuous variables. Categorizing continuous variables will reduce the statistical power. In this case, we used the MFP approach to model continuous variables, which failed to detect significance. Although inappropriate cutoff points may provide spurious results, categorization is more clinically meaningful, with easy application. We employed quarterly categorization, which is commonly applied in the literature. In addition, the patient population from Clemens's study seemed different from ours, since the overall survival is slightly lower in this study, which we believed might be related to the more advanced stages in our study. Third, since the protocol of this present study did not mandate the collection of recurrence information and most patients were followed up with telephone interviews, recurrence information was absent. For post-operative cancer patients, prognostic factors predicting recurrence may be more clinically meaningful than predicting overall survival.

In summary, elevated pre-operative serum ferritin appeared as an independent mortality risk factor in non-metastatic colorectal cancer, especially in stage III patients. While the interactions of serum ferritin with PNI status, anemic status, and TNM stages were statistically significant, caution is advised in interpreting

the results. Further studies are warranted to investigate those possible associations.

References

1. Manz DH, Blanchette NL, Paul BT, Torti FM, Torti SV. Iron and cancer: recent insights. ***Annals of the New York Academy of Sciences*** 2016; 1368: 149-161.
2. Urano S, Ohara T, Noma K, Katsube R, Ninomiya T, Tomono Y, et al. Iron depletion enhances the effect of sorafenib in hepatocarcinoma. ***Cancer Biol Ther*** 2016; 17: 648-656.
3. Li Z, Tanaka H, Galiano F, Glass J. Anticancer activity of the iron facilitator LS081. ***J Exp Clin Cancer Res*** 2011; 30: 34.
4. Alkhateeb AA, Connor JR. The significance of ferritin in cancer: anti-oxidation, inflammation and tumorigenesis. ***Biochim Biophys Acta*** 2013; 1836: 245-254.
5. Chekhun VF, Lukyanova NY, Burlaka CA, Bezdenezhnykh NA, Shpyleva SI, Tryndyak VP, et al. Iron metabolism disturbances in the MCF-7 human breast cancer cells with acquired resistance to doxorubicin and cisplatin. ***Int J Oncol*** 2013; 43: 1481-1486.
6. Alkhateeb AA, Leitzel K, Ali SM, Campbell-Baird C, Evans M, Fuchs EM, et al. Elevation in inflammatory serum biomarkers predicts response to trastuzumab-containing therapy. ***PloS One*** 2012; 7: e51379.
7. Carpagnano GE, Lacedonia D, Palladino GP, Koutelou A, Martinelli D, Orlando S, et al. Could exhaled ferritin and SOD be used as markers for lung cancer and prognosis prediction purposes? ***Eur J Clin Invest*** 2012; 42: 478-486.
8. Shi HB, Li XD, Jiang JT, Zhao WQ, Ji M, Wu CP. Serum ferritin is elevated in advanced non-small cell lung cancer patients and is associated with efficacy of platinum-based chemotherapy. ***J Cancer Res Ther*** 2014; 10: 681-685.
9. Ji M, Li XD, Shi HB, Ning ZH, Zhao WQ, Wang Q, et al. Clinical significance of serum ferritin in elderly patients with primary lung carcinoma. ***Tumour Biol*** 2014; 35: 10195-10199.
10. Kalousova M, Krechler T, Jachymova M, Kubena AA, Zak A, Zima T. Ferritin as an independent mortality predictor in patients with pancreas cancer. Results of a pilot study. ***Tumour Biol*** 2012; 33: 1695-1700.
11. Wang SL, Cao S, Wu R, Chi F, Tang MY, Jin XY, et al. Serum ferritin predicted prognosis in patients with locally advanced pancreatic cancer. ***Future Oncol*** 2015; 11: 2905-2910.
12. Strasser-Weippl K, Ludwig H. Ferritin as prognostic marker in multiple myeloma patients undergoing autologous transplantation. ***Leuk Lymphoma*** 2014; 55: 2520-2524.
13. Facciorusso A, Del Prete V, Antonino M, Neve V, Crucinio N, Di Leo A, et al. Serum ferritin as a new prognostic factor in hepatocellular carcinoma patients treated with radiofrequency ablation. ***J Gastroenterol Hepatol*** 2014; 29: 1905-1910.
14. Morgenstern DA, London WB, Stephens D, Volchenboum SL, Simon T, Nakagawara A, et al. Prognostic significance of pattern and burden of metastatic disease in patients with stage 4 neuroblastoma: A study from the International Neuroblastoma Risk Group database. ***Eur J Cancer*** 2016; 65: 1-10.
15. Lee S, Song A, Eo W. Serum ferritin as a prognostic biomarker for survival in relapsed or refractory metastatic colorectal cancer. ***J Cancer*** 2016; 7: 957-964.
16. Bouwhuis MG, Collette S, Suciu S, de Groot ER, Kruit WH, Ten Hagen TL, et al. Changes of ferritin and CRP levels in melanoma patients treated with adjuvant interferon-alpha (EORTC 18952) and prognostic value on treatment outcome. ***Melanoma Research*** 2011; 21: 344-351.
17. Petekkaya I, Aksoy S, Roach EC, Okoh AK, Gecmez G, Gezgen G, et al. Impact of inflammatory markers on the prognosis of patients with operable breast cancer. ***J BUON*** 2014; 19: 673-680.
18. Kim KS, Son HG, Hong NS, Lee DH. Associations of serum ferritin and transferrin % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National Health and Nutrition Examination Survey follow-up study. ***J Prev Med Public Health*** 2012; 45: 196-203.
19. Chua AC, Knuiman MW, Trinder D, Divitini ML, Olynyk JK. Higher concentrations of serum iron and transferrin saturation but not serum ferritin are associated with cancer outcomes. ***Am J Clin Nutr*** 2016; 104: 736-742.
20. Giessen-Jung C, Nagel D, Glas M, Spelsberg F, Lau-Werner U, Modest DP, et al. Preoperative serum markers for individual patient prognosis in stage I-III colon cancer. ***Tumour Biol*** 2015; 36: 7897-7906.
21. Giessen C, Nagel D, Glas M, Spelsberg F, Lau-Werner U, Modest DP, et al. Evaluation of preoperative serum markers for individual patient prognosis in stage I-III rectal cancer. ***Tumour Biol*** 2014; 35: 10237-10248.
22. Buranrat B, Connor JR. Cytoprotective effects of ferritin on doxorubicin-induced breast cancer cell death. ***Oncology Reports*** 2015; 34: 2790-2796.
23. Chekhun SV, Lukyanova NY, Shvets YV, Burlaka AP, Buchinska LG. Significance of ferritin expression in formation of malignant phenotype of human breast cancer cells. ***Exp Oncol*** 2014; 36: 179-183.
24. Usnarska-Zubkiewicz L, Strutynska-Karpinska M, Zubkiewicz-Kucharska A, Zarebski P, Grabowski K. Soluble urokinase-type plasminogen activator receptor and ferritin concentration in patients with advanced alimentary tract carcinoma. Relationship to localization, surgical treatment and the stage of the disease--preliminary report. ***Adv Clin Exp Med*** 2014; 23: 959-967.
25. Prutki M, Poljak-Blazi M, Jakopovic M, Tomas D, Stipancic I, Zarkovic N. Altered iron metabolism, transferrin receptor 1 and ferritin in patients with colon cancer. ***Cancer Letters*** 2006; 238: 188-196.
26. Ma Y, Zhao M, Zhong J, Shi L, Luo Q, Liu J, et al. Proteomic profiling of proteins associated with lymph node metastasis in colorectal cancer. ***J Cell Biochem*** 2010; 110: 1512-1519.
27. Jezequel P, Campion L, Spyratos F, Loussouarn D, Campone M, Guerin-Charbonnel C, et al. Validation of tumor-associated macrophage ferritin light chain as a prognostic biomarker in node-negative breast cancer tumors: A multicentric 2004 national PHRC study. ***Int J Cancer*** 2012; 131: 426-437.
28. Alkhateeb AA, Han B, Connor JR. Ferritin stimulates breast cancer cells through an iron-independent mechanism and is localized within tumor-associated macrophages. ***Breast Cancer Res Treat*** 2013; 137: 733-744.
29. Tesfay L, Huhn AJ, Hatcher H, Torti FM, Torti SV. Ferritin blocks inhibitory effects of two-chain high molecular weight kininogen (HKa) on adhesion and survival signaling in endothelial cells. ***PloS One*** 2012; 7: e40030.