

Associations of reticulocyte hemoglobin equivalent with erythrocyte indices and serum ferritin in anemic patients with hepatocellular carcinoma: A retrospective study

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Abstract. Cancer-related anemia is common in patients with hepatocellular carcinoma (HCC), and its pathogenesis is complex due to the coexistence of chronic liver disease, portal hypertension, hypersplenism, inflammation, nutritional disorders and tumor-related factors. The present retrospective study characterized anemia morphology in 96 anemic patients with HCC before initiation of their first antitumor therapy, and examined the associations of reticulocyte hemoglobin equivalent (RET-He) with erythrocyte indices and serum ferritin (SF), with a focus on whether RET-He and SF displayed distinct patterns of association with hematological parameters. The data collected included sex, age, Barcelona Clinic Liver Cancer stage, Child-Pugh grade, splenomegaly/hypersplenism, hemoglobin (Hb) concentration, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), MCH concentration (MCHC), absolute reticulocyte count (RET#), RET-He and SF. Patients were compared by anemia severity, morphological type and RET-He level, and Spearman's correlation was used to assess relationships between RET-He and other parameters. Mild, moderate and severe anemia occurred in 41 (42.71%), 52 (54.17%) and 3 (3.13%) patients, respectively. Macrocytic, normocytic, simple microcytic and microcytic hypochromic anemia were found in 29 (30.21%), 40 (41.67%), 5 (5.21%) and 22 (22.92%) patients, respectively. A total of 26 patients (27.08%) had RET-He ≤ 29 pg, and the median SF was 248.5 ng/ml

(interquartile range, 146.8-346.0 ng/ml). Compared with mild anemia, moderate-to-severe anemia was associated with lower MCV, MCH, MCHC and RET-He, and higher proportions of RET-He ≤ 29 pg and microcytic hypochromic anemia (all $P < 0.05$). RET-He was lowest in the microcytic group ($P < 0.001$). Patients with RET-He ≤ 29 pg had lower Hb concentration, MCV, MCH, MCHC and RET# (all $P < 0.05$). Spearman's analysis indicated that RET-He was positively correlated with Hb concentration, MCV, MCH, MCHC and RET# (all $P < 0.05$), but not with SF ($P = 0.408$). In conclusion, anemia in patients with HCC demonstrated heterogeneous morphological patterns, and microcytic anemia was associated with lower RET-He levels. RET-He was associated with erythrocyte indices related to hemoglobinization, whereas no such association was observed with SF. The dissociation between RET-He and SF suggests that these markers may reflect different aspects of iron metabolism and erythropoiesis in HCC. RET-He may offer complementary information on erythroid hemoglobinization not directly captured by SF, but its clinical utility requires further validation. SF should be interpreted cautiously in the context of chronic liver disease, inflammation and tumor-related factors.

Introduction

Cancer-related anemia is common in patients with malignant tumors and is associated with impaired performance status, reduced quality of life and lower tolerance to antitumor treatment (1,2). Hepatocellular carcinoma (HCC) usually develops in the setting of chronic liver disease and cirrhosis, and anemia in these patients is influenced by both tumor- and liver-related factors. In a recent cohort of 1,262 patients with HCC, anemia was present in 44.0% of patients and was associated with impaired liver function, portal hypertension, more advanced Barcelona Clinic Liver Cancer (BCLC) stage (3), systemic inflammation and shorter overall survival time (4). These findings indicate the clinical relevance of characterizing anemia in patients with HCC before antitumor treatment.

The pathogenesis of anemia in HCC is multifactorial. In addition to tumor-related inflammation and nutritional deficiencies, cirrhosis and portal hypertension may contribute to anemia through chronic gastrointestinal blood loss and hypersplenism (5-7). Gastroesophageal varices and portal

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Abbreviations: HCC, hepatocellular carcinoma; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET-He, reticulocyte hemoglobin equivalent; SF, serum ferritin; BCLC, Barcelona Clinic Liver Cancer

Key words: HCC, anemia, morphology, RET-He, ferritin

hypertensive gastropathy may cause acute or chronic gastrointestinal blood loss, while splenomegaly and hypersplenism may increase splenic sequestration and destruction of red blood cells (6,7). Hepatic dysfunction, impaired nutritional status and a reduced marrow response may further contribute to anemia (6). As several mechanisms can coexist, anemia in HCC may present with microcytic, normocytic or macrocytic features (6,8). Hemoglobin (Hb) concentration is used to determine anemia severity, whereas red blood cell indices are used to characterize morphological patterns (6,8).

Morphological classification based on mature red blood cell indices, including mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and MCH concentration (MCHC), provides useful information on anemia patterns (6,8). However, these indices describe the circulating mature red blood cell population and may not adequately reflect hemoglobinization in newly generated erythrocytes. Reticulocyte hemoglobin equivalent (RET-He) measures the Hb content of circulating reticulocytes and therefore reflects recent erythroid hemoglobinization (9,10). Previous studies have evaluated RET-He for the assessment of iron-restricted erythropoiesis and iron deficiency, including assessment in mixed cancer populations (9,10). Nevertheless, HCC-specific data on the relationship between RET-He, anemia severity and morphological anemia patterns remain limited.

The interpretation of iron-related parameters is also complicated in HCC as the liver is the principal source of hepcidin and a major site of iron storage. Inflammation, hepatocyte injury and HCC-associated alterations in hepatic iron regulation may affect systemic iron homeostasis (11,12). Serum ferritin (SF), although commonly used to estimate iron stores, is an acute-phase reactant and may also be influenced by inflammation, hepatocyte injury, malignancy and altered hepatic iron storage (13). Therefore, ferritin levels should not be interpreted in isolation as a direct indicator of iron availability or iron deficiency in this complex clinical setting (11,14). RET-He and SF may therefore provide information on different aspects of iron metabolism and erythroid hemoglobinization in patients with HCC.

At present, HCC-specific evidence integrating anemia morphology, RET-He and SF remains insufficient. The present retrospective study aimed to analyze the clinical and laboratory anemia profiles in patients with HCC prior to initial antitumor therapy. The correlation of RET-He with anemia severity, morphological subtypes and hematological indices, together with the correlation between RET-He and SF, were primarily assessed to characterize anemia phenotypes and compare the distinct association patterns of RET-He and SF with blood parameters in the present cohort.

Materials and methods

Study subjects. The present single-center retrospective study included consecutive anemic patients diagnosed with HCC who were scheduled to receive their first antitumor therapy at Xingtai People's Hospital (Xingtai, China) between January 2023 and December 2025. The study cohort comprised 61 male and 35 female patients, with a median age of 68 years (range, 50–82 years). The inclusion criteria were as follows: i) Diagnosis conforming to the Practical Guidelines for Pathological Diagnosis of Primary Liver Cancer (2015

updated edition) (15); ii) patients receiving first antitumor therapy; iii) presence of anemia before treatment, with completion of complete blood count, reticulocyte parameter and SF testing prior to treatment; and iv) complete clinical data and main laboratory results. The exclusion criteria were as follows: i) Concurrent definite autoimmune disease, hematological malignancy, aplastic anemia, hemolytic anemia or other hematological disorders that could independently cause anemia; ii) prior history of definite myelosuppression or receipt of radiotherapy, chemotherapy or other treatments that may affect bone marrow hematopoiesis shortly before testing; iii) active bleeding shortly before testing, especially upper gastrointestinal bleeding, gross hematuria, hemoptysis or other definite bleeding events; iv) receipt of red blood cell transfusion, whole blood transfusion, iron therapy, erythropoietin or other hematopoietic treatments that may affect Hb concentration, reticulocyte count or RET-He levels shortly before testing; v) concurrent severe infection, acute inflammatory reaction or renal failure that could markedly interfere with anemia assessment; and vi) missing key clinical or laboratory data. The present study was approved by The Ethics Committee of Xingtai People's Hospital (approval no. 2026121), which waived the requirement for informed consent. The present study was carried out in strict accordance with the Declaration of Helsinki.

Clinical data collection. General patient data and HCC-related clinical data were collected, including sex, age, BCLC stage, Child-Pugh grade (16) and the presence of splenomegaly and/or hypersplenism.

Laboratory tests. Results of complete blood count, reticulocyte parameters and SF testing performed before treatment were collected. Complete blood count parameters included Hb concentration, MCV, MCH and MCHC. Reticulocyte parameters included absolute reticulocyte count (RET#) and RET-He. Complete blood count and reticulocyte parameters were measured using the Sysmex XN series automated hematology analyzer (Sysmex Corporation). SF was measured using the Beckman AU5800 automated biochemical analyzer (Beckman Coulter, Inc.).

Diagnostic criteria for anemia and grouping. Anemia was diagnosed as Hb <120 g/l in adult men and Hb <110 g/l in adult women. According to anemia severity, patients were classified as having mild, moderate or severe anemia. Mild anemia was defined as Hb 90–120 g/l in men and Hb 90–110 g/l in women. For both men and women, moderate anemia was defined as Hb 61–90 g/l and severe anemia as Hb 31–60 g/l. Owing to the small number of patients with severe anemia, moderate and severe anemia were combined only for the comparison of anemia severity groups. In all other subgroup analyses, the original three severity categories (mild, moderate and severe) were retained.

Anemia was classified based on red blood cell morphology as follows: i) Macrocytic anemia, with MCV >100 fl, MCH >34 pg and MCHC 320–360 g/l; ii) normocytic anemia, with MCV 80–100 fl, MCH 27–34 pg and MCHC 320–360 g/l; iii) simple microcytic anemia, with MCV <80 fl, MCH <27 pg and MCHC 320–360 g/l; and iv) microcytic hypochromic anemia, with MCV <80 fl, MCH <27 pg and MCHC <320 g/l.

As no validated RET-He threshold exists for patients with chronic liver disease or HCC, ≤ 29 pg was adopted as the cut-off for exploratory subgroup analysis. This value was derived from the adult reference range of the laboratory, published reference data for healthy Chinese adults (17) and nephrology clinical guidelines (18,19). Given population-specific differences in RET-He thresholds (9,10), this cut-off only screens for relatively low RET-He levels and cannot serve as a validated diagnostic standard for iron deficiency in HCC.

Statistical analysis. Statistical analyses were performed using SPSS version 22.0 software (IBM Corp.). The normality of continuous data was assessed using the Shapiro-Wilk test. Normally distributed continuous data are presented as the mean $\pm$ SD. Comparisons between two groups were performed using the independent-samples t-test, and comparisons among multiple groups were performed using one-way ANOVA, with Tukey's HSD post hoc test applied for significant omnibus results. When variances were unequal, Welch's one-way ANOVA was used, followed by Dunnett's T3 test for post hoc pairwise comparisons. Non-normally distributed continuous data are presented as the median [interquartile range (IQR)]. Comparisons between two groups were performed using the Mann-Whitney U test, and comparisons among multiple groups were performed using the Kruskal-Wallis H test. When the Kruskal-Wallis H test was significant, pairwise comparisons were performed using Dunn's post hoc test with Bonferroni adjustment. Categorical data are presented as numbers and percentages, and group comparisons were performed using the χ^2 test or Fisher's exact test. For post hoc pairwise comparisons of categorical variables, P-values were adjusted using the Bonferroni method.

Spearman's correlation analysis was used to evaluate the correlations of RET-He and SF with Hb concentration, MCV, MCH, MCHC and RET#, as well as the correlation between RET-He and SF. For exploratory analyses, patients were stratified according to SF quartiles. Continuous and categorical characteristics were compared across the four quartile groups using the aforementioned tests. As the SF quartile groups had a natural order, the Jonckheere-Terpstra test was additionally used to assess a monotonic trend in RET-He across increasing SF quartiles.

Multivariable binary logistic regression analysis was performed to identify factors associated with low RET-He (≤ 29 pg). Owing to the limited number of low-RET-He events and the biological overlap among candidate variables, clinical and hematological factors were evaluated in separate models. The clinical model included age, BCLC stage, Child-Pugh grade and splenomegaly/hypersplenism. In the primary hematological model, Hb concentration and MCV were included. As substantial multicollinearity was identified among MCV, MCH and MCHC, sensitivity analyses were performed by replacing MCV with MCH or MCHC while retaining Hb concentration in the model. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were reported. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Baseline characteristics of the study population. A total of 96 patients were included in the present study. The median Hb concentration was 84 g/l (IQR, 73-99 g/l). Among the

Table I. Baseline clinical and laboratory characteristics of anemic patients with hepatocellular carcinoma (n=96).

Variable	Value
Sex, n (%)	
Male	61 (63.54)
Female	35 (36.46)
Age, years ^a	68.50 $\pm$ 7.17
BCLC stage, n (%)	
B	69 (71.88)
C	27 (28.13)
Child-Pugh grade, n (%)	
A	62 (64.58)
B	34 (35.42)
Splenomegaly and/or hypersplenism, n (%)	40 (41.67)
SF, ng/ml ^b	248.5 (146.8-346.0)
Hb concentration, g/l ^b	84 (73-99)
MCV, fl ^b	93.4 (78.5-106.3)
MCH, pg ^b	33.8 (24.6-35.4)
MCHC, g/l ^b	330 (321-340)
RET#, $\times 10^{12}/l^a$	0.053 $\pm$ 0.0097
RET-He, pg ^a	31.78 $\pm$ 6.39
RET-He level, n (%)	
≤ 29 pg	26 (27.08)
> 29 pg	70 (72.92)
Anemia severity, n (%)	
Mild anemia	41 (42.71)
Moderate anemia	52 (54.17)
Severe anemia	3 (3.13)
Morphological classification of anemia, n (%)	
Macrocytic anemia	29 (30.21)
Normocytic anemia	40 (41.67)
Simple microcytic anemia	5 (5.21)
Microcytic hypochromic anemia	22 (22.92)

Data are presented as ^amean $\pm$ SD and ^bmedian (interquartile range). BCLC, Barcelona Clinic Liver Cancer; SF, serum ferritin; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET#, absolute reticulocyte count; RET-He, reticulocyte hemoglobin equivalent.

cohort, 41 patients (42.71%) had mild anemia, 52 (54.17%) had moderate anemia and 3 (3.13%) had severe anemia. Low RET-He (≤ 29 pg) was observed in 26 patients (27.08%). According to the morphological classification, 29 patients (30.21%) had macrocytic anemia, 40 (41.67%) had normocytic anemia, 5 (5.21%) had simple microcytic anemia and 22 (22.92%) had microcytic hypochromic anemia (Table I).

Comparison according to anemia severity. Using Hb level as the grouping criterion for anemia severity, patients were divided

Table II. Comparison of parameters by anemia severity in anemic patients with hepatocellular carcinoma.

Variable	Anemia severity		Statistic	P-value
	Mild anemia (n=41)	Moderate-to-severe anemia (n=55)		
Male, n (%)	28 (68.29)	33 (60.00)	$\chi^2=0.697$	0.404
Age, years ^a	67.88±8.31	68.96±6.22	t=0.732	0.466
BCLC stage, n			$\chi^2=2.626$	0.105
B	33	36		
C	8	19		
Child-Pugh grade, n			$\chi^2=0.430$	0.512
A	28	34		
B	13	21		
Splenomegaly and/or hypersplenism, n			$\chi^2=1.665$	0.197
No	27	29		
Yes	14	26		
SF, ng/ml ^b	265 (120-363)	238 (150-334)	Z=-0.463	0.643
Hb concentration, g/l ^b	100 (96-108)	73 (68-81)	Z=-8.084	<0.001
MCV, fl ^b	98.4 (88.9-108.0)	89.5 (76.4-98.5)	Z=-3.422	<0.001
MCH, pg ^b	32.6 (29.6-37.0)	29.2 (22.5-34.0)	Z=-3.907	<0.001
MCHC, g/l ^b	336 (327-347)	327 (306-334)	Z=-3.483	<0.001
RET#, $\times 10^{12}/l^a$	0.054±0.0093	0.052±0.0100	t=0.727	0.469
RET-He, pg ^a	33.30±6.09	30.65±6.43	t=2.043	0.044
RET-He level, n			$\chi^2=5.616$	0.018
≤29 pg	6	20		
>29 pg	35	35		
Morphological classification of anemia, n				
Macrocytic anemia	16	13	$\chi^2=2.638$	0.104
Normocytic anemia	20	20	$\chi^2=1.490$	0.222
Simple microcytic anemia	1	4		0.389 ^c
Microcytic hypochromic anemia	4	18	$\chi^2=7.017$	0.008

Data are presented as ^amean ± SD and ^bmedian (interquartile range). ^cFisher's exact test. BCLC, Barcelona Clinic Liver Cancer; SF, serum ferritin; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET#, absolute reticulocyte count; RET-He, reticulocyte hemoglobin equivalent.

into a mild anemia group (n=41) and a moderate-to-severe anemia group (n=55). Compared with the mild anemia group, the moderate-to-severe anemia group had lower Hb concentration, MCV, MCH, MCHC and RET-He, together with higher proportions of low RET-He (≤29 pg) and microcytic hypochromic anemia (all P<0.05). No significant difference in SF levels was observed between the two groups (P>0.05; Table II).

Comparison according to morphological classification of anemia. Due to the small number of patients with simple microcytic anemia, the simple microcytic and microcytic hypochromic categories were combined into a microcytic anemia group for comparative analyses. The resulting morphological groups were macrocytic anemia, normocytic anemia and microcytic anemia. Significant differences in Hb concentration, MCV, MCH, MCHC, RET# and RET-He were observed among the three morphological groups. The proportion of patients with RET-He ≤29 pg also differed significantly among the three morphological groups (1/29 macrocytic, 7/40

normocytic and 18/27 microcytic; P<0.001). After adjustment for multiple comparisons, the macrocytic anemia group had higher Hb concentration, MCV, MCH, MCHC, RET# and RET-He than the microcytic anemia group, whereas the normocytic anemia group had higher MCV, MCH, MCHC, RET# and RET-He than the microcytic anemia group. The macrocytic group also had higher MCV and MCH than the normocytic group (both adjusted P<0.05) (Table III). The proportions of patients with mild and moderate anemia differed significantly among the three morphological groups (both P<0.05), whereas the proportion with severe anemia did not differ significantly (P>0.05). After Bonferroni adjustment, mild anemia was less frequent in the microcytic group than in the macrocytic and normocytic groups (both adjusted P<0.05), whereas none of the pairwise comparisons for moderate anemia remained statistically significant.

Comparison according to RET-He level. Compared with patients with RET-He >29 pg, those with low RET-He (≤29 pg)

Table III. Comparison of parameters by morphological type in anemic patients with hepatocellular carcinoma.

Variable	Morphological type			Statistic	P-value
	Macrocytic (n=29)	Normocytic (n=40)	Microcytic (n=27)		
Male, n (%)	22 (75.86)	21 (52.50)	18 (66.67)	$\chi^2=4.119$	0.128
Age, years ^a	68.03±8.02	68.00±6.15	69.74±7.70	F=0.558	0.574
BCLC stage, n				$\chi^2=0.848$	0.654
B	21	27	21		
C	8	13	6		
Child-Pugh grade, n				$\chi^2=1.881$	0.390
A	17	29	16		
B	12	11	11		
Splenomegaly and/or hypersplenism, n				$\chi^2=0.884$	0.643
No	19	22	15		
Yes	10	18	12		
Median SF (IQR), ng/ml	230 (137-369)	261 (174-355)	201 (103-309)	H=2.739	0.254
Median Hb concentration (IQR), g/l	95 (74-104)	88 (73-101)	75 (64-86) ^b	H=8.43	0.015
Median MCV (IQR), fl	108.1 (106.0-110.7)	92.85 (88.7-95.2) ^a	74.3 (72.3-78.1) ^{b,c}	H=83.42	<0.001
Median MCH (IQR), pg	36.7 (35.4-37.7)	30.5 (29.4-32.1) ^a	22.4 (20.3-24.1) ^{b,c}	H=82.93	<0.001
Median MCHC (IQR), g/l	336 (329-343)	332 (327-340)	304 (279-313) ^{b,c}	H=38.08	<0.001
Mean RET# ± SD, ×10 ¹² /l	0.055±0.0094	0.056±0.0084	0.047±0.0094 ^{b,c}	F=8.711	<0.001
Mean RET-He ± SD, pg	35.60±4.03	32.67±6.18	26.36±5.12 ^{b,c}	F=22.081	<0.001
RET-He level, n					<0.001 ^d
≤29 pg	1	7	18		
>29 pg	28	33	9		
Anemia severity, n					
Mild	16	20	5 ^{b,c}	$\chi^2=9.167$	0.010
Moderate	13	19	20	$\chi^2=6.045$	0.049
Severe	0	1	2		0.361 ^d

^aMacrocytic vs. normocytic, adjusted P<0.05; ^bmacrocytic vs. microcytic, adjusted P<0.05; ^cnormocytic vs. microcytic, adjusted P<0.05; ^dFisher's exact test. BCLC, Barcelona Clinic Liver Cancer; SF, serum ferritin; Hb, hemoglobin; IQR, interquartile range; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET#, absolute reticulocyte count; RET-He, reticulocyte hemoglobin equivalent.

had lower Hb concentration, MCV, MCH, MCHC and RET#, and were less likely to have mild or macrocytic anemia, but more likely to have microcytic hypochromic anemia (all P<0.05). Median RET-He was lower in the ≤29 pg group than in the >29 pg group (23.70 vs. 34.30 pg; P<0.001). No significant difference in SF was observed between the two groups (P>0.05). Differences in age, sex, BCLC stage, Child-Pugh grade and splenomegaly/hypersplenism are presented in Table IV.

Correlation between RET-He and laboratory parameters. Spearman's correlation analysis demonstrated that RET-He was positively correlated with Hb concentration, MCV, MCH, MCHC and RET# (all P<0.05), but not with SF (P>0.05). By contrast, SF indicated no significant correlation with any of the evaluated hematological parameters (all P>0.05) (Table V).

Comparisons according to SF quartiles. Patients were divided into four groups according to SF quartiles, with

24 patients in each group. Mean RET-He levels were 32.44±6.57, 32.08±6.78, 31.18±5.63 and 31.43±6.83 pg in Q₁, Q₂, Q₃ and Q₄, respectively, with no statistically significant difference among the four groups (P>0.05). The proportions of patients with low RET-He were also similar across the quartiles (33.3, 20.8, 25.0 and 29.2%; P>0.05). The Jonckheere-Terpstra test presented no evidence of a monotonic trend in RET-He across increasing SF quartiles (Z=-0.658; P>0.05). Apart from the defining differences in SF concentrations, no statistically significant differences were detected in the other evaluated clinical or hematological characteristics (Table VI).

Factors associated with low RET-He. In the clinical model, age, BCLC stage, Child-Pugh grade and splenomegaly/hypersplenism were included as potential clinical factors associated with low RET-He. None of these variables demonstrated a statistically significant association with low RET-He after multivariable adjustment (all P>0.05; Table VII).

Table IV. Comparison of parameters by RET-He level in patients with hepatocellular carcinoma.

Variable	RET-He level		Statistic	P-value
	RET-He ≤ 29 pg (n=26)	RET-He > 29 pg (n=70)		
Male, n (%)	15 (57.69)	46 (65.71)	$\chi^2=0.527$	0.468
Age, years ^a	70.08 $\pm$ 6.57	67.91 $\pm$ 7.33	t=1.319	0.190
BCLC stage, n			$\chi^2=0.123$	0.725
B	18	51		
C	8	19		
Child-Pugh grade, n			$\chi^2=0.337$	0.562
A	18	44		
B	8	26		
Splenomegaly and/or hypersplenism, n			$\chi^2=0.006$	0.938
No	15	41		
Yes	11	29		
SF, ng/ml ^b	266 (125-367)	249 (155-343)	Z=-0.029	0.977
Hb concentration, g/l ^b	75 (64-89)	89 (75-99)	Z=-2.635	0.008
MCV, fl ^b	76.9 (72.4-88.7)	96.5 (88.7-106.3)	Z=-5.050	<0.001
MCH, pg ^b	22.8 (20.8-30.0)	32.5 (29.4-35.7)	Z=-5.248	<0.001
MCHC, g/l ^b	310.0 (282.8-327.0)	333.0 (327.0-341.0)	Z=-4.994	<0.001
RET#, $\times 10^{12}/l^a$	0.043 $\pm$ 0.0091	0.056 $\pm$ 0.0072	t=-7.416	<0.001
RET-He, pg ^b	23.70 (20.40-26.40)	34.30 (31.45-37.68)	Z=-7.504	<0.001
Anemia severity, n				
Mild	6	35	$\chi^2=5.616$	0.018
Moderate	18	34	$\chi^2=3.259$	0.071
Severe	2	1		0.177 ^c
Morphological classification of anemia, n				
Macrocytic anemia	1	28		<0.001 ^c
Normocytic anemia	7	33	$\chi^2=3.189$	0.074
Simple microcytic anemia	0	5		0.319 ^c
Microcytic hypochromic anemia	18	4	$\chi^2=43.297$	<0.001

Data are presented as ^amean $\pm$ SD and ^bmedian (interquartile range). ^cFisher's exact test. BCLC, Barcelona Clinic Liver Cancer; SF, serum ferritin; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET#, absolute reticulocyte count; RET-He, reticulocyte hemoglobin equivalent.

In the primary hematological model, lower MCV was associated with low RET-He after adjustment for Hb concentration (OR, 0.902; 95% CI, 0.859-0.947; $P<0.001$), whereas Hb concentration was not statistically significant. Sensitivity analyses produced similar findings when MCV was replaced by MCH (OR, 0.762; 95% CI, 0.676-0.859; $P<0.001$) or MCHC (OR, 0.932; 95% CI, 0.900-0.966; $P<0.001$; Table VII).

Discussion

HCC predominantly arises against a backdrop of chronic liver disease and cirrhosis, where tumor-associated inflammation, gastrointestinal hemorrhage, portal hypertension, hypersplenism, malnutrition and hepatic dysfunction overlap to induce anemia via diverse pathogenic pathways (1,3,5,6). Prior research has indicated that chronic liver disease-related anemia can manifest as microcytic, normocytic or macrocytic phenotypes (6,8). A recent HCC cohort study further verified

that anemia was associated with compromised liver function, portal hypertension, advanced BCLC staging, systemic inflammation and unfavorable survival outcomes (4). Such overlapping hepatic and malignant pathological features distinguish HCC-related anemia from anemia associated with other solid malignancies (1,20). Clinically, iron deficiency cannot be regarded as the sole etiology of anemia in patients with HCC, and comprehensive assessment covering anemia severity, red cell morphology, hepatic reserve and key laboratory biomarkers is mandatory (1,21).

In the present cohort, RET-He was consistently associated with anemia severity and morphological subtype. Compared with patients with mild anemia, those with moderate-to-severe anemia had lower MCV, MCH, MCHC and RET-He, and a higher proportion of microcytic hypochromic anemia. These findings indicate that greater anemia severity in this cohort was accompanied by smaller erythrocyte size, reduced erythrocyte hemoglobinization and lower Hb concentration in

Table V. Correlation of RET-He and SF with laboratory parameters.

Variable	Correlation with RET-He, r_s	P-value	Correlation with SF, r_s	P-value
Hb concentration	0.342	<0.001	0.032	0.759
MCV	0.539	<0.001	0.104	0.311
MCH	0.591	<0.001	0.109	0.290
MCHC	0.545	<0.001	0.124	0.228
RET#	0.748	<0.001	-0.021	0.840
SF	-0.085	0.408	-	-

RET-He, reticulocyte hemoglobin equivalent; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET#, absolute reticulocyte count; SF, serum ferritin.

newly produced reticulocytes. Potential contributing mechanisms may include chronic blood loss, nutritional deficiencies, inflammation-associated alterations in iron metabolism and impaired erythropoietic activity (6,12). Cancer-related anemia has also been associated with impaired quality of life, reduced treatment tolerance and adverse clinical outcomes (2,22). However, these mechanisms were not directly assessed in the present study.

Consistent with the aforementioned results, patients assigned to the microcytic anemia group displayed reduced Hb concentration, MCV, MCH, MCHC, RET# and RET-He, accompanied by a larger share of patients with low RET-He. However, reduced RET-He could still be detected in partial normocytic and macrocytic anemic cases. Routine mature erythrocyte parameters only describe the size and Hb load of circulating mature red cells, while RET-He quantifies Hb levels in newly generated reticulocytes. Accordingly, morphological typing relying solely on mature erythrocytes fails to fully capture real-time erythroid hemoglobinization. Notably, simple microcytic anemia only involved 5 patients in the present cohort. Although this subgroup was combined with microcytic hypochromic anemia for comparative analysis, both subtypes presented markedly reduced RET-He levels, which validated the stability of differences within the merged microcytic group.

The consistent positive correlations between RET-He and Hb concentration, MCV, MCH, MCHC and RET# further suggest a biologically rational link between real-time erythroid hemoglobinization and routine hematological markers. Prior clinical research across oncology cohorts has demonstrated that RET-He delivers supplementary evidence for evaluating anemia and iron-limited erythropoiesis (9,10). These correlative relationships cannot confirm definite anemia etiologies, yet they demonstrate that RET-He is associated with red cell morphology and Hb synthetic capacity.

Multivariate logistic regression revealed that age, BCLC stage, Child-Pugh grade and splenomegaly/hypersplenism exhibited no independent correlation with low RET-He within the clinical covariate model. By comparison, all tested erythrocyte indices exhibited statistically meaningful correlations with low RET-He in separate hematological regression models. After adjusting for Hb in the primary model, decreased MCV remained independently linked to low RET-He; identical results emerged after substituting MCV with MCH or MCHC

for sensitivity testing. Marked multicollinearity existed among MCV, MCH and MCHC, hence each indicator was fitted into an individual regression framework. Uniform correlation directions across all sensitivity models verify the stable association between suppressed RET-He and abnormal erythrocyte morphology/Hb synthesis. Nevertheless, MCV, MCHC and MCH cannot be viewed as independent predictive factors for low RET-He, and the present retrospective cross-sectional design cannot draw causal conclusions. Non-significant clinical covariate associations do not eliminate their biological effects on anemia and erythropoiesis, and narrow clinical spectrum, limited overall sample and scarce low-RET-He cases jointly lowered the statistical power of the clinical regression model. Mechanistically, splenomegaly and hypersplenism mainly mediate red blood cell peripheral destruction (6,23), while advanced BCLC or poor Child-Pugh status alters systemic iron reserves and inflammatory burden (4,24). None of these factors directly interfere with intramedullary reticulocyte Hb synthesis, which explains their failure to independently predict low RET-He.

In contrast to the stable correlations between RET-He and erythrocyte parameters, SF displayed entirely divergent results. SF concentrations exhibited no intergroup disparities stratified by anemia severity, red cell morphology or RET-He status, indicating that SF fails to discriminate clinically meaningful anemia subtypes among patients with HCC. When subjects were categorized into four SF quartile subgroups, neither mean RET-He values nor the proportion of low RET-He carriers differed statistically between groups. Jonckheere-Terpstra trend testing additionally ruled out a monotonic change in RET-He alongside elevated SF quartiles. Collectively, SF bore no detectable correlation with RET-He whether treated as a continuous variable or ordered quartile grouping. The two biomarkers possessed completely distinct correlative patterns, whereby RET-He maintained stable positive links with Hb, MCV, MCH, MCHC and RET#, while SF lacked any meaningful correlation with the aforementioned hematological markers.

Such divergent correlative signatures demonstrate that RET-He and SF mirror separate biological pathways in HCC-related anemia. RET-He directly mirrors intramedullary erythroid Hb synthesis, while SF cannot reflect erythropoietic status; its concentration is predominantly driven by systemic inflammation, hepatocyte damage and disordered hepatic

Table VI. Clinical and hematological characteristics according to SF quartiles.

Variable	SF quartile group				Statistic	P-value
	Q ₁ (n=24)	Q ₂ , (n=24)	Q ₃ , (n=24)	Q ₄ , n=24		
Male, n (%)	14 (58.3)	17 (70.8)	15 (62.5)	15 (62.5)	$\chi^2=0.854$	0.836
Age, years ^a	69.58±7.92	69.71±7.65	67.21±5.78	67.50±7.21	F=0.821	0.485
BCLC stage, n					$\chi^2=1.804$	0.614
B	18	17	19	15		
C	6	7	5	9		
Child-Pugh grade, n					$\chi^2=0.911$	0.823
A	16	14	17	15		
B	8	10	7	9		
Splenomegaly and/or hypersplenism, n					$\chi^2=1.029$	0.794
No	15	12	15	14		
Yes	9	12	9	10		
SF, ng/ml ^b	88 (69-111)	187 (167-230)	305 (276-331)	384 (365-403)	H=89.076	<0.001
Hb, g/l ^b	91 (74-103)	79 (69-91)	78 (70-100)	93 (76-100)	H=5.829	0.120
MCV, fl ^b	91.6 (76.8-105.8)	93.4 (75.3-106.5)	89.9 (78.7-99.2)	95.4 (85.6-106.1)	H=1.867	0.600
MCH, pg ^b	30.0 (23.8-35.3)	30.6 (23.0-34.7)	30.2 (25.3-34.7)	31.9 (28.9-36.4)	H=1.610	0.657
MCHC, g/l ^b	330.5 (313.0-339.3)	326.5 (304.5-333.0)	334.0 (322.3-342.5)	330.0 (324.5-339.8)	H=4.773	0.189
RET#, x10 ¹² /l ^a	0.052±0.010	0.054±0.011	0.052±0.010	0.053±0.008	F=0.118	0.949
RET-He, pg ^a	32.44±6.57	32.08±6.78	31.18±5.63	31.43±6.83	F=0.193	0.901
RET-He level, n					$\chi^2=1.055$	0.788
≤29 pg	8	5	6	7	$\chi^2=1.055$	0.788
>29 pg	16	19	18	17		
Anemia severity, n						
Mild	12	6	10	13	$\chi^2=4.896$	0.180
Moderate	12	17	13	10	$\chi^2=4.364$	0.225
Severe	0	1	1	1		0.999 ^c
Morphological classification of anemia, n						
Macrocytic anemia	8	7	5	9	$\chi^2=1.729$	0.630
Normocytic anemia	7	10	12	11	$\chi^2=2.400$	0.494
Simple microcytic anemia	2	0	3	0		0.326 ^c
Microcytic hypochromic anemia	7	7	4	4		0.715 ^c

Data are presented as ^amean ± SD and ^bmedian (interquartile range). ^cFisher's exact test. BCLC, Barcelona Clinic Liver Cancer; SF, serum ferritin; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RET#, absolute reticulocyte count; RET-He, reticulocyte hemoglobin equivalent.

iron deposition (24,25), none of which were directly quantified in the present research. This viewpoint aligns with the widely acknowledged difficulty of interpreting SF values in patients with chronic liver disease and HCC. Ferritin levels are jointly modulated by systemic iron reserves, inflammatory status, hepatocellular damage, malignant lesions and disrupted hepatic iron regulatory pathways (11,14). Tumor-related inflammation and HCC-induced dysregulation of hepcidin further disturb intestinal iron uptake, macrophage iron recycling and iron allocation toward bone marrow erythropoiesis (11,12).

Meanwhile, ferritin acts as an acute-phase reactant and rises under inflammatory stimulation. Prior research has documented extensive overlapping SF ranges between patients with cirrhosis and patients with HCC, greatly reducing the diagnostic specificity of isolated ferritin testing (26). Under inflammatory anemia status, ferritin can remain at normal or elevated levels even when iron supply for erythroid progenitor cells is insufficient (12). The present study lacked the detection of inflammatory markers, hepcidin and iron utilization indicators. Accordingly, the absent correlation between SF

Table VII. Multivariable logistic regression analyses of factors associated with low RET-He (≤ 29 pg).

Variable	Adjusted OR	95% CI	P-value
Model 1: Clinical factors			
Age	1.044	0.979-1.115	0.191
BCLC stage	1.251	0.458-3.419	0.662
Child-Pugh grade	0.754	0.284-2.006	0.572
Splenomegaly/hypersplenism	0.970	0.383-2.460	0.949
Model 2A: Primary hematological model			
Hb concentration	0.976	0.942-1.011	0.194
MCV	0.902	0.859-0.947	<0.001
Model 2B: Sensitivity model			
Hb concentration	0.989	0.951-1.029	0.559
MCH	0.762	0.676-0.859	<0.001
Model 2C: Sensitivity model			
Hb concentration	0.993	0.957-1.031	0.709
MCHC	0.932	0.900-0.966	<0.001

The dependent variable was low RET-He, defined as RET-He ≤ 29 pg and coded as 1. Model 2A was the primary hematological model. Models 2B and 2C were sensitivity analyses in which MCV was replaced by MCH or MCHC, respectively, as substantial multicollinearity was observed among the erythrocyte indices. OR, odds ratio; CI, confidence interval; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; BCLC, Barcelona Clinic Liver Cancer; RET-He, reticulocyte hemoglobin equivalent.

and RET-He reminds clinicians to interpret ferritin cautiously in the present cohort, yet this finding neither confirms that SF has no clinical value nor verifies the existence of functional iron deficiency. Such dissociation between RET-He and SF is less prominent in patients with non-hepatic solid tumors without underlying cirrhosis (9,27). In malignancies such as lung and breast cancer, alterations in ferritin have been associated with tumor-related inflammation and disturbed iron homeostasis (28,29). By contrast, patients with HCC carry dual disturbances of chronic liver damage and malignant lesions, which synergistically distort SF concentrations and decouple SF from erythroid hemoglobinization markers such as RET-He (24,25). This distinction highlights the unique limitation of SF as a hematological biomarker specific to cirrhotic HCC populations.

Collectively, RET-He and SF deliver partially overlapping yet non-interchangeable diagnostic information for anemic patients with HCC. RET-He closely associates with red cell morphology and Hb synthetic markers, while multiple analytical strategies failed to detect any meaningful link between SF and RET-He. The decoupling of the two biomarkers indicates they reflect distinct segments of iron metabolism and erythropoiesis within patients with HCC, and RET-He can supply supplementary data on real-time reticulocyte Hb synthesis that cannot be acquired from SF testing alone. Still, the lack of correlation does not mean that the two biomarkers regulate fully independent biological cascades. Complete anemia evaluation requires integrated analysis of red cell morphology, iron metabolism, inflammatory state, nutritional condition, hepatic function and potential chronic hemorrhage.

RET-He cannot serve as an independent diagnostic biomarker for iron deficiency among patients with HCC.

The cut-off value of ≤ 29 pg adopted in the present study was only designed for exploratory subgroup stratification, and was derived from an institutional reference range and published clinical experience. This threshold has not been validated exclusively for chronic liver disease or HCC populations. Decreased RET-He merely reflects lowered Hb load in peripheral reticulocytes and cannot differentiate absolute iron deficiency, inflammation-induced iron sequestration, suppressed erythropoiesis or nutritional anemia as a single indicator. Prior investigations have demonstrated that RET-He possesses auxiliary value for evaluating iron supply to erythroid cells, yet optimal cut-offs and diagnostic efficacy differ widely across disease cohorts (9,10). Researchers have also warned against over-reliance on a single RET-He threshold under complicated multi-pathology clinical backgrounds (30). Accordingly, RET-He is best deployed as an auxiliary index alongside routine blood work and full iron panel testing, rather than a replacement for standard anemia evaluation. From a clinical perspective, relying solely on SF to judge iron availability for erythropoiesis could lead to misjudgment in anemic patients newly diagnosed with HCC prior to antitumor therapy. Combined detection of RET-He can more accurately identify insufficient Hb synthesis in erythroid progenitor cells, providing objective laboratory evidence to guide rational iron supplementation or erythropoietin intervention (19,31).

Multiple limitations of the present retrospective single-center study need to be addressed. First, only anemic patients with HCC were enrolled with a limited total sample, which may trigger selection bias and restrict extrapolation of the results to non-anemic patients with HCC or cohorts with disparate tumor staging and hepatic function profiles. Second, severe anemia ($n=3$) and isolated simple microcytic anemia

(n=5) subgroups contained a small number of cases, forcing subgroup combination and weakening the stability and statistical power of intergroup comparisons. Only 26 subjects met the exploratory low RET-He criterion, limiting the quantity of covariates stably incorporated into logistic regression models. Third, serum iron, transferrin saturation, total iron-binding capacity, C-reactive protein, hepcidin, folate and vitamin B12 were not detected, making it impossible to separately diagnose absolute iron deficiency, functional iron restriction, inflammatory anemia and nutritional anemia subtypes. Fourth, the 29 pg RET-He cut-off lacks HCC-specific verification; converting continuous RET-He into categorical subgroups inevitably loses partial quantitative data. The present research was not designed to assess the diagnostic performance of this threshold; hence receiver operating characteristic, sensitivity and specificity analyses were not conducted. SF quartile grouping was purely exploratory, with dividing values unique to the sample without unified clinical reference standards. Last, the cross-sectional retrospective design prohibits causal deduction, and the correlations between the two biomarkers and treatment response, transfusion demand, anemia intervention schemes and long-term survival were not analyzed. Large-scale multi-center prospective trials integrating complete iron metabolism, inflammatory, nutritional and long-term prognostic indicators are warranted to further confirm the clinical application value of RET-He in HCC populations.

In conclusion, anemia in patients with HCC showed heterogeneous morphological patterns, and microcytic anemia was associated with lower RET-He levels. RET-He was associated with erythrocyte indices reflecting cell size and hemoglobinization, whereas no significant correlation was observed between RET-He and SF. The dissociation between RET-He and SF suggests that these two markers reflect different aspects of iron metabolism and erythropoiesis in HCC. RET-He may provide complementary information on recent erythroid hemoglobinization that is not directly captured by ferritin, but should not be used as a stand-alone marker of iron deficiency. SF should be interpreted cautiously in the context of chronic liver disease, inflammation and tumor factors.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

DL and ZL contributed to the study conceptualization. ZL was responsible for the data curation, formal analysis, investigation and software. DL contributed to the methodology and provided supervision and validation; ZL wrote the original draft of the

manuscript. DL and ZL participated in the reviewing and editing of the manuscript. Both authors have read and approved the final version of the manuscript. DL and ZL confirm the authenticity of all the raw data.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of Xingtai People's Hospital (Xingtai, China; approval no. 2026121) and was carried out in strict accordance with the Declaration of Helsinki. As this is a retrospective study and strict privacy measures were in place, the Ethics Committee waived the requirement for informed consent.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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