

Letter to the Editor

**Verification of the Cut-off Value of the Reticulocyte Hemoglobin Content to Diagnose Iron Deficiency***

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Iron deficiency anemia (IDA) is a common nutrient deficiency, and it has become a global public health problem identified by the World Health Organization. Studies have shown that IDA remains prevalent in both developing and developed countries, such as Australia and China, and that IDA may be associated with poorer cognitive and neurophysiologic maturation in early life and causes labor productivity losses, developmental delays, mental and behavior disorders, and perinatal complications in pregnant and premenopausal women^[1]. Additionally, the monthly loss of iron with menstrual bleeding may be the reason IDA occurs more frequently in women. Accurate detection of IDA in its early phase can help patients determine the causes in a timely manner, achieve early intervention and treatment, and thus shorten the disease course, decrease complications, and greatly reduce the disease and its economic burden.

There are some general indices to evaluate iron status, but these are not satisfactory. Based on studies in different countries, the most commonly used test is the bone marrow biopsy with Prussian blue staining. Bone marrow iron staining can precisely assess iron status, but it causes trauma and a financial burden to patients, and it is also considered to be cumbersome and invasive. Serum ferritin (SF), which is the accepted gold standard to detect storage iron depletion, is now the main surrogate marker used in daily clinical practice^[2]. However, because SF is susceptible to inflammation, such as malignant tumors, acute and chronic liver diseases, and hyperthyreosis, it is still not an ideal

indicator for IDA diagnosis. Hemoglobin (Hb) is a widely used marker for anemia. The Hb content better reflects iron status. When erythropoiesis is affected, the amount of Hb is insufficient, resulting in microcytic hypochromic anemia with low Hb^[3]. Mean cellular volume (MCV) was used to classify anemia and exclude iron deficiency. There is no international recommended marker to assess a patient's iron status, and more studies are now focusing on exploring the advantages of using reticulocyte hemoglobin content (CHr) in this role^[4].

Reticulocytes are transition cells in the process of erythrocyte maturation. The number of reticulocytes in peripheral blood can reflect the hematopoietic function of the bone marrow. CHr, with its short lifespan, high sensitivity and specificity, and easy acquisition, is now a proper predictor of IDA. Measuring CHr can reflect a negative iron balance before Hb, and mature red cell parameters start to decrease^[5]. Because of these advantages, CHr is used to screen iron status, especially in pregnant women and children who have difficulties with blood collection and community-acquired pneumonia and dialysis patients. But now, because of the variation in pre-analysis, the comparison is hard to be done. The standardized reference intervals are lacking. Our previous research has already explored a suitable CHr cut-off value of 27.2 pg in Chinese adults with bone marrow iron staining as the gold standard^[6] and a reliable MCV concentration of 76.6 fL. In this study, SF, MCV, and Hb, which are the frequently used hematological indicators of IDA diagnosis, were used to assess the diagnostic efficiency of CHr. This

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study aimed to determine whether the CHr cut-off value of 27.2 pg, which was obtained from our former study, is suitable for diagnosing IDA in Chinese adults.

We selected patients from the Hematology Department at Beijing Peking Union Medical College Hospital between January 2015 and March 2017. The prospective inclusion criteria for patients were as follows: (1) patients must be adults (18–60 years old), (2) no bone marrow aspiration, and (3) no blood transfusion or oral or intravenous iron supplement within 1 month of the study's start date. We excluded patients who were pregnant and had leukemia, myeloma, myelodysplastic syndrome, and hemoglobinopathy. Patients provided written informed consent before the study. As recommended by the International Federation of Clinical Chemistry, the minimum sample size for the detection is 120. In this study, there were 182 patients enrolled, including 42 (23%) men and 140 (77%) women, with a mean age of 43.1 years.

Basic information and venous blood were collected from all subjects to detect the complete blood cell count and iron metabolism indices. All venous blood samples were inserted into a K2-EDTA anticoagulant tube (BD, Franklin Lakes, NJ, USA) after collection. Then, a centrifugal speed-freezing centrifuge (KDC-2046, Zhongke Zhongjia, Hefei, China) was used to centrifuge blood samples. The hemogram standard parameters included CHr, Hb, hematocrit (HCT), MCV, mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), transferrin (TRF), total iron-binding capacity (TIBC), single cell hemoglobin (CH), red cell distribution width (RDW), reticulocyte percentage (RET), serum iron (Fe), iron saturation (IS), transferrin saturation (TS), and SF. These were performed using an automatic blood cell analyzer (ADVIA120, Bayer, Leverkusen, Germany) in the laboratories at the Beijing Peking Union Medical College Hospital.

CHr (27.2 pg), which is based on the previous study of our research group, was used as the cut-off value for diagnosing IDA in this study. An SF of 12 ng/mL, which implies that iron stores are absent^[7], and MCV (76.6 fL) or Hb (110 g/L for women and 120 g/L for men) were used as diagnostic standards to assess the diagnostic efficiency of CHr. The 182 selected samples were divided into two groups based on the different diagnostic standards as follows: (1) IDA group: CHr ≤ 27.2 pg, SF < 12 ng/mL and MCV ≤ 76.6 fL, and SF < 12 ng/mL and Hb < 110 g/L (women) or 120 g/L (men) and (2) non-IDA (NIDA) group CHr > 27.2 pg,

SF ≥ 12 ng/mL and MCV > 76.6 fL, and SF ≥ 12 ng/mL and Hb ≥ 110 g/L (women) or 120 g/L (men). SF + MCV, SF + Hb, or SF + MCV + Hb, which were used to make the clinical diagnosis, were also used to screen for IDA. The consistency between these methods was compared.

Statistical analysis was performed using SPSS version 19.0. Receiver operator curves (ROCs) were performed using MedCalc13.0. The Kolmogorov–Smirnov normality test was used to evaluate the distribution of all indexes. Quantitative variables (such as Hb, HCT, MCV, MCHC, MCH, TRF, and TIBC), which were distributed normally, were expressed as mean ± standard deviation ($\bar{x} \pm SD$). Others such as CH, RDW, RET, Fe, IS, and TS, which did not have a normal distribution, were expressed as median (P25–P75). The average CHr of the samples was 27.63 pg, and the mean values of MCV and Hb were 81.65 fL and 97.23 g/L, respectively. The value of SF was 17 ng/mL with a range of 5 to 104 ng/mL. The demographic characteristics are presented in Table 1. Despite the growing number of studies on CHr worldwide, few data are available about Chinese adults and the CHr cut-off value in different subjects, and using different analytical methods may give various results. A study by Mast et al.^[8] in 2002 conducted in the US showed the cut-off point of CHr to be 28 pg, which was higher than in our study.

A Mann–Whitney *U* test was used to detect statistical differences between the CHr and SF + MCV or SF + Hb groups in the IDA and NIDA groups. *P*-values less than 0.05 were considered to be statistically significant. The analysis of different indices is shown in Table 2. Mean values, SD, and median (P25–P75) show the characteristics of biochemical and hematological parameters. In the comparison between the CHr and SF + MCV groups, most of the parameters have a significant difference

Table 1. Demographic characteristics of the patients

Variables	Value (N = 182)
Age (years), mean (SD)	43 (15.7)
Gender (female), n (%)	140 (76.9)
CHr (pg), mean (SD)	27.63 (5.47)
MCV (fL), mean (SD)	81.65 (11.42)
Hb (g/L), mean (SD)	97.23 (25.18)
SF (ng/mL), median (range)	17 (5–104)

Note. SD, standard deviation; CHr, reticulocyte hemoglobin content; Hb, hemoglobin; MCV, mean cellular volume; SF, serum ferritin.

($P < 0.05$) except RDW and RET in both the IDA and NIDA groups. When the CHr group was compared with the SF + Hb group, there was a significant difference in Fe, IS, TS, TRF, and TIBC in the IDA group. Meanwhile, in the NIDA group, the values of MCV, MCH, and CH were significantly different between the CHr and SF + Hb groups, whereas other indices showed no difference. The prevalence rates of IDA in the CHr, SF + MCV, and SF + Hb groups were 51%, 24%, and 31%, respectively. Because our team had already evaluated the CHr cut-off value, in this study, all subjects were evaluated by a CHr value of 27.2 pg. The observed results of our comparison are in accordance with those of previous studies. The progression of IDA occurs gradually, with decreased MCV and MCH levels that are present initially. Then, CHr is decreased, and SI and TIBC are affected by inflammation and infectious stimuli^[9]. If the condition has not been alleviated, IDA will occur. In our study, the CHr \leq 27.2 pg group had significantly lower Hb, MCV, MCHC, MCH, CH, TS, IS, and Fe and higher TRF and TIBC levels compared with the CHr $>$ 27.2 pg group ($P < 0.05$). Karlsson et al.^[9] showed that the mean values of Hb, MCV, MCH, Fe, and TS were significantly lower in the IDA group, which is consistent with the results of our research.

ROC curve analysis was used to illustrate the performance of the medical diagnostic tests. The diagnostic value of CHr was estimated using

different diagnostic standards. The area under the ROC curve (AUC) was used to evaluate the ability to identify the disease for two or more diagnostic tests. Areas under the ROC curve (AUC) of 0.5 to 0.7 have low diagnostic accuracy, 0.7 to 0.9 moderate, and 0.9 to 1.0 high. Table 3 summarizes the analytical data from the ROC curve. The AUC ranged from 0.876 to 0.935. Using SF 12 ng/mL and MCV 76.6 fL as the criteria, the AUC was 0.920 (95% confidence interval: 0.870–0.955). Because Hb has a different cut-off value based on sex, the criteria were divided into two parts. For men, the AUC was 0.935, which is higher than the value of 0.876 for women. When the criteria included SF, MCV (76.6 fL), and Hb, the AUC was 0.923. The sensitivity, specificity, and Youden's index for the three criteria are also listed in Table 3. SF + Hb in men had the highest sensitivity. SF + MCV (76.6 fL) + Hb had the highest specificity, and the largest Youden's index was also observed in SF + Hb in men. Although CHr's specificity may be influenced by thalassemia and anemia in chronic disease and by clinical IDA (small cell hypochrome) features, the use of traditional indicators, such as SF, Hb, and MCV analyzed together, can obtain a more accurate diagnostic efficiency for IDA. The ROC curve was used to find the best cut-off point for MCV in Cai et al.'s study, and the value was 76.6 fL^[6]. However, the shortcoming of MCV was its low specificity. The AUC for different diagnostic standards was higher than

Table 2. Analysis of biochemical and hematologic parameters of CHr, SF + MCV, and SF + Hb

Indexes	IDA			NIDA		
	CHr (n = 93)	SF + MCV (n = 44)	SF + Hb (n = 56)	CHr (n = 89)	SF + MCV (n = 138)	SSF + Hb (n = 126)
Hb (g/L)	92.2 \pm 21.6 ^a	84.0 \pm 20.1		102.4 \pm 27.6 ^a	101.4 \pm 25.3	
MCV (fL)	73.3 \pm 7.1 ^a		72.5 \pm 6.9	90.2 \pm 8.3 ^{a,b}		85.8 \pm 10.6
MCHC (g/L)	297.6 \pm 26.2 ^a	285.7 \pm 26.2	289.8 \pm 25.7	332.3 \pm 25.6 ^a	323.8 \pm 26.7	325.9 \pm 26.5
MCH (og)	21.9 \pm 3.5 ^a	20.1 \pm 3.2	21.2 \pm 3.6	30.1 \pm 3.7 ^{a,b}	27.8 \pm 4.6	28.2 \pm 4.7
TRF (g/L)	3.1 \pm 0.6 ^{a,b}	3.3 \pm 0.4	3.3 \pm 0.4	2.4 \pm 0.6 ^a	2.5 \pm 0.7	2.5 \pm 0.6
TIBC (ug/dL)	417.2 \pm 86.5 ^{a,b}	453.8 \pm 68.7	453.1 \pm 62.3	315.8 \pm 80.2 ^a	341.6 \pm 89.9	329.6 \pm 85.6
CH (pg)	21.8 (19.8, 24.4) ^a	20.1 (17.4, 22.1)	21.2 (18.5, 23.5)	30.3 (28.3, 32.6) ^{a,b}	28.3 (24.6, 31.0)	29.2 (25.1, 31.35)
Fe (ug/dL)	22.3 (14.0, 41.9) ^{a,b}	15.5 (10.4, 21.6)	15.3 (12, 22.3)	88.4 (51.3, 161.5) ^a	69.9 (29.1, 130.2)	78.3 (35.4, 133.7)
IS (%)	5.7 (3.0, 11.1) ^{a,b}	3.4 (2.5, 5.4)	3.5 (2.6, 4.8)	28.4 (14.5, 55.0) ^a	22.3 (7.2, 42.6)	24.7 (9.1, 48.5)
TS (%)	5.3 (2.7, 10.3) ^{a,b}	3.2 (2.3, 5.1)	3.3 (2.5, 4.7)	26.2 (14.1, 53.3) ^a	20.8 (7.0, 40.9)	23.7 (8.8, 45.4)
HCT (%)	30.7 \pm 5.3 ^a	29.1 \pm 4.9	22.8 \pm 4.6	30.7 \pm 8.1 ^a	31.21 \pm 7.21	31.5 \pm 7.4
RDW (%)	16.4 (15.2, 18.2)	16.9 (15.8, 18.7)	16.7 (15.4, 18.6)	16.3 (13.9, 20.1)	15.8 (14.0, 18.6)	15.8 (14, 19.2)
RET% (%)	1.50 (1.1, 2.1)	1.43 (1.0, 1.9)	1.40(1.1, 1.9)	1.50 (1.1, 2.7)	1.55 (1.04, 2.54)	1.60 (1.1, 2.7)

Note. ^a $P < 0.05$ between the CHr and SF + MCV groups. ^b $P < 0.05$ between the CHr and SF + Hb groups. Hb, hemoglobin; MCV, mean cellular volume; MCHC, mean corpuscular hemoglobin concentration; MCH, mean corpuscular hemoglobin; TRF, transferrin; TIBC, total iron-binding capacity; CH, single cell hemoglobin; Fe, serum iron; IS, iron saturation; TS, transferrin saturation; SF, serum ferritin; CHr, reticulocyte hemoglobin content; HCT, hematocrit; RDW, red cell distribution width; RET, reticulocyte.

Table 3. The comparison of clinical diagnostic values for CHr

Diagnostic criteria	AUC (95% CI)	Sensitivity (%)	Specificity (%)	Youden's index (%)
SF + MCV (76.6)	0.920 (0.870–0.955)	73.70	95.30	69.00
SF + Hb				
Total	0.895 (0.841–0.936)	70.60	96.40	67.00
Men	0.935 (0.814–0.988)	94.44	83.33	77.77
Women	0.876 (0.810–0.926)	68.89	94.00	62.89
SF + MCV (76.6) + Hb	0.923 (0.874–0.957)	72.00	97.40	69.40

Note. AUC, area under curve; CI, confidence interval; SF, serum ferritin; MCV, mean cellular volume; Hb, hemoglobin.

0.895, which suggests a high diagnostic accuracy. The value of AUC also indicated that CHr has the same ability as other criteria to diagnose IDA.

In summary, CHr has the characteristics of a new and reliable detector for IDA. Advantages of CHr are that the application can be better used in infants, pregnant women, and elderly people, who have difficulties with blood draws. Analysis of CHr should be used more frequently in clinical examination in the future. Although CHr has many advantages over other indices, there are also studies showing that CHr has only limited value for detecting iron depletion and that it is not a good indicator of early stages of ID^[10]. Meanwhile, for different age groups, the role of CHr is also the lack of systematic and effective discussion. Therefore, in future research, we should evaluate the role of CHr in the whole process of iron deficiency more comprehensively. Because of the disease distribution by sex, more women were enrolled, which is also a shortcoming of this study. Including more male samples in the future will solve the problem of the sex-based proportional imbalance.

In conclusion, using CHr \leq 27.2 pg to diagnose IDA in Chinese adults shows a high consistency with the three diagnostic criteria that are widely used in clinical practice. CHr with a cut-off value of 27.2 pg has a high diagnostic efficiency that is needed to detect IDA.

YANG Li Chen and HAN Bing conceived and designed the experiments; ZHANG Hui Di and CAI Jie performed the experiments and wrote the paper; WU Meng and REN Jie analyzed the data; LONG Zhang Biao and DU Ya Li collected the data; LI Guo Xun drew the tables.

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