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## Retrospective Study

# Ratio of hemoglobin to mean corpuscular volume: A new index for discriminating between iron deficiency anemia and thalassemia trait

Qing-Chun Yao, Hui-Li Zhai, Hou-Cai Wang

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## Abstract

### BACKGROUND

Iron deficiency anemia (IDA) and thalassemia trait (TT) are the most common microcytic and hypochromic anemias. Differentiation between mild TT and early IDA is still a clinical challenge.

### AIM

To develop and validate a new index for discriminating between IDA and TT.

### METHODS

Blood count data from 126 patients, consisting of 43 TT patients and 83 IDA patients, was retrospectively analyzed to develop a new index formula. This formula was further validated in another 61 patients, consisting of 48 TT patients and 13 IDA patients.

### RESULTS

The new index is the ratio of hemoglobin to mean corpuscular volume. Its sensitivity, specificity, accuracy, Youden's Index, area under the receiver operating characteristic curve, and Kappa coefficient in discriminating between IDA and TT were 93.5%, 78.4%, 83.3%, 0.72, 0.97, and 0.65, respectively.

### CONCLUSION

This new index has good diagnostic performance in discriminating between mild TT and early IDA. It requires only two results of complete blood count, which can be a very desirable feature in under-resourced scenarios.

**Key Words:** Iron deficiency anemia; Thalassemia trait; Hemoglobin; Mean corpuscular volume; Thalassemia trait; Gene sequencing

**Core Tip:** The ratio of hemoglobin to mean corpuscular volume was proposed and validated as a new index for discriminating between iron deficiency anemia and thalassemia trait, with a sensitivity and a specificity of 93.5% and 78.4%, respectively. This new index has good diagnostic performance and is useful in under-resourced scenarios.

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## INTRODUCTION

Anemia is still a global health burden with an estimated prevalence of 32.9% in 2010[1]. Iron deficiency anemia (IDA) and thalassemia trait (TT) are the most common microcytic and hypochromic anemias worldwide, primarily affecting people living in underdeveloped countries[2].

Correct diagnosis of IDA and TT is essential for choosing the right treatment method, which is distinctively different between these two anemias. Diagnosing IDA requires measuring the levels of serum ferritin and iron, and total iron binding capacity.  $\beta$ -Thalassemia trait can be diagnosed by a level of hemoglobin subunit alpha 2 (HbA2) higher than 3.5% using hemoglobin electrophoresis. Whereas  $\alpha$ -thalassemia trait is indicated by the presence of  $\alpha$ -gene mutations using genetic testing. However, these gold standard tests are time-consuming and expensive, which significantly restricts their use and access in scenarios with limited medical resources.

By using red blood cell (RBC) parameters, various mathematical formulas have been constructed as indexes to discriminate between IDA and TT[3-5]. Although many of these indexes showed good diagnostic performance, parameters such as red cell distribution width (RDW) that is not available on low-end automated blood cell counters are still required [6]. The Matos & Carvalho Index demonstrates good performance in differentiating between IDA and TT, particularly in patients with higher hemoglobin levels (average of 10.9 g/dL), even without the inclusion of RDW[7].

The present study aimed to propose and validate a novel index to discriminate between IDA and TT using the most common RBC parameters.

## MATERIALS AND METHODS

### Patients

This was a retrospective study analyzing the clinical data of the patients diagnosed with IDA or TT during January 2019 through December 2021 at our hospital. The inclusion criteria were: (1) Age  $\geq$  18 years; and (2) mild TT [mean corpuscular volume (MCV) 55-85 fL, HbA2 levels  $>$  3.5%] or early IDA (Hb  $>$  90 g/L, MCV 55-85 fL, ferritin  $<$  12 ng/mL)[8]. Patients were excluded if they had malignant diseases or active infections. IDA patients with  $\alpha$ -TT were also excluded. The study was approved by the Shanghai Tenth People's Hospital, Tongji University School of Medicine.

### Laboratory tests

Two milliliters of venous blood were drawn from each participant using ethylenediaminetetraacetic acid-containing tubes. Blood cells were counted by using an automated blood cell counter (Sysmex, XS-500i, Japan). Hemoglobin electrophoresis was performed at pH 8.6. Levels of HbA2 were measured by the elution method using a cellulose acetate strip. Mutations that cause  $\alpha$ -TT were identified using gene sequencing. The serum levels of ferritin were measured using another 5 mL uncoagulated venous blood using the chemiluminescent immunoassay method.

### Development and validation of the new index

To develop a new index for discriminating IDA and TT, the clinical data of 126 consecutive patients were reviewed. Linear and nonlinear fisher discriminant curves and receiver operating characteristic (ROC) curves were drawn. Then the formula of the index was determined according to the largest area under the curve. This new index was further validated in another 110 consecutive patients.

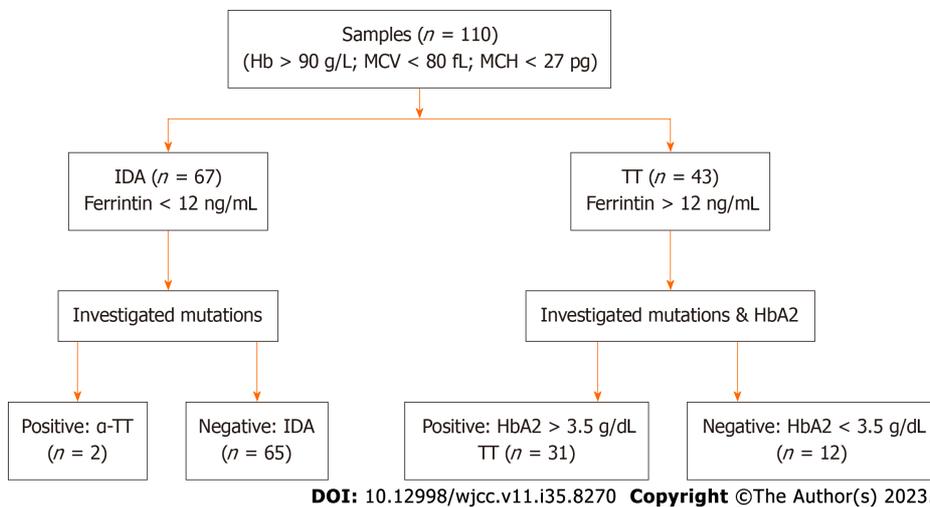
### Statistical analysis

The variables between patients with IDA and patients with TT were compared using the Mann-Whitney test due to the non-normal distribution of the data. The degree of agreement between the new index and the gold standards in diagnosing IDA and TT was analyzed using the Kappa coefficient. ROC curves were drawn using a non-parametric method. All statistical analyses were performed using SPSS Statistics 20 software (IBM Corp, Armonk, NY, United States). A *P* value less than 0.05 was considered statistically significant.

**Table 1 Complete blood count results of patients for the validation of the new index**

Variables	Iron deficiency anemia (n = 65)	Thalassemia trait (n = 31)	P value
RBC (10 <sup>12</sup> /L)	4.5 (0.4)	5.7 (0.6)	< 0.01
Hb (g/L)	104 (11)	115 (11)	< 0.01
HCT	34.2 (3.2)	37.3 (3.8)	< 0.01
MCV (fl)	76.2 (4.3)	65.1 (4.5)	< 0.01
MCH (pg)	23.4 (1.8)	20.7 (2.0)	< 0.01
MCHC (g/L)	305 (11)	312 (21)	0.22
RDW (%)	17.1 (3.8)	16.6 (1.9)	0.522
Platelet (10 <sup>9</sup> /L)	298 (72)	258 (87)	0.21
WBC (10 <sup>9</sup> /L)	6.03 (2.03)	7.19 (2.48)	0.16

RBC: Red blood count; Hb: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; RDW: Red blood cell distribution width.



**Figure 1 Patients for the validation of the new index.** Hb: Hemoglobin; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; IDA: Iron deficiency anemia; TT: Thalassemia trait.

## RESULTS

### Patient characteristics

Inclusion and exclusion of the patients for the validation of the new index are shown in **Figure 1**. The final analysis included 96 patients, consisting of 65 IDA patients and 31 TT patients. The complete blood count results were compared between the IDA patients and the TT patients (**Table 1**). Compared to the IDA patients, the TT patients had significantly higher levels of RBC, Hb, and hematocrit, and significantly lower levels of MCV and mean corpuscular hemoglobin. No significant difference was noticed in the levels of mean corpuscular hemoglobin concentration, red blood cell distribution width, platelet count, and white blood cells between the TT patients and the IDA patients.

### Formula of the new index

The largest area under the ROC curve for the Hb/MCV indicated that this index had the best performance in discriminating between IDA and TT (**Figure 2**). Thus, the Hb/MCV ratio was used as the new index formula. The validity of the Hb/MCV index was compared to other gold diagnostic methods, such as molecular techniques for β-TT, HbA2 measurement for α-TT, and ferritin measurement for IDA. As shown by the ROC curve, the cut-off value of the Hb/MCV index is 1.512, with IDA indicated by a value < 1.512 and TT by a value ≥ 1.512.

### Validation of the Hb/MCV index

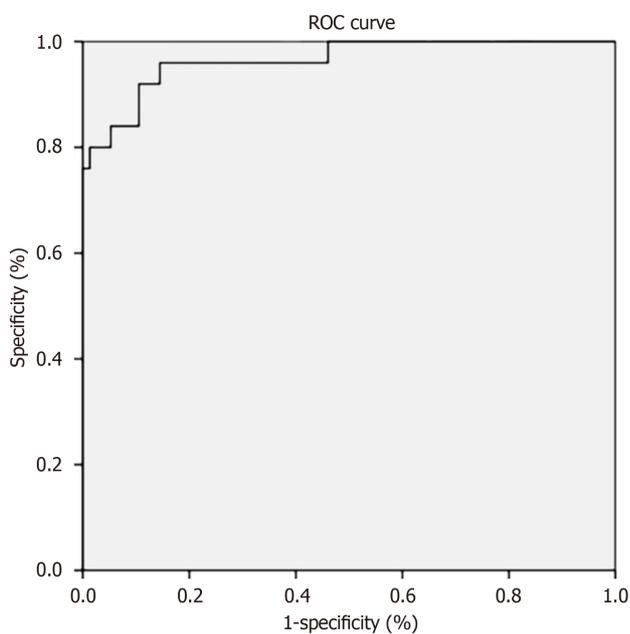
The new index showed good diagnostic performance in discriminating between IDA and TT against gold standard diagnostic methods, with a sensitivity and a specificity of 93.5% and 78.4%, respectively (**Table 2**).

**Table 2** Diagnostic performance of the hemoglobin/mean corpuscular volume index in differentiating between iron deficiency anemia and thalassemia trait against gold standard diagnostic methods<sup>1</sup>

Diagnostic parameters	TT, % (95%CI)	IDA, % (95%CI)
Sensitivity	93.5 (77.2-98.9)	78.4 (66.2-87.3)
Specificity	78.4 (66.2-87.3)	93.5 (77.2-98.9)
Accuracy	83.3 (74.5-89.6)	
Youden's index	0.72	
AUC	0.97 (0.93-1.00)	
Kappa coefficient	0.65 (0.51-0.80)	

<sup>1</sup> $\alpha$ -TT was confirmed by the presence of mutations.  $\beta$ -TT carriers were confirmed by hemoglobin electrophoresis at pH 8.6 and HbA2 levels > 3.5%. And ferritin measurement was confirmed for iron deficiency anemia.

IDA: Iron deficiency anemia; TT: Thalassemia trait; 95%CI: 95% confidence interval; AUC: Area under the receiver operating characteristic curve.



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**Figure 2** Receiver operating characteristic curve for the new index. ROC: Receiver operating characteristic.

## DISCUSSION

TT is caused by genetic mutations and tends to cluster in small geographical regions[9]. In these areas with a high incidence of TT, clinicians are more likely to order genetic testing for diagnosis. However, in regions with complex genetic backgrounds, such as Shanghai, there is a great need of a simple preliminary diagnostic screening index for discriminating between microcytic and hypochromic anemias.

IDA is caused by exhaustion of iron storage or insufficient raw materials for hemoglobin synthesis. Iron is also an essential component for many critical cellular processes[6,10]. TT is an anemia caused by abnormal hemoglobin synthesis induced by genetic mutations. The clinical manifestations of both these diseases present as microcytic and hypochromic anemia.

The Hb/MCV index has a high sensitivity of 93.5% in diagnosing TT. Misdiagnosis of IDA as TT is associated with unnecessary medical burdens on patients. Therefore, early screening for TT is of great importance. The new index requires only two basic results of complete blood count and is very convenient for outpatient settings. An Hb/MCV ratio less than 1.512 indicates low risk of TT, which warrants observation and monitoring of Hb and iron indicators over time. Conversely, if the clinical manifestations also fit, an Hb/MCV ratio  $\geq 1.512$  indicates relatively high risk of TT; genetic testing should then be ordered to confirm the diagnosis.

Despite the convenience and good diagnostic performance of the Hb/MCV index, it is only a screening tool for discriminating between IDA and TT and is not intended to replace the gold standard diagnostic tests for these two anemias.

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## CONCLUSION

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The Hb/MCV index has good diagnostic performance in discriminating between IDA and TT in patients with high hemoglobin concentration (Hb > 90 g/L). This new index requires only two basic results of complete blood count, which can be a desirable feature in areas with insufficient medical resources.

## ARTICLE HIGHLIGHTS

### **Research background**

Iron deficiency anemia (IDA) and thalassemia trait (TT) are common causes of microcytic anemia that are challenging to differentiate, especially in resource-limited settings. A simple screening index using basic complete blood count parameters could facilitate diagnosis.

### **Research motivation**

To develop and validate a new index for discriminating between IDA and TT which only requires two routine complete blood count results.

### **Research objectives**

To determine the optimal formula for a new screening index to differentiate between IDA and TT and validate its performance against gold standard tests.

### **Research methods**

Retrospective analysis of complete blood counts from 126 patients to develop the index formula based on receiver operating characteristic curves. Prospective validation in 61 additional patients using sensitivity, specificity, and other metrics.

### **Research results**

The hemoglobin to mean corpuscular volume ratio (Hb/MCV) showed the best discrimination. An index cut-off of 1.512 differentiated IDA from TT with 93.5% sensitivity and 78.4% specificity.

### **Research conclusions**

The Hb/MCV index successfully differentiated between IDA and TT. As it only requires hemoglobin and MCV, which is convenient for use in resource-limited settings.

### **Research perspectives**

The Hb/MCV index could facilitate screening and early diagnosis of TT in areas without access to genetic testing. Future studies should evaluate it prospectively across diverse populations.

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## FOOTNOTES

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**Author contributions:** Yao QC performed the study and draft the manuscript; Zhai HL collected the data and drafted the manuscript; Wang HC designed the study and analyzed the data. All authors have read and approved the final manuscript.

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