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A PROSPECTIVE EVALUATION OF THE RETICULOCYTE HAEMOGLOBIN CONTENT (CHR) AT THE CHARLOTTE MAXEKE JOHANNESBURG ACADEMIC HOSPITAL

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ABSTRACT

Introduction: The diagnosis of iron deficiency anaemia (IDA) in hospitalised patients with chronic infection and inflammation presents a challenge. Recently laboratory tests such as the reticulocyte haemoglobin content (CHR), which are independent of infection and inflammation, have become available for routine diagnostic use.

Methods: A study was conducted at the Charlotte Maxeke Johannesburg Academic Hospital in order to compare the accuracy of the CHr with that of standard haematological tests for the diagnosis of IDA. The study population included 74 adult and paediatric inpatients that were anaemic. There were 20 patients with IDA, 44 patients with anaemia of chronic disease (ACD) and 10 patients with IDA/ACD as defined according to bone marrow iron stores, supporting iron studies and markers of inflammation.

Results: CHR, mean cell volume, mean cell haemoglobin (MCH) levels were significantly lower in the IDA and IDA/ACD groups as compared to the ACD group (p<0.001). A CHr of >28 pg reliably distinguished IDA and ACD with a sensitivity of 77.78% and a specificity of 79.55%. On ROC analysis, however, the diagnostic performance of the CHr (0.84, 95% CI 0.74-0.94) was not superior to the MCH (0.84, 95% CI 0.73-0.95).

Conclusion: The CHr is a simple, cost-effective, reliable test for the diagnosis of IDA in hospitalised patients. The CHr parameter, however, is not superior to standard haematological tests.

KEYWORDS
Iron deficiency anaemia; anaemia of chronic disorders; reticulocyte haemoglobin content; hospital patients; diagnosis

INTRODUCTION

Iron deficiency is one of the most common nutritional problems in the world and the leading cause of anaemia in children and pregnant women.[1] In South Africa (SA), iron deficiency and anaemia constitute a significant disease burden. A South African population study found the prevalence of iron deficiency to be 5.1% in children under 5 years and 9-12% in pregnant women.[2]

Iron deficiency is a treatable condition. Successful management of iron deficiency anaemia (IDA) requires accurate diagnosis followed by investigation of the underlying cause of iron loss and treatment with iron supplementation. Accurate diagnosis demands differentiation of IDA from the anaemia of chronic disease (ACD), also referred to as anaemia of inflammation.[3] In hospitalised patients, chronic infection(s) and inflammation often coexist with iron deficiency. The distinction between concomitant IDA and ACD is often difficult. In everyday clinical practice, IDA and ACD are traditionally differentiated by assessment of iron studies, which include serum iron, transferrin, transferrin saturation and ferritin.[4] A low ferritin level is highly sensitive for the diagnosis of IDA, but ferritin is also an acute-phase reactant showing an increase in the presence of infection or inflammation when iron is sequestered in reticulo-endothelial system macrophages. A normal ferritin level therefore does not exclude accompanying IDA.[5]

More recently, automated analysers can perform tests that are reported to be independent of infection and inflammation. These include biochemical parameters, namely zinc protoporphyrin and soluble transferrin receptor concentration (sTfR), as well as haematological parameters, namely the percentage of hypochromic red blood cells, and reticulocyte parameters such as the reticulocyte haemoglobin content (CHR) and reticulocyte haemoglobin equivalent (Ret-He). The CHr is calculated from the reticulocyte haemoglobin concentration and the mean cell volume of the reticulocytes on the Advia haematology analysers. If iron stores are low, newly formed reticulocytes will have a low haemoglobin content as iron is required for haemoglobin synthesis. The advantage of this parameter is that the reticulocytes have a shorter lifespan (1 - 2 days) than mature red cells and thus provide an early indication of iron deficiency.[6] Measurement of the CHr can be done on the same specimen used for full blood count (FBC) analysis. This therefore represents an attractive alternative for the diagnosis of iron deficiency in the hospitalised patient. Several recent studies have confirmed the diagnostic performance of the CHr, which is routinely available.[6-9]

However, the diagnostic CHr cut-off values vary according to the study population and diagnostic inclusion criteria, empha-
MATERIALS AND METHODS

Study Design

A laboratory based prospective comparative study was performed at National Health Laboratory Service Haematology Laboratory at the Charlotte Maxeke Johannesburg Academic Hospital, South Africa. This is a quaternary facility which offers specialist medical and surgical treatment including haematology and oncology. The study was approved by the Human Research Ethics Committee of the University of the Witwatersrand (approval number M09-06-88).

Study Population

Seventy four anaemic hospitalised adult and paediatric patients (defined as haemoglobin <116 g/l for women; <134 g/l for men) requiring a bone marrow (BM) examination as part of their diagnostic workup, were enrolled in this study. These participants needed to have a FBC, iron studies (serum iron, transferrin, transferrin saturation, and ferritin), C-reactive protein (CRP), or other biochemical markers of inflammation and BM iron stores results before they were included in the study. The reticulocyte MCV directly affects the CHr. Participants were therefore excluded if they had a diagnosis of thalassemia or any other haemoglobinopathy (falsey lowers the CHr) or a mean cell volume (MCV) >100 fl (falsey elevates the CHr).

Analytical methods

BM aspirates were stained for iron with Perl's Prussian Blue reaction. Absence of stainable BM iron confirmed by two independent pathologists was in keeping with the diagnosis of IDA.

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RESULTS

Comparison of parameters

Mean (SD) values for biochemical and haematological parameters in the IDA, IDA/ACD and ACD groups are presented in Table 2. The CHr levels were normally distributed with mean±SD values of 25.83±3.74, 24.72±4.10 and 31.42±3.91 pg in the IDA, IDA/ACD and ACD groups respectively (P<0.001). In addition, the mean values for MCV, MCH, iron, ferritin and transferrin saturation were significantly lower in the IDA and IDA/ACD groups as compared to the ACD group (P<0.001). The transferrin was significantly higher in the IDA group whereas there was no difference for RDW and Hb. Markers of inflammation were significantly raised in the ACD and combined IDA/ACD groups (P<0.002) when compared to the IDA group. The median (range) CRP, in the ACD and combined IDA/ACD groups, was 46 (4-298) and 142 (69-208) mg/l respectively.

Figure 1 shows the ROC curve for the CHr test for the diagnosis of IDA. The AUC was 0.84 with a 95% confidence interval of 0.74-0.94 (P<0.001). The CHr cut-off with the most optimal sensitivity and specificity to distinguish ACD from IDA was 46 (range) CRP, in the ACD and combined IDA/ACD groups, was 46 (4-298) and 142 (69-208) mg/l respectively.

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<table>
<thead>
<tr>
<th>Clinical Diagnoses</th>
<th>%</th>
</tr>
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<tbody>
<tr>
<td>Chronic infection</td>
<td>59</td>
</tr>
<tr>
<td>Malignancy</td>
<td>23</td>
</tr>
<tr>
<td>Haematologic</td>
<td>21</td>
</tr>
<tr>
<td>Non-haematologic</td>
<td>2</td>
</tr>
<tr>
<td>Autoimmune Disorders</td>
<td>5</td>
</tr>
<tr>
<td>Chronic kidney disease</td>
<td>2</td>
</tr>
<tr>
<td>Benign haematologic conditions</td>
<td>14</td>
</tr>
</tbody>
</table>

* More than one clinical diagnosis per patient
The optimal cut-off values of the MCV and MCH are presented along with sensitivity, specificity and AUC in Table 3. Analysis of the IDA and the IDA/ACD groups was not significant for CHr, MCV and MCH by ROC analysis.

**DISCUSSION**

This study conducted in 74 hospitalised patients, evaluated the performance of the CHr parameter for the diagnosis of IDA using the BM iron stain as the reference. BM biopsy and iron staining is considered the gold standard test for the diagnosis of iron deficiency. However, BM biopsy is an invasive procedure and is no longer considered the standard of care for assessment of iron stores. More recently, markers, such as the CHr, which are independent of infection or inflammation, are available for routine diagnostic use.

In this study, a CHr cut-off of >28pg reliably distinguished IDA and ACD with a sensitivity of 77.78% and a specificity of 79.55%. The AUC for the CHr (0.84, 95% CI 0.74-0.94) indicates that the CHr is a good discriminator of IDA, which can be used as an alternative test in hospitalised patients with chronic infection and inflammation. The CHr was compared with standard haematological tests for the diagnosis of IDA. The sensitivity of the CHr, however, was not superior to the MCH (AUC 0.84, 95% CI 0.73-0.95). This compares with the results of two other studies performed in hospitalised patients where the authors also concluded that the CHr does not perform better than standard tests for IDA.

The reported diagnostic CHr cut-off values vary according to the study population and diagnostic inclusion criteria. In this study, the BM iron stain was used as the reference. Studies, however, using BM iron stores as the gold standard in hospitalised patients with pathologic conditions are limited. In a study by Mast et al. a comparable CHr cut-off of 28pg, corresponding to a sensitivity of 73.9% and a specificity of 73.3%, was shown to be indicative of IDA in 106 patients. Karlsson et al. reported a higher CHr cut-off of 30.5pg, corresponding to a sensitivity of 93% and a specificity of 69%, in 54 elderly patients. Studies performed in elderly patients have reported a higher MCV and MCH in the IDA and ACD groups. Studies to date have not been performed in children using BM iron stores as the diagnostic criterion.

A local study performed at Pelonomi Regional Hospital, Bloemfontein, in 100 infants and children aged 6 months - 6 years showed that the optimal CHr cut-off for the diagnosis of iron

**Table 2:** Comparison of haematological and biochemical laboratory parameters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IDA (n=20)</th>
<th>Combined IDA/ACD group (n=10)</th>
<th>ACD group (n=44)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb, g/l</td>
<td>77.25 ± 30.66</td>
<td>92.1 ± 18.29</td>
<td>75.95 ± 18.70</td>
</tr>
<tr>
<td>MCH, pg</td>
<td>23.81 ± 3.42</td>
<td>23.66 ± 3.91</td>
<td>28.22 ± 2.75</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>79.18 ± 10.15</td>
<td>76.23 ± 8.81</td>
<td>87.97 ± 5.90</td>
</tr>
<tr>
<td>RDW, (%)</td>
<td>19.47 ± 4.60</td>
<td>18.37 ± 3.40</td>
<td>18.57 ± 3.24</td>
</tr>
<tr>
<td>CHr, pg</td>
<td>25.83 ± 3.74</td>
<td>24.72 ± 4.10</td>
<td>31.42 ± 3.91</td>
</tr>
<tr>
<td>Fe, umol/l</td>
<td>5.12 ± 3.51</td>
<td>4.63 ± 6.72</td>
<td>19.13 ± 16.50</td>
</tr>
<tr>
<td>Transferrin, g/l</td>
<td>2.83 ± 0.91</td>
<td>1.83 ± 0.67</td>
<td>1.79 ± 0.55</td>
</tr>
<tr>
<td>Transferrin saturation, (%)</td>
<td>7.58 ± 5.37</td>
<td>9.30 ±8.93</td>
<td>41.56 ± 30.88</td>
</tr>
<tr>
<td>Ferritin, ug/l</td>
<td>14 (6-244)</td>
<td>115 (10-292)</td>
<td>1188 (165-53290)</td>
</tr>
</tbody>
</table>

**Table 3:** Test characteristics based on optimal cut-off values for the diagnosis of iron deficiency anaemia and anaemia of chronic disease determined by receiver operating characteristic curve analysis.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>AUC</th>
<th>Cut-off</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCH, pg</td>
<td>78.95</td>
<td>85.71</td>
<td>0.84</td>
<td>&lt;25.95</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MCV, fl</td>
<td>89.47</td>
<td>74.42</td>
<td>0.82</td>
<td>&lt;85.15</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CHr, pg</td>
<td>77.78</td>
<td>79.55</td>
<td>0.84</td>
<td>&lt;28.35</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Key: IDA, iron deficiency anaemia; ACD, anaemia of chronic disorders; Hb, haemoglobin; MCH, mean cell haemoglobin; MCV, mean cell volume; RDW, red cell distribution width; CHr, reticulocyte haemoglobin content; Fe, iron. Parametric tests are expressed as mean ± SD and nonparametric tests are expressed as median (range).
deficiency was 29 pg. This corresponded to a sensitivity of 86% and a specificity of 50%, using a transferrin saturation of <25% as the diagnostic criterion for iron deficiency.19

The CHr cut-off of 28 pg obtained in this study is specific to this patient population and cannot be generalised to other patient populations. ACD described in other studies is characteristically a mild to moderate anaemia (Hb 80-90.5 g/l).19 In contrast, the mean ±SD Hb levels in this study population were moderate to severe (IDA group, 77.25±30.66; combined IDA/ACD group, 92.10±18.30; ACD group, 75.90±18.70).

In SA there is a high burden of chronic infections such as tuberculosis and HIV.19 More than half of the patients with ACD had a clinical diagnosis of chronic infection. Furthermore, the prevalence of HIV infection in the ACD and combined IDA/ACD groups was 50%. Anaemia has been reported in up to 95% of HIV patients during the course of disease, reflecting cytokine dysregulation, (IL-6, IL-10) drug therapy, presence of malignancy, presence of infection and/or nutritional deficiencies.10–14

In this study, patients with isolated IDA were analysed separately from those with combined IDA/ACD. In hospitalised patients, chronic infection(s) and inflammation often coexist with iron deficiency. The distinction between concomitant IDA and ACD is often difficult. Determination of the CHr, however, was not helpful for distinguishing IDA from the combined state of IDA/ACD (P=0.375). However, a low number of patients with IDA/ACD were investigated (n=10) which is a limitation of this study. Further, this study was laboratory based without access to clinical details such as whether patients in the IDA group started iron supplementation as the CHr corrects much sooner than BM iron stores. In addition, Thomas and Thomas have demonstrated that the CHr in conjunction with laboratory measurements such as the STfR index and the proportion of hypochromic red cells in combination with diagnostic plots were able to diagnose IDA/ACD more accurately.10,14 These tests, however, were not performed, which is a further limitation of this study.

CONCLUSION

The findings of this study indicate the CHr parameter is not superior to standard haematological tests for the diagnosis of IDA in a group of hospitalised patients when using BM iron stores as the gold standard. The CHr test is however a simple, cost-effective reliable test which can be determined from the sample used for FBC analysis and therefore is a good alternative test to diagnose IDA in hospitalised patients.

DECLARATION OF CONFLICT OF INTERESTS

The author(s) declare no conflicts of interest with respect to the authorship and/or publication of this article.

REFERENCES