Reticulocyte hemoglobin content in 13 critically ill patients: a preliminary study

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SUMMARY

In the presence of inflammation, iron availability for erythropoiesis is decreased, and hemoglobin production is reduced in reticulocytes. The reticulocyte hemoglobin content (CHr) provides a real picture of bone marrow status and could be useful to evaluate iron metabolism in critically ill patients. We conducted a preliminary study to evaluate the feasibility to measure the CHr in the intensive care units of two university hospitals and to evaluate the impact of C-reactive protein on CHr values. The CHr was measured in 14 consecutive critically ill patients hospitalized in the intensive care unit between 48 and 96 hours. One patient with a ferritin concentration < 100 µg/L was excluded to eliminate a possible coexistence of iron deficiency with inflammation. A statistically significant correlation was observed between C-reactive protein concentrations and CHr values ($r = -0.588; P = 0.03, n = 13$). The results observed in this preliminary study are interesting and could be useful to establish the research protocol for a future study evaluating iron metabolism in critically ill patients. A larger study is feasible and warranted given the results observed in this preliminary study.

At the time of this study, Geneviève Préfontaine was MSc student.

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- Anemia
- CHr
- Critical illness
- Inflammation
- Iron metabolism
INTRODUCTION

Anemia is a frequent complication in the intensive care unit (ICU).\(^1,2\) Several causes may be at the source of this anemia such as many blood samples for laboratory analyses, surgical procedures, blunted erythropoietin secretion\(^3–5\), as well as malnutrition and alteration of iron metabolism.\(^6\) It has been demonstrated that inflammation alters the usual markers of iron metabolism, making it difficult to evaluate iron stores.\(^7,8\) The anemia observed in the ICU is similar to the anemia in chronic inflammatory diseases, except that it occurs in acute situations.\(^9–11\) Serum iron and transferrin saturation are lower in inflammatory states, whereas the synthesis of ferritin is increased by the presence of inflammatory mediators.\(^10,12\) Consequently, increased ferritin in the presence of inflammation does not necessarily correspond to adequate iron stores. The clinical interpretation of these usual markers is therefore not reliable in critically ill patients.

As the lifespan of hemoglobin is about 120 days, hematological tests based on the hemoglobin do not reflect the actual iron availability for erythropoiesis.\(^13,14\) The reticulocyte hemoglobin content (CHr) is a new hematologic marker of interest. CHr is a more reliable parameter and is an early marker for iron deficiency because the reticulocytes remain in the blood stream for only 1–2 days.\(^15\) In the presence of inflammation, iron availability for erythropoiesis is decreased, and hemoglobin production is reduced in reticulocytes. Thus, CHr provides a real picture of bone marrow status and could be useful to evaluate iron metabolism in critically ill anemic patients.\(^13,15\)

This study was a preliminary study to evaluate whether it would be feasible to measure CHr in critically ill patients. The objective of this study was to evaluate the impact of elevated C-reactive protein (CRP) concentrations on CHr values in critically ill patients in order to explore the mechanisms leading to anemia in the presence of acute inflammation.

MATERIALS AND METHODS

This was a prospective observational study conducted in ICU at the Hôtel-Dieu de Lévis and at the Centre Hospitalier Universitaire de Sherbrooke between December 2006 and March 2007. The study was approved by the local ethics committee at both institutions, and informed consent was signed by the patient or a member of the family. The CHr is measured using an Advia 120 automated Haematology System (Siemens Healthcare Diagnostics, Tarrytown, NY, USA) and was not available at the study sites. Blood samples for the measurements of the CHr had to be sent to the CRED laboratory (Collaborative Research for Effective Diagnostics, Sherbrooke, Québec, Canada). Therefore, this study was a preliminary study and for this reason, we enrolled only 14 consecutive critically ill patients. Inclusion criteria were patients with a hemoglobin concentration \(< 13.0 \text{ g/dL}\) and age \(> 18\) years old. To better reflect the changes on iron availability induced by inflammation, only patients with an ICU stay \(\geq 48\) hours were enrolled. To provide a high-inflammatory state in the study population, the patients were not selected if they were in the ICU for more than 96 hours at the time of recruitment. Other exclusion criteria were: chronic hemodialysis, administration of erythropoiesis-stimulating agents or an iron supplement in the last 30 days before being selected for the study and known chronic inflammatory disease. Surgical patients in ICU for uncomplicated elective surgery and patients with chronic alcoholism were also excluded. The iron metabolism status of the 14 patients was then assessed by measuring serum iron concentration, ferritin concentration, transferrin saturation, soluble transferrin receptor concentration (sTFR), zinc protoporphyrin (ZPP) and CHr. Patients with a ferritin concentration \(< 100 \text{ mg/L}\) were excluded to eliminate a possible coexistence of iron deficiency with inflammation.\(^16\)

Data collection

The blood samples were taken at the same time in the morning in order to minimize the impact of possible circadian variations. The Acute Physiology and Chronic Health Evaluation II score was calculated within the first 24 hours after being admitted to the ICU. The laboratory analyses were all centralized and conducted using the same devices. Ferritin, vitamin B\(_12\) and folates were measured by chemiluminescence on an Access2 (Beckman Coulter, Brea, CA, USA). Serum iron (by spectrophotometry), the percentage of iron saturation (derived from the unsaturated iron binding capacity), CRP (by immunoturbidimetry) and sTFR (by immunoturbidimetry) were measured on a Modular (Roche Diagnostics, Indianapolis, IN, USA). Hemoglobin was measured by spectrophotometry on a Gen-S (Beckman Coulter). CHr and reticulocytes count were measured in
whole blood collected in EDTA using Advia 120 automated Haematology System (Siemens Medical Solutions Diagnostics). Blood samples for the measurements of CHr and reticulocytes were stored on ice at 4°C and had to be sent to the CRED laboratory within 72 hours. One blood sample was analyzed within 12 hours, nine blood samples within 24 hours and four blood samples within 48 hours. CHr is derived from the simultaneous measurement of volume and hemoglobin concentration in reticulocytes. The CHr value is an indication of the hemoglobin content of each reticulocyte. The corrected reticulocyte index = reticulocyte (%) x hematocrit x 100/40. ZPP was measured using an Aviv 206 hematofluorimeter (Aviv Biomedica Inc., Lakewood, NJ, USA).

Statistical analysis
Statistical analysis was conducted using the SPSS software (version 12 for Windows; SPSS Inc., Chicago, IL, USA). Means ± standard deviation are presented for continuous variables. Spearman correlation test was applied to establish a correlation between two variables. All testing was two-sided and conducted at a 0.05 significance level.

RESULTS
Among the 14 critically ill patients recruited, one patient presented a possible coexistence of iron deficiency with inflammation (ferritin = 83 µg/L) and was excluded. The characteristics of the 13 remaining patients are presented in Table 1. All patients presented a high-inflammatory status as measured by CRP concentrations >5 mg/L (Table 2), and 11 patients were septic at enrollment. No patient had a history of hepatic disease, and all patients had normal levels of serum transaminases. However, one patient presented a folate deficiency (<5.7 nmol/L), and one patient presented both vitamin B₁₂ (<108 pmol/L) and folate (<5.7 nmol/L) deficiencies.

Hematologic and iron parameters are presented in Table 2. Despite significant anemia, erythropoiesis measured by the corrected reticulocyte index was low in all patients. Two patients were transfused in the days preceding the collection of the blood samples for the study. One patient received one unit of blood, and the other patient received four units of blood. CHr values were <28 pg in six patients, and all patients presented low serum iron concentrations. Ferritin concentrations were elevated in all patients as expected in the presence of inflammation.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>APACHE II score</th>
<th>Primary diagnosis</th>
<th>Intensive care unit stay before study entry (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Male</td>
<td>81</td>
<td>13</td>
<td>Esophageal perforation</td>
<td>3.5</td>
</tr>
<tr>
<td>2</td>
<td>Male</td>
<td>63</td>
<td>19</td>
<td>Septic shock</td>
<td>2.0</td>
</tr>
<tr>
<td>3</td>
<td>Female</td>
<td>49</td>
<td>21</td>
<td>Meningitis</td>
<td>2.4</td>
</tr>
<tr>
<td>4</td>
<td>Male</td>
<td>78</td>
<td>14</td>
<td>Abdominal aortic aneurysm repair</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>Male</td>
<td>77</td>
<td>16</td>
<td>Septic shock</td>
<td>4.0</td>
</tr>
<tr>
<td>6</td>
<td>Female</td>
<td>66</td>
<td>19</td>
<td>Necrotizing fasciitis</td>
<td>3.9</td>
</tr>
<tr>
<td>7</td>
<td>Male</td>
<td>34</td>
<td>9</td>
<td>Necrotizing fasciitis</td>
<td>2.4</td>
</tr>
<tr>
<td>8</td>
<td>Female</td>
<td>89</td>
<td>20</td>
<td>Pneumonia</td>
<td>2.0</td>
</tr>
<tr>
<td>9</td>
<td>Male</td>
<td>53</td>
<td>12</td>
<td>Pneumonia</td>
<td>2.8</td>
</tr>
<tr>
<td>10</td>
<td>Male</td>
<td>35</td>
<td>22</td>
<td>Status epilepticus</td>
<td>2.1</td>
</tr>
<tr>
<td>11</td>
<td>Female</td>
<td>85</td>
<td>32</td>
<td>Pneumonia</td>
<td>2.8</td>
</tr>
<tr>
<td>12</td>
<td>Male</td>
<td>74</td>
<td>20</td>
<td>Septic shock</td>
<td>3.8</td>
</tr>
<tr>
<td>13</td>
<td>Male</td>
<td>60</td>
<td>15</td>
<td>Septic shock</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Mean ± standard deviation 65 ± 18 18 ± 6 2.9 ± 0.8

APACHE, Acute Physiology and Chronic Health Evaluation.
of inflammation. All patients presented low or normal sTFR values. The ZPP values were increased in all patients (the ZPP measurement was omitted in one patient).

A statistically significant correlation was observed between CRP concentrations and CHr values (\( r = -0.588; \) \( P = 0.03, \) \( n = 13; \) Figure 1). Two patients had a mean cellular volume > 100 fL that may have affected the CHr value. When these patients are excluded from the analysis, the correlation between CRP and CHr (\( r = -0.90; \) \( P = 0.001) is even more significant.

There was also a statistically significant correlation between CRP concentrations and the percentage of iron saturation (\( r = -0.641; \) \( P = 0.02, \) \( n = 13)\), as well as between CRP concentrations and serum iron concentrations (\( r = -0.580; \) \( P = 0.04, \) \( n = 13)\).

DISCUSSION

In this small cohort of critically ill patients with no evidence of real iron deficiency, three strong negative correlations were obtained between CRP and serum iron, between CRP and iron saturation, as well as between CRP and CHr values. These results suggest that the greater the inflammation, the less iron there is available for erythropoiesis. The inability to use iron efficiently for erythropoiesis in spite of adequate iron stores is called functional iron deficiency (FID). In the presence of inflammation, patients may develop FID, and it has been described in critically ill patients.\(^9\) Observational studies in critically ill patients all reported a decrease in serum iron and iron saturation along with an increase in ferritin.\(^9\) However, there is no data on CHr in this population of patients. The CHr corresponds to the hemoglobin content in reticulocytes.\(^11\) It is a direct and early marker of the FID as the reticulocytes remain in circulation for 24–48 hours.\(^11\) Thus, the CHr makes it possible to reveal in real time the iron availability for the bone marrow. In the presence of inflammation, the marrow cannot have access to the iron stores, and this situation leads to iron-restricted erythropoiesis despite the presence of adequate iron. A CHr value < 28 pg has been

Table 2. Laboratory parameters at the time of inclusion into the study (\( n = 13)\)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± standard deviation</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammatory status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CRP [mg/L]*</td>
<td>164 ± 116</td>
<td>35–463</td>
</tr>
<tr>
<td>Hematologic parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin [g/dL]</td>
<td>10.6 ± 12</td>
<td>89–128</td>
</tr>
<tr>
<td>Absolute reticulocyte count</td>
<td>41 ± 17</td>
<td>10–61</td>
</tr>
<tr>
<td>Corrected reticulocyte index (%)</td>
<td>0.96 ± 0.41</td>
<td>0.26–1.50</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>94 ± 6</td>
<td>88–110</td>
</tr>
<tr>
<td>CHr [pg]†</td>
<td>29.6 ± 3.6</td>
<td>25.3–35.8</td>
</tr>
<tr>
<td>Iron parameters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ferritin [µg/L]</td>
<td>581 ± 572</td>
<td>108–2281</td>
</tr>
<tr>
<td>Serum iron [µmol/L]</td>
<td>6.8 ± 4.3</td>
<td>2–14</td>
</tr>
<tr>
<td>Iron saturation (%)</td>
<td>22 ± 14</td>
<td>5–45</td>
</tr>
<tr>
<td>ZPP [µg/g Hb]‡</td>
<td>6.4 ± 2.6</td>
<td>3.4–12.0</td>
</tr>
<tr>
<td>sTFR [mg/L]§</td>
<td>2.3 ± 0.9</td>
<td>1.3–3.8</td>
</tr>
</tbody>
</table>

*CRP: 0–3 mg/L. †CHr: 28–35 pg. ‡ZPP: 0–3.6 µg/g Hb. §sTFR: 1.9–4.4 mg/L (women) and 2.2–5.0 mg/L (men).

CHr, reticulocyte hemoglobin content; CRP, C-reactive protein; MCV, mean cellular volume; sTFR, soluble transferrin receptor; ZPP, zinc protoporphyrin.

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shown to be a predictor for iron deficiency.\textsuperscript{14,20} In the present study, we tried to eliminate the coexistence of iron deficiency with inflammation, and therefore one patient with a ferritin < 100 µg/L was excluded. Despite no evidence of inadequate iron stores in the remaining patients, six patients presented a CHr value < 28 pg (Figure 1) suggesting FID.

Several studies have been conducted on the use of CHr in the monitoring of iron metabolism in hemodialysis patients receiving recombinant human erythropoietin (EPO).\textsuperscript{21–25} In a study conducted in hemodialysis patients with a respiratory tract infection, it was observed that the decrease in CHr values was better correlated with the decrease in hematocrit and the increase in CRP concentrations compared with the iron saturation.\textsuperscript{26} Another study conducted by Fishbane et al. reported that the use of CHr compared with ferritin and iron saturation reduced the needs of iron by hemodialysis patients.\textsuperscript{21} In these patients, the CHr was also a more stable marker, having a less variation throughout the study. Mittman et al. monitored the CHr of 364 hemodialysis patients, and the CHr values were in correlation with hemoglobin concentrations and hematocrit values.\textsuperscript{23} The use of CHr as an early marker of iron-deficient erythropoiesis is well documented in hemodialysis patients. However, there is a lack of data on the use of CHr in patients with chronic or acute inflammatory diseases. To our knowledge, our study is the first to present data on CHr in anemic ICU patients.

In this study, the ZPP values were elevated in all patients. ZPP is a sensitive marker that increases in the presence of iron-deficient erythropoiesis, even before the hemoglobin decreases and iron deficiency anemia occurs.\textsuperscript{27} The ZPP increases also in the presence of inflammatory anemia as the transportation of iron is altered and insufficient for erythropoiesis.\textsuperscript{27} The synthesis of sTFR is not directly affected by the presence of inflammation, which makes it particularly interesting for distinguishing iron deficiency anemia from anemia of inflammation.\textsuperscript{28–30} The circulating sTFR is the reflection of erythropoietic activity in the marrow, and it increases only in the presence of iron deficiency or during increased erythropoiesis.\textsuperscript{31} In this study, all sTFR concentrations were low or within the normal range indicating no real iron deficiency.

This study has several limits. First, only 14 patients were recruited. Consequently, the significant correlation observed between CHr and CRP could simply be attributable to chance or to a third variable related to both of these variables. Also, this small ICU population had various diagnostics, and the results observed are difficult to generalize. However, all patients had an important inflammatory status, and there was no history of hepatic disease or chronic inflammatory disease that could have affected the iron metabolism. Other issues such as blood transfusions may have influenced the hematologic parameters. However, only two patients were transfused in the days preceding the collection of the blood samples. It is also possible that folate deficiency coexists with the anemia observed in critical illness, and this should be addressed in future research protocols.\textsuperscript{32} Indeed, the CHr is the product of the cellular volume and cellular hemoglobin concentration. The second issue with regard to the study design was that blood samples were collected only once. It would have been interesting to evaluate the course of CHr during the ICU stay. The blood samples were collected between 48 and 96 hours after admission to the ICU. This delay was necessary to organize the transport of blood samples to the CRED laboratory and ensure the analysis within 72 hours. For stability reasons, blood samples stored for 72 hours at 4°C may be suitable for the analysis of the CHr. In this study, almost all blood samples were analyzed within 24 hours, and the others were analyzed within 48 hours.

The administration of EPO in critically ill patients has been shown to reduce the need for blood transfusions.\textsuperscript{33–35} The amplification of erythropoiesis that results from the administration of EPO increases the need for iron. Future studies using CHr values during EPO administration and following ICU discharge would be useful to prevent iron deficiency anemia\textsuperscript{36,37} and to optimize EPO effect.

Despite normal or elevated ferritin concentrations, the significant negative correlation between CRP concentrations and CHr values observed in this small cohort of critically ill anemic patients suggests that inflammation reduces iron availability for erythropoiesis in ICU.

**CONCLUSION**

The results observed in this preliminary study are interesting and could be useful to establish the research protocol for a future study evaluating iron metabolism in critically ill patients. A larger study is feasible and warranted given the results observed in this preliminary study.
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