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Is iron deficiency anemia always microcytic?

Short title: Hypochromic or microcytic anemia

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Introduction:
Iron Deficiency Anemia (IDA) is the most common form of anemia worldwide. [1, 2] In developed countries, 6-8% of adult population has anemia including 40–45% suffering from iron deficiency. Iron Deficiency (ID) is typically developed on a background of insufficient dietary intake, malabsorption, hemorrhage/hemoglobinuria or increased demand. As ID progresses it turns into IDA. IDA can be diagnosed on a combined evidence of anemia and iron deficiency.
Anemia of inflammation (AI) is an important differential diagnostic problem of IDA in many cases. AI is a frequent (35% of anemia) and very special form of anemia. In AI the inhibition of erythropoiesis (by Tumor necrosis factor (TNF) and TNF releated apoptosis inducing ligand (TRAIL)) is combined with the blockaged iron expression into the blood (from splenic macrophages, duodenal enterocyte and hepatocyte), because of elevated hepcidine (Interleukine 6 (IL6) - acute phase reaction). In AI the serum iron is generally low, the transferrin level is low (reciproc acute phase reaction) and so the transferrin saturation can be normal or in few cases - low. The low transferrin level in AI vs high/normal in IDA helps us to differentiate between the two forms of anemia. In AI the inhibition of erythropoiesis and the blockaged iron metabolism is generally proportional, so the anemia is normochromic and hyporegenerative (low reticulocyte count).

Iron is essential for hemoglobin (hem) production and only a very severe ID would directly inhibit erythropoiesis; hence, anemia associated with IDA is characterized by suppressed hemoglobin production and a more or less well-sustained red blood cell count. [3]

Reduction of mean corpuscular hemoglobin (MCH) content also results in reduction of their volume (MCV). Commonly used laboratory automated analyzers calculate MCV (hematocrit (Htc) / red blood cell (RBC)) and MCH (Hemoglobin (Hgb) / RBC) from the measured parameters (RBC, Hct, Hgb). [4] Previous generations of laboratory analyzers used to measure MCV directly, and IDA was defined as anemia with low MCV or microcytic anemia.

Red cell volume constantly decreases during their 100-120-days lifespan from spheroid reticulocyte with an initial 150-170 fl to the late form of aged red cells. [5] Red blood cells in IDA are characterized by reduced cell volume and also reduced hemoglobin content generally called hypochromasia. Presuming no double pathology including associated advanced mechanic intravasal injury or hemolysis, red blood cell hemoglobin content is constant during
their life span (it develops at late reticulocyte stage, which is measured). All the above highlight the role of MCH, and IDA can be characterized by hypochromic feature rather than mirocytic phenotype. [5-6]

The laboratory of Somogy Country Kaposi Mór Teaching Hospital analyzes samples from several thousands of patients with anemia per year and we found strong correlations between reduced MCH and iron deficiency. We analyzed our data and report the results in this article to demonstrate the significance of MCH compared to MCV in the field of diagnose ID. We analyzed MCV and MCH results in 2,503 patients with ID including 1,733 patients with IDA.

**Patients and methods:**

The patient population of Kaposi Mór Multidisciplinary County Teaching Hospital (with 1,000 beds) was evaluated based on 644,556 samples tested for complete blood count (CBC) over a 5-years period (2013-2017) (excluding patients below 18 years and/or receiving hemodialysis therapy). (The vast majority of the tested population was Caucasian-Hungarian with nearly no cases of moderate to severe hemoglobinopathies). Iron deficiency was defined as transferrin saturation (TRFsat) ≤ 20% or reticulocyte hemoglobin (Ret Hgb) ≤ 28 pg [7, 8, 9] or ferritin ≤ 30 µg/L in males, and ≤ 20 µg/L in females.

CBC was tested on Sysmex XN-2100i (Hoffmann-LaRoche) laboratory automated analyzer including hemoglobin measured by sodium lauryl sulfate (SLS)-method, red blood cell count measured by impedance test, reticulocyte measured by flow cytometry, respectively. Iron parameters were tested on COBAS 8000 c502-es (Roche) immunochemistry analyzer. Serum iron levels were tested by FerroZine method at 552 nm, and results were provided in mmol/L. The first threshold was 6.6 mmol/L for females, and 11 mmol/L for males (later the threshold
for females was also changed to 11 mmol/L). Ferritin levels were measured by electrochemiluminescence immunoassay – ECLIA, and transferrin was tested by immunoturbidimetry. Transferrin saturation was calculated based on the following formula: serum iron/transferrin × 3.98. The results were provided in percentage, and values ≤ 20 % were considered as abnormal.

Anemia was diagnosed in patients with hemoglobin levels ≤ 130 g/L in males and ≤ 120 g/L in females. [10]

Statistical analysis: In addition to the descriptive statistical method, the chi-square test was used to compare dichotomous variables. Statistical analysis was performed using the open-source R statistical software package, version 3.1.2 (The R Foundation for Statistical Computing); statistical tests were interpreted at a 5% significance level.

Ethical approval was granted by the Research Ethics Committee of the Somogy County Moritz Kaposi General Hospital. As it is a retrospective data analysis, written consent of patients is not required.

Results:

Over the 5 years of this retrospective study 644,556 samples were tested for CBC, with gender ratio of 365,271 females/279,285 males. Iron parameters were concurrently tested in 35,398 cases, 23,321 females/12,077 males.

In the 35,398 cases with measured iron parameters, MCH and MCV were compared: MCH were ≤ 28 pg in 8,915 patients, among them 4,039 (45.3%) having MCV ≤ 80 fl. In the 26,483 cases with MCH>28 pg, 150 cases (0.6%) having MCV ≤ 80 fl.

Serum Iron levels were tested in 30,830 cases (20,867 females/9,963 males).

Transferrin saturation was also tested in 2,938 cases (1,972 females/966 males).

Ferritin results were available in 2,551 cases (1,680 females/871 males)
Reticulocyte hemoglobin was tested in 5,117 cases (2,835 females/2,282 males)

Table 1

Among 2,503 patients with confirmed iron deficiency 1,733 (69.4%) were identified as having iron deficiency anemia (IDA). 1,241 females (67.6%) and 492 males (73.7%) were diagnosed with IDA.

1,529 IDA patients (88.2%) had MCH ≤ 28 pg, 1,108 females (89.3%) and 421 males (85.6%). 916 IDA patients (52.9%) had MCV ≤ 80 fL 707 females (57%) and 209 males (42.5%). Hypochromia was significant compared to microcytosis p< 0.001 in term IDA.

**Discussion:**

Currently, IDA is primarily defined as microcytic anemia. [11] The results of our retrospective analysis highlight the importance of MCH in the measurements with commonly used hematology automated analyzers. While hypochromia occurred in nearly 90% of IDA cases, microcytosis was found only in 53%. Ret Hgb was shown to be essential among the measured reticulocyte parameters. MCH results are secondary to its alterations, and theoretically it should have an important role amongst other CBC parameters. This retrospective analysis has provided significant evidence supporting the previous assumption of importance of MCH in the diagnosis of IDA.
References:


Table 1 Summary of Complete Blood Count results in patients with iron deficiency and Iron deficiency anemia

<table>
<thead>
<tr>
<th>No. of patients with ID</th>
<th>Female (1836)</th>
<th>Male (667)</th>
<th>Total (2503)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferritin (male ≤ 30 µg/L, female≤ 20 µg/L) or, Trf sat. (&lt;20%) or, Ret Hgb (&lt; 28 pg)</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>MCH ≤ 28 pg</td>
<td>1464</td>
<td>79.7</td>
<td>527</td>
</tr>
<tr>
<td>MCV ≤ 80 fL</td>
<td>857</td>
<td>46.7</td>
<td>267</td>
</tr>
<tr>
<td>IDA</td>
<td>1241</td>
<td>67.6</td>
<td>492</td>
</tr>
<tr>
<td>-- MCH ≤ 28 pg</td>
<td>1108</td>
<td>89.3</td>
<td>421</td>
</tr>
<tr>
<td>-- MCV ≤ 80 fL</td>
<td>707</td>
<td>57.0</td>
<td>209</td>
</tr>
<tr>
<td>ID without anemia</td>
<td>595</td>
<td>32.4</td>
<td>175</td>
</tr>
<tr>
<td>-- MCH ≤ 28 pg</td>
<td>357</td>
<td>60.0</td>
<td>106</td>
</tr>
<tr>
<td>-- MCV ≤ 80 fL</td>
<td>151</td>
<td>25.4</td>
<td>58</td>
</tr>
</tbody>
</table>

MCH: Mean Corpuscular Hemoglobin, MCV: Mean Corpuscular Volume, ID: Iron deficiency, IDA: Iron Deficiency Anemia, Trf sat.: Transferrin saturation