Iron deficiency in sepsis patients based on reticulocyte hemoglobin and hepcidin concentration: a prospective cohort study

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Abstract

Introduction: Iron tests are deranged in sepsis; therefore new biomarkers should be used for diagnosis of iron deficiency (ID)/ID anemia (IDA).
Methods: Diagnosis of ID/IDA was based on reticulocyte (RET) hemoglobin (Hb) equivalent (RET-He) and Hb concentration, with hepcidin (Hep) determined retrospectively.
Results: The prevalence of ID and IDA was 7% and 47%, respectively. The AUROCs for RETs number and Hep in prediction of ID/IDA were 0.69 and 0.62, respectively.
Conclusions: Approximately half of sepsis patients are iron-deficient. Number of RETs may be a predictor of ID/IDA when RET-He is not available. Hepcidin is a poor IDA predictor.

Key words: hepcidin, intensive care unit, iron deficiency anemia, reticulocyte hemoglobin equivalent, sepsis.

Iron is crucial for host immunity and hemoglobin (Hb) synthesis. The ability to fight an infection and provide aerobic metabolism is especially important for sepsis patients; however, iron is also used by certain bacteria and fungi for growth. Bacteria evolved transport systems for both free iron and its organic forms [1]. Therefore iron concentration has to be kept at optimal levels in sepsis patients.

Ferritin and transferrin, used in standard iron tests, are acute phase proteins and cannot be used for accurate iron deficiency (ID)/ID anemia (IDA) diagnosis in critically ill patients with systemic inflammation [2]; therefore there are no reliable data regarding prevalence of ID/IDA in sepsis patients.

The aim of the study was to determine the prevalence of ID/IDA in patients with sepsis or septic shock hospitalized in the intensive care unit (ICU) using new laboratory biomarkers devoid of aforementioned limitations, namely reticulocyte (RET) Hb equivalent (RET-He) and hepcidin (Hep) concentration.

Methods. This prospective clinical study was carried out in a 10-bed mixed medical-surgical ICU between September 24th 2021 and August 31st 2022. We enrolled consecutive patients diagnosed with sepsis or

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septic shock using the third international definition and appropriate diagnostic criteria [3]. Additionally procalcitonin (PCT) was used to improve the accuracy of sepsis diagnosis. We set the PCT cut-off value at > 0.5 ng/ml, as it has been shown that with lower concentrations systemic infection is unlikely [4].

The detailed description of the methodology is covered in Appendix 1.

Results. We analyzed data of 90 study subjects. The median age in the study group was 65 (IQR 51–72) years. Acute kidney injury was present in 21% of study subjects, and 11% required renal replacement therapy. Common anatomical sites of infection were pulmonary (36%), abdominal (21%), and urinary (21%).

There were 6 (6.7%) study subjects diagnosed with ID and 42 (46.7%) with IDA. Comparison of hematological indices and Hep concentration in patients with ID/IDA and normal iron status (NIS) is presented in Table I. RET-He in the ID/IDA and NIS group was 26.5 (IQR 25.6–27.3) and 33.4 (IQR 32.7–34.1) pg, respectively ($p < 0.01$). Statistically significant differences between ID/IDA and NIS groups were noted for Hb, hematocrit, mean corpuscular volume (MCV), mean cell Hb, percentage and number of RETs. AUROCs for these hematological parameters in prediction of ID/IDA were 0.64–0.69. The most accurate in this context was number of RETs, with AUROC of 0.69 (95% CI: 0.58–0.78, $p < 0.01$) and the optimal cut-off value at ≤ 0.07 10^6/µl.

The difference in Hep concentration between ID/IDA and NIS groups was close to statistical significance; therefore we decided to analyze this biomarker in the separate subgroups of ID and IDA patients. A significant difference in Hep concentration was noted only between IDA and NIS patients (Me 750 (IQR 413–22870) vs. Me 1184 (IQR 664–2462) pg/ml, $p = 0.05$). AUROC for Hep concentration in prediction of IDA was 0.62 (95% CI: 0.51–0.72, $p = 0.049$), with the optimal cut-off value at ≤ 493 pg/ml. Comparison of hematological indices and Hep concentration in ID and IDA groups is presented in Table II. RBCs, Hb, and hematocrit were significantly lower in the IDA compared to the ID group (p for all < 0.01).

Discussion. Diagnosis of ID/IDA in our study was based on RET-He. Different RET-He cut-off values have been used for diagnosis of IDA in the past: 25 [5], 28 [6], 29 [7], and 30 [8] pg. We decided to use as the cut-off the lower limit of the local laboratory reference range (i.e. < 30.2 pg) as our intention was to identify even mild ID. Based on our diagnostic approach we identified high prevalence of ID/IDA in sepsis patients hospitalized in the ICU – 53.3%. The only study that used RET-He for ID diagnosis in the ICU was carried out in a general ICU population. The reported ID prevalence was 37% [9]. This result was lower than in our study, but the cut-off value used was also lower (< 28 vs. < 30.2 pg).

Another new biomarker that we researched was Hep. There is however no gold standard meth-

Table I. Hematological indices and hepcidin concentration in the study subjects with and without iron deficiency/iron deficiency anemia

Parameter	ID/IDA (n = 48, 53.3%)	NIS (n = 42, 46.7%)	P-value
RBC [10^6/µl]	3.2 (2.9–3.9)	3.6 (3.0–4.2)	0.13
Hb [g/l]	**9.9 (9.3–10.5)**	**10.9 (10.3–11.6)**	**0.01**
Hematocrit (%)	**30.1 (26.7–33.5)**	**33.6 (29.4–38.9)**	**0.02**
Mean cell volume [fl]	**90.2 (87.9–92.5)**	**93.4 (91.5–95.3)**	**0.04**
Mean cell Hb [pg]	**29.3 (27.7–30.4)**	**30.2 (29.3–31.7)**	**0.02**
Mean cell Hb concentration [g/dl]	32.1 (31.2–33.2)	32.6 (31.6–33.6)	0.22
RBC distribution width-SD [fl]	51.9 (47.0–56.1)	50.2 (45.8–55.0)	0.60
RBC distribution width-CV (%)	15.4 (14.0–17.1)	15.1 (14.0–16.8)	0.46
RETs (%)	**1.4 (1.1–2.1)**	**2.2 (1.4–2.8)**	**< 0.01**
RETs [10^6/µl]	**0.05 (0.04–0.07)**	**0.08 (0.05–0.09)**	**< 0.01**
Immature RETs fraction (%)	19.5 (16.9–21.9)	19.2 (16.1–22.4)	0.91
Low fluorescence ratio RETs (%)	81.6 (73.9–87.8)	83.0 (73.6–87.5)	0.84
Medium fluorescence ratio RETs (%)	13.2 (11.9–14.5)	12.2 (10.9–13.6)	0.29
High fluorescence ratio RETs (%)	3.4 (2.0–10.3)	5.0 (1.8–10.1)	0.83
RET-He [pg]	26.5 (25.6–27.3)	33.4 (32.7–34.1)	< 0.01
Hepcidin [pg/ml]	813 (423–2287)	1184 (664–2462)	0.09

ID – iron deficiency, IDA – iron deficiency anemia, RBC – red blood cells, Hb – hemoglobin, RETs – reticulocyte/s, RET-He – reticulocyte hemoglobin equivalent, NIS – normal iron status, statistically significant values in bold.

Table II. Hematological indices and hepcidin concentration in the study subjects with iron deficiency and iron deficiency anemia

Parameter	ID (n = 6)	IDA (n = 42)	P-value
RBC [10^6/µl]	**4.7 (4.2–4.8)**	**3.2 (2.9–3.6)**	**< 0.01**
Hb [g/l]	**13.4 (12.2–14.7)**	**9.4 (8.9–9.9)**	**< 0.01**
Hematocrit (%)	**41.9 (40.1–43.0)**	**29.5 (26.1–31.7)**	**< 0.01**
Mean cell volume [fl]	87.6 (73.6–101.7)	90.6 (88.4–92.8)	0.39
Mean cell Hb [pg]	29.3 (25.2–30.3)	29.3 (27.8–30.4)	0.66
Mean cell Hb concentration [g/dl]	32.0 (30.2–32.8)	32.1 (31.3–33.5)	0.46
RBC distribution width-SD [fl]	52.5 (48.5–56.5)	51.9 (45.1–55.8)	0.66
RBC distribution width-CV (%)	15.7 (14.4–16.9)	15.4 (14.0–17.2)	0.71
RETs (%)	1.6 (0.7–2.3)	1.4 (1.1–2.0)	0.95
RETs [10^6/µl]	0.07 (0.03–0.09)	0.05 (0.04–0.06)	0.38
Immature RETs fraction (%)	21.3 (11.4–31.2)	19.2 (16.5–21.9)	0.58
Low fluorescence ratio RETs (%)	78.0 (73.4–87.7)	81.8 (73.9–87.9)	0.54
Medium fluorescence ratio RETs (%)	14.8 (9.8–19.8)	12.9 (11.7–14.3)	0.34
High fluorescence ratio RETs (%)	5.4 (2.0–11.9)	3.4 (2.1–10.3)	0.96
RET-He [pg]	26.9 (22.9–30.8)	26.4 (25.5–27.3)	0.69
Hepcidin [pg/ml]	1462 (762–2498)	750 (413–2287)	0.30

ID – iron deficiency, IDA – iron deficiency anemia, RBC – red blood cells, HB – hemoglobin, RET – reticulocyte/s, RET-He – reticulocyte hemoglobin equivalent; statistically significant values in bold.

od for measuring Hep concentration and there is no standard reference. Moreover, Olinder et al. observed variability in Hep concentration over the first 4 days of hospitalization in the ICU and a negative correlation with RET-He [10]. Despite the enthusiastic approach towards Hep exploitation, the wide array of clinical conditions influencing its levels may limit its value as an IDA biomarker. In order to compare Hep concentrations obtained in our study to the results of other authors we searched for studies that used the ELISA test for Hep determination. In the study investigating Hep concentration in patients with hepatitis C virus (HCV) infection, the authors reported median baseline Hep concentration of 75 pg/ml in the study group and 60 pg/ml in the control group [11]. The results obtained in our study were much higher than those obtained in patients with HCV or healthy controls. In the study performed in an inflammatory bowel disease population, mean Hep concentration in ulcerative colitis and Crohn's disease patients was 4090 ±1005 pg/ml and 3798 ±1337 pg/ml, respectively [12]. This result on the other hand was much higher than that obtained in our study; however, concentrations in our study were in the wide range 32.0–32,628.6 pg/ml.

Our study is not without limitations. The first limitation is the number of study subjects. However, we performed a posteriori power calculations for a sample size and we discovered that we would require a minimum of 86 patients to verify the differences in values between NIS, ID, or IDA, with an $\alpha \leq 0.05$ and a β 0.20. Therefore, we consider that our sample size is sufficient to draw conclusions, and our study was not underpowered. The other limitation is the fact that ID/IDA diagnosis was not confirmed by the diagnostic gold standard – staining of a bone marrow sample with Prussian blue [13]. However, this method is highly invasive and routine aspiration of the bone marrow is rarely performed. Lastly we did not analyze markers of hemolysis, which could have had an impact on RET number.

In conclusion, based on RET-He, approximately half of sepsis patients hospitalized in the ICU may have either ID or IDA. Number of Rets may be used for diagnosis of ID/IDA when RET-He is not available. Hepcidin concentration seems to be a poor predictor of IDA in sepsis patients.

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Conflict of interest

The authors declare no conflict of interest.

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Appendix 1.

The initial diagnosis of ID/IDA was based on Ret-He and Hb concentration: ID (RET-He < 30.2 pg, Hb ≥ 120/130 g/l); IDA (RET-He < 30.2 pg, Hb < 120/130 g/l). Reticulocyte Hb equivalent reflects current bone marrow iron availability and changes well before red blood cell (RBC) indices become abnormal [1]. The unquestionable advantage of Ret-He is that it allows ID/IDA diagnosis at an early stage. The exclusion criteria were factors that could potentially influence the results of parameters that were determined: active bleeding, RBC transfusion in the last 3 months, iron supplementation in the last 3 months, pregnancy. Mean cell volume (MCV) above the upper range overestimates RET-He, whereas thalassemia underestimates its value; therefore both constituted exclusion. Suspicion of thalassemia was based on the Mentzer index value < 13 [2]. The Mentzer index is calculated as MCV expressed in fl divided by RBC expressed in millions per µl. The complete blood count and Ret indices were determined from a single 2 ml test tube using a central laboratory hematology analyzer (XN-1000, Sysmex, Japan). Blood for Hep determination was centrifuged, frozen, stored at a temperature of –80°C, hepcidin was detremined using enzyme-linked immunosorbent assay (ELISA) (Cloud-Clone Corporation, Katy, United States of America). Statistical analysis was performed using MedCalc v.18 software (MedCalc Software, Ostend, Belgium). Between-group differences for continuous variables with normal distribution were assessed with independent samples Student's *t*-test, whereas those for continuous variables with non-normal distribution were assessed using the Kruskal-Wallis test. The χ^2 test was applied for categorical variables. Receiver operating characteristic (ROC) curves were drawn and areas under ROC curves (AUROCs) were calculated to determine the predictive value of studied parameters. A *p*-value ≤ 0.05 was considered significant.

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