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Iron depletion in blood donors – Have extended erythrocyte and reticulocyte parameters diagnostic utility?

Elisabeth Aardal Eriksson [a], Caroline Mobäck [a], Sandra Jakobsson [a], Johannes J.M.L. Hoffmann [b,*]

[a] Department of Clinical Chemistry, University Hospital, Linköping, Sweden
[b] Abbott Diagnostics, Department of Medical & Scientific Affairs, Abbott GmbH & Co. KG, Wiesbaden, Germany

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ABSTRACT

Background: Blood donation is associated with iron depletion, but donor iron status is not usually investigated, as such tests are cumbersome and costly. It would therefore be desirable to have simple, fast and inexpensive tests that give information on a donor's risk of developing iron depletion. In a pilot study we investigated whether novel erythrocyte and reticulocyte parameters can serve this goal.
Methods: In regular blood donors extended red cell parameters were measured using the Abbott CELL-DYN Sapphire hematology analyzer and conventional biochemical tests of iron status. Donors were compared with a regionally matched group of non-donating controls.
Results: In the controls, the reference ranges of extended RBC parameters were well comparable to published data. Donors had significantly more microcytic RBC than controls (median 0.9 vs 0.6%), lower serum ferritin concentration (median 43 vs 91 mg/L) and higher soluble transferrin receptor/ferritin index (median 1.60 vs 1.27). Overall 18–28% of the donors were iron depleted. Moreover, 3.3% of donors had iron-restricted erythropoiesis. Microcytic RBC and reticulocyte mean cell hemoglobin content predicted iron depletion with 70% and 64% sensitivities and specificities of 72% and 78%, respectively. When combined these two parameters increased the sensitivity to 82%.
Conclusions: Our results in Swedish blood donors confirm a high prevalence of iron depletion, despite iron supplementation used by about half of the donors. Microcytic RBC and MCHr appeared to be helpful in identifying iron-depleted donors, who might benefit from iron supplementation. We recommend larger prospective investigations in order to confirm and extend the findings of this pilot study.

1. Introduction

Donation of a standard unit of whole blood implies a loss of 200–250 mg iron from the donor's body iron stores [1,2]. As iron intake is normally only 1–2 mg per day, it is evident that long-term blood donation may be associated with iron depletion and indeed a high prevalence of iron depletion among blood donors has been described [3–6]. Iron depletion cannot only cause iron deficiency anemia, but has also been associated with physical and cognitive disorders [1,7,8]. In order to prevent or mitigate the deleterious effects of blood donation due to iron deficiency, in some countries blood centers offer iron supplementation to their donors [9,10]. However, compliance is a challenge due to gastrointestinal side-effects of this treatment [11]. In addition, blood centers generally only measure hemoglobin and/or hematocrit and these tests are poor indicators of body iron stores [12], meaning that some donors taking iron actually may not need it because their iron stores are not depleted. Serum ferritin reflects body iron stores much better, at least

* Corresponding author. Abbott Diagnostics, Department of Medical and Scientific Affairs Hematology Europe, Max-Planck-Ring 2, D-65205 Wiesbaden-Delkenheim, Germany. Tel.: +31 65162 1735; fax: +49 612 2581668.
E-mail address: hans.hoffmann@abbott.com (J.J.M.L. Hoffmann).

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in the absence of an acute phase reaction, but few blood centers have the possibility to measure this analyte. It would therefore be valuable if a simple test were available to assess iron depletion in blood donors and to monitor the effect of iron supplementation in donors who have developed iron depletion.

Over the last years some hematology analyzers obtained the capability of measuring extended erythrocyte and reticulocyte parameters by multi-angle optical analysis using the principles of the Mie scatter theory [13,14]. Two of these parameters are directly reflecting iron incorporation during erythropoiesis. Mean cell hemoglobin content of reticulocytes (MCHr) is the most sensitive indicator of current iron availability to the erythropoiesis, due to the short lifespan of reticulocytes in the circulation. Hypochromic red blood cells (RBC) reflect iron supply to the erythropoiesis over the previous weeks to months [15,16]. There are only few publications on the use of these parameters for investigating the iron status of blood donors [2,17]. Moreover, the results of these studies cannot be generalized, as different types of hematology analyzers show systematic differences in the extended RBC and reticulocyte parameters [18]. Therefore we designed a pilot study with the aim to investigate MCHr and related erythrocyte parameters, in a group of Swedish donors in relation to their donation history and iron supplementation treatment. A set of conventional iron status tests was used as a reference for assessing the diagnostic characteristics of the new RBC parameters hypochromic RBC, microcytic RBC and MCHr.

2. Materials and methods

2.1. Study subjects

After informed consent we enrolled regular blood donors of the Linköping Blood Bank in a 6-week period in April and May 2012. During a scheduled visit for blood donation, each participant donated two additional tubes of venous blood: one with K_2-EDTA as the anticoagulant for hematological analysis and one with a clotting activator for obtaining serum for chemical analysis. In addition, we asked the donors to complete a questionnaire regarding their health condition, lifestyle and medication history. It was also noted whether the donor took oral iron offered by the Blood Bank (at donation each donor is provided with 20 tablets of Duroferon®, containing 100 mg Fe^{2+} in the form of ferrous sulphate). The donor's donation history was retrieved from the Blood Bank archives. All data and blood samples were identified in such a way that the individual source was no longer traceable. Ethical approval for the study was obtained according to the local guidelines.

During the same period, we recruited subjectively healthy individuals as control subjects. These were non-donating volunteers, mainly students and staff members of the Linköping University Hospital and Linköping University who gave two tubes of venous blood as described earlier and supplied information on their health condition, all after informed consent. Some of the control women used oral anticonceptives, none was pregnant.

2.2. Analytical methods

Serum was used for measuring ferritin (using an immunochemiluminescence method) on a Cobas e602 analyzer (Roche Diagnostics, Basel, Switzerland), soluble transferrin receptor (sTfR; Roche Diagnostics) and high sensitive C-reactive protein (hs-CRP; Siemens Healthcare, Munich, Germany). The latter two are immunoturbidimetric methods and were performed on an Advia 1800 analyzer (Siemens). The sTfR/F index was calculated as the ratio of sTfR and log ferritin concentrations [19]. This index was used for defining iron depletion; the 97.5th percentile of male controls (2.09) was used as the cut-off value [5]. Hematological parameters were determined using a CELL-DYN Sapphire hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA) always within 6 hours from the time of blood draw [18]. A set of 7 extended parameters is automatically measured in a complete blood count with reticulocytes, but we only used hypochromic RBC, microcutic RBC and MCHr as these are related to iron status. In this analyzer, microcytic RBC are defined as RBC with a volume <60 fL and hypochromic RBC as erythrocytes having cellular hemoglobin concentration <28 g dL^{-1}.

2.3. Statistical analysis

Reference intervals were calculated as mean ± 2 SD or as the 2.5 and 97.5 percentile values, depending on the data distribution [20]; normality was checked using the Kolmogorov–Smirnov test. Groups were compared using Student's t-test for Gaussian distributed parameters and Mann–Whitney U-test for the other parameters. Optimal cut-off points were determined using receiver–operator–characteristics (ROC) curves with sTfR/ferritin index as a reference for iron depletion. We used the statistical software MedCalc (version 12.7; MedCalc, Ostende, Belgium) and regarded P values <0.05 as statistically significant.

3. Results

Our study group comprised 150 blood donors, 66 women (44%) and 84 men (56%). Their age ranged from 18 to 69 y (median 43.5 y). Whole blood donors had a history of between 1 and 159 donations (median 16) and plasma donors between 1 and 249 (median 12). Male donors had donated more frequently than female donors (medians 18 and 14, respectively), but the difference was not statistically significant (P = 0.814). Slightly more than half of the donors (n = 79; 52.7%) reported to use oral iron supplementation provided by the Blood Bank. The donors taking supplemental iron were not different from those who did not, with respect to age, gender or donation history.

The control group consisted of 104 subjects, 58 women and 46 men. Their age ranged from 17 to 69 y (median 36.5 y); they were somewhat younger than the donors, but the difference was not significant (P = 0.272). We used this group for verifying whether the reference ranges of the extended RBC parameters were comparable to published data. We found that MCHr ranged from 28.4 to 33.7 pg; microcytic RBC were up to 1.2%; and hypochromic RBC up to 4.1%;