ACCEPTED MANUSCRIPT

Accepted manuscripts are the articles in press that have been peer reviewed and accepted for publication by the Editorial Board of the Vojnosanitetski Pregled. They have not yet been copy edited and/or formatted in the publication house style, and the text could still be changed before final publication.

Although accepted manuscripts do not yet have all bibliographic details available, they can already be cited using the year of online publication and the DOI, as follows: article title, the author(s), publication (year), the DOI.

Please cite this article IRON STATUS AMONG BLOOD DONORS REFERRED DUE TO LOW HAEMOGLOBIN LEVEL

ISPITIVANJE STATUSA GVOŽĐA KOD DAVALACA KRVI VRAĆENIH ZBOG NISKOG HEMOGLOBINA

Authors Mirjana Kovac*,† MD PhD, Bojana Eric* MD, Jelena Stojneva Istatkov* MD, Vojislav Lukic* MD, Ana Milic* MD, Dragana Vukicevic * MD, Dusan Orlic* MD, Branko Tomic‡ PhD, Vojnosanitetski pregled (2019); Online First May, 2019.

UDC:

DOI: https://doi.org/10.2298/VSP190327063K

When the final article is assigned to volumes/issues of the Journal, the Article in Press version will be removed and the final version appear in the associated published volumes/issues of the Journal. The date the article was made available online first will be carried over.
IRON STATUS AMONG BLOOD DONORS REFERRED DUE TO LOW HAEMOGLOBIN LEVEL

ISPITIVANJE STATUSA GVOŽĐA KOD DAVALACA KRVI VRAĆENIH ZBOG NISKOG HEMOGLOBINA

Mirjana Kovac*,† MD PhD, Bojana Eric* MD, Jelena Stojneva Istatkov* MD, Vojislav Lukic* MD, Ana Milic* MD, Dragana Vukicevic * MD, Dusan Orlic* MD, Branko Tomic‡ PhD,

Blood Transfusion Institute of Serbia, Belgrade*, Faculty of Medicine, University of Belgrade, Serbia†, Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Serbia‡

Corresponding author:

Mirjana Kovac, MD PhD

Blood Transfusion Institute of Serbia, Belgrade, Serbia

Sv. Save 39, 1 000 Belgrade, Serbia

Email: mkovac008@gmail.com

Tel: +381 11 3812 804

Fax: +381 11 2458 328

Running title: Iron status in blood donors with low Hb
Abstract

Background/Aim. Haemoglobin determination is a routine part of the blood donor selection process. Previously reported studies have revealed that iron deficiency is common in frequent donors. This prospective investigation was aimed at examining iron status among blood donors with low circulating haemoglobin and evaluating capillary methods for haemoglobin determination in relation to reference values from venous blood count (BC) and ferritin level. Methods. Between February 2017 and December 2018, 200 consecutively recruited regular blood donors with low haemoglobin, aged 19 to 64 years (median 39) were included. Haemoglobin was determined using copper sulphate and HemoCue capillary methods as well as in venous blood samples on a haematology analyzer. Plasma ferritin was determined turbidimetrically. Results. In 42.7% of the men and 57.3% of the women ferritin concentration was low (P = 0.008). The relative numbers of males and females with levels <12 μg/L (P=0.023) or >50 μg/L (P=0.022) differed. Comparison of the values obtained with the capillary methods with reference haemoglobin obtained from the blood count (BC) showed that the copper sulphate procedure gave false fails in 10.5% cases (P<0.001). Values from HemoCue were significantly correlated with haemoglobin from BC, but no correlation was observed between ferritin levels and either capillary method. Conclusion. In 51.5% of Serbian blood donors referred due to low haemoglobin a low ferritin was observed. Based on our study results the determination of the algorithm in the iron deficiency detection is necessary, while capillary method (HemoCue) represents more convenient method for haemoglobin testing prior to donation.

Keywords: blood donors, haemoglobin determination, capillary method, ferritin.

Apstrakt

Uvod/Cilj. Određivanje nivoa hemoglobina je rutinski deo selekcije dobrovoljnih davalaca krvi. Prethodno publikovane studije su pokazale da se nedostatak gvožđa javlja kod redovnih davalaca krvi. Cilj ove prospektivne studije je da se utvrdi status gvožđa kod davalaca kod kojih je pre davanja utvrđen nizak nivo hemoglobina i da se proceni stepen korelacije kapilarnih metoda, sa referentnom metodom iz venske krvi i nivoom feritina.
Metode. U periodu od februara 2017. do decembra 2018, uključeno je 200 dobrovoljnih davalaca sa niskim hemoglobinom, starosne dobi 19-64 (medijana 39 godina). Hemoglobin je određivan primenom metode bakar sulfat, kapilarnom metodom HemoCue i iz venske krvi u sklopu krvne slike (KKS). Nivo feritina je određivan primenom turbodimetrijske metode. Rezultati. Nizak novo feritina je utvrđen kod 42.7% muškaraca i 57.3% žena, (P= 0.008). U odnosu na nivo feritina <12 μg/L odnosno >50 μg/L u odnosu na pol je zabeležena značajna razlika, (P=0.023, P=0.022). Poređenje vrednosti hemoglobina dobijenih kapilarnim metodama u odnosu na referentni hemoglobin iz KKS, pokazalo je da metoda bakar sulfata daje lažno niske vrednosti hemoglobina kod 10,5% slučajeva (P <0,001). Vrednosti hemoglobina dobijene metodom HemoCue-a su značajno korelirale sa hemoglobinom iz KKS, dok korelacija između nivoa feritina i hemoglobin obe kapilarnih metoda nije uočena. Zaključak. Kod 51,5% naših davalaca krvi koji su vraćeni zbog niskog hemoglobin utvrđen je snižen nivo feritina. Na osnovu rezultata naših istraživanja neophodno je odrediti algoritam za detekciju nedostatka gvožđa, dok je kapilarna metoda (HemoCue) pogodnija metoda za testiranje hemoglobina pre donacije.

Ključne reči:
dobrovoljni davaoci krvi, određivanje hemoglobin, kapilarna metoda, ferritin.

Introduction

Blood donor selection is one of the most important measures used in blood transfusion centres in order to ensure blood safety. Determination of haemoglobin (Hb) level is a routine part of the donor selection process in order to ensure high quality of the red cell concentrates collected and at the same time to protect the potential donor's health. However, iron deficiency has been found to be common in frequent blood donors, particularly women, while the Hb level measured may not accurately reflect iron stores.

Determination of Hb during the donor selection process using capillary methods, distinctly saves time and expenditure without endangering blood donors. Data from the forum investigation state that the capillary copper sulphate procedure is used to determine Hb level before donation in three European countries (United Kingdom, Spain and Croatia), in one country both capillary methods are employed, while in the remaining European countries, the capillary photometric method is preferred, most often HemoCue. In Serbian
transfusion centres we use the capillary copper sulphate method, considering the minimum acceptable Hb level to be >135 g/L for male and >125 g/L for female donors. Low circulating Hb is globally the most common reason for deferral of prospective blood donors. During 2017 the total rate of all deferrals in our centre was 14.2%, among which 30.5% were due to low Hb level. Considering the relatively high proportion of deferrals due to low Hb among our voluntary blood donors, we performed a prospective study aimed to determine iron status among such blood donors. The second aim was to evaluate agreement between values for Hb obtained using capillary methods with reference haemoglobin from BC and their association with ferritin level, in order to indicate the most appropriate procedures in our blood transfusion centre.

Methods
Between February 2017 and December 2018, this prospective study included 200 consecutively recruited regular blood donors (102 male and 98 female) with low Hb, aged 19 to 64 years (median 39). The term regular blood donor was defined as someone who had routinely donated blood in the same centre within the previous 2 years in accordance with minimum time intervals. The total number of previous blood donations in the study group was 3340 and the median time interval between them was 5.3 months. All study participants were recruited in the Blood Transfusion Institute of Serbia, Belgrade. In the Serbian transfusion service, copper sulphate is used as the standard method for Hb determination in a finger prick sample. This method was applied during recruitment of the study participants. Another capillary method for a finger prick sample was applied and Hb measured photometrically using the HemoCue® Hb 201 System, (Mission Viejo, Ca, USA). In addition, Hb was determined in a venous blood sample taken into EDTA tubes, using a haematology analyzer (Horiba Medical ABX Micros ES 60 blood counter, France). Ferritin concentration was determined turbidimetrically in a second venous blood sample collected in plastic tubes for biochemical analysis, using test reagents from Linear Chemicals, Spain. The reference range designated as normal by the manufacturer was 20-250 μg/L for males and 20-200 μg/L for females. In particular, a plasma ferritin level <12 μg/L is defined as absent iron stores (AIS) and ferritin concentration <29 μg/L is defined as depletion. These were used in statistical analyses in order to point to the iron status of blood donors referred due to low Hb.
All study participants were approached with the standard questionnaires for voluntary blood donors. Data related to age, gender, number of previous donations, date of the last donation, dietary regime, health problems since the last donation, such as haemorrhage, menstrual bleeding, fever, respiratory infection, stomach problems were analyzed.

Institutional approval for the study was granted by the Local Research Ethics Committee (EK-number 7767/2016) in accordance with internationally accepted ethical standards and each participant signed the informed consent form.

**Statistical methods**

The Statistical Package for Social Sciences 20.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used for statistical analysis. The Mann-Whitney U-test, Fisher’s exact test and Pearson Chi-square test were employed to evaluate differences in the demographic and haemoglobin/ferritin test characteristics among the study participants. The probability $P<0.05$ was considered as statistically significant.

Spearman tests were used for correlation analysis and $P<0.01$ was taken as statistically significant.

**Results**

Referring to their state of health, 25% of our subjects reported fatigue, 6.5% nutrition changes (diets, fasts), 7% bleeding episodes, and 61.5% good health. Considering the lower limit of the reference range to be 20 $\mu$g/L, a decreased ferritin level was found in 103/200 (51.5%) subjects (Table 1).

Equal frequencies of reduced Hb levels for the genders were indicated with all three methods ($P=0.323$, $P=0.796$ and $P=0.422$ respectively). Statistically significant differences were found between male and female donors regarding age, total number of donations, and the time interval following the previous donation ($P<0.001$). Concerning ferritin level, 44 (42.7%) males and 59 (57.3%) females had low ferritin concentrations, the difference being statistically significant ($P=0.008$). Moreover, the median plasma ferritin for females (15.0 $\mu$g/L) was lower than that for males (22.5 $\mu$g/L; $P=0.002$). For ferritin levels defined as $<12$ $\mu$g/L or $>50$ $\mu$g/L significant differences between male and female donors were also observed ($P=0.023$ and $P=0.022$ respectively; Table 3).

Comparison of the results from the capillary methods with the reference Hb values obtained from BC, showed that the copper sulphate method pointed to false fails in 21 (10.5%) donors, 13 male and 8 female ($P<0.001$; Table 2). Values for Hb obtained with the
capillary HemoCue method were positively correlated with those from BC (P<0.001) but not with plasma ferritin level (P=0.393). However, in relation to ferritin level defined as AIS (<12 μg/L), significant correlation was observed in the case of the capillary HemoCue method and ferritin level in female donors (P<0.001), but not for male donors (P=0.148; Figure 1). There were no significant correlations when results for Hb using the copper sulphate method were compared with those from BC (P=0.209) or ferritin level (P=0.855).

**Discussion**

Our evaluation of iron status among blood donors referred due to low Hb level, showed that half of them had decreased plasma ferritin concentrations, while a quarter of them reported fatigue prior to donation. The frequency of low ferritin levels was significantly higher in females and a greater proportion of women had ferritin levels lower than 12 μg/L, defined as absent iron stores (AIS).

A negative correlation between blood donation and total iron reserve was demonstrated over three decades ago and has been confirmed in recently published studies. Our results pointed to the same. Based on all these data, additional measures are needed to improve the health of blood donors in order to prevent iron deficiency anemia. Several studies have aimed to determine the best approach for preventing iron deficiency in blood donors. Their findings point to a need to re-evaluate current criteria in blood donor selection concerning the interval between donations, optimal testing strategy for measuring iron stores, and the necessity of iron supplementation. A plasma ferritin level below 12 μg/L, defined as absent iron stores (AIS), or less than 29 μg/L defined as depletion require deferral of blood donation and confirmation. In Italy donors with iron deficiency are invited to lengthen the interval between whole blood donations. In Denmark, if plasma ferritin is less than 15 μg/L the donor is given 100 iron tablets by post. When ferritin level is 15-40 μg/L the donor is given 60 iron tablets for supplementation.

In our study 60% of male and 73.5% of female donors had ferritin values that required deferral of blood donation for three to six months. We should point out that almost half of our low Hb female donors (45%) were observed in the group with ferritin <12 μg/L, while only 11.5% of them had a ferritin concentration that indicated optimal iron status.

It should be emphasized that donor selection criteria have generally been adopted through health system regulations, but practices in different transfusion centers vary in current approaches. Although, the demand for supplies of blood is decreasing in many countries
due to the implementation of Patient Blood Management, in Serbia it is constantly increasing due to aging of the population and the relatively high incidence of malignant diseases. Additionally, emigration of young people during the last few decades for economic reasons is also a problem of Serbian society. Therefore, it is crucial to define a rational evidence based donor selection process in our transfusion services in order to minimize unnecessary rejection of voluntary blood donors, and to prepare them for future donation in order to maintain a continuous blood supply.

Determination Hb level before donation with two capillary methods and comparison with the value obtained from BC, showed that the copper sulphate method gave false fails in 10.5% donors. On the other hand, the capillary HemoCue method provided Hb values comparable with those from BC. Moreover, in female donors with ferritin below 12 μg/L there was a significant association with Hb level obtained with the capillary HemoCue method. With regard to that every effort should be made to improve the accuracy of Hb screening in our centres. This implies a recommendation that the capillary photometric method should be introduced to replace the copper sulphate procedure for Hb determination. In addition, taking into consideration our results for iron status, determination of the algorithm for detection of iron deficiency together with iron supplementation should be included in Serbian transfusion services.

Our study has limitations that should be considered. Namely the number of participants was relatively small. With regard to the study design, no selection was made during recruitment, so all donors with identified low Hb were included. Therefore, differences between donors with regard to age and number of previous donations could have an implication that resulted in recall bias. However, this is the first study conducted among Serbian voluntary donors and the results obtained need to be confirmed in further investigations involving a larger number of participants.

**Conclusion**

The HemoCue capillary method is more suitable than the copper sulphate method for determining haemoglobin prior to donation. In 51.5% of Serbian blood donors referred due to low haemoglobin a low plasma ferritin concentration was observed. Our findings indicated that determination of the algorithm for detection of iron deficiency is necessary. However, in addition to optimal testing strategy for measuring iron stores, the necessity of
iron supplementation and treatment of iron deficiency anemia among blood donors are extremely important.

Acknowledgements
This study was supported by grant 173008 from the Ministry of Education, Science and Technological Development, Serbia.

References

10. Gorlin J. Iron man pentathlon or "we have met the enemy and they is us!" Transfusion 2014; 54:747-9.


### Table 1

**Demographic characteristics**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>200</td>
</tr>
<tr>
<td>Age, median (range), [years]</td>
<td>39 (19-64)</td>
</tr>
<tr>
<td>Gender M/F</td>
<td>102/98</td>
</tr>
<tr>
<td>Total number of previous donations</td>
<td>3340</td>
</tr>
<tr>
<td>Interval from last donation, median (range), [months]</td>
<td>5.3 (3.0-9.5)</td>
</tr>
<tr>
<td>Condition status</td>
<td>n (%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>50 (25)</td>
</tr>
<tr>
<td>Diet</td>
<td>13 (6.5)</td>
</tr>
<tr>
<td>Recently bleeding</td>
<td>14 (7)</td>
</tr>
<tr>
<td>Good condition</td>
<td>123 (61.5)</td>
</tr>
<tr>
<td>Number with low ferritin level</td>
<td>103 (51.5)</td>
</tr>
</tbody>
</table>

M- male, F- female

### Table 2

**Haemoglobin and ferritin levels in relation to gender**

<table>
<thead>
<tr>
<th></th>
<th>Male N=102</th>
<th>Female N=98</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range [years])</td>
<td>43 (22-64)</td>
<td>30 (19-64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total number of previous donations, median (range)</td>
<td>16 (2-120)</td>
<td>5 (2-48)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interval from last donation, mean (range [months])</td>
<td>4 (3-9.5)</td>
<td>5 (4-17)</td>
<td>0.001</td>
</tr>
<tr>
<td>Number with low Hb level by copper sulphate &lt;135 g/L M, &lt;125 g/L F</td>
<td>102</td>
<td>98</td>
<td>0.323*</td>
</tr>
<tr>
<td>Number with low Hb level by HemoCue</td>
<td>90</td>
<td>89</td>
<td>0.796</td>
</tr>
<tr>
<td>Number with low Hb level from BC</td>
<td>89</td>
<td>90</td>
<td>0.422</td>
</tr>
<tr>
<td>Hb level median (range) by HemoCue [g/L]</td>
<td>128 (92-145)</td>
<td>118 (84-136)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>from BC [g/L]</td>
<td>127 (93-149)</td>
<td>114 (83-136)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Number with low ferritin n (%)</td>
<td>44 (42.7)</td>
<td>59 (57.3)</td>
<td>0.008</td>
</tr>
<tr>
<td>Ferritin level [μg/L] median (range)</td>
<td>22.5 (4-209)</td>
<td>15 (2-349)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

M- male, F- female, BC - Blood count, P- Mann-Whitney test; *Fisher test,
Difference with regard to number with low Hb - copper sulphate vs BC (P<0.001). The reference ranges for ferritin designated as normal by the manufacturer were 20-250 μg/L for males and 20-200 μg/L for females.

Table 3

Iron status in relation to the different ferritin levels and gender

<table>
<thead>
<tr>
<th>Ferritin level</th>
<th>Male N=102 n (%)</th>
<th>Female N=98 n (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;12 μg/L</td>
<td>30 (29.4)</td>
<td>44 (45)</td>
<td>0.023</td>
</tr>
<tr>
<td>&lt;29 μg/L</td>
<td>32 (31)</td>
<td>28 (28.5)</td>
<td>0.659</td>
</tr>
<tr>
<td>30-50 μg/L</td>
<td>16 (15.6)</td>
<td>15 (15)</td>
<td>0.942</td>
</tr>
<tr>
<td>&gt; 50 μg/L</td>
<td>24 (23.5)</td>
<td>11 (11.5)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

Pearson Chi-square test

Fig. 1 - Correlation of Hb level measured by the HemoCue capillary method with reference Hb from BC

P<0.001
For correlation analysis, Spearman tests were used.
Fig. 2- Correlation of Hb level measured by the HemoCue capillary method with ferritin level

\[ P=0.393 \]

For correlation analysis, Spearman tests were used.

Received on March 27, 2019.
Revised on May 10, 2019.
Accepted May 10, 2019.
Online First May, 2019.