Feto-maternal haemorrhage assessment in a woman with a large population of red blood cells containing fetal haemoglobin

Ocena przecieku płodowo-matczynego u kobiety z dużą populacją krwinek czerwonych zawierających hemoglobinę płodową

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Abstract

**Background:** FMH quantification is necessary to calculate an individual dose of prophylactic anti-RhD immunoglobulin and to diagnose fetal anaemia causes. We encountered a healthy woman with a numerous RBCs containing fetal haemoglobin (HbF).

**Aims:** To investigate the cause of this sign and the correct evaluation of fetal RBCs in maternal circulation.

**Materials and Methods:** Patient’s samples and artificial mixtures were tested by microscopic Kleihaur-Betke (KB) and flow cytometric (FC) tests with anti-HbF + anti-CA (carbonic anhydrase), and with anti-D. The patient’s blood count with reticulocyte parameters, and concentration of bilirubin, haptoglobin, iron, transferrin, ferritin, hepcidin, sTR, HbF, HbA2 were measured. Genes coding the β- and γ-globin were sequenced.

**Results:** It was impossible to distinguish the population of fetal and maternal HbF positive cells using KBT and FC with anti-HbF. Application of anti-CA and anti-D allowed to separate them. Maternal blood haematological and biochemical parameters were normal but HbF was 3.3% of total Hb concentration (normal <1%). There were no mutations in the β- and γ-globin genes, but Xmn I polymorphism at –158 position in γ-globin gene was detected in the homozygous state.

**Conclusion:** A very large population of HbF positive cells sometimes can be detect in a healthy woman. Implementation of the various procedures for FMH assessment is necessary in the such case, otherwise, the detection of fetal erythrocytes may not be possible or can give false results.

Key words: feto-maternal haemorrhage (FMH) / Kleihauer-Betke test (KBT) / flow cytometry tests (FCTs) / F cells / fetal haemoglobin (HbF) / carbonic anhydrase (CA) / β- and γ-globin genes /
Słowa kluczowe: przeciek płodowo-matczyny / test Kleihauera-Betke / cytometry przepływowa / komórki F / hemoglobinina płodowa / anhydraza węglanowa / geny β- i γ-globiny /
parameters such as mean corpuscular volume; mean content haemoglobin (CHr); mean corpuscular haemoglobin concentration (CHCMr) were measured by the haematological analyzer, biochemical indicators of haemolysis and iron metabolism: concentration of bilirubin, haptoglobin, iron, transferrin, ferritin by biochemical analyzers, hepcidin and soluble transferrin receptor (sTR) by enzyme linked immunosorbent assay (R&D Systems Europe, Ltd., UK) and percentage of HbF and HbA2 by haemoglobin electrophoresis.

**DNA analysis**

Genomic DNA was isolated using the MagNA Pure Compact Nucleic Acid Isolation Kit I (Roche, Germany). Polymerase chain reaction (PCR) was used to amplify the promoter region, entire coding sequence and surrounding sequences of the β-globin and both γ-globin (γβ and γγ) genes. The sequences of all primers and the annealing temperatures used for PCR are available upon request. DNA fragments generated by PCR amplification were purified using the QIAquick PCR Purification Kit (QIAGEN, Germany) and directly sequenced with BigDye Terminators and the appropriate primers using an ABI Prism 377 sequencer (Applied Biosystems, CA, USA). The XmnI polymorphism at the -158 site upstream of the γγ globin gene was detected by PCR amplification and restriction enzyme digestion [10].

**Results**

Results of KB and FC tests in the maternal blood sample were the same before and immediately after childbirth and one year later. Using KBT, approximately 10% of the RBCs were well stained, other cells were stained very weakly, and about 70% looked like typical adult “ghost” cells.

Figure 1 shows cytograms of the patient's and newborn's (cord) blood mixture, tested with anti-HbF-PE antibody and compared to the control mixture. Population of about 30% HbF+ cells, separated from the HbF- ones is clearly visible. It was impossible to distinguish the population of fetal and maternal RBCs in the patient sample, while it was visible in the control one. Application of anti-HbF-PE+anti-CA-FITC and anti-RhD-PE allowed to separate maternal and fetal cells (Figure 2).

Morphological and biochemical parameters are shown in Table 1. The concentration of HbF accounted for 3.3% of total haemoglobin. Hepcidin and sTR concentration, markers of haemolysis compensation by increased erythropoiesis, were normal. Slightly increased haemoglobin content in reticulocytes (CHCMr and CHr) was noted.

Direct sequencing showed no mutations in the β- and γ-globin genes. However, XmnI polymorphism at -158 position in γ-globin gene was detected in the homozygous state.

**Discussion**

It is estimated that in healthy adults usually less than 1% of HbF is present among total Hb concentration and values from 1% up to 5% are considered as high [11]. Very high level of HbF concentration, from 50% to 100% can be detected in some congenital diseases such as sickle cell anaemia, haemoglobinopathies or β-thalassemia, and it compensates the lack of normal adult HbA (HbA1 + HbA2) [12-14]. In such cases, HbF usually is homogenously distributed among the red blood cells and it is named pancellular hereditary persistence.
of fetal haemoglobin (HPFH) syndrome. In thalassemias the concentration of HbA2 is often increased. In HPFH cases without congenital haemoglobinopathies or thalassemias HbF usually appears as 15% - 30% of total haemoglobin and it also can be pancellular. In healthy individuals with elevated HbF concentration it is irregularly distributed among erythrocytes and correlated with low percentage (< 1%) of so-called F cells [8]. Mundee et al indicated the correlation between concentration of HbF and percentage of F cells. However, they noted that the concentration of HbF in F cells may vary from 10% to 40% [6]. Our patient showed different result. Her number of F cells was unexpectedly high (30%) in relation to the level of her HbF (3.3%). Thorpe et al described four donors with large population of F cells and moderately increased HbF concentration, but they did not study them in relation to FMH assessment [15]. In three described cases of pregnant women, in which F cells complicated assessment of FMH, the concentration of HbF was 2%, 5.4% or 15% of total HbF and F cells were <4% among all erythrocytes in those samples [16, 17]. Results in those patients varied from day to day, while in our patient we were stable even after a year. In contrast to the report by Janssen and Hoffman or Kush et al [9, 17] our FCT with anti-HbF was insufficient to distinguish fetal from maternal RBCs. Our opinion is rather consistent with Leers et al, Iyer et al., Porra et al, and Kumpel et al [16, 18-20] that adult F cells in some cases can be differentiated from the fetal cells when additional antibodies are used. Prus and Fibach have just presented study of 12 thalasemic patients, women and men, tested with anti-HbF+CA. They observed additional population of RBCs resembling fetal cells because of the lack of CA, and concluded the coexistence of F cells from two types of stem cell, adult and fetal, lineages. For such women anti-HbF+CA may lead to misinterpretation [21]. For our patient these antibodies were useful, as we have examined a mixture of her and cord blood.

Our patient did not have any thalassemia β signs, including concentration of HbA2, which was normal, as well as morphological and biochemical results. Molecular analyses have not showed the presence of possible mutation responsible for thalassemia β and HPFH syndrome [22-24]. Slightly increased haemoglobin content in reticulocytes (CHCMr and CHr) could be due to a genetic variant, C→T at position -158 of the βγ globin gene detected in our patient. The Xinn I polymorphism is widespread in all populations and is present at frequency of 0.32 - 0.35 [24]. It has been shown that this variant is associated with elevated HbF level [25].

Summary

In a healthy mother we sometimes can detect a large population of RBCs containing HbF. It is important to implement different FMH assessment procedures, otherwise the distinction between HbF positive maternal and fetal erythrocytes in such cases may not be possible or may give false results.

Table 1. Patient’s morphology of RBCs and parameters of haemolysis and erythropoiesis /*slightly increased, **increased

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Reference range</th>
<th>Patient's result</th>
</tr>
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<tbody>
<tr>
<td>Red Blood Cell count</td>
<td>x 10^12/l</td>
<td>3.5-5.4</td>
<td>4.71</td>
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<tr>
<td>Haemoglobin concentration</td>
<td>g/dl</td>
<td>12.0-15.0</td>
<td>14.2</td>
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<tr>
<td>Haematocrit</td>
<td>%</td>
<td>37.0-47.0</td>
<td>42.2</td>
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<tr>
<td>Mean Corpuscular Volume</td>
<td>fl</td>
<td>81.0-99.0</td>
<td>89.6</td>
</tr>
<tr>
<td>Mean Corpuscular Haemoglobin</td>
<td>pg</td>
<td>26.0-34.0</td>
<td>30.1</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration</td>
<td>g/dl</td>
<td>31.0-36.0</td>
<td>33.0</td>
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<tr>
<td>Red Blood Cell Distribution Width</td>
<td>%</td>
<td>11.5-14.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Reticulocyte count</td>
<td>%</td>
<td>0.50-2.50</td>
<td>1.87</td>
</tr>
<tr>
<td>Mean Corpuscular Volume r (reticulocyte)</td>
<td>fl</td>
<td>101-119</td>
<td>104.6</td>
</tr>
<tr>
<td>Mean Corpuscular Hemoglobin Concentration r</td>
<td>g/dl</td>
<td>23-29</td>
<td>29.9*</td>
</tr>
<tr>
<td>Content of Haemoglobin r</td>
<td>pg</td>
<td>25-30</td>
<td>31.2*</td>
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<tr>
<td>Haemoglobin F</td>
<td>%</td>
<td>&lt; 1</td>
<td>3.3 **</td>
</tr>
<tr>
<td>Haemoglobin A2</td>
<td>%</td>
<td>1.9-3.5</td>
<td>2.2</td>
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<tr>
<td>Bilirubin</td>
<td>mg/dl</td>
<td>&lt; 0.70</td>
<td>0.28</td>
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<tr>
<td>Haptoglobin</td>
<td>mg/dl</td>
<td>&gt; 30</td>
<td>224.9</td>
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<tr>
<td>Iron</td>
<td>µg/dl</td>
<td>37.0-145.0</td>
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<tr>
<td>Transferrin</td>
<td>mg/dl</td>
<td>200.0-360.0</td>
<td>256.00</td>
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<td>Ferritin</td>
<td>ng/dl</td>
<td>13.0-150.0</td>
<td>64.50</td>
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<td>Hecpin</td>
<td>ng/ml</td>
<td>13.3-54.4</td>
<td>20.70</td>
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<tr>
<td>Soluble Transferrin Receptor</td>
<td>nmol/l</td>
<td>8.7-28.1</td>
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References:


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