RETICULOCYTE INDICES IN THE DIAGNOSIS OF IRON DEFICIENCY IN SUCKLING PIGLETS

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Abstract

The aim of the study was to evaluate the usefulness of the reticulocyte indices in the diagnosis of anaemia in piglets. The piglets in group I (n=16) were treated with iron dextran (200 mg of Fe, i.m.) at the age of 16 d. In group II (n=16), piglets at the age of 3 d received 100 mg of Fe and then at the age of 22 d - 200 mg of Fe. The piglets in group III (n=16) received 200 mg of Fe at the age of 3 d. From the 7 d of age, all the piglets had access to creep feed. In group I, there was a decrease in red blood cell indices and the piglets developed anaemia. These piglets had a lower growth intensity compared to piglets of groups II and III. In comparison with Hb, PCV, and RBC, the reticulocyte indices (haemoglobin concentration in reticulocyte and index RET-Y) reflexed more rapidly impaired erythropoiesis and in the case of mild iron deficiency (group II) they reacted more effectively to iron treatment.

Key words: piglets, iron, haemoglobin, reticulocytes, anaemia.

Iron deficiency anaemia represents a serious problem in swine production. The reserve of iron in newborn piglets is limited (50 mg of Fe) (26). The sow’s milk provides the piglet only with 1-2 mg of Fe per day (3), while the daily requirement of iron for normal development is ca 7-10 mg (24). Therefore, without additional iron supplementation, the piglets develop anaemia within 10-14 d after birth (10). The most common method of anaemia prevention is i.m. administration of 200 mg of Fe^{3+} in the form of iron dextran to two to three-day-old piglets (25).

The primary diagnosis of iron deficiency anaemia involves the examination of red blood cell indices, i.e. haemoglobin concentration, haematocrit, and erythrocyte count. The diagnosis of the latent iron deficiency is based on the determination of biochemical indices in blood plasma. However, these indices can be influenced also by other factors than iron deficiency (8, 18). With the introduction of automated flow cytometry, human laboratories started to use new reticulocyte indices (haemoglobin concentration in reticulocytes and index Ret-Y) as sensitive indicators of iron-deficient erythropoiesis (2). These indices reflect the actual state of marrow erythroid activity, and are not influenced by factors affecting traditional biochemical markers (14).

To the authors’ knowledge, the new reticulocyte indices have not been yet tested as indicators of iron deficient erythropoiesis in piglets. The aim of our study was to evaluate these indices in the diagnosis of iron deficiency anaemia in suckling piglets.

Material and Methods

Experimental design. The piglets were divided into three groups using split litters. All the groups received iron in the form of iron dextran at different doses. The piglets in group I (n=16) serving as the control anaemic group were given intramuscularly (i.m.) 200 mg of Fe^{3+} at the age of 16 d. The piglets in group II (n=16) were injected i.m. with 100 mg of Fe^{3+} at the age of 3 d and then, at the age of 22 d, were given the second dose of iron (200 mg of Fe^{3+}). The piglets in group III (n=16) received 200 mg of Fe^{3+} at the age of 3 d.

The clinical status of the animals was monitored daily. Clinical signs of anaemia (pale mucosal membranes, pale skin on the whole body surface) in piglets of group I were apparent at the age of 16 d. The piglets received sow’s milk until they were weaned. From day 7 of the age until the end of the trial (day 35), the piglets had access to creep feed (SKS weaning pellets, Slavkovské krmné směsi a. s). This creep feed contained 220 mg of Fe/kg and was offered to the piglets ad libitum. The piglets were weaned at the age of 29 d.

Sampling and analyses. Blood was collected from the vena cava cranialis on days 3, 8, 16, 22, 29, and 36 of the piglets life. In groups I and II, the blood was also taken 48 h after iron administration. EDTA (ethylenediaminetetraacetic acid) was used as an anticoagulant for the haematological examination and heparin was used as an anticoagulant for the determination of iron concentration in blood plasma. The piglets were weighed on the days of the blood collection.
The haematological indices were measured using automatic analyser Sysmex EX-2100 and included: haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), reticulocyte count (RET), percentage of reticulocytes (RET %), haemoglobin concentration in reticulocytes (RET-He), and index RET-Y.

The iron concentration in the blood plasma was determined photometrically by measuring the iron complex with ferrozine (Iron liquid 917, Roche Diagnostic, Germany).

The differences between groups were evaluated by non-parametric Kruskal-Wallis ANOVA test at the level of significance P<0.05. The changes within groups were evaluated by non-parametric Friedman ANOVA and subsequently by a paired Wilcox test at the level of significance P<0.01.

Results

Differences among groups. At the age of 16 and 22 d, the indices of Hb, PCV, MCV, MCH, RET-He, RET-Y, and Fe in group II (100 mg of Fe) were significantly lower than in group III (200 mg of Fe). In group I, the indices of Hb, PCV, RBC, MCV, MCH, RET, RET %, RET-He, RET-Y, and Fe started to be significantly lower as early as from the 8 d of age.

At the age of 16 d, the body weight in group I was significantly lower than in groups II and III.

Changes in the examined indices within groups:

- **Group I.** The indices of Hb, PCV, MCV, MCH, MCHC, RET, and RET % decreased significantly at the age of 16 d. After iron administration at the age of 16 d, Hb, PCV, MCV, MCH, RET, RET %, RET-He, RET-Y, and Fe increased significantly within 48 h.

- **Group II.** At the age of 16 d, there was a decline in the indices of MCV, MCH, MCHC, RET, RET %, Ret-He, RET-Y, and Fe. After iron administration at the age of 22 d, there was a significant rise of RET, RET %, RET-He, RET-Y, and Fe indices within 48 h.

- **Group III.** At the age of 22 d, the indices of MCV, MCH, RET-He, and RET-Y declined significantly. The iron concentration in blood plasma declined earlier, i.e. at the age of 16 d. One week after weaning, the indices of RET, RET %, RET-He, RET-Y, and Fe increased significantly.

<table>
<thead>
<tr>
<th>Table 1</th>
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<tr>
<td>Mean iron concentration in blood plasma (µmol/L)</td>
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<table>
<thead>
<tr>
<th>Days of age</th>
<th>3</th>
<th>8</th>
<th>16</th>
<th>22</th>
<th>29</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>4.9 ± 2.2a</td>
<td>3 ± 0.7a</td>
<td>3.1 ± 0.6a</td>
<td>24.0 ± 7a</td>
<td>23.6 ± 7.2a</td>
<td>27.1 ± 5.6a</td>
</tr>
<tr>
<td>Group II</td>
<td>4.8 ± 2.6a</td>
<td>24.8 ± 5.1b</td>
<td>7.6 ± 3.9b</td>
<td>4.6± 1.6b</td>
<td>22.2 ± 7.5c</td>
<td>26.2 ± 6.4c</td>
</tr>
<tr>
<td>Group III</td>
<td>4.0 ± 1.6a</td>
<td>26.0 ± 5.4b</td>
<td>15.8± 5.2c</td>
<td>13.0 ± 6.9a</td>
<td>13.5 ± 7.4a</td>
<td>23.5 ± 7.3a</td>
</tr>
</tbody>
</table>

48 h after treatment (day 16 in group. I, day 22 in group. II): Group I 36.6 ± 12.2, Group II 20.8 ± 6.9

Letters a, b, and c express significant differences between the groups (P<0.05). ± SD.

<table>
<thead>
<tr>
<th>Table 2</th>
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<td>Mean body weight during the trial (kg)</td>
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<table>
<thead>
<tr>
<th>Days of age</th>
<th>3</th>
<th>8</th>
<th>16</th>
<th>22</th>
<th>29</th>
<th>36</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>2.2 ± 0.5a</td>
<td>3.3 ± 0.8a</td>
<td>5.2 ± 1.2a</td>
<td>6.4 ± 1.4a</td>
<td>7.1 ± 1.5a</td>
<td>9.1 ± 2.1a</td>
</tr>
<tr>
<td>Group II</td>
<td>2.5 ± 0.5a</td>
<td>3.8 ± 0.8a</td>
<td>6.4 ± 1.4b</td>
<td>8.1± 1.9b</td>
<td>8.9 ± 1.7b</td>
<td>11 ± 2.2b</td>
</tr>
<tr>
<td>Group III</td>
<td>2.4 ± 0.4a</td>
<td>3.9 ± 0.7a</td>
<td>6.4± 1.5b</td>
<td>8.7 ± 1.9b</td>
<td>9.5 ± 1.5b</td>
<td>11.1 ± 1.5b</td>
</tr>
</tbody>
</table>

Letters a, b, and c express significant differences between the groups (P<0.05). ± SD.
Fig. 1. Haemoglobin concentration.

Fig. 2. Packed cell volume.

Fig. 3. Red blood cell count.

Fig. 4. MCV.

Fig. 5. MCH.

Fig. 6. MCHC.

Fig. 7. Reticulocyte count.

Fig. 8. Percentage of reticulocytes.
Discussion

Iron deficiency is recognised by correlating the red blood cell indices with biochemical markers of a negative iron imbalance. Traditionally, the standard biochemical markers of iron metabolism have been serum or plasma iron, transferring (Tf), transferring saturation (TfS), ferritin, and more recently, measurement of serum soluble Tf receptor (sTfR). Iron deficiency anaemia is characterised by hypochromia and microcytosis in conjunction with low serum iron and ferritin concentrations, decreased TfS, or increased sTfR (12, 23).

When inflammatory disorders occur, they occur concomitantly with iron deficiency, serum iron, transferring, and ferritin values may be unreliable (8, 19). Prussian blue staining of the marrow biopsy is considered as the gold standard for the assessment of iron status, but this invasive test is rarely used. An increased transferring receptor has been used as a sensitive indicator of iron deficiency. As soluble transferring receptor is not affected by acute or chronic inflammation, the test can be used as a reliable index of marrow iron stores (21, 23). However, for screening purposes, methods of measuring ferritin and serum transferring receptors are relatively expensive, compared with RBC indices.

As erythrocytes have a lifespan of ca 120 d, the traditional red blood cell indices cannot provide information on the rapid change in erythropoietic activity (16). Therefore, there is a need to seek an earlier and more sensitive marker for functional iron deficiency. Haemoglobin concentration in reticulocytes reflects the amount of available iron during RBC production, as reticulocytes exist in the circulation system for only 1-2 d. The usefulness of this index as an early marker of iron deficiency was demonstrated by the studies evaluating the iron status in human patients (1, 4).

With the introduction of automated flow cytometry and fluorescent dyes, it is possible to analyse reticulocyte indices including their volume (1, 5). The parameter Ret-Y measured on the Sysmex XE 2100 provides a relative measure of the equivalent of a mean corpuscular volume of reticulocytes. Kickler et al. (14) found in human patients that Ret-Y parameter has the highest sensitivity and specificity among tests used for the diagnosis of iron deficiency anaemia. The reticulocyte indices can be used also for an early recognition of “non-responsiveness” to iron administration.

Fishbane et al. (9) and Macdougall et al. (15) reported in human patients that at least 4 or 8 weeks are necessary to observe a significant response to iron administration by monitoring the erythrocyte indices, including haemoglobin and haematocrit. The main reason for the late diagnosis is the long life span of mature RBC. In the case of reticulocyte indices, Fishbane et al. (9) and Mittman et al. (17) found in human patients that these indices increase significantly within 48 h after iron administration.

The anaemic limit, i.e., the point when anaemia begins to exert a detrimental effect on weight gain and gives clinical symptoms, is set by most authors at Hb concentration below 80 g/L (7, 11). In group I (200 mg of Fe3+, day 16), there was a decrease in RBC indices and the piglets developed anaemia (Hb below 80 g/L). These piglets had a lower growth intensity compared to piglets of groups II and III. This is in agreement with findings of other authors (7, 22). Forty-eight hours after iron administration, there was a significant increase in the indices of RET, RET %, RET-He, RET-Y, and Fe increased significantly; while the indices of RBC, Hb, PCV, MCV, and MCH did not. However, for screening purposes, methods of measuring ferritin and serum transferring receptors are relatively expensive, compared with RBC indices.
indicates their lower reaction to iron treatment in the case of mild iron deficiency.

There are conflicting data concerning the efficacy of 100 mg of Fe<sup>3+</sup> dose. Kay et al. (13) found this dose as appropriate for the normal development of suckling piglets. On the contrary, Daykin et al. (6) do not consider such a low dose as sufficient and recommend the dose of 200 mg of Fe<sup>3+</sup>. It is obvious from our results that the dose of 100 mg of Fe<sup>3+</sup> does not provide sufficient haemoglobin concentrations (days 16 and 22) and thus it should not be used in the practice.

Even in group III (200 mg of Fe<sup>3+</sup>, day 3) there was a decline in MCV, MCH, RET-He, RET-Y, and Fe indices. The lack of a decrease in Hb, PCV, and RBC indicates a low sensitivity of these indices for the detection of impaired erythropoiesis. The rise of reticulocyte indices one week after weaning could be explained by the fact that piglets started to feed intensively on creep feed.

Relatively rapid increase in haemoglobin concentration after iron treatment (in group I after 48 h in group II after 1 week) differs from the data obtained in human patients. Fishbane et al. (9) and Macdougall et al. (15) reported a significant rise of haemoglobin as late as after 4-8 weeks after iron administration. The rapid increase in haemoglobin content after iron treatment could be explained by an intensive erythropoiesis, which is related to high growth intensity of suckling piglets (20).

In comparison with Hb, PCV, and RBC values, the reticulocyte indices reflect more rapidly an impaired erythropoiesis. The reaction of MCV and MCH to impaired erythropoiesis is comparable with reticulocyte indices. When the iron was given to piglets with severe iron deficiency (group I), there was a significant increase after 48 h, both in traditional (Hb, PCV, MCV, and MCH) and reticulocyte indices. In the case of mild iron deficiency (group II), the reticulocyte indices reacted to iron treatment more rapidly than traditional RBC indices.

It is concluded that reticulocyte indices can be considered as very sensitive indicators of iron deficiency in sucking piglets.

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References
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