Selected arterial blood gasometry parameters as indicators of blood transfusion effectiveness in foals with haemolytic disease

Artur Stopyra, Anna Snarska

Department of Internal Diseases, Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn, 10-719 Olsztyn, Poland
anna.snarska@onet.eu

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Abstract

The aim of the study was to determine the suitability of basic haematological, biochemical, and gasometric tests in checking the effectiveness of transfusion therapy in foals during isoerythrolysis. The number of red blood cells, haemoglobin, haematocrit, and partial pressure of carbon dioxide, oxygen, and blood pH was determined immediately before and several times after blood transfusion. The concentration of serum free bilirubin was also measured to confirm haemolysis. Fluids (0.9% NaCl, multielectrolytic fluid, 5% glucose) and antibiotics (penicillin, amikacin) were provided to the foals. The lowest values of haematological parameters were observed before transfusion. This was accompanied by decreased partial pressure of oxygen, low pH, and increased arterial carbon dioxide tension. Transfusion of whole blood led to a gradual normalisation of the haematological parameters, also accompanied by the normalisation of gasometric indicators (decrease in pCO₂ and pO₂ and pH increase). Monitoring of selected haematological and gasometric parameters allows to evaluate the efficacy of blood transfusion during treatment of haemolytic disease of foals.

Keywords: foals, isoerythrolysis, blood transfusion, blood, gasometry.

Introduction

Blood transfusions are rarely required during therapy in horses. They are generally performed in life-threatening haemorrhage after perinatal complications, massive trauma, and in foals with severe haemolytic disease (7, 15). Haemolytic disease of the newborn, also known as isoerythrolysis, neonatal jaundice (icterus neonatorum), affects foals in the first week of life. It results from the uptake of colostrum containing antibodies directed against the newborn’s red blood cells (RBC). Haemolytic disease relatively frequently leads to death (11). The serological conflict occurs when a foal inherits RBC antigens, which the mare does not have. RBC antibodies are produced when the mare’s immune system is exposed to a foreign antigen (transfusion, difficult parturition, pregnancy). This process generally concerns antibodies against Aa and Qa antigens. Colostrum immunoglobulins are absorbed in an unmodified form during pinocytosis in the first 18-72 h of the foal’s life. Then they are transported to the blood stream where they bind to red blood cells and lead to their disintegration. Serological incompatibility is generally observed in multiparous mares, in particular in the third and successive pregnancies, but it has also been noted in primiparous mares (16).

Foals affected by haemolytic disease are born healthy. Concentration of RBC antibodies in the blood, the pathological process initiated by the uptake of colostrum containing RBC antibodies, is manifested by inhibited sucking reflex, dyspnea, anaemia with subsequent jaundice or, in cases of hyperacute disease progression, acute failure and sudden death. Haemolytic disease leads to various life-threatening complications, including secondary sepsis, nervous system disorders, nephropathy, diarrhoea, and pneumonia. The disease should be diagnosed and treatment should begin in the first three days of the foal’s life. The foal is weaned and fed milk replacers or colostrum from a non-sensitised mare. Blood is transfused when haematocrit decreases below 0.12 l/l and acute symptoms of the disease are observed. Up to 2 L of blood are routinely transfused. The exact blood volume (in dm³) can be determined based on the
following formula proposed by Orsini and Divers (9):
(expected Ht – present Ht) x body weight x 0.09/donor's Ht leve.

Transfusion increases RBC counts in circulating blood and improves tissue oxidation (2). Geldings, which have never undergone a blood transfusion are the ideal donors. The blood from such donor should not contain RBC antibodies. Despite above, the donor's and recipient's blood is subjected to cross-matching and agglutination tests (15). It is recommended that the transfusion is preceded by a biological test during which 25-50 mL of blood is administered over a period of 15-30 min and the foal’s responses are monitored. In a foal's circulatory system, the life span of the donor's RBC is estimated at 4-6 d, and it is significantly shorter than in adult horses (5). An improvement in clinical condition is generally observed after 3 d, but it may take as long as 30 to 60 d for normal haematocrit values to be restored.

Blood transfusions are associated with various complications (3, 10). In addition to anaphylactic shock, acute haemolytic transfusion reaction and disseminated intravascular coagulation, blood transfusion may also lead to pulmonary oedema, referred to as transfusion-related acute lung injury (TRALI) in human medicine (4, 13). The resulting impaired function of the blood-air barrier inhibits gas exchange in the lungs, leading to hypoxia and hypercapnia, and may obliterate the anticipated effects of blood transfusion despite an increase in the recipient’s RBC counts (6, 12).

The aim of this study was to evaluate the usefulness of basic haematological and gasometrical parameters to monitor the effectiveness of blood transfusion in foals affected by haemolytic disease. The chosen tests are possible to perform in the majority of veterinary laboratories, and their price will not raise significantly the total costs of therapy.

**Material and Methods**

Evaluation was performed in eleven foals, aged 2 to 5 d, with uncomplicated form of haemolytic disease. The animals were the offspring of multiparous mares that had not previously undergone blood transfusions. The disease was diagnosed on the basis of clinical symptoms, including apathy, inhibited sucking reflex, tachycardia, elevated respiratory rate, and pallor and yellow discoloration of mucous membranes, as well as the results of laboratory analyses: low haematocrit values, haemoglobin concentration, and erythrocyte count, elevated serum unconjugated bilirubin levels and agglutination of the foal’s erythrocytes by the mother’s serum in a cross-matching test.

Blood transfusions were performed when haematocrit decreased below 0.2 l/L. Blood for transfusions was obtained from geldings that had never undergone a blood transfusion. Blood was collected into pre-sterilised polyethylene bags containing sodium citrate and stored for up to 14 d at 3-4°C. Cross-matching was performed before transfusion to decrease the risk of adverse reactions. The transfusion was started slowly, and approximately 35 mL of blood was transfused in the first 30 min. If no adverse reactions were observed, blood was transfused in the amount calculated based on the foal’s body weight and the donor’s and recipient's haematocrit values with an average of 1040 mL per animal (788-1356 mL), 20 ±5 mL/kg b.w. per hour. The foals were administered 60 mL/kg b.w. of fluid infusions (0.9% NaCl, multielectrolytic fluid, 5% glucose) daily, and antibiotics (penicillin 22 000 iu/kg i.v. two times daily, amikacin 20 mg/kg i.v. once daily). The animals were muzzled to prevent them from sucking sensitised mares and fed fresh colostrum or milk from other mares, supplemented with frozen colostrum preheated to 38°C.

Selected peripheral blood parameters (RBC counts, haemoglobin levels – Hb, haematocrit – Ht) were determined in venous blood samples collected from the jugular vein into plastic test-tubes with EDTA K2, using haematological analyser (ADVIA 2120i, Siemens). Arterial blood gasometry parameters (partial pressure of carbon dioxide – pCO2, partial pressure of oxygen - pO2) were determined in samples collected from the common carotid artery to glass capillaries with heparin, using acid-base equilibrium analyser (Rapidlab 348, Siemens). Concentration of serum unconjugated bilirubin was determined by biochemical analyser (ACCENT-200, Cormay). Blood was sampled directly before transfusion (sample 0), 15 min after transfusion (sample I), 3, 12, 24, 48, and 96 h after transfusion (samples II – VI), and 6 and 8 d after transfusion (samples VII and VIII). Venous blood was taken from opposite jugular vein to the vein used to transfusion. Results from 11 foals in nine examinations were compiled by Student’s t-test and shown in Table 1.

**Results**

The foals’ general condition and results of clinical examinations were indicative for haemolytic disease. High serum unconjugated bilirubin levels with an average of 143.9 µmol/L provided an additional evidence for the above diagnosis. Cross-matching of the foals’ erythrocytes with the mothers’ serum confirmed the presence of a serological conflict in each case.

Haematological parameters determined before transfusion were below the physiological norm for foals aged 2-5 d. RBC counts, with an average of 4.42 × 1012/L, reached the lowest, statistically significant level (Table 1, sample 0). Hb levels with an average of 4.025 mmol/L and Ht of 0.182 /l were also significantly lower in comparison with the values of successive samples.
Gasometry parameters determined before transfusion (Table 1, sample 0) were also below the physiological norm for young healthy foals. The only parameter within the physiological norm was pCO₂ at 44.467 mmHg. pO₂ reached the lowest value of 32.672, which was significantly below the reference range for healthy foals aged 2-5 d. Arterial blood pH was also below the norm (7.264).

Blood transfusions changed all of the analysed arterial blood gasometry parameters and supported their return to normal physiological levels (Table 1, samples I-VIII).

RBC counts increased to 5.34 × 10¹²/L in sample II (3 h after transfusion) and decreased to 4.97 × 10¹²/L 12 h after transfusion (sample III). In successive samples, RBC counts gradually increased to reach the highest, statistically significant level of 7.01 × 10¹²/L 6 d after transfusion (sample VII). The last sample collected 8 d after treatment (sample VIII) revealed an insignificant drop in RBC counts relative to the highest value. After an initial increase to 6.003 mmol/L in sample I (15 min after transfusion), haemoglobin levels decreased to 4.563 mmol/L in two successive samples and increased significantly to 6.799 mmol/L 8 d after transfusion (sample VIII). Haematocrit increased to 0.234 1/1 in sample I, decreased to 0.201 1/1 12 h after transfusion (sample III), and increased significantly to the highest level of 0.240 1/1 6 d after treatment (sample VII).

Partial pressure of carbon dioxide decreased gradually after transfusion to reach the lowest, statistically significant, value of 41.903 mmHg in sample VIII (8 d after transfusion). An insignificant increase in pCO₂ (45.269 mmHg) was observed 12 h after treatment (sample III) in comparison with the values noted in samples II (3 h after transfusion) - 44.812 mmHg and IV (24 h after treatment) - 44.561 mmHg. Partial pressure of oxygen increased steadily

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**Table 1. Haematological and blood gasometry parameters in foals before and after blood transfusion (n = 11)**

<table>
<thead>
<tr>
<th>Time after transfusion</th>
<th>0</th>
<th>15 min</th>
<th>3 h</th>
<th>12 h</th>
<th>24 h</th>
<th>48 h</th>
<th>96 h</th>
<th>6 d</th>
<th>8 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC 10¹²/L</td>
<td>average</td>
<td>4.42*</td>
<td>5.98</td>
<td>5.34</td>
<td>4.97</td>
<td>5.81</td>
<td>5.88</td>
<td>6.45</td>
<td>7.01*</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>2.812</td>
<td>2.981</td>
<td>3.002</td>
<td>2.403</td>
<td>2.627</td>
<td>2.411</td>
<td>2.729</td>
<td>2.689</td>
</tr>
<tr>
<td>Hb mmol/L</td>
<td>average</td>
<td>4.025*</td>
<td>6.003</td>
<td>5.121</td>
<td>4.563</td>
<td>4.978</td>
<td>5.081</td>
<td>5.763</td>
<td>6.542</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>1.746</td>
<td>2.126</td>
<td>2.093</td>
<td>3.001</td>
<td>2.094</td>
<td>2.045</td>
<td>3.012</td>
<td>2.228</td>
</tr>
<tr>
<td>Ht 1/1</td>
<td>average</td>
<td>0.182*</td>
<td>0.234</td>
<td>0.228</td>
<td>0.201</td>
<td>0.231</td>
<td>0.233</td>
<td>0.238</td>
<td>0.240*</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.423</td>
<td>0.393</td>
<td>0.402</td>
<td>0.383</td>
<td>0.451</td>
<td>0.413</td>
<td>0.356</td>
<td>0.418</td>
</tr>
<tr>
<td>pCO₂ mmHg</td>
<td>average</td>
<td>48.467*</td>
<td>46.763</td>
<td>44.812</td>
<td>45.269</td>
<td>45.561</td>
<td>44.607</td>
<td>43.614</td>
<td>42.185</td>
</tr>
<tr>
<td>pO₂ mmHg</td>
<td>average</td>
<td>32.672*</td>
<td>39.485</td>
<td>46.375</td>
<td>38.461</td>
<td>50.264</td>
<td>54.863</td>
<td>61.472</td>
<td>72.637</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>0.131</td>
<td>0.211</td>
<td>0.153</td>
<td>0.114</td>
<td>0.108</td>
<td>0.113</td>
<td>0.128</td>
<td>0.124</td>
</tr>
</tbody>
</table>
| Bil µmol/L             | average | 143.9
|                        | SD     | 19.78 |

* P ≤ 0.05
after treatment to reach the highest, statistically significant value of 76.026 mmHg in sample VIII (8 d after transfusion). The pH of arterial blood decreased significantly to the lowest value of 7.208 3 h after transfusion (sample II), after which it increased significantly to 7.469 on day six (sample VII) and decreased to 7.438 8 d after treatment (sample VIII).

Discussion

One of the main indications for blood transfusion is to increase RBC counts in circulating blood to reduce hypoxia and restore normal tissue oxygen levels (2). The improvement in RBC counts and the time required for an improvement in haematological parameters are determined by various factors, mainly the recipient's immune function, spleen's ability to store RBC, the life span of erythrocytes, RBC breakdown by the reticuloendothelial system, RBC recovery rate, and elimination of the causes of erythrocyte loss (8).

Before transfusion, haematological parameters were significantly below the lower reference limits for healthy foals. The analysed animals revealed haemolytic disease of the newborn, also referred to as neonatal isoerythrolysis. The pathogenesis of the disease involves the breakdown of the newborn's erythrocytes by antibodies present in the colostrum of sensitised mothers, which decreases RBC counts, haematocrit and haemoglobin levels in the blood. Similar results were reported by Polkes et al. (11) in a study of isoerythrolysis in foals. A decrease in RBC counts and haemoglobin levels lowers the amount of oxygen supplied to body tissues. In hypoxia, diagnosed on the basis of low partial pressure of oxygen in arterial blood, many cells switch from aerobic to anaerobic metabolism, which lowers blood pH (1). Hypoxia and acidosis provoke hyperventilation, increase oxygen supply to the lungs, speed up the evacuation of carbon dioxide from the body, and maintain partial pressure of carbon dioxide at an acceptable level (14). Those physiological mechanisms support a rapid body response to acidosis and promote the maintenance of the acid-base balance. They do not guarantee adequate levels of oxygen transport or tissue oxidation in a haemoglobin deficiency (2).

According to Whiting and David (16), and Polkes et al. (11), haematocrit values lower than 0.2 l/l and poor health pose a threat to the foal's life, and constitute indications for blood transfusion. In this study, foals received whole blood in the amount and at the rates recommended for horses by Orsini and Divers (9). Transfusions improved haematological parameters and tissue oxidation, and increased partial pressure of oxygen. A gradual increase in pO2 values with a progressive decrease in pCO2 values rules out respiratory complications of blood transfusion described by Hurcombe et al. (3). In the second half of the first day after transfusion, a minor decrease was noted in the analysed haematological parameters, accompanied by an insignificant increase in pCO2 values. The animals' overall condition continued to improve, the respiratory rate was lowered, and a healthy pink colour of mucous membranes was noted. Similar changes in haematological parameters were reported by Whiting and David (16) and Boyle et al. (1), who attributed the observed improvements to the equine spleen's ability to store erythrocytes. The further improvement in haematological and gasometry parameters 6 and 8 d after transfusion is associated with the recovery of RBC in foals. Similar observations were made by Hauser et al. (2).

In conclusion, in foals affected by isoerythrolysis, blood transfusion improves tissue oxygenation and reduces systemic acidosis. The beneficial effects of whole blood transfusion were observed even 8 d after treatment. Due to the spleen's ability to store erythrocytes, these changes were not clearly manifested on the second and third day after transfusion, which does not minimize the benefits of the treatment for foals. In foals which received blood transfusions, blood gasometry parameters were monitored to evaluate the effectiveness of the applied treatment, contributing to an early detection of complications that impair gas exchange in the lungs, and supporting the initiation of life-saving treatment.

References