Value of Crossmatch Tests for Verifying the Compatibility in Dog Blood Transfusion Therapy

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Abstract. In case of pets, dogs and cats transfusions are already a current practice, being an intensive therapy procedure. In a group of dogs that underwent intensive therapy whole blood transfusion was used based on major and minor Crossmatch test results in order to establishing blood compatibility. The slide and tube techniques used were original and taken from different authors. Using 15 potential donors, 5 patients were transfused, one of them diagnosed with osteosarcoma. In 4 of the patients, all donor tested were compatible and they recovered after blood transfusion. Incompatibility problems appeared in the patient with osteosarcoma, for which 11 potential donors were tested and among them only 2 were compatible, the others showed positive agglutination reaction in major crossmatch. In patients with positive reactions of incompatibility, the results were relevant in both variants (slide, tube), meaning the high level of plasmatic alloantibodies. After the evaluation of blood compatibility using major and minor crossmatch tests it was decided to opt for blood transfusion which made possible the recovery of 4 out of 5 dogs undergoing intensive therapy. The development was fatal for the patient with osteosarcoma because of the severe complications caused by the cancer. The highly positive major crossmatch results obtained in the patient with osteosarcoma and 9 potential donors, who never received a transfusion, suggest the possibility of producing alloantibodies to some foreign cancer induced proteins, cross-reactant with common erythrocyte antigens.

Key words: pretransfusion evaluation, incompatibility, blood agglutination, osteosarcoma

INTRODUCTION

Establishing the blood transfusion compatibility in dogs can be done by using crossmatch testing alone or in addition to blood typing of the donor and receiver. There are cases when the crossmatch test can be sufficient for a safe transfusion therapy while in other cases blood typing is necessary.

The large diversity of the erythrocytes antigenic profiles is characterized by the fact that dogs do not have naturally occurring antibodies to the 13 clinically significant erythrocyte antigens, as we can see in cats and other species. (Giger and col, 2005)

It is notable that the crossmatch tests only the presence or the absence of the plasmatic anti-erythrocytes antibodies in the tested partners (Giger and Blais, 2005) and for this reason doesn’t exclude the possibility of the immunogenic transfusion reaction, so-called febrile non-hemolytic transfusion reactions, generated by the leukocyte/platelet.
MATERIALS AND METHODS

For the evaluation of the blood transfusion compatibility and detection of the anti-erythrocyte alloantibodies present in the donor or recipient plasma we resorted to determining the relevance of agglutination and hemolysis reactions, using different variants of crossmatch.

Five patients subjected to intensive therapy procedures, who also needed blood transfusions, were tested before transfusion. Three of them were mixed breeds, one was a Siberian husky and the other Rotweiller. 4 patients were compatible with the first tested donors, but the 5-th patient, a Rotweiller male, diagnosed with left scapular osteosarcoma, and needed more tests.

The patients were suffering of progressive states of anemia (a total number of erythrocytes under 5 mil/ml of blood) and after clinical and hematological evaluation (18 parameters determined with automatic analyzer-Abacus junior vet) we opted for transfusion therapy. Worsening trends in the patient with osteosarcoma required completing the haematological investigations with biochemical and immunological tests (plasmatic level of IgG and IgM).

The group of potential donors included 15 healthy dogs, vaccinated and parasite free; from different breeds and ages (between 5 months to 10 years). We also tested, as a potential donor, a dog with lymphoma, to distinguish the likelihood of incompatibility reactions generated by the development of cancer. None of the tested dog had been transfused with blood products. Among the tested potential donors there were 3 females that had one or more series of puppies.

The methods for testing the transfusion compatibility included different techniques of major and minor crossmatch. Some of them were based on adaptations of crossmatch techniques from reference literature and the others were based on the elaboration of original methods on slide and in tube rapid tests. The tests were performed between patients and potential donors and also between donors. The autoagglutination was checked in the same time in every dog.

The majority of samples, needed for testing and the obtaining of plasma, were collected on EDTA, beside these, for some we used citrate/oxalate. The serums were obtained from samples collected in tubes containing clotting activator.

Following the work protocol, first of all we began with a rapid slide test variant, elaborated by us. This consist of: the mixture of 3 µl donor erythrocyte concentrate with 12µl plasma/serum from the recipient, in the case of major crossmatch, in the case of the minor crossmatch, the same quantities from recipient erythrocyte with donor plasma/serum. The autoagglutination control was done using reagents from the same dog, mixing the above mentioned quantities of erythrocytes with plasma/serum.

The macro agglutination was interpreted after slow rotation movements of the slide and the micro agglutination was checked at microscope with 10X.

For the patient with osteosarcoma we also proceeded to a test variant for erythrocytes using human blood group Hemotests. The procedure consists in checking the slide agglutination of the tested erythrocytes with antiA, antiB and antiD serums. (Ogonean and col., 2008)

Nine positive results, represented by slide agglutination, were noticed only in case of the patient with osteosarcoma, who was also tested using tube methods as we describe below. Tube test crossmatch, after Bernard F. Feldman 2006, with the following steps:

- Preparation of the erythrocyte suspension from donor and recipient by placing 0,2 ml of erythrocyte in 4,8 ml normal saline;
• Preparation of the plasma/serum from the two partners using known methods (Ognean and col, 2008)
• Testing major crossmatch by mixing, in three small test tubes, 0,1 ml donors erythrocyte suspension with 0,1 ml recipient serum/plasma;
• Testing minor crossmatch by using the same quantities of recipient erythrocytes and donor plasma/serum;
• Incubation of the samples, for 15 minutes at 3 various temperatures: 37°C, 4°C and room temperature;
• Reading results, after a preliminary centrifugation for 1 minute at 3500rpm – examination of the supernatant for any hemolysis, slowly shake to observe the presence of agglutination, like a compact mass or small parts of this;
• Performing the control test for the autoagglutination using the plasma and erythrocytes from the same dog in the above mentioned technique and quantities;

Modified Crossmatch tube test presented by Dodds, W. Jean at the 30th World Small Animal Veterinary Association World Congress, Mexico, 2005. After the adaptation of this test the following stages have resulted:
• Preparation of red blood concentrate (pRBC), plasma and blood serum, using the usual techniques;
• Washing donor and recipient pRBC: in two small tubes test add one drop of pRBC and 2 ml saline, spin at 2500 rpm, pour off saline, add fresh saline and spin as before. Repeat 3 times, leaving cells in tubes;
• Major crossmatch: add two drops of recipient plasma to washed donor RBC;
• Minor crossmatch: add two drops of donor plasma to washed recipient RBC;
• Autoagglutination control using reagents from the same dog in the quantities mentioned above. Both controls should be negative;
• Checking the results on slide-mix tubes, place one drop from each tube on separate microscope slides to check for agglutination or haemolysis;

Tube test crossmatch:
• Major crossmatch: adding of a drop from donor pRBC in a small test tube to a drop of recipient plasma/serum and then fill the tube with saline
• Minor crossmatch: mix recipient pRBC with donor plasma as before
• After 5 minutes at room temperature centrifuge the tubes 3 min at 3500rpm
• Reading of the results: like we previously mentioned.

RESULTS AND DISCUSSION

In 9 cases we obtained positive results in major crossmatch test, using slide technique, and these were represented by agglutination. Minor crossmatch tests were all negative. The agglutination intensity varied from + to ++++ (Tab1).

It is also mentionable that in all cases positive agglutination results were expressed very clear in macroscopic and microscopic examination of the slides (Fig1,2)

Crossmatch tests performed in tube showed similar results, but the incubation at 37°C of the samples provided a clearer expression of the intensity of agglutination reactions, and a differentiation based on color, of the haemolysis.
The evolution of the agglutination intensity reaction in crossmatch compatibility tests

<table>
<thead>
<tr>
<th>TEST (no.)</th>
<th>DONOR</th>
<th>RECIPIENT</th>
<th>MAJOR CROSSMATCH</th>
<th>MINOR CROSSMATCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rotweiller</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Romanian Shepherd</td>
<td></td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Mixed breed</td>
<td></td>
<td>++++</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Labrador retriever</td>
<td>Patient (Rotweiller with osteosarcoma)</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>German Shepherd</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Mixed breed</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>German Shepherd</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>German Shepherd</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Mixed breed</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Fox terrier</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Mixed breed</td>
<td></td>
<td>+++</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Rotweiller (1)</td>
<td>Romanian Shepherd (2)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Mixed breed (3)</td>
<td>Labrador retriever (4)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Fox terrier (10)</td>
<td>Mixed breed (9)</td>
<td>++++</td>
<td>-</td>
</tr>
</tbody>
</table>

Fig.1. Macroscopic agglutination  
Fig.2. Microscopic agglutination

All minor crossmatch tests showed negative reactions in the tube, like on slide. All incompatibilities were detected between the patient with osteosarcoma and 9 of the 11 donors tested. In this dog the serum titre of immunoglobulins has exceeded the physiological limits (IgG-2017 mg/dl and IgM-408 mg/dl) and the biochemical changes consisted in increased alkaline phosphatase (640 U/L), ASAT (141 U/L), creatinine (5.6 mg/dl) and blood glucose (541 g/dl).

Note also the appearance of incompatibility between this patient with osteosarcoma and a donor with lymphoma.

Only 2 potential donors were compatible with this patient with osteosarcoma, both being German shepherd dogs.

The major Crossmatch test results between donors showed the incompatibility only in one case, for dog with lymphoma, the agglutination intensity was assessed +++. On the basis of negative results recorded in all performed autoagglutination tests, the evolution of autoimmune hemolytic anemia in these dogs was excluded.
The tests with anti-human erythrocyte antigens we obtained negative reactions, which could exclude dogs belonging to group test DEA 7. Regarding this, Bowdler and col., implies the existence of a similarity between the Tr antigen (DEA7) and the A antigen from human red blood cells. (Bull and Slating, 1971)

According to the obtained results, detecting elevated levels of canine anti-erythrocyte alloantibody can also be done on with the use of major Crossmatch slide techniques which can be as relevant as the tube methods.

As most researchers in the field have found, the crossmatch test is absolutely necessary to verify the pretransfusion compatibility in dogs that have received a previous blood transfusion and females that have had puppies.

Starting from the fact that there are no known specific natural alloantibodies which cause important posttransfusion reactions, there are authors who consider that the first blood transfusion should not create problems. (Slatter, 2003) Moreover, some of them consider that crossmatch test may be omitted in practice before the first transfusion. (Giger and Blais, 2005)

Although in our study no dog has received blood transfusion, we observed a number of 9 agglutination, some of them having a very strong intensity. In case of the dog suffering from osteosarcoma we can assume the formation of antibodies against some cancer induced foreign proteins, which give cross-reactions with the erythrocytes of other healthy dogs. For this reason we resorted to testing another patient with cancer, respectively lymphoma, after which we obtained similar results to the first case. This may support the hypothesis that some plasmatic alloantibodies found in dogs with cancer can agglutinate canine red blood cells. To better support such findings, the plasma from lymphoma patient should also agglutinate the erythrocytes from osteosarcoma patient and obtaining a positive minor crossmatch.

As we mentioned, obtaining an incompatible major crossmatch test reveals the destruction of the transfused erythrocytes by the plasmatic antibodies and this can result in very severe consequences for the patient being transfused. (Giger and Blais, 2005) Such incompatibilities are included in the mechanism of the type 2 hypersensitivity reaction (antigen-antibody).

Aloantibodies involved in the positive crossmatch reactions are hemolysins or haemagglutinins and can be directed against antigens of known blood type or other antigens on the surface of erythrocytes. (Giger and Blais, 2005)

Giger and col. also mentions that in patients with a very low hematocrit (below 10%) the crossmatch test cannot highlight a possible positive response to incompatibility. According to the same author, major crosmatch test also give errors in the event of autoagglutination, when it’s recommended the use of washed (at least 3 times) red blood cells. In this context, is estimated that in cases of autoagglutination intensity reactions from + to ++++, the patient will receive only DEA1 negative blood until the disappearance of the autoagglutination and then repetition the crossmatch test.

Our results also reveal an important fact in testing the hematotransfusion compatibility of patients with cancer, recommending crossmatch test even before the first transfusion. We also mention that crossmatch test do not replaces blood typing and doesn’t exclude a possible sensitization of the recipient.
CONCLUSIONS
Blood compatibility testing with different crossmatch techniques in a group of dogs that underwent intensive therapy was the basis for the following conclusions:

1. Major Crossmatch slide techniques may be as relevant as those in tube, for the detection of elevated alloantibodies levels in dogs, the use of blood typing is needed for a more complex assessments of compatibility;

2. Increased frequency of negative results to major Crossmatch tests confirm the lack of preformed plasmatic alloantibodies in most tested cases, because they have not gone through any previous form of hemotherapy;

3. Assessing the compatibility using Crossmatch tests has made possible the transfusion of whole blood, with recovery of 4 of the 5 dogs underwent intensive therapy, the evolution being fatal for the patient with osteosarcoma, because of severe complications caused by cancer;

4. Getting positive reactions to major Crossmatch tests confirms once again the possibility of posttransfusion alloantibodies formation in dogs, even if initial tests were consistent with several donors. In this case a reliable source of compatible blood remaining the whelps.

5. First blood transfusion in dog should not create compatibility issues, although there may be cases with high levels of natural antibodies, detected by major Crossmatch test. The test is recommended for all patients who have already undergone a blood transfusion and in females who have had puppies.

6. Intense positive reactions of major Crossmatch tests found in the patient with osteosarcoma and the 9 potential donors, without any of these having underwent any blood transfusions, suggests the possibility of antibodies formation, similar to blood group isoaglutinins, to certain foreign proteins induced by cancer;

7. Agglutination of the lymphoma dog erythrocytes with osteosarcoma dog plasma, but not vice versa, reveals the need for compatibility testing of all patients with cancer undergoing transfusion therapy with blood products.

REFERENCES