Using Crossmatch Tests for Serological Compatibility Assessment Intra- And Interspecific at Dogs and Cats

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Abstract

Selecting compatible blood is essential for the safety and efficiency of transfusion therapy. Correct performing and repeating Crossmatch tests can prevent immediate and delayed immune reactions caused by sensitization of subjects. In the cats' case, it is called into question solving low availability of sources of compatible blood donors by using canines. The aim was to comparatively analyze serological intraspecific and interspecific compatibility on samples of dogs and cats in order to evaluate the possibility of implementing transfusion therapy with canine blood to feline subjects.

There were conducted Crossmatch tests on blood samples (n=42) collected on anticoagulant substance from dogs (n=36) and cats (n=6) from the FMV Cluj-Napoca clinics. There were performed 156 Crossmatch tests, predominantly through quick technique on smears and in some cases (15%) the method based on separating the plasma and preparing hematies suspension.

Intraspecific compatibility on dogs was predominantly negative. Remarkable is the case of a canine patient on which we registered all 24 Crossmatch major tests high positive intensity (3+), without a historical therapy with blood products. Regarding the intraspecific compatibility tests, all the xenocompatibility dog-cat tests showed high positive reactions, both for major and minor Crossmatch (3+/4+).

The intraspecific compatibility at dogs is very high before the first contact with blood products, without excluding the possibility of some atypical sensitivization for clinical interest. The evaluation of the post-transfusional risk. Regarding the interspecific compatibility dog-cat, all the tests were highly positive and we can not sustain a probable xenotransfusion.

Keywords: *cat, Crossmatch, dog, quick tests, xenocompatibility*

INTRODUCTION

For transfusion therapy to be safe and effective it must be done with compatible blood products. The compatibility assessment is based on Crossmatch tests and blood type tests. Relevance of Crossmatch tests require plenty of accuracy and attention in individualization, in handling and in investigating samples because the uncertain reactions are quite common (Kisielewich and Self, 2014). These tests are essential for preventing possible side effects consecutive to transfusion of incompatible blood products. In clinics such reactions can be induced frequently at cat, dog and horse. Dogs, although they have the blood group system outnumber that of cats, do not have formed naturally anti-erythrocyte antigens (alloantibodies) (Sanchez *et al*, 2014). They are formed only after a contact between the patient's immune system with RBC (red blood cells) foreign on whose surface there are different antigens than those on the surface of the red blood cells of the recipient. The absence of preformed

alloantibodies would enable a single blood transfusion without knowing the blood group of the donor or receiver but with the risk of sensitization of the receiver. On the other hand, cats have 3 groups (A, B, AB), and they also have performed alloantibodies, which requires us to test the blood compatibility and find the blood type (Schneider *et al*, 2000).

The current problem in feline subjects is the low availability of blood products for this species. Domestic animals have blood volume about 7-8% of body weight (BW), and cats have below 6.5% of BW. It follows that an adult cat that has an average BW between 3.6 and 4.5 kg has a total of 230 mL - 290 mL blood. Therefore, the amount of blood collected from a cat is 50mL, maximum of 60 mL, to not endanger the health of the donor. So the availability of the required cat's blood group A, B or AB is very low. Cats with certain pathologies who would use an administration of whole blood or another blood product were the pioneers of the interspecific dog-cat transfusions in the early 1960s (Bovens and Jones, 2013). This is the main reason why there were clinicians who have hoped and tried a xenotransfusion with blood from dog to cat. In literature, no publications were found to support that cats should have natural antibodies against dog's erythrocyte. However, antibodies against canine red blood cells develop in the first 5-7 days after the first administration and the transfused red cells are lysed and removed, and the symptomatology is of the delayed hemolytic reaction (decreased hemoglobin, fever, jaundice, or hemoglobinuria) (Ognean *et al.*, 2009). If the administration of canine red blood cells is repeated after a period larger than 7 days after the first transfusion, occurred anaphylactic reaction is often fatal (Owens *et a1*., 2001)

The aim of this study had 3 main directions: intra- and interspecific serological compatibility analysis, using the Crossmatch tests on blood samples from dogs and cats; the post-transfusion risk assessment; possibility of implementing the xenotransfusion with canine blood products to feline subjects.

MATERIALS AND METHOD:

The biological material used was the blood samples collected from canine (n=36) and felines (n=6), in tubes with anticoagulant (EDTA). Investigated animals came from FMV Cluj clinics. Samples collection followed the common protocol, resorting to the punture of the jugular vein in the middle third, brachial cephalic or saphenous. Canine subjects were frequently placed in lateral decubitus and cats by immobilizing with a towel to limit violent movements and aggression.

There were conducted 156 Crossmatch tests, among which 132 tests by rapid method of the slide (Ognean and Cernea, 2011), and 24 by a new method based on the separation of plasma and red blood cells to prepare a suspension of 5%. Regarding the rapid method on slide were performed Crossmatch tests: major and minor Crossmatch and Autoagglutination, using a ratio of ¼ between reactants.

The working protocol in the newly introduced method consisted in separation of plasma by centrifuge at 1500 g for 5 minutes and the red cell concentrate taking in 0.9% saline solution, followed by centrifuging it for 5 minutes at 1500 g. This procedure was repeated 3 times, and after three washes to 5% reconstituted red blood cell suspension in serum. Testing itself consisted of homogenizing the two reactants in equal proportions to assess, after 30 minutes at thermostat, the major, minor Crossmatch and Autoagglutination.

RESULTS AND DISCUSSIONS:

The assessment of intraspecific serological compatibility in the tested heterogeneous dogs sample revealed a high level of compatibility. The high level was given by the predominance of negative reactions from major and minor Crossmatch tests and by the control of Autoagglutination, except for the case of a patient with an atypical reactivity, which gave positive or strongly positive reactions from major Crossmatch (3+) with all partners, respectively negative from the minor test (Tab. 1; Fig.1 A, B).

Unlike the previous, from the interspecific serological compatibility testing we found strong

positive reaction both to major and minor Crossmatch tests. The clear appearance on the slide of agglutination reactions, expressed by differentiating plasma from the clusters of red blood cells, indirectly confirms the presence of anti-erythrocyte antibodies in the plasma of cats.

The results of xenocompatibility evaluation by major and minor Crossmatch tests on the slide were conducted between partners grouped in three lots, each including eight dogs and a cat. Thus, the lot 2 the major Crossmatch tests were mostly strongly positive (4+), with one exception in which we reported a mild positive reaction (2+) (Tab. 2). As it can be noted from the same table, the minor Crossmatch tests showed only moderate positive reactions (2+ and 3+).

Similar results were also obtained from assessing the lot 3, which revealed strongly positive reactions (4+) both in major and minor compatibility tests, except for a sample for which the reaction remained positive (3+) but not of the same intensity (Tab. 3). In this context can be framed the data obtained from tests of lot 3, indicating predominance of strong positive reactions (4+) to major

Tab. 1. Crossmatch test results performed between the dogs from the studied samples

Number of Tests	Partner 1 (Donor)	Partner 2 (Receptor)	Major Crossmatch	Minor Crossmatch	Autoagglutination
96	C1-C24	C1-C4	**-**	**-**	**-**
24	C1-C24	Cr	+++	**-**	**-**

C1-C24-tested dogs;
Cr-dog with atypical reactivity.

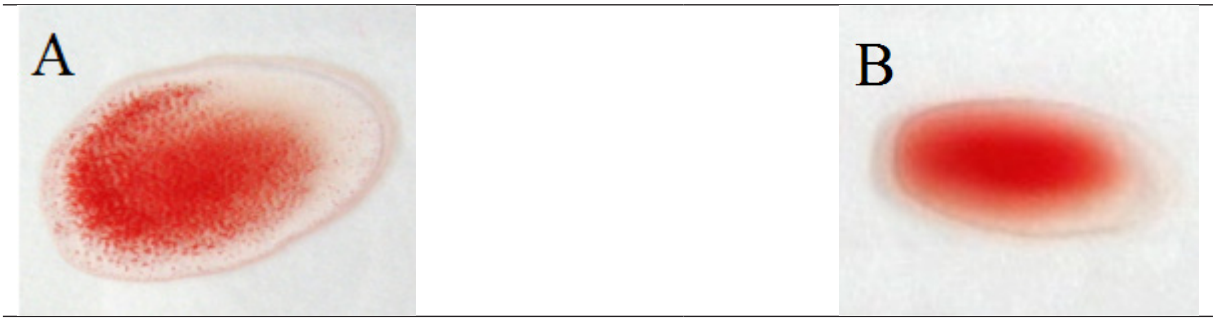

Fig. 1. The detailed appearance of intensely positive (3+) major Crossmatch reaction (A) and the minor Crossmatch negative (-) (B) concerning the dog (Cr) with atypical plasma reactivity.

Tab. 2. Results of interspecific Crossmatch tests made between dogs in lot 1 (C1-C8) and cat 1 (P1)

Number of Tests	Partner 1 (Donor)	Partner 2 (Receptor)	Major Crossmatch	Minor Crossmatch	Autoagglutination
1	C1	P1	++++	++	-
2	C2		++++	+++	-
3	C3		++++	++	-
4	C4		++	++	-
5	C5		++++	++	-
6	C6		++++	+++	-
7	C7		++++	+++	-
8	C8		++++	+++	-
Average	8	1	++++	+++	-

compatibility test and for the minor test 4 strongly positive intense reactions (4+), 3 positive reactions (3+) and a negative reaction (-) (Tab. 4).

Overall analysis of the data presented shows a good level of effectiveness of Crossmatch tests on the slide used in this study due to all positive tests (n=24) responses being sufficiently uniform and expressed significantly (3+) (Tab. 5.). The fact that they were recorded only in combinations of a single patient (Cr), suggests the need of new investigations in order to ensure the accuracy

Tab. 3. Results of interspecific Crossmatch tests performed between dogs in lot 2 (C1-C8) and cat 2 (P2)

Number of Tests	Partner 1 (Donor)	Partner 2 (Receptor)	Major Crossmatch	Minor Crossmatch	Autoagglutination
1	C1	P2	++++	++++	-
2	C2		++++	++++	-
3	C3		++++	++++	-
4	C4		++++	++++	-
5	C5		+++	++++	-
6	C6		++++	++++	-
7	C7		++++	+++	-
8	C8		+++	++	-
Average	8	1	++++	++++	-

Tab. 4. Results of interspecific Crossmatch tests performed between dogs in lot 3 (C1-C8) and cat 3 (P3)

Number of Tests	Partner 1 (Donor)	Partner 2 (Receptor)	Major Crossmatch	Minor Crossmatch	Autoagglutination
1	C1	P3	++++	++++	-
2	C2		++++	+++	-
3	C3		++++	++++	-
4	C4		++++	+++	-
5	C5		++++	+++	-
6	C6		+++	-	-
7	C7		++++	++++	-
8	C8		++++	++++	-
Average	8	1	++++	+++	-

Tab. 5. The frequencies of agglutination at the Crossmatch tests registered on the slide, conducted between dogs and dogs and also between dogs and cats

Crossmatch Test	**No.**	**Number of agglutination**					**The total number of Agglutinations**
		0/+	1+	2+	3+	4+	
					Dog X Dog		
Major	120	0	0	0	24	0	24
Minor	120	0	0	0	0	0	0
					Dog X Cat		
Major	24	0	0	1	3	20	24
Minor	24	0	0	5	8	10	23

0/+ = uncertain outcome; No= Number of conducted tests

necessary for quick tests on the slide. In contrast, interspecific dog-cat testing gave only positive reactions, but of different intensities. As it can be noted from analysis of data in Table 5, in the case of Crossmatch major tests (20 of 24) prevailed strongly positive reactions, but not the same regarding the minor test (10 of 23). Although the score recorded from the evaluation of these tests indicated graded intensity, we can conclude that the method used is of good efficacy.

Summarizing the results of intraspecific compatibility tests performed on the dogs sample we find that most of them were negative, except for a patient who had positive reactions with all 24 partners tested. We believe that in the case of this patient an anterior sensitization, to one or more canine erythrocyte antigens, took place. This kind of aspects was less reported by researchers in the field, because the natural isoimmunization to erythrocyte antigens are extremely rare in canine populations. For this reason, the incompatibility of blood at first transfusion is almost nonexistent (Ognean *et al*, 2009). On the other hand, the fact that all interspecific dog-cat tests showed exclusively positive or strongly positive reactions confirmed the existence of a real blood incompatibility between individuals of various species, which indicates the uncertainty of xenotransfusion and its major risk with severe consequences on the recipient.

Most of the blood compatibility tests on dogs are negative, especially regarding the first transfusion but it is not excluded the sensitization of some animals. The high level of blood compatibility in unsensitized dogs justifies, but not fully, the clinical conduct of performing the first blood transfusion without risk, since this species has no preformed alloantibodies.

CONCLUSIONS

The level of sanguine compatibility at the investigated dogs was 72.72% negative and the rest of test was conducted between animals that were previously sensitized.

Regarding the xenotransfusion (dog-cat), our survey's results confirm the blood incompatibility between these partners and the existence of a major risk of inducing immediate adverse reactions with severe consequences.

Based on the results and consulted data we recommend the testing of pretransfusional compatibility (by Crossmatch tests or blood group) of canine patients, including for the first transfusion in order to exclude any risk of sensitization or delayed adverse reaction.

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