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Morphological Changes in Blood Cells After Implantation of Titanium and Plastic Clips in the Neurocranium - Experimental Study on Dogs

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ABSTRACT

Introduction: Various studies confirm the biocompatibility and efficacy of clips for certain target tissues, but without any comparative analysis of hematological parameters. Therefore, we conducted a study to assess the possible association of the implantation of titanium and plastic clips in the neurocranium with possible morphological changes in the blood cells of experimental animals. **Materials and Methods:** As a control, the peripheral blood smears were taken before surgery from 12 adult dogs that were divided into two experimental groups. After placing titanium and plastic clips in the neurocranium, the peripheral blood of the first group was analyzed on the seventh postoperative day, while the peripheral blood of the second group was analyzed on the sixtieth day. By microscopy of the blood smears, the following parameters were analyzed: the presence of poikilocytosis of the red blood cells, degenerative changes in the leukocytes and leukogram. **Results:** There were no statistically significant differences between the mean values of the groups. Monocytosis was detected (first group 22.83 % and second 16.30 %), as well as neutropenia (46.80 %, in the second group). Degenerative changes to neutrophils and the occurrence of atypical lymphocytes were observed in the second experimental group (60th postoperative day). **Conclusion:** A mild adverse effect from the biomaterials present in the neurocranium of dogs was detected, affecting the majority of leukocytic cells. A chronic recurrent inflammatory process was caused by the presence of the plastic and titanium clips in the brain tissue. No adverse effect of biomaterials on erythrocytes in the neurocranium was detected in the dogs studied. Further studies are necessary to explain the occurrence of degenerative changes in the neutrophils and lymphocytes.

Keywords: Plastic and titanium clips, dog, atypical lymphocytes, degenerative neutrophils, inflammation, poikilocytosis erythrocytes.

1. INTRODUCTION

Biomaterials are made up of various artificially synthesized compounds, which may affect local tissue or the whole organism, in general.

Titanium clips cause mild inflammation (1) but are the standard in neurosurgery. In contrast, plastic clips have radiological advantages in usage in the neurocranium (2). Their biocompatibility, both in peritoneal cavity (3) and the neurocranium (1) has also been confirmed, but they are not yet being used in neurosurgery.

Various studies have confirmed the biocompatibility and efficiency of clips for certain target tissues, but without any comparative analysis of hematological parameters. (4-7) except by a few authors who, in the assessment of the toxicity or biocompatibility of implanted materials, have taken into consideration the hematotoxicity of the tested materials (8, 9).

Analysis of the overall hematological profile of the patient is a practically indispensable activity within every clinical examination of patients. Assessment of possible risks, after care and treatment of patients, as well as a final evaluation of treatment outcomes, are possible with a detailed analysis of hematological parameters (10). The use of hematological parameters, as tools for assessing the toxicity of biomaterials, would open the possibility of the efficient selection of appropriate materials for implantation.

Therefore, the study was conducted in order to assess the possible connection between the implantation of titanium and plastic clips in the neurocranium and possible morphological changes in the blood cells of

experimental animals. The assessment of the proportion of poikilocytosis forms of red blood cells was discussed, as well as morphological and degenerative changes in the neutrophils and lymphocytes. An analysis of the obtained leukogram percentage was also performed.

2. MATERIAL AND METHODS

The procedure for obtaining of approval for experimental part of the study was described in the previous paper (1). 12 adult crossbreed dogs, aged 8-10 years, with body weight 10-15 kg, were used in the study. They were placed in standard cages with ambient temperature of 20-24°C, with a 12 hour light/dark cycle.

They were divided into two equal groups. Before surgery, peripheral blood was taken from both groups of dogs to be used as a control. Then a titanium and plastic clip was placed within the neurocranium of each dog in the experiment. After seven days, the peripheral blood of the first group was analyzed, while the peripheral blood of the second group was analyzed after sixty postoperative days.

2.1. PREOPERATIVE AND OPERATIVE PROCEDURES

A few days before the surgery, the dogs provided for the experiment were clinically healthy. They were subjected to appropriate ecto and endoantihelmintics. General anesthesia and surgical procedure for the dogs was carried out according to the protocol described in the work of Delibegović et al. (1).

2.2. HEMATOLOGY PROCEDURES

Peripheral blood was taken and blood smears were made in accordance with the normal laboratory procedures (11). For each original stained smear, 2000 erythrocytes and 1000 leukocytes were counted and analyzed, using a Motic Type 102M binocular light microscope, with a magnification of 900 times.

Poikilocytes were defined on the basis of the standard morphology (12), while counting was limited to a representative one-tier visual fields in which about half of the erythrocytes touched, but did not overlap (11, 13, 14). The most representative fields of vision were stored in electronic form using Motic Images Plus 2.0.computer-software.

The numbers and types of poikilocytes were recorded and expressed as a percentage (%) of the red blood cells. Poikilocytosis was classified semi-quantitatively according to similar studies, using the following criteria: non-existent (0%), rare (0.05-0.5%), mild (>0.5-3%), moderate (>3-10%), or expressed (>10%) (11).

The leukogram was determined, and the values were expressed as percentages, after analysis of 1,000 such cells from each animal in the experiment. The assessment of semi-quantitative degenerative changes in leukocytes, such as toxic granulation in the cytoplasm of neutrophils, and an increased number of reactive lymphocytes, were recorded according to the following criteria (12): a few (5%–10%), moderate (11%–30%), and a large number (>30%)

2.3. STATISTICAL DATA PROCESSING

Minimum and maximum values, mean and standard deviation of the results were determined. Statistical significance of the results was tested using T-test and Fisher's test.

3. RESULTS

The results of the leukogram of the dogs in the first group, (7th day, Table 1) correspond to the norm-referenced physiological intervals of stray dogs (10), as well as to leukogram values of other categories of dogs: dogs in shelters and fostered dogs (15). The exceptions were monocytes; the percentage values measured from the blood smears taken beforehand were significantly higher (15.33%) than the upper physiological limit (9%) for adult dogs (10). The value of monocytes on the seventh day was twice higher (22.83%) than the results of Khan et al, (10). The results of T-test and Fisher's test did not show statistically significant differences between the mean values ($p>0.05$) of the control results and the results of the first experimental group.

Analyzing the leukogram of the second experimental group (60th day, Table 2), an increase in lymphocytes (38.80%) in agranulated leukocytes was noticed when compared to the same in the first experimental group, and it reaches the upper physiological limit in dogs (10). Monocytosis was also noticed in the control of the second experimental group (14.80%), and in the second experimental group(16.20%).

In the first experimental group (7th day), the number of monocytes had a tendency to decline. The number of neutrophils in the control, as well as in the first experimental group (7th day) was within normal limits, while

Parameter (%)	Minimum		Maximum		Mean		SD	
(n=6)	control	7 th day	control	7 thday	control	7th day	control	7th day
Lymphocytes	20	16	33	37	26.33	26.17	4.72	8.56
Monocytes	3	14	24	35	15.33	22.83	7.78.	8.21
Mature neutr.	49	38	67	68	56.33	50.83	6.31	12.86
Band neutr	0	1	14	15	4.83	6.50	5.46	4.85
Acidophils	0	0	30	2	5.17	0.50	12.20	0.83
Basophils	0	0	3	0	1.00	0.00	1.55	0.00

Table 1. Leukogram (%) of the first group of dogs after implantation of plastic and titanium clips on the 7th postoperative day

Parameter (%)	Minimum		Maximum		Mean		SD	
(n=6)	control	60 th day	control	60 th day	control	60th day	control	60th day
Lymphocytes	14	26	32	67	23.20	38.80	6.76	16.36
Monocytes	3	1	29	44	14.80	16.20	10.85.	18.06
Mature neutr.	37	23	70	73	56.60	43.20	12.62	20.70
Band neutr	1	0	19	11	9.20	3.60	6.57	4.39
Acidophils	0	0	9	3	3.40	0.60	3.91	1.34
Basophils	1	0	2	1	1.40	0.20	0.54	0.45

Table 2. Leukogram (%) of the second group of dogs after implantation of titanium and plastic clips on the 60th postoperative day

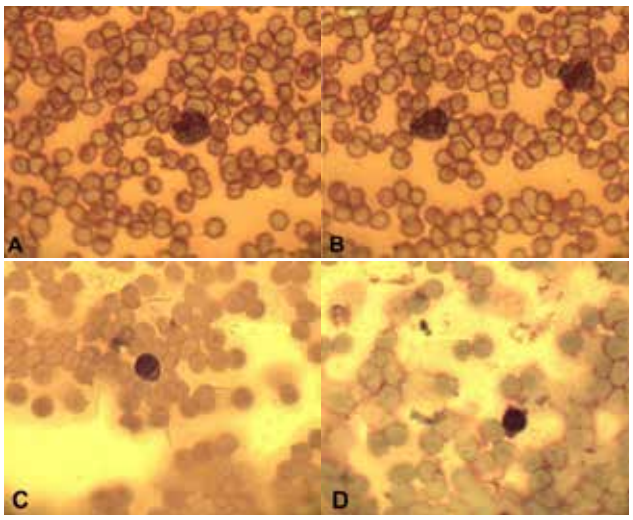

Figure 1. Morphological changes in neutrophils (A and B) and lymphocytes (C and D)

cytes was 12%, which represents apparent echinocytosis (Chart 1 and Chart 2).

There are obvious differences in the percentage values of spherocytes in the control blood smears; they amounted to 13% (control of 7th day group), which corresponds to apparent spherocytosis. In both experimental groups, spherocytes ranged from 4% to 6%, which corresponds to moderate spherocytosis (Chart 1 and Chart 2).

Acanthocytes were mild to moderately abundant, from 2% to 6%. Dacrocytes (Figure 2 B), anulocytes, schizocytes, elliptocytes, stomatocytes, and meta-cells had a negligible percentage share, which ranged from 0-2% (Chart 1 and Chart 2), i.e. from rare to mild representation (11).

4. DISCUSSION

Automated hematology equipment does not detect immature erythroid precursors, orpoikilocytotic forms of differentiated red blood cells in the peripheral blood.

Cells type	Dog 1		Dog 2		Dog 3		Dog 4		Dog 5		Dog 6	
	A	B	A	B	A	B	A	B	A	B	A	B
Degenerat. neutrophils (%)	0	0	0	0	0	24,6	0	0	0	0	0	1,6
Atypical lymphocytes (%)	0	10,4	0	6,6	0	0	3,1	0	0	7,0	0	0

Table 3. Atypical leukocytes (%) after implantation of titanium and plastic clips on the 60th postoperative day (Group 2.). A -Control before treatment, B-Values after 60th day

the results of the second group (60th day), showed mild neutropenia. Acidophils and basophils were dormant in the second experimental group (Table 2). Results of the T-test and Fisher's test also showed no statistically significant difference between the mean values ($p > 0.05$) of the control results and the results of the second group. In addition, Fisher's test showed no significant difference between the results of the first and the second group.

Microscopic analysis revealed atypical neutrophils as well as atypical lymphocytes in the observed groups, especially in the second experimental group (Table 3), with obvious blue granules in the cytoplasm.

Figure 1 shows: A) a neutrophils in the center of the blue granules distributed throughout most of the cytoplasm; B) two neutrophils with blue granules in the cytoplasm; C) atypical lymphocytes with dark blue cytoplasm; D) a lymphocyte with wavy edges of the cytoplasm. According to the semi-quantitative assessment of degenerative changes, leukocytes were classified in percentages as follows: a few (5% - 10%), moderate (11% - 30%), a large number (>30%) (12). Morphological changes in red blood cells were noticed; the presence of echinocytes occurred (Figure 2 A) in the peripheral blood smears taken for control in both experimental groups. In the control samples of the first experimental group, the presence of echinocytes accounted for 3% (Chart 1), which represents the boundary between mild and moderate echinocytosis, while in the control sample of the second experimental group echinocytes accounted for 15%, what represents apparent echynocytosis (11). In the blood smears of both experimental groups of dogs (7th day and 60th day), the percentage value of echino-

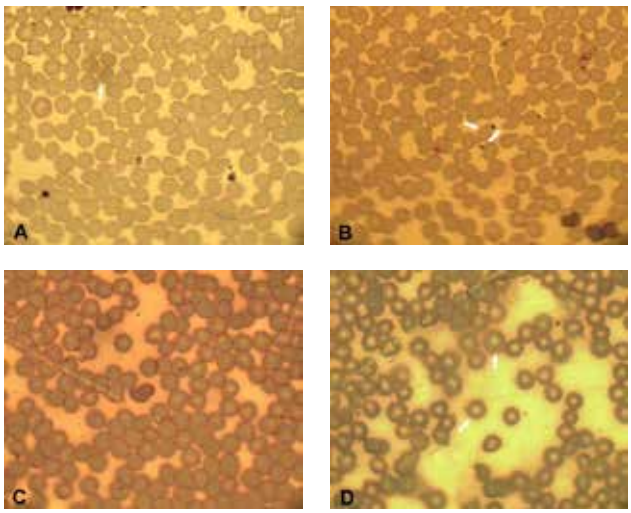

Figure 2. Poikilocytotic forms of erythrocytes

Apart from these, it also cannot identify immature and toxic changes in neutrophils, atypical lymphocytes and leukemic cells. Therefore, it is advisable to analyze blood smears microscopically to obtain more accurate results of leukogram and resized forms of red blood cells and platelets (16). Blood smears can provide insights into any possible anisocytosis, the presence of abnormal cells or inclusion, as well as the possible presence of blood parasites (17-19).

The presence of monocytosis in the control results in both groups of dogs (Table 1 and Table 2) can be explained by the effect of prolonged stress, pain, and limited space during the experiment, and during hospitalization, all under the influence of endogenous glucocorticoids,

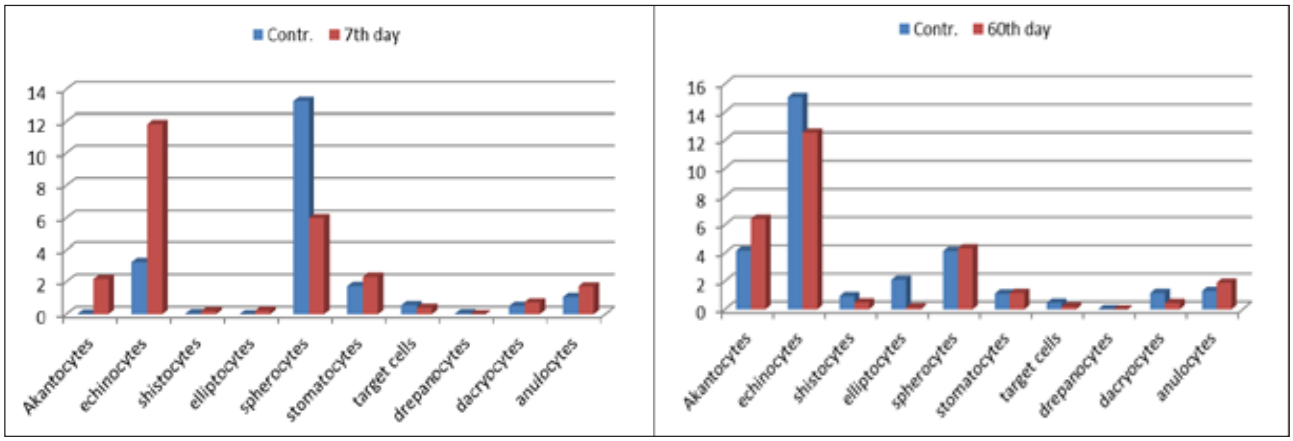

Chart 1. and Chart 2. Poikilocytotic forms of erythrocytes after implantation of titanium and plastic clips on the 7th and 60th postoperative days

what results in, among other things, monocytosis (19). Also, a possible reason for the monocytosis in the control results is the age of the tested dogs, which amounted to 8-10 years (15, 20). The very apparent monocytosis in the experimental dogs in the first group (22.83% -Table 1) and the second group (16.20% -Table 2) is a sign of infectious or chronic inflammatory processes, or tissue damage (15, 20, 21, 22, 23).

The functions of mononuclear phagocytes, amongst other things, are the secretion of mediators and regulators of the inflammatory response, interactions with antigens and lymphocytes, and removal of the tissue debris through phagocytosis. In order to perform these functions, they are arranged in all the places where they could come into contact with infectious and other agents (21).

Dogs have a neutrophils blood count (10, 12, 15, 17); the dominant trend of the percentage values of neutrophils in relation to other leukocitic cells was also recorded in our research. The control results from the first group did not exceed the physiological framework. The number of immature neutrophils was slightly elevated and amounted to 6.5% in the first group (Table 1), while the upper physiological limit was 4% (10), which indicates enhanced granulopoesis in the bone marrow. Neutropenia was obvious in the second experimental group (43.2% - Table 2), while the lower physiological limit in adult dogs is 51% (10). The immature neutrophils were dormant in physiological terms. The presence of neutrophils with morphological changes was very indicative (Figure 1A and Figure 1B), but only in the second experimental group. Degenerative changes in neutrophils were determined (Table 3) in two out of six dogs in the second group of tested dogs. In dog number 3 of the second experimental group, the percentage of degenerative neutrophils amounted to 24.6%, which, according to the semi-quantitative evaluation of degenerative changes on neutrophils (12), belongs to the "moderate" group. Dog number 6 had 1.6% of degenerative neutrophils, which belongs to the "few" group (Table 3).

The changes were manifested in the presence of pronounced blue granules, which filled more than 50% of the cytoplasm of neutrophils (Figure 1 A and Figure 1 B). When the cytoplasm of neutrophils is filled with basophilic sparkling vacuolation, called Dohle bodies, it is considered that the neutrophils have suffered degenerative changes due to the effect of toxins. These morphological abnormalities develop in neutrophils in the bone marrow, before they reach the circulation (12). No degenerative neutrophils were detected in the first experimental group. Neutropenia and the observed degenerative neutrophils in the second experimental group, may be connected to the subsequent development of inflammatory processes. Morphological changes to neutrophils were found in the blood smears in two dogs (Table 3), which indicates the necessity for the conduct of additional, more complex research, with the use of electron microscopy.

The lymphocytes of the second experimental group were on the upper physiological limit (Table 2). Also, atypical lymphocytes were only determined in the second experimental group (Figure 1C and 1D), and they are associated with the reactive lymphocytes (Figure 1C) with highly basophilic cytoplasm. Lymphocytes with wavy edges with generally scarce cytoplasm were also noticed (Figure 1D) (28). Atypical lymphocytes were identified on the blood smears of three dogs in the second experimental group (10.4%, 6.6% and 7%), which belong to the "few" group. Their presence indicates a response to antigenic stimulation. Lymphocytes exhibit increased activity through magnifying the basophilic cytoplasm (12, 24).

Acidophils and basophils were in the normal range in both experimental groups, indicating the absence of allergic reactions in the tested animals, and confirms the cleansing of parasites in the animals previously conducted.

Fisher's test found no statistically significant difference between the mean values of the control and experimental results within the groups ($p > 0.05\%$), although monocytosis, neutropenia and slight lymphocytosis were detected, with the evident presence of atypical lymphocytes and degenerative neutrophils. The results are fully in line with the results Delibegović et al., 2016[a].

Healthy erythrocytes in dogs (Figure 3C) are larger in diameter when compared to the red blood cells of other domesticated animals, and they have a biconcave disc shape (21). The life span of canine erythrocytes varies from 110 to 120 days. They often change their shape as a result of biochemical and pathological changes, due to

the impact of toxins or physical damage to the red blood cells (12, 25).

When analyzing the presence of poikilocytotic forms of red blood cells in the study (Figure 2D), a negligible presence of anulocytes (25), schizocytes, dacrocytes (Figure 2 B) elliptocytes, stomatocytes and meta-cells (12) was found. Their scarce percentage presence was seen in the context of the artifacts; the mechanical trauma to the cell during the preparation of blood smears, and the effect of anticoagulants or microscopy on the outskirts of the blood smear (25).

In contrast, a more significant percentage distribution of echinocytes (Figure 2A) and spherocytes (Figure 1 and Figure 2) was determined. The greater presence of spherocytes in blood smears is associated most often with anemia and immune-type hereditary spherocytosis. Significant percentages of echinocytes are associated with artifacts or longer contact of erythrocytes with an anticoagulant. According to Božić, 2012, echinocytosis was found in dogs diagnosed with lymphoma, and in a number of other animals with kidney disorders, most often glomerulonephritis. Taking into consideration that our experiment showed about the same presence of spherocytes and echinocytes in the control and experimental blood smears of both experimental groups, it cannot be claimed that these poikilocytotic forms of erythrocytes are the result of the unfavorable impact of the plastic and titanium clips placed in the neurocranium.

5. CONCLUSION

Preliminary hematological results confirm the adverse impact on the majority of leukocitic cells of plastic and titanium clips placed in the neurocranium of dogs in terms of monocytosis and neutropenia, as well as the occurrence of degenerative changes in the neutrophils and atypical lymphocytes in the second experimental group (60^{th} day). Chronic inflammatory processes, resulting from the presence of different biomaterials in the brain tissue, were detected. No adverse effects on the shape of red blood cells were identified in treated dogs in either group. Poikilocytotic forms of red blood cells found in blood smears can be linked to artifacts or other pathophysiological disorders in the body. Further studies and more complex hematological analysis are necessary for an explanation of the occurrence of degenerative changes in the neutrophils and lymphocytes.

- Conflict of interest: the authors declare no conflict of interest.

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