

Haematological Profile After Use of Titanium Double Shanked Ligation Clips in Laparoscopic Appendectomy: An Experimental Study on Rat Model

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Abstract

The aim of the study was to determine possible adverse effects on haematological parameters of the rats, using different surgical techniques, i.e. titanium double shanked ligation (DS) clips and endoloop polyglactin 910 suture material in laparoscopic appendectomy. The use of DS clips and other similar methods is not widely represented in veterinary surgery, as is the case in humans. Fifty rats were randomized into three groups: group 1 in which the appendiceal base was closed with a endoloop polyglactin 910 ligatures, 20 rats, group 2 in which the appendectomy was closed with DS titanium clips, 20 rats. Group 3 was the control, in which the animals were not subjected to the surgical procedure, 10 rats. Blood was drawn from the caudal vein of ten animals from each experimental group, 7 and 28 days post-surgery. Thrombocytopenia was found in the DS clips group, and hypochromia with hypochromic erythrocytes, as well as neutrophilia and lymphopenia in both experimental groups. From a haematological standpoint, DS clips are more applicable for potential patients than endoloop polyglactin 910 ligatures.

Keywords: Comparison with suture material, Hypochromic anaemia, Lymphopenia, Neutrophilia, Rat

Laparoskopik Apendektomide Titanyum Çift Uçlu Ligasyon Klipslerinin Kullanımı Sonrası Hematolojik Profil: Ratlar Modelinde Deneysel Bir Çalışma

Öz

Bu çalışmada, ratlarda laparoskopik apendektomide titanyum çift uçlu ligasyon (DS) klipsleri ve endoloop poliglaktin 910 suture materyali gibi farklı cerrahi teknikler kullanılarak hematolojik parametreler üzerine olası olumsuz etkilerinin belirlenmesi amaçlanmıştır. DS klipslerinin ve diğer benzer yöntemlerin kullanımı, insanlarda olduğu gibi veteriner cerrahisinde yaygın kullanım alanı bulmamaktadır. Elli rat rastgele üç gruba ayrılmış: Grup 1'de (n=20 rat), apendiks tabanının bir endoloop poliglaktin 910 ligatürü ile kapatıldığı apendektomi, Grup 2'de (n=20 rat) DS titanyum klipslerle kapatıldığı apendektomi uygulanmıştır. Grup 3 (n=10 rat), ratların cerrahi işleme tabi tutulmadığı kontrol grubunu oluşturmuştur. Cerrahi işlemden 7 ile 28 gün sonra her deney grubundan on hayvanın kaudal damarlarından kan alınmıştır. DS klips uygulanan grupta trombositopeni ve her iki deney grubunda hipokromik eritrositlerle birlikte hipokromi ve ayrıca nötrofil ve lenfopeni saptanmıştır. Hematolojik açıdan, DS klipsleri potansiyel hastalar için endoloop poliglaktin 910 ligatürlerinden daha uygundur.

Anahtar sözcükler: Suture materyali ile karşılaştırma, Hipokromik anemi, Lenfopeni, Nötrofil, Rat

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INTRODUCTION

Resection of the appendix may be performed routinely using several well-known techniques. One of the standard and cheap technique used is endoloop polyglactin 910 ligatures [1]. A more expensive technique is the use of staplers with titanium clips, which has advantages in laparoscopic surgery [2,3] as a possible alternative when other techniques are not able to close the appendiceal stump [4,5]. Using non-resorptive Hem-o-lok plastic clips is also another alternative method which has some advantages [1,6]. The effect of each of these techniques on haematological parameters has already been described [1,3].

Titanium DS clips are made of the same material as stapler clips, but they are constructed differently [7,8]. Their effect on haematological parameters after the application is unknown. Since DS clips and stapler clips are made of the same material, it may be presumed that their effect on the haematological profile will be similar. Any possibly undesirable effect of DS clips on haematological parameters may have implications in their clinical use in the end.

In general, monitoring the complete haematological status of patients is an extremely important aspect during postoperative care, through which is possible faster prevention of unwanted potential health disorders [3].

For this reason, we conducted an experimental study using rodents, in order to establish any undesirable effect of DS clips on haematological parameters and compare them with endoloop polyglactin 910 ligatures, following laparoscopic appendectomy.

MATERIAL AND METHODS

Ethical Statement

The research study was compatible with the Law on Animal Welfare of Bosnia and Herzegovina for experiments involving animals, and the Ethics Committee of the Veterinary Faculty of the University of Sarajevo issued a positive opinion about it, number 01-02-18-16/19.

Animals

Wistar rats were used in the study, aged 12-14 weeks and weighing between 200 and 300 g. The animals were housed in a vivarium with a natural light-dark cycle, with appropriate environmental temperature and humidity. They were given pellet feed for rodents and water *ad libitum*. Food was removed 24 h before the surgical procedures. Fifty rats were randomized into three groups: Group 1 (n=20 rats) in which the appendiceal base was closed with an endoloop polyglactin 910 (Vicryl®, ETHICON) ligatures, Group 2 (n=20 rats) in which the appendectomy was closed with DS titanium clips. In Group 3 with 10 rats,

actually the control and the animals were not subjected to any surgical procedures.

Surgical Procedure

The rats were anaesthetized using the combination of 10 mg/kg xylazine HCl (Rompun® 2%, Bayer) and Ketamine HCl 50 mg/kg (International B.V. Boxmeer, The Netherlands), intramuscularly. The fur covering of the abdomen was shaved, and the skin was disinfected with a povidone-iodine solution. They were laid on the operation table on their backs. Their extremities were fixed with sticky tape. An incision was made along the medial line. The large caecum sac was located in the lower third of the abdominal cavity. The large caecum sac in rats has a closed end and is equivalent to the appendix in humans. That part was resected.

Laparotomy and skin closure were performed with a 3-0 continuous suture. No antibiotic therapy was used during or after the experiment. Throughout the observation period, all animals were monitored and subjected to clinical trials [1].

Parameters of Monitoring

Blood was drawn from the caudal vein of ten animals from each experimental group, 7- and 28-days post-surgery. Rats in the control group (n=10) also had blood drawn and it was used for comparison.

Haematological Tests

An "Idexx Laser Cyte" flow haemocytometer was used, and the following parameters were analysed: Erythrocytes (count) (RBC) ($10^{12}/L$) and Leukocytes (count) (WBC) ($10^9/L$), Platelets (count) (PLT), Haemoglobin (HGB) (g/dL), Haematocrits (HCT) (%), Mean Corpuscular Volume (MCV) (fl), Mean Corpuscular Haemoglobin (MCH) (pg) and Mean Corpuscular Haemoglobin Concentration (MCHC) (g/dL).

Microscopic analysis of poikilocytic forms of erythrocytes involved microscope examination of blood smears, previously stained by the Giemsa method. Poikilocytosis was classified semi-quantitatively according to similar research [9,10], using the following criteria: absent (0%), rare (0.05% - 0.5%), mild (>0.5% - 3%), moderate (>3% - 10%) or marked (>10%).

The number and type of poikilocytic forms of erythrocytes were recorded as the percentage of erythrocytes. Within each stained blood smear from the test animals, 1000 erythrocytes were counted and analysed using a binocular light microscope, Motic Type 102 M, with 900 x magnification [1,3].

The relative values of the leukogram for lymphocytes (L), neutrophils (N), monocytes (M), basophils (B) and acidophiles (A) are shown as percentages (%), after microscopic differentiation of 1000 of them within each blood smear

from the test animals, from the experimental and the control group, using a Motic Type102 M binocular light microscope with 900 x magnification [1].

Statistical Analysis

Statistical analysis of data was performed using IBM SPSS Statistics for Windows, Version 24. The data were processed using a parameter (ANOVA) test or a non-parameter test (Kruskal Wallis) depending on whether the data were normally distributed or not. The Shapiro-Wilk test was used to test normality, and $P < 0.05$ was taken as statistically significance. If the result showed statistical significance, post hoc tests were conducted (Tuckey, Dunett) to establish between which groups that difference existed [11].

RESULTS

Fig. 1-8 show the mean values and standard deviations of RBC, HCT, HGB, MCV, MCH, MCHC, WBC and PLT, respectively. The highest values of RBC, HCT and WBC were noticed in the Polyglactin 910 group on day 28, whilst the MCHC and PLT values were the highest in the Polyglactin 910 group on day 7. What is extremely interesting is that the values of HGB, MCV and MCH were lower in the test groups than in the control. The standard deviations calculated for all the test groups and for all parameters were quite large, which indicates the large dispersion of results in relation to the mean values calculated.

Fig. 9, which shows the mean cell values in the leucogram, clearly shows that lymphocytes (L) and neutrophils (N) dominate the total number in terms of percentage. Although in all the test groups the L values was above 55%, its highest value was in the control group (82.56%) and its lowest in the Polyglactin 910 group on day 7 (56.7%). In contrast to the situation, we can see with L, the presence

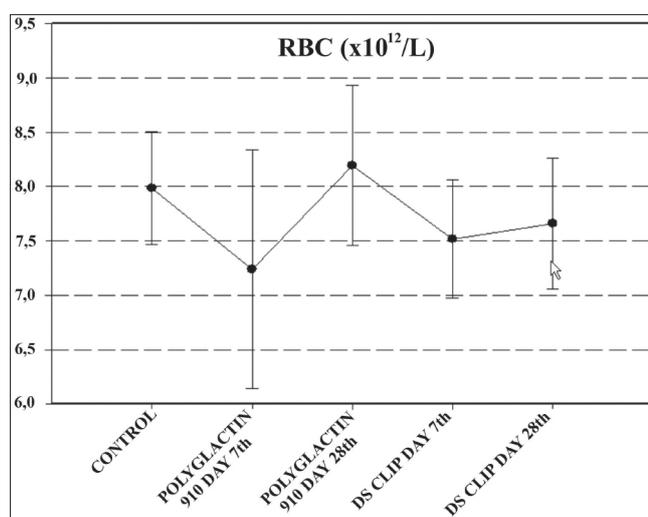


Fig 1. Changes in the number of erythrocytes (RBC) (mean value \pm standard deviation)

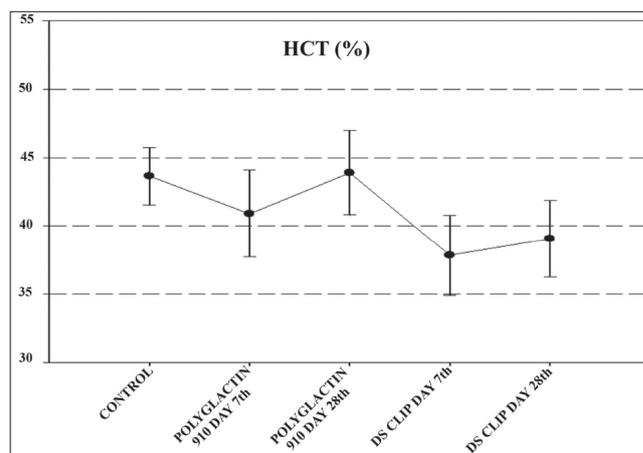


Fig 2. Changes in the haematocrit values obtained (HCT) (mean value \pm standard deviation)

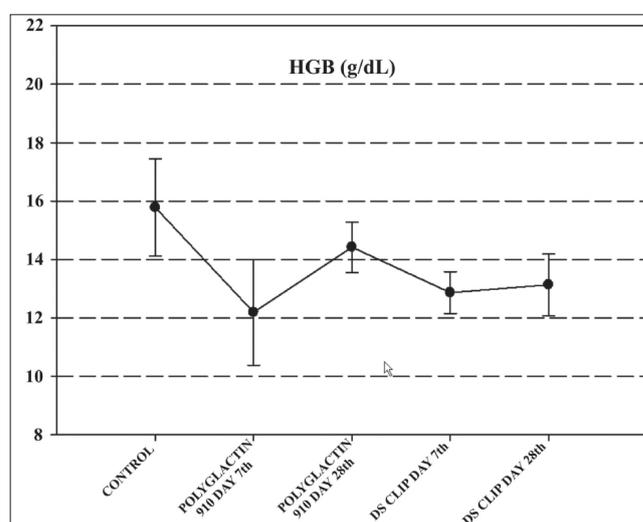


Fig 3. Changes in the haemoglobin values obtained (HGB) (mean value \pm standard deviation)

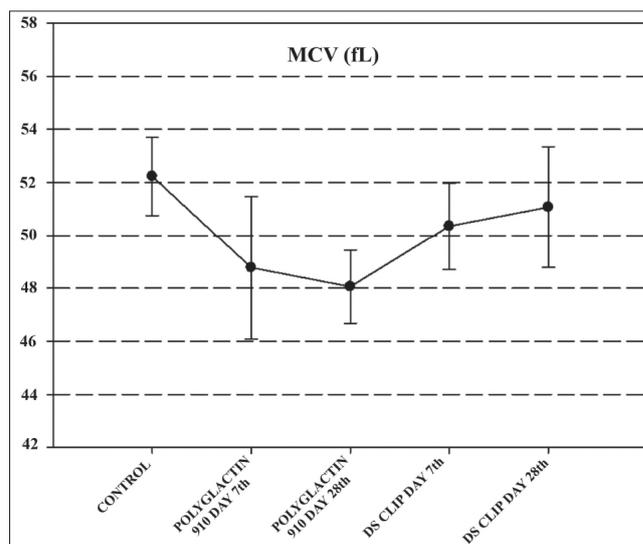


Fig 4. Changes in the MCV values obtained (mean value \pm standard deviation)

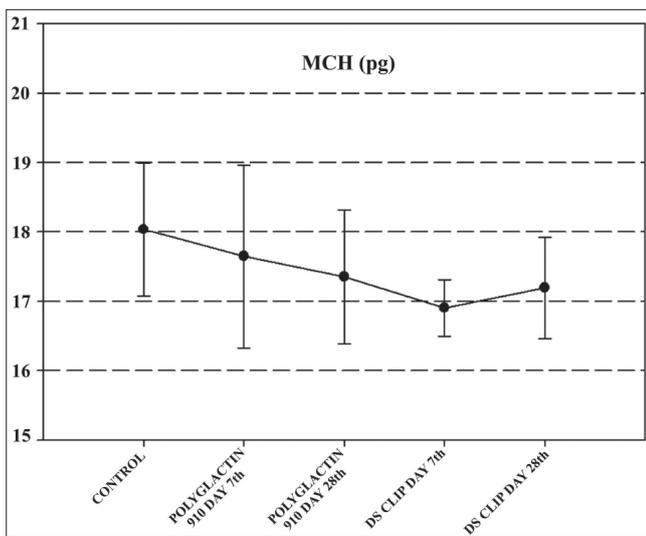


Fig 5. Changes in the values obtained (MCH) (mean value ± standard deviation)

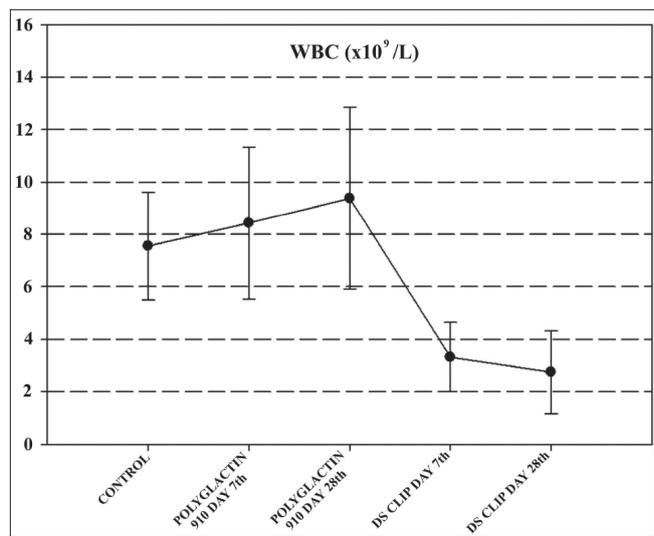


Fig 7. Changes in the haemoglobin leukocyte values obtained (WBC) (mean value ± standard deviation)

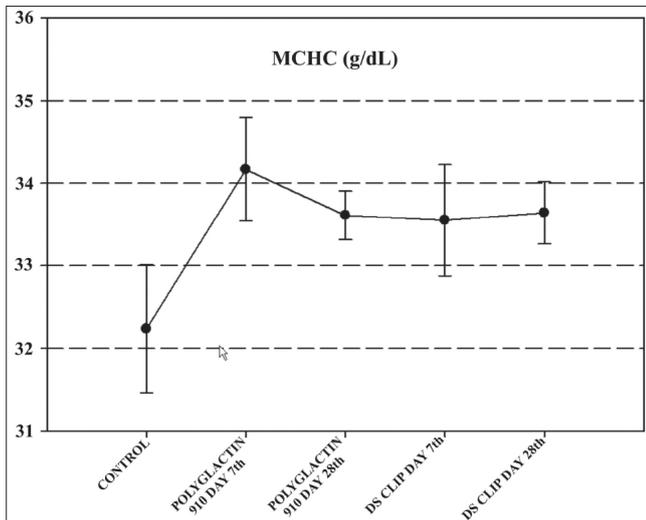


Fig 6. Changes in the values obtained (MCHC) (mean value ± standard deviation)

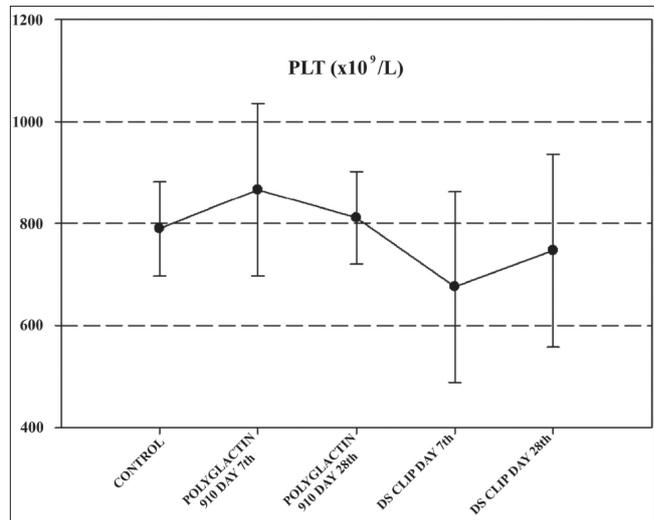


Fig 8. Changes in the thrombocyte values obtained (PTL) (mean value ± standard deviation)

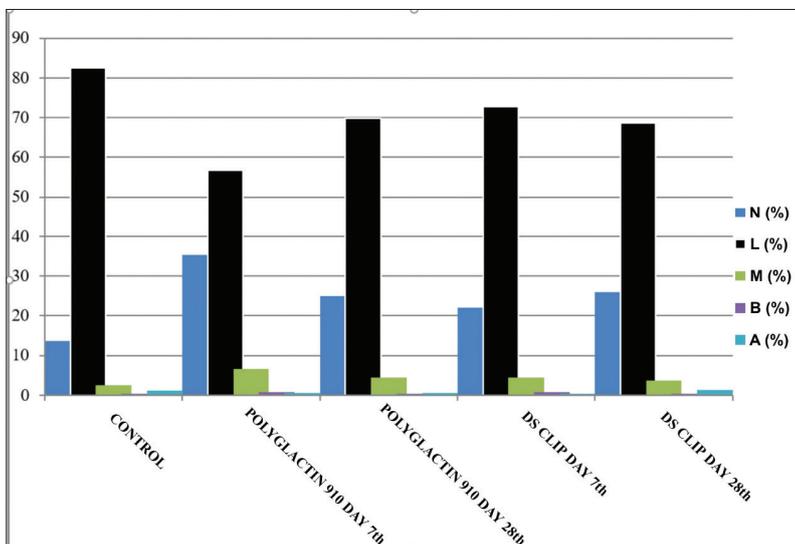
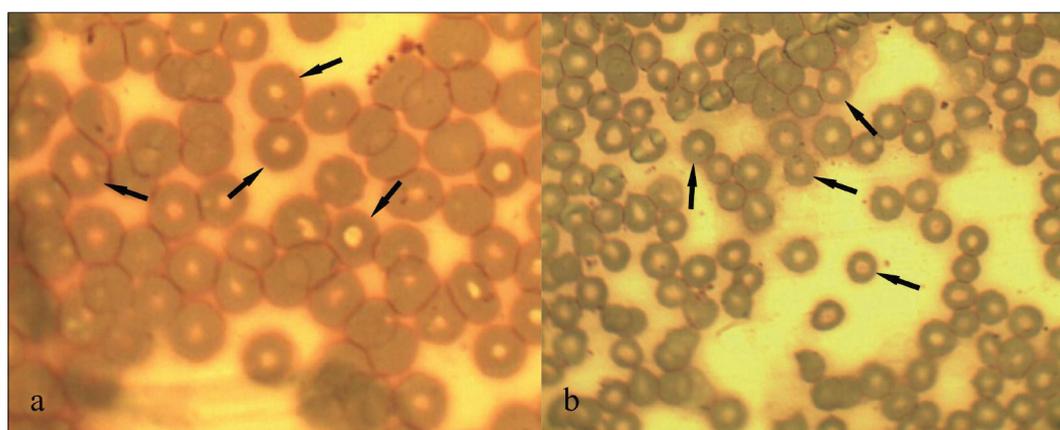


Fig 9. Leucogram (%) for the control and two experimental groups

Table 1. The type of poikilocytic forms in peripheral blood smears in the control and experimental groups. The values represent the percentage (%) of poikilocytic forms in 1000 erythrocytes analysed

Poikilocytic Forms of Erythrocytes	Control	Polyglactin 910 Day 7	Polyglactin 910 Day 28	DS Clip Day 7	DS Clip Day 28
OVALOCYTES	0.195	0.49	0.49	1.09	1.37
DACRIOCYTES	0.16	1.11	0.54	0.685	1.285
ANULOCYTES	1.025	3.45	4.56	2.435	11.105
ECHINOCYTES	0.495	1.09	1.46	0.17	1.37
STOMATOCYTES	0.27	0.50	1.77	1.11	0.155
DREPANOCYTES	0	0.02	0.00	0.07	0.08
SCHIZOCYTES	0.035	0.14	0.08	0.115	0.075
TARGET CELLS	0	3.74	0.82	0.44	1.405
ACANTOCYTES	0	3.03	0.00	0	1.015
SPHEROCYTES	0.01	2.56	1.57	0.98	2.335
RETICULOCYTES	0.24	0.64	0.12	0.64	0.12

**Fig 10.** Hypochromic erythrocytes (anulocytes), Polyglactin 910 day 28 (black arrow) (a), and also hypochromic erythrocytes (anulocytes), DS clips day 28 (black arrow) (b)**Table 2.** Statistical analysis of the results obtained. The calculated values are the result of post hoc analysis after the ANOVA test disproved the null hypothesis

Parameter	P-Value (ANOVA/Kruskal Wallis)	P-Value (post hoc)									
		Polyglactin 910 Day 7-DS Clip Day 7	Polyglactin 910 Day 7-DS Clip Day 28	Polyglactin 910 Day 7-Control	Polyglactin 910 Day 7-910 Day 28	DS Clip Day 7-DS Clip Day 28	DS Clip Day 7-Control	DS Clip Day 7-Polyglactin 910 Day 28	DS Clip Day 28-Control	DS Clip Day 28-Polyglactin 910 Day 28	Control-Polyglactin 910 Day 28
RBC ($\times 10^{12}/L$)	0.012	1.0	1.0	0.593	0.024	1.0	0.9	0.045	1.0	0.29	1.0
HCT (%)	0.000	0.774	1.0	0.824	0.684	1.0	0.004	0.002	0.037	0.025	0.684
HGB (g/dL)	0.000	1.0	1.0	0.000	0.043	1.0	0.000	0.037	0.006	0.385	1.0
MCV (fL)	0.001	1.0	0.37	0.045	1.0	1.0	0.785	0.329	1.0	0.02	0.001
MCH (pg)	0.118	-	-	-	-	-	-	-	-	-	-
MCHC (g/dL)	0.000	0.153	0.284	0.000	0.234	0.997	0.000	0.999	0.000	1.0	0.000
WBC ($\times 10^9/L$)	0.000	0.000	0.000	0.936	0.909	0.983	0.004	0.000	0.001	0.000	0.469
PLT ($\times 10^9/L$)	0.085	-	-	-	-	-	-	-	-	-	-
N (%)	0.001	0.034	0.237	0.000	0.156	0.897	0.365	0.962	0.032	0.999	0.111
L (%)	0.000	0.01	0.092	0.000	0.051	0.902	0.264	0.970	0.044	0.999	0.079
M (%)	0.143	-	-	-	-	-	-	-	-	-	-
B (%)	0.321	-	-	-	-	-	-	-	-	-	-
A (%)	0.071	-	-	-	-	-	-	-	-	-	-

A statistically significant result is $P < 0.05$

of N and monocytes (M) is highest in the Polyglactin 910 group on day 7 (35.4%; 6.6%, respectively), and lowest in the control group (13.56%; 2.56%, respectively). The values of basophils (B) and acidophiles (A) were without major deviations in all the groups.

In all groups, poikilocytic forms were present (Table 1), with the marked presence of anulocytosis in the DS clip experimental group on day 28 (11.105%) (Fig. 10-b) and moderate anulocytosis in the Polyglactin 910 group on day 28 (4.56%) (Fig. 10-a), and in the Polyglactin 910 group on day 7 (3.45%). A moderate presence of target cells was noticed in the Polyglactin 910 group on day 7 (3.74%). All the other poikilocytotic forms were mildly expressed (0.05-3%) within all the experimental groups. A complete absence of drepanocytes was noticed in the control group and the Polyglactin 910 group on day 28, of target cells in the control group, of acantocytes in the control and Polyglactin 910 groups on day 28, and in the DS clip group on day 7.

Multivariate analysis of data ($P < 0.05$) showed a statistically significant difference between the groups for all parameters except MCH and PLT (Table 2). Also, the values of monocytes, basophils, and eosinophils did not differ significantly between the groups. Post hoc analysis showed between which groups there was a statistically significant difference, as presented below. A statistically significant difference in RBC values was observed in the Polyglactin 910 group on day 7 and day 28, as well as between the DS clip group on day 7 and the Polyglactin 910 group on day 28. A statistically significant difference in HCT values was observed between the control group and the DS clip group on day 7 and the DS clip group on day 28. There was also a difference between the Polyglactin 910 group on day 28 and the DS clip group on day 7 and DS clip on day 28.

A difference in HGB values was observed between the control group and the Polyglactin 910 group on day 7, the DS clip group on day 7, and the DS clip group on day 28. There was also a statistically significant difference between the Polyglactin 910 group on day 28 and the Polyglactin 910 and DS clip groups on day 7.

A statistically significant difference in MCV values was observed between the control group and the Polyglactin 910 group on day 7 and the Polyglactin 910 group on day 28, as well as between the DS clip group on day 28 and the Polyglactin 910 group on day 28.

There was a statistically significant difference in the MCHC levels between the control group and all the experimental groups, that is Polyglactin 910 day 7, Polyglactin 910 day 28, DS clip day 7 and DS clip day 28.

A statistically significant difference was observed in the WBC measured between the control group and the DS clip group on day 7 and the DS clip group on day 28, as well as

between the Polyglactin 910 group on day 7 and the DS clip group on day 7 and day 28. The WBC values measured in the Polyglactin 910 group on day 28 differed statistically significantly from the WBC values measured in the DS clip group on day 7, and the DS clip group on day 28.

There were statistically significant differences in the values of lymphocytes and neutrophils measured between the same groups, that is, between the control group and the Polyglactin 910 group on day 7 and the DS clip group on day 28, as well as between the Polyglactin 910 group on day 7 and the DS clip group on day 7.

DISCUSSION

Rats are laboratory animals with low blood volume values in their cardiovascular system in proportion to their body weight^[12]. A small loss of blood can lead to a lethal outcome in this animal. As a result, healthy rodents have higher PLT values than other larger species^[13]. Their task is to react promptly to maintain normal haemostasis of the blood, which represents a form of physiological adaptation of the organism^[14]. In our study, the PLT count in the DS clips group on days 7 and 28 was significantly lower than in the control group, as well as the Polyglactin 910 experimental group. The values obtained were even lower than the reference intervals^[13].

Recent research into non-resorbable clips, titanium stapler clips and plastic hemo-o-lok clips regarding haematological parameters after laparoscopic appendectomy indicated the beginnings of hypochromic anaemia with the evident presence of anulocytes, or hypochromic erythrocytes^[1,3]. Our study with the use of titanium DS clips in appendectomy also confirmed the beginnings of hypochromic anaemia. The most probable reason for this minimal loss of HGB, RBC and HCT was the course of the surgical procedure. There was clearly controlled bleeding, and the *regio abdominalis* in rats is extremely well vascularized^[15]. Other erythrocyte indexes, such as MCV, MCH and MCHC, moved within a wide range, also with broad standard deviations, whereby the values established indicated hypochromic anaemia. The haematological values obtained from the DS stapler group indicated the very beginnings of anaemia, whilst the values in the Polyglactin 910 group indicated a somewhat more advanced stage of the same form of anaemia.

Recent reports have indicated that placing bioactive materials in tissue parts in an *in vivo* situation leads to a biological response^[16]. In the practice of laparoscopic surgery, these are most frequently endoloop polyglactin 910 ligature sutures, as well as, for example, plastic and/or metal material^[17]. In these circumstances, a certain reaction by the organism through leukocyte cells is expected. Recent research by Bajrić et al.^[3] showed that non-resorbable titanium linear stapler clips cause a smaller reaction in the surrounding tissue than resorbable endoloop ligatures.

Placing DS titanium clips to close the appendiceal base in our study did not cause an increase in the total WBC count, in fact, the values obtained on days 7 and 28 were even lower than the control values. Although the values for WBC in the Polyglactin 910 group on days 7 and 28 were somewhat higher than the control values, they did not go beyond the upper physiological threshold for rats ^[18].

The leucogram results in our study correspond completely with the results of Bajrić et al.^[3]. Neutrophilia was recorded in both experimental groups, where it was somewhat more marked in the Polyglactin 910 group.

This neutrophilia is an expected reaction in the peripheral blood in the post-operative period, due to the possible occurrence of an inflammatory process, the partial loss of blood, and the emotional stress in the operated experimental animals. All of this leads to the redistribution of neutrophils from the bone marrow reserves into the bloodstream ^[19].

Since rats have an extremely lymphocytic blood count ^[20], these values were in a slight decline in our study. The values obtained from both experimental groups were significantly lower than the control and were also below the lower physiological threshold ^[12].

The lymphopenia found in our study is completely in line with similar researches by Bajrić et al.^[1,3]. The reasons for the occurrence of lymphopenia may be seen in the redistribution of lymphocytes as a response to the stressful situation for the experimental rates during and after the appendectomy ^[1,3]. The values obtained for monocytes, basophils, and acidophils were low and appropriate to their physiological variations, and there was no significant deviation.

It is important to point out the limitations of this study since it was performed on a rat model. The important question is how far these results may be applied to humans. It is also not possible to determine how far the short incision on the anterior abdominal wall and closing it provoked an increased loss of HGB and RBC. Another limitation of this study is the small sample, that is, the very likely possible repercussions on the results obtained due to the small sample.

However, we can conclude that the results of this study indicate that titanium DS clips, in comparison with the results obtained recently for linear titanium staplers, from the point of view of haematological indicators, are equally less unfavourable, in comparison with other methods such as plastic Hemo-o-lok clips and resorbable endoloop polyglactin 910 ligatures, used for laparoscopic closure of the appendiceal stump, or even some other surgical procedures on the abdominal tissue.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Experimental Design was conceived by A.B., M.K. and S.D. Data was collected by A.K., M.Č., M.K. and Aj.B. Statistical analysis was conducted by A.S. and A.K. Original draft was written by M.K. S.D, and N.H.A.

A.Z., D.R., M.S., M.Č., A.B., S.D., M.K. A.K. and Aj.B. are the executors of the experimental design and experimental research of this study. A.S. and N.H.A. finished data analysis and writing the first draft of the paper. M.Č. and M.S. participated in analysis of experimental results. S.D. and A.B. are the designers and leaders of the project, guiding experimental design, data analysis, thesis writing and revision. All authors have contributed to the revision and final proof-reading of the manuscript.

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