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The Effects of Splenectomy and Autologous Spleen Transplantation on Complete Blood Count and Cell Morphology in a Porcine Model

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1. Introduction

Spleen, as a part of hematopoietic and immune system, plays an important role in the life cycle of blood cells. There are three major functions of the spleen and these are handled by three different tissues within the spleen. Reticuloendothelial tissue is responsible for removing old or damaged erythrocytes and cell debris from the blood stream. This same tissue may participate in hematopoiesis when there is an increased need for red blood cells and is a place where young erythrocytes produced in bone marrow undergo the process of maturation before releasing into the blood stream. Venous sinusoids along with the ability of the spleen to contract, provides a means for expelling the contained blood to meet increased circulatory demands in certain animals. White pulp provides lymphocytes and a source of plasma cells and hence antibodies for the cellular and humoral specific immune defenses (Dyce et al., 2002; Teske, 2000).

Splenectomy is a surgical removal of the spleen that may be carried out in patients whose spleen has been ruptured by trauma or damaged by other pathological processes such as cancer, infections or some autoimmune diseases (Tillson, 2003). However, total removal of the spleen may lead to side effects such as postsplenectomy infections and sepsis, due to the decreased production of antibodies and phagocytes or thrombosis, due to elevated platelet count in blood (Bessler et al., 2004; Khan et al., 2009; Miko et al., 2003; Timens & Leemans, 1992). Also, many studies report increased count of morphologically abnormal erythrocytes, immature red blood cells and pathologic erythrocyte inclusions in the peripheral blood of various species following splenectomy as a result of the loss of splenic filtering function (Haklar et al., 1997; Resende et al., 2002; Traub et al., 1987). In addition, it has been reported that removal of the spleen causes significantly higher increase of reticulocyte count than other surgeries. This suggests that spleen may somehow hormonally regulate the release of red blood cells into circulation, thus after removal of spleen bone marrow releases more red

blood cells as well as more immature erythrocytes into the blood stream (Knežević et al., 2002; Lorber, 1958). Splenectomy also causes changes in number of white blood cells with subsequent leukocytosis (Bessler et al., 2004; Karagülle et al., 2007). Initial transient neutrophilia is followed by the persistent lymphocytosis and monocytosis. Increased leukocytosis accompanied by the significant left shift is found in patients, and often there are myelocytes or other precursor in the granulocytic series in their peripheral blood (Labar & Hauptmann, 1998; Tang et al., 2003; Zhang et al., 2002).

Severe postoperative infections after removal of the spleen prompted a development of alternative methods to conserve functions of the spleen. Autologous spleen transplantation is a method of choice after total splenectomy in order to preserve splenic immune and hematopoietic functions (Marques et al., 2003; Patel et al., 1981). The effectiveness of splenic autotransplant depends on many factors and is still controversial (Theodorou et al., 2007). Studies done on rats, mice, rabbits and men report that autotransplantat's capability of recovering its primary function highly depends on the volume of transplanted spleen tissue (Karagülle et al., 2007; Miko et al., 2003; Resende & Petroianu, 2003; Tang et al., 2003).

In the recent years there is a close cooperation between Faculty of Veterinary Medicine and Medical faculty in University of Zagreb. Because of close interests that both faculties have, combined projects and seminars have been established. One of the best examples was education of Medical faculty surgeons for laparoscopic cholecystectomy, liver lobectomy, laminectomy, and for experimental wounds surgery on our faculty, in the Clinic for Surgery, Orthopedics and Ophthalmology. These operative procedures were carried out on pigs because of its similarity in organs size. In recent years, a xenotransplantation of pig organs to nonhuman primates is being investigated. In order to prolong survival of primates that have received porcine xenografts, the same animals underwent a splenectomy to prevent humorally mediated immunological damage (Cozzi et al., 2000). In order to save human lives, it is important not only to master the precise surgical technique, but also to recognize all the factors that may affect the rejection or return of physiological functions of organs after transplantation.

The aim of this study was to evaluate the effects of total splenectomy and autologous spleen transplantation in a porcine model on complete blood count and cell morphology. Also, we aimed to determine the functional effectiveness of autotransplanted splenic tissue by its capacity to remove erythrocyte having Howell-Jolly bodies from the blood stream.

2. Materials and methods

2.1 Animals, anaesthesia and surgery

The experimental protocol was approved by the Department of Veterinary Science, Ministry of Agriculture, Republic of Croatia and was conducted in accordance with the guidelines for the treatment of laboratory animals. Nineteen pigs of either sex, aged three months, weighing 19-26 kg were used in the experiment. Food was withheld from all the pigs 12 h and water 2 h before the experiment. All animals were premedicated with 2 mg/kg i.m. of xylazine (Xylapan, Vetoquinol, Bern, Switzerland), and left auricular vein was catheterized percutaneously for continuous infusion of lactated Ringer's solution at a rate of 10 ml/kg/h (Infusion pump BIOF 3000, Biotron CO, Kangwondo, South Korea) during surgical procedures and for the administration of drugs. Anaesthesia was induced with 5 mg/kg i.v.

of ketamine (Ketaminol 10, Intervet, Boxmeer, The Netherlands) and 10 µg/kg i.v. fentanyl (Fentanyl-Janssen, Janssen Pharmaceutica, Beerse, Belgium), and animals were intubated, connected to a circle system and maintained on spontaneous ventilation. Anaesthesia was maintained with 1.5% isoflurane (Forane, Abbott, Queenborough, UK) and oxygen and continuous intravenous infusion of fentanyl in a dose of 0.8 µg/kg/min. Supplemental doses of ketamine were applied during surgery to maintain sufficient anaesthesia depth. Preoperative antibiotic prophylaxis was administered using 20 mg/kg ampicillin and subactam *i.v.* (Penactam, Krka, Novo Mesto, Slovenia).

After anaesthesia induction, animals were randomly divided into three groups: sham-operated pigs with spleens intact (control group, n=6), splenectomized pigs (n=6), and splenectomized pigs with small fragments of 20% mass of the spleen autotransplanted into the greater omentum (n=7).

2.2 Blood sampling and experimental protocol

Two blood samples of each pig were taken from the *v. auricularis lateralis* just before surgery and on the first, fifth, twelfth and twenty sixth day postoperatively. Exceptionally, blood samples for reticulocyte, and differential white blood cell counting were also taken on the fortieth postoperative day. First sample was collected into the Vacutainer® tubes containing K₃EDTA anticoagulant (BD-Vacutainer, Plymouth PL6 7BP UK) and the other was taken without anticoagulant and was used to make blood smears. Hematological parameters: red blood cell count (RBC), white blood cell count (WBC), hemoglobin concentration (Hgb), hematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), platelet count (PLT) and mean platelet volume (MPV) were determined using automated blood analyzer (SERONO-9120 Baker System). Reticulocyte counting was done on brilliant cresyl blue stained blood smears, and differential leukocyte count and morphological changes of blood cells were determined by identifying 200 consecutive leukocytes on May Grünwald stained blood smears using immersion objective with 1000x enlargement of the light microscope (Olympus BX 41). The frequency of blood cells immature precursor, degenerative neutrophils or increased reactive lymphocytes is reported as a few (5% to 10%) or moderate (11% to 30%). Similarly, semi quantitative evaluation of red blood cell morphology based on average number of abnormal cells per 1000x microscopic monolayer field was used to assess morphological changes in erythrocyte (Weiss, 1984).

The results were statistically analyzed by calculating mean values, standard deviation, and coefficient of variability, and were presented in tables as the mean values ± standard deviation. The significance of the differences between the results was verified using the Student *t*-test and Statistica 7.1 computer programme.

3. Results

3.1 Red blood cell count (RBC), hemoglobin (Hgb) and hematocrit (Htc)

Total red blood cell count in sham-operated pigs was significantly decreased only on the fifth day postoperatively compared to the value before surgery. In splenectomized pigs red blood cell count was significantly lower on the first, fifth, twelfth and twenty sixth day

postoperatively compared to the value before surgery. In pigs with autologous splenic transplants, red blood cell count was significantly decreased on the fifth and twelfth day after surgery compared to the value before surgery. Hemoglobin and hematocrit values in sham-operated pigs were significantly decreased on the fifth day postoperatively compared to the values before surgery. Splenectomized pigs showed significantly lower values of these parameters on the fifth, twelfth and twenty sixth day postoperatively compared to the values before surgery. In pigs with splenic autotransplants, hemoglobin and hematocrit values were significantly decreased on the first, fifth and twelfth day after surgery compared to the values before surgery (Table 1.).

3.2 Mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and red blood cell distribution width (RDW)

There were no statistical differences of mean corpuscular volume value in sham-operated pigs before and after surgery. Compared to the value before surgery mean corpuscular volume value showed significant raise in splenectomized pigs on the twelfth and twenty sixth day postoperatively, and in contrast autotransplanted pigs showed significantly decreased value of mean corpuscular volume on the first, fifth and twelfth day postoperatively. There were no statistical differences of mean corpuscular hemoglobin value in sham-operated pigs before and after surgery while mean corpuscular hemoglobin concentration value was significantly higher on the twelfth day after surgery compared to preoperative value. In splenectomized pigs significant raise in values of both parameters was noted on the fifth day postoperatively when compared to the values of these parameters before surgery. In pigs with splenic autotransplants mean corpuscular hemoglobin value was significantly decreased on the first and twelfth day after surgery, but the mean corpuscular hemoglobin concentration value was significantly raised on the fifth day after surgery when compared to preoperative values. There were no noted significant changes of red blood cell distribution width values except in sham-operated pigs on the twenty sixth day postoperatively when compared to the same value before surgery (Table 1.).

3.3 Total and differential white blood cell count

In group with autotransplanted splenic tissue on the fifth postoperative day total white blood cell count dropped significantly in comparison with the preoperative value as well as in comparison with the value measured in the control group on the same day of experiment. After this, on the twelfth and twenty sixth day of the experiment, significant increase of white blood cell count in comparison with the preoperative value was noted in all experimental groups of pigs (Table 1.). Changes in absolute differential count of segmented neutrophils followed the same pattern as those in the total white blood cell count during whole experimental period in each of the groups of pigs.

Compared to the value right before surgery, rise in absolute count of unsegmented neutrophils was noted, with significant increase on the first and twelfth day in the control group, first and fifth day in splenectomized group and first, twelfth and twenty sixth day in group with autotransplanted splenic tissue (Table 2.).

Although absolute number of lymphocytes was decreasing postoperatively in all experimental groups of pigs, it was significantly decreased only on the fifth day in splenectomized group and in this group it remained at low values until the end of experimental period. In contrast, on the twelfth postoperative day absolute number of lymphocytes in control and autotransplanted group started to rise, even exceeding preoperative levels.

Relative differential number of monocytes ranged from one to eight percent in all blood smears. Still, statistical analysis showed significant decrease of absolute number of monocytes on the fifth day after the surgery in control group compared to the value before operation and twelfth day after splenectomy compared to the control group of the same day. Relative differential number of eosinophils on blood smears ranged from zero to twelve percent in all groups. Only statistically significant shift in the absolute number of eosinophils occurred on the first day after the surgery in control group compared to values before the surgery. Relative number of basophils in all groups ranged from one and three percent and statistically significant increase in number was found on the fifth day in the group with autotransplanted tissue compared to the values before surgery and control group of the same day (Table 2.).

3.4 Ratio of absolute differential number of neutrophils and lymphocytes (N/L)

Compared with the preoperative values, significantly elevated neutrophil/lymphocyte ratio was recorded in the control group on the first, fifth and twelfth postoperative day. In splenectomized pigs significant elevation of neutrophil/lymphocyte ratio in respect to preoperative value, as well as in respect to the value of control group on the same day of the experiment, appeared on the fifth day postoperatively and it remained on the significantly higher values till the end of experimental period. On the twelfth day of the experiment significant decrease of neutrophil/lymphocyte ratio in comparison with the sham-operated pigs on the same day was reported in group with autotransplanted splenic tissue (Table 3.).

		Before surgery	Days after surgery			
			1st	5th	12th	26th
WBC 10 ⁹ /L	Sham-operation	22.26±3.82	27.33±9.26	24.94±11.60	**38.95±5.88	**28.14±0.42
	Splenectomy		20.26±3.32	26.37±9.01	*39.23±12.09	*27.13±7.57
	Autotransplantation		23.87±0.21	*18.13±4.53+	***34.9±6.75	**28.91±3.18
RBC E*10 ¹² /L	Sham-operation	6.89±0.11	7.22±0,52	***5.85±0.55	6.84±0.16	5.09±1.79
	Splenectomy		*6.33±0.42 ⁺	***5.19±0.44 ⁺	**3.01±1.49 ⁺	*4.73±0.97
	Autotransplantation		6.35±0.29 ⁺	**5.20±1.12	*5.92±0.94 ⁺	4.55±1.18
Hgb g/L	Sham-operation	126.71±5.66	134.5±14.14	**109.4±9.19	125.75±4.27	99.5±29.44
	Splenectomy		120.6±10.61 ⁺	***101.83±7.22	**69.75±18.53 ⁺⁺	*106.33±8.96
	Autotransplantation		*112.57±4.95 ⁺⁺	**93.57±19.61 ⁺	**106.57±16.29 ⁺⁺	85.5±13.28

		Before surgery	Days after surgery			
			1st	5th	12th	26th
Htc L/L	Sham-operation	0.39±0.01	0.42±0.05	***0.33±0.03	0.38±0.01	0.30±0.09
	Splenectomy		0.37±0.03 ⁺	***0.30±0.03 ⁺	*0.21±0.07 ⁺	*0.32±0.04
	Autotransplantation		*0.35±0.02 ⁺	**0.28±0.07	**0.33±0.05 ⁺	0.26±0.04
MCV fL	Sham-operation	56.92±0.78	57.87±2.33	56.56±1.13	55.7±1.3	59.8±2.66
	Splenectomy		59.04±1.48	58.57±2.54	*69.7±8.58 ⁺	*68.67±6.22
	Autotransplantation		*54.6±1.34 ⁺⁺	**53.84±1.93 ⁺	*54.57±1.58	57.25±3.14
MCH pg	Sham-operation	18.41±0.57	18.63±0.49	18.74±0.28	18.4±0.65	19.85±1.21
	Splenectomy		19.1±0.42	***19.65±0.57 ⁺	22.13±2.81	23±3.01
	Autotransplantation		*17.74±0.26 ⁺	17.99±0.62	*17.81±0.59	19.2±2.08
MCHC g/L	Sham-operation	323.57±4.95	322.67±2.83	330.8±1.41	*330.25±4.11	331.5±5.19
	Splenectomy		323.6±1.41	**335.5±8.19	344.48±13.08	333.33±6.59
	Autotransplantation		324.86±8.49	*334.71±12.45	326.29±5.5	335.5±6.35
RDW %	Sham-operation	23.01±0.07	23.2±3.39	22.54±2.83	22.7±2.14	*21.85±0.17
	Splenectomy		23.08±3.54	23.62±3.5	24.48±4.43	23.4±1.17
	Autotransplantation		23.87±2.69	23.43±1.32	22.93±1.11	22.15±0.87
PLT E*10 ⁹ /L	Sham-operation	551.92±359.72	509.33±16.26	554±46.67	389.33±19.43	272±125.73
	Splenectomy		400±147.35	534.25±381.81	***96±25.94 ⁺⁺⁺	286.33±219.21
	Autotransplantation		587.67±326.51	690±431.74	445.53±405.88	223±114.28
MPV fL	Sham-operation	10.49±1.2	10.8±2.12	10.5±0.21	11.4±1.08	12.6±1.42
	Splenectomy		11.33±0.79	***11.65±0.87 ⁺	*12.63±1.08	10.97±1.37
	Autotransplantation		***12.43±0.42 ⁺	***12.5±0.57 ⁺⁺	12.45±0.92	12.7±1.63

All values are presented as mean values ± standard deviation.
Statistical difference with respect to the value before surgery: *P<0.05; **P<0.01; ***P<0.001.
Statistical difference with respect to the value in sham operated pigs on the same day of experiment: +P<0.05; ++P<0.01; +++P<0.001.

Table 1. White blood cell count (WBC), red blood cell count (RBC), blood hemoglobin concentration (Hgb), hematocrit (Htc), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), mean platelet volume (MPV) and red blood cell distribution width (RDW) in the peripheral blood of observed pigs during the experiment

		Before surgery	Days after surgery			
			1st	5th	12th	26th
Segmented neutrophils	Sham-operation	7.52±3.93 31.3% (6-50%)	13.12±6.85 44.7% (7-64%)	12.15±9.85 47.2% (39-63%)	**19.10±3.80 48.5% (41-54%)	**10.99±3.21 39.17% (31-49)
	Splenectomy		5.67±7.08 27.4% (14-61%)	11.32±10.00 41.3% (22-54%)	**24.38±13.99 61.75% (55-68%)	*14.00±3.28 48.5% (40-60%)
	Auto-transplantation		8.34±4.27 34.3% (18-56%)	*4.20±0.74++ 22.1% (8-36%)	*14.07±5.08 38.9% (11-55%)	*11.77±4.51 39.9% (18-57%)
Band neutrophils	Sham-operation	0.58±0.40 2.6% (1-5%)	*1.57±0.19 5.5% (4-8%)	0.63±0.13 2.8% (0-8%)	**1.53±0.05 4.0% (3-6%)	0.32±0.22 1.2% (0-3%)
	Splenectomy		**1.85±0.80 9.8% (5-15%)	**3.67±2.83++ 14.5% (5-28%)	1.58±2.18 4.5% (0-10%)	0.67±0.40 2.25% (0-4%)
	Auto-transplantation		**1.84±0.21 7.9% (4-18%)	1.00±0.39 5.7% (1-14%)	*1.41±0.06 3.9% (2-8%)	*1.64±0.08+ 5.43% (0-12%)
Lymphocytes	Sham-operation	13.06±2.88 61.2% (43-86%)	11.94±2.04 47.2% (28-85%)	11.61±2.29 47.6% (37-54%)	*16.81±2.67 43.7% (36-51%)	*15.54±3.82 55.2% (45-63%)
	Splenectomy		11.62±3.44 57.0% (29-70%)	**9.39±4.88 36.3% (29-49%)	12.52±6.33 31.5% (28-36%)	12.50±0.63+ 45.0% (36-56%)
	Auto-transplantation		12.93±3.51 54.3% (36-69%)	11.96±1.77 66.7% (51-86%)	**17.86±6.51 52.6% (36-79%)	14.22±1.50 50.1% (29-70%)
Monocytes	Sham-operation	0.33±0.27 1.4% (0-3%)	0.36±0.03 1.3% (1-2%)	**0.09±0.13 0.4% (0-1%)	0.48±0.06 1.2% (1-2%)	0.56±0.43 1.8% (0-7%)
	Splenectomy		0.36±0.03 2.2% (0-8%)	0.57±0.75 2.0% (1-3%)	0.18±0.22+ 0.5% (0-1%)	0.49±0.20 1.7% (0-5%)
	Auto-transplantation		0.28±0.38 1.3% (0-3%)	0.36±0.12 2.0% (0-5%)	0.53±0.51 1.6% (0-4%)	0.28±0.32 1.0% (0-3%)
Eozynophils	Sham-operation	0.75±0.60 3.3% (0-9%)	**0.21±0.18 1.0% (0-3%)	0.41±0.27 1.8% (0-4%)	0.95±0.45 2.2% (0-6%)	0.63±1.05 2.3% (0-5%)
	Splenectomy		0.71±0.31 3.4% (1-5%)	1.29±0.22 5.3% (1-12%)	0.48±0.66 1.5% (0-3%)	0.58±0.48 2.3% (0-4%)
	Auto-transplantation		0.48±0.38 2.3% (0-5%)	0.44±0.24 2.3% (0-3%)	0.83±0.06 2.6% (1-5%)	0.85±0.72 3.0% (1-6%)
Basophyls	Sham-operation	0.03±0.07 (0-1%)	0.10±0.02 (0-1%)	0.04±0.02 (0-1%)	0.08±0.23 (0-1%)	0.10±0.21 (0-1%)
	Splenectomy		0.04±0.14 (0-1%)	0.13±0.03 (0-2%)	0.08±0.21 (0-1%)	0.07±0.02 (0-1%)
	Auto-transplantation		0.00±0 (0%)	*0.18±0.14+ (0-3%)	0.20±0.22 (0-2%)	0.15±0.14 (0-2%)

Absolute differential values (10⁹/L) are presented as mean values ± standard deviation
Relative differential values (%) are presented as mean value, and minimum and maximum values in the brackets
Statistical difference with respect to the value before surgery:*P<0.05; **P<0.01; ***P<0.001.
Statistical difference with respect to the value in sham operated pigs on the same day of experiment:
+P<0.05; ++P<0.01; +++P<0.001.

Table 2. Absolute and relative white blood cell differential count in the peripheral blood of observed pigs during the experiment

	N/L ratio				
	Day 0	Day 1	Day 5	Day 12	Day 26
Sham-operation	0.61	*1.39	*1.09	*1.25	0.76
Splenectomy	0.61	0.83	**1.62+	***2.13++	*1.18
Autotransplantation	0.61	0.89	0.45++	0.93	1.09

Statistical difference with respect to the value before surgery: *P<0.05; **P<0.01; ***P<0.001.
Statistical difference with respect to the value in sham operated pigs on the same day of experiment:
+P<0.05; ++P<0.01; +++P<0.001.

Table 3. Ratio of absolute differential number of neutrophils and lymphocytes (N/L) in the peripheral blood of observed pigs during the experiment

3.5 Platelet number (PLT) and mean platelet volume (MPV)

There were no statistical differences in platelet count in sham-operated and autotransplanted pigs, while on the twelfth postoperative day platelet count in splenectomized pigs was significantly lower in comparison with the platelet count before surgery, and to the value in the control group at the same experimental day. There were no statistical differences of mean platelet volume value in sham-operated pigs before surgery and the value of mean platelet volume on days after surgery. In splenectomized pigs significantly higher mean platelet volume value was noted on the fifth and twelfth day postoperatively. In autotransplanted pigs statistical differences were noted on the first and fifth day after the surgery in comparison with the value before surgery (Table 1.).

3.6 Reticulocyte count (RTC)

Prior to surgeries, reticulocyte count in all experimental pigs ranged within 0.5 to 1.5 %. On the first postoperative day reticulocyte count was significantly increased (2 to 4 %) when compared to the value before surgeries and it continued to grow simultaneously in all experimental groups on the fifth (4 to 8 %), twelfth (6 to 8 %) and twenty sixth (6 to 9 %) postoperative day. On the fortieth day after the surgery, reticulocyte count continued to increase in splenectomized (7 to 16 %) and autotransplanted pigs (7 to 18 %), while at the same time it began to decrease in sham-operated pigs (2 to 3 %), although was still significantly higher when compared to the preoperative value. Corrected reticulocyte count (reticulocyte production index - RPI) is shown in Table 4., and was extremely high on twenty sixth experimental day in splenectomized and autotransplanted pigs (Table 4.).

	RPI				
	Day 0	Day 1	Day 5	Day 12	Day 26
Sham-operation	0.5	1.2	2.3	3.3	1.1
Splenectomy	0.5	1.0	2.0	1.8	4.5
Autotransplantation	0.5	1.0	2.0	1.9	4.0

Table 4. Reticulocyte production index (RPI) in experimental pigs

3.7 Morphological changes of red blood cells

Polychromasia (the heterogeneous staining of red blood cells), as well as increased number of Howell-Jolly bodies (nuclear remnants found in red cells) were present on the blood films of all experimental groups, regardless of the surgical procedures, although both of these morphological changes were more manifested and frequent in pigs with total splenectomy. Five to seven erythrocytes containing Howell-Jolly bodies were found per 1000x microscopic monolayer field on the blood smears of splenectomized pigs. Erythroblasts (immature, nucleated red cells) sporadically appeared on the blood films of splenectomized pigs and pigs with transplanted autologous splenic tissue on all postoperative days. Abnormally shaped erythrocytes, such as leptocytes and codocytes, were found only on the blood smears of splenectomized pigs from the twelfth to the fortieth postoperative day.

3.8 Morphological changes of white blood cells

Neither morphological changes nor precursor cells were found on the blood films of experimental pigs prior to surgeries. On the first postoperative day a few (one to three %) reactive lymphocytes and a few (two to five %) metamyelocytes were found on each smear of control pigs. Similar results were found in the splenectomized group, but number of metamyelocytes was higher than in the control group (three to five %). Results found in group with autotransplanted tissue were almost identical to those in splenectomized group of pigs.

On the fifth day after the surgery reactive lymphocytes were found at only one blood smear from the control group, but blood smears of other two groups contained averagely three to four reactive lymphocytes. Splenectomized group had the largest number of metamyelocytes (four to six %), and also contained dividing cells, while in the group with autotransplanted tissue number of metamyelocytes was smaller (two to five %).

On the twelfth day after the surgery reactive lymphocytes became rarer, and were found only on one smear of splenectomized group, but still on almost all smears (one to two %) in the group with autotransplanted tissue. Metamyelocytes appeared sporadically on the blood smears of each experimental group. Twenty-six days after surgeries reactive lymphocytes were no longer noted on blood smears, and number of found metamyelocytes was decreasing until the fortieth postoperative day when they completely disappeared.

4. Discussion

4.1 Hematocrit, hemoglobin and erythrocyte count

Sham operated pigs exhibited the fastest recovery of hematologic values after surgery. Although red blood cell values of autotransplanted group were significantly lower when compared to sham operated pigs, postoperative blood regeneration took less time than in splenectomized pigs. In contrast to other two surgical procedures, total splenectomy resulted in a greater decrease of red blood cell values, even below physiological values (according to Jain (1993)), which persisted for a longer period (Diagram 1.). Hemoglobin and hematocrit values changed codependently with the changes of red blood cell count in all experimental groups on all postoperative days (Diagram 2., 3.). Postoperative oligocythemia, followed by decrease of hemoglobin and hematocrit, as the result of blood

loss is a very well known founding. Durance of postoperative blood regeneration depends on many factors (e.g. degree of tissue lesion and trauma, blood loss and availability of hematopoiesis activating substances). The significant decrease of erythrocyte values and long postoperative recovery after total splenectomy and autotransplantation of splenic tissue have been documented in mice (Sipka et al., 2006), dogs (Lorber, 1958) and humans (Knežević et al., 2002). Results of this study suggest that observed decrease of erythrocyte values and postoperative recovery in each experimental group were in accordance with severity of surgical traumas (sham-operation, splenectomy and transplantation of autologous splenic tissue).

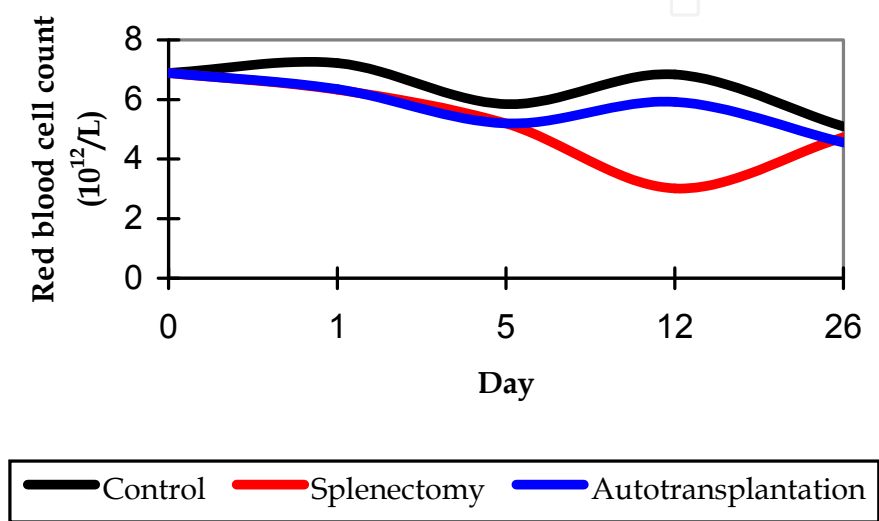


Diagram 1. Changes in red blood cell count in the blood of experimental pigs during the experiment

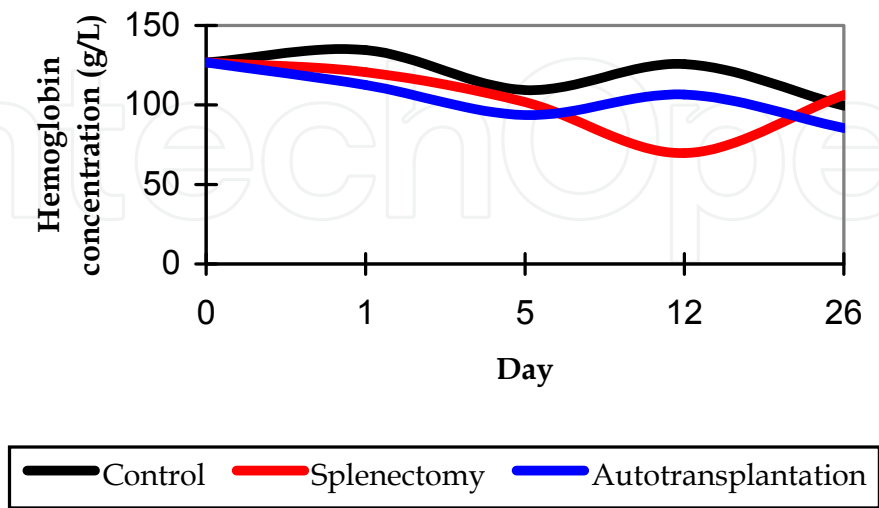


Diagram 2. Changes in blood hemoglobin concentration of experimental pigs during the experiment

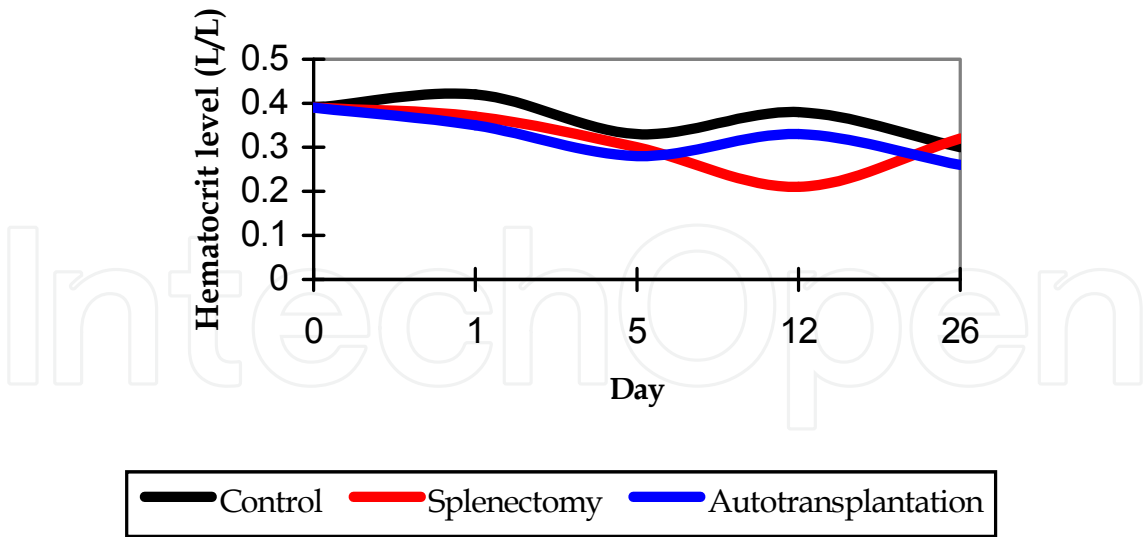


Diagram 3. Changes in hematocrit level in the blood of experimental pigs during the experiment

4.2 Mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration

As previous experiments report, splenectomized patients of different species have higher mean corpuscular volume values than autotransplanted and sham-operated patients (Knežević at al., 2002, Lorber, 1958). The aging erythrocytes undergo changes in their plasma membrane which make them retain the fluid inside the cell, thus aged erythrocytes have higher mean corpuscular volume values. Total splenectomy leads to increased number of circulating old red blood cells. This, combined with significant reticulocytosis, led to high mean corpuscular volume values of splenectomized pigs in this study (Diagram 4.). Lower postoperative value of mean corpuscular volume in autotransplanted pigs was expected as the result of significantly lower concentration of hemoglobin. Surgical trauma and blood loss led to inadequate iron supply for the developing erythroblasts and consequently to limited hemoglobin synthesis. The red blood cell membrane shrinks to fit its hemoglobin content, thus volume of the cell decreases.

4.3 Total and differential white blood cell count

Leukocytosis, characterized by neutrophilia, initial lymphocytopenia and later recovery of lymphocyte count, was recorded postoperatively in all experimental groups (Diagram 5., 6., 7.). Increase in total leukocyte count after splenectomy and autotransplantation of splenic tissue as well as persistent leukocytosis are main characteristics of white blood cell count in mice (Bessler et al., 2004), rabbits (Karagülle et al., 2007) and humans (Zhang et al., 2002). However, in present study the differences in the degree of leukocytosis among the groups were not detected, except on the fifth postoperative day when a significant decline in the total number of leukocytes in pigs with a transplanted tissue was established compared to the control group at the same day of the experiment. Therefore, present leukocytosis has not been regarded as a change specific for splenectomy or autotransplantation rather than a post-injury inflammatory response due to tissue lesions during operation.

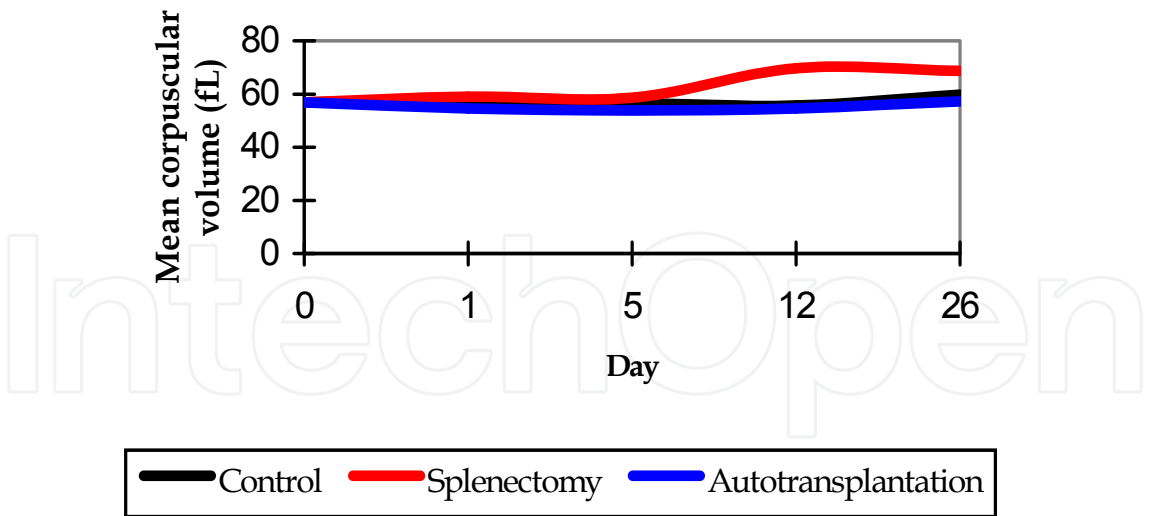


Diagram 4. Changes in erythrocyte mean corpuscular level of experimental pigs during the experiment

Due to differential leukocyte count in various species following splenectomy diverse reports were published. Some authors find an increase neutrophil and lymphocyte count Tarnuzi & Smiley (1967), other significantly higher lymphocyte count while neutrophil count remained unchanged (Bessler at al., 2004). Opposite to that, Sipka at al. (2006) found a significant increase in neutrophil count while lymphocyte count remained unchanged. However, some researches found none significant changes in differential neutrophil and lymphocyte count in blood after splenectomy or autotransplantation of splenic tissue (Resende & Petroianu, 2003; Shokouh-Amiri at al., 1990). In present study there does not seem to be a unique form of changes in differential blood count after splenectomy and autotransplantation of the spleen so we can conclude that the pattern of recorded changes in each experimental group corresponded with the degree of immune response of circulating white blood cells and stress caused by the surgical procedure.

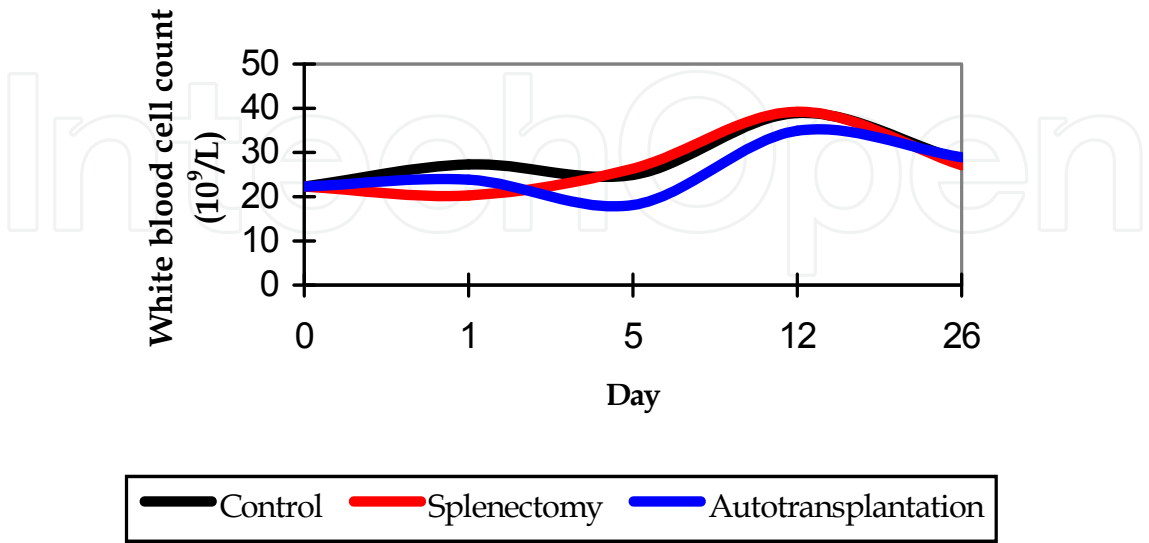


Diagram 5. Changes in total leukocyte count in the blood of experimental pigs during the experiment

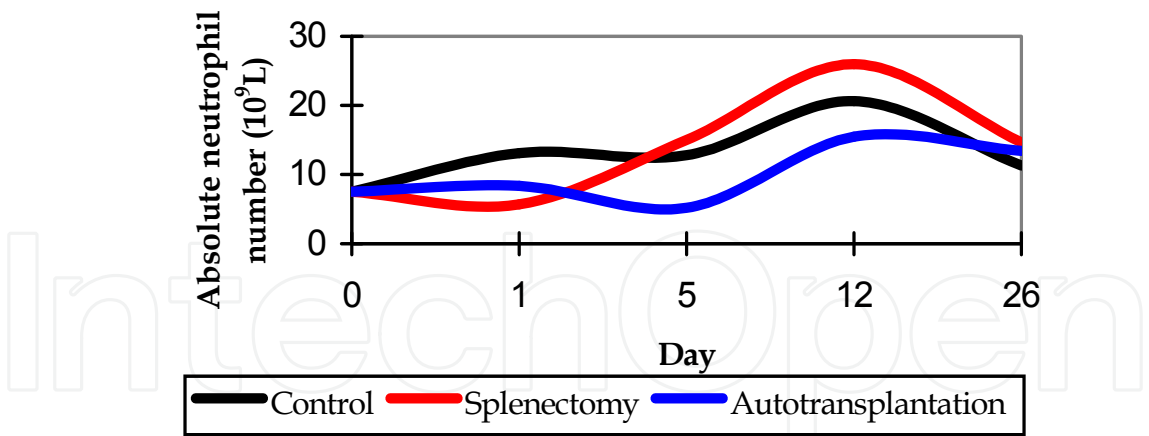


Diagram 6. Changes in absolute neutrophil number in the blood of experimental pigs during the experiment

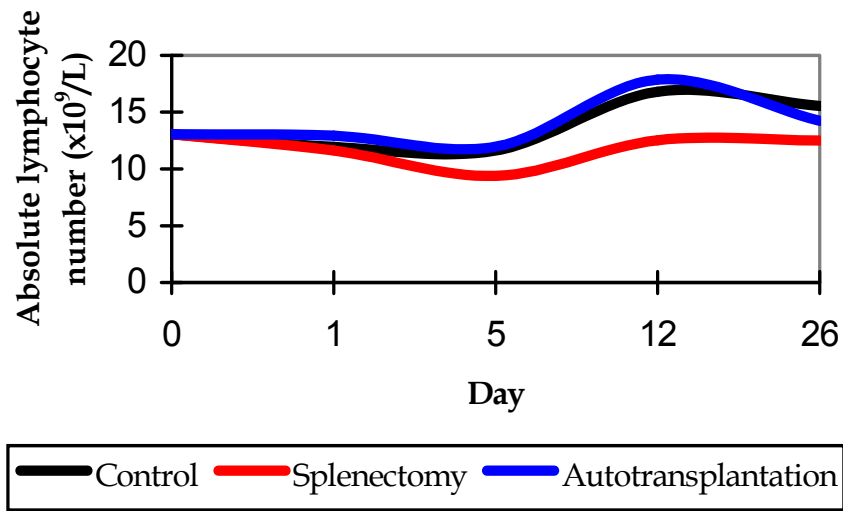


Diagram 7. Changes in absolute lymphocyte number in the blood of experimental pigs during the experiment

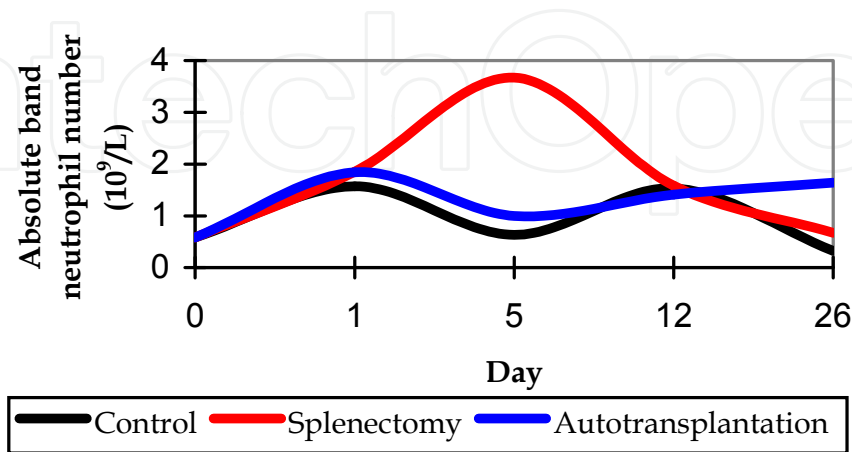


Diagram 8. Changes in absolute band neutrophil number in the blood of experimental pigs during the experiment

Apart from changes found in absolute neutrophil count, there were also changes in number of band neutrophils. Before the surgeries, relative band neutrophil count found on blood smears was 2.6 % in average. Compared to that value, it was evident that amount of band neutrophil increased during postoperative period in all groups. The largest increase was found in group of splenectomized pigs, where up to 28 % band neutrophils per smear were found (Diagram 8.). Increased number of band neutrophils, subsequent with findings of granulocyte precursors and dividing cells, suggest an increased bone marrow activity and release of immature cells, as well as their mobilization from the marginal pool.

4.4 Ratio of absolute differential number of neutrophils and lymphocytes

As it is well known, significant neutrophilia and lymphocytopenia occur as an immediate immune response following multiple traumas, surgical procedures, endotoxemia and sepsis. Since duration, pattern and degree of this immune response highly depend on the extensiveness and severity of surgical procedure, ratio of neutrophils and lymphocytes (N/L) can be considered as a reliable indicator of the immune response progress (Zahorec, 2001). Although both, sham-operated and splenectomized group of pigs in our study had significant postoperative increases in neutrophil/lymphocyte ratio (Diagram 9.), the change was more pronounced in splenectomized group indicating that splenectomy imposed greater stress on the organism than sham operation. The lowest value of neutrophil/lymphocyte ratio during the research was recorded on the fifth postoperative day in the group of autotransplanted piglets. Described decrease came as a result of concurrent lymphocytopenia and neutropenia on the fifth day of the experiment in the blood of piglets with splenic autotransplants.

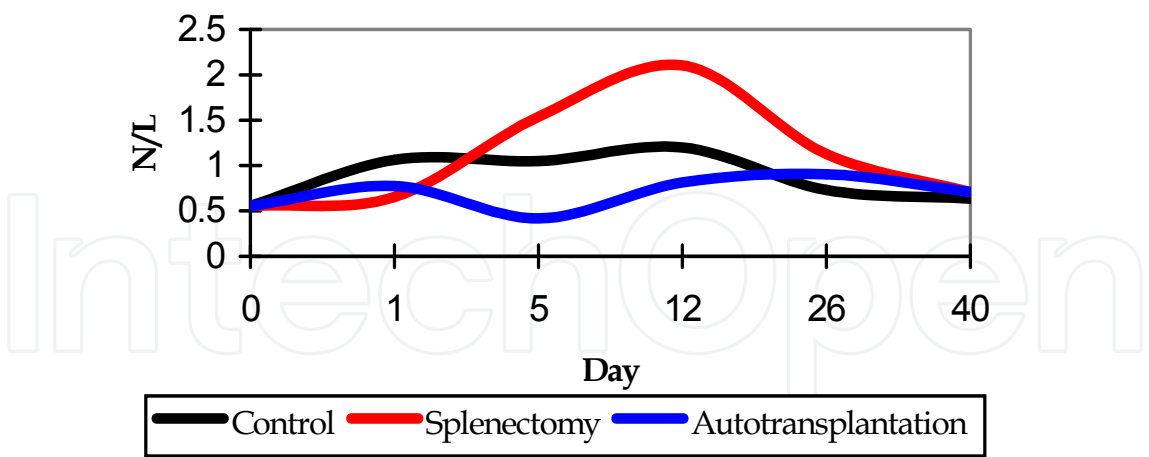


Diagram 9. Changes in ratio of absolute number of segmented neutrophils and lymphocytes (N/L) in the blood of experimental pigs during the experiment

4.5 Platelets

Diverse reports on the platelet level in various species following splenectomy have been published. The response has been reported to be either unchanged (Resende & Petroianu,

2003; Resende et al., 2002) or increased (Karagülle et al., 2007; Knežević et al., 2002; Miko et al., 2003). As one third of total platelets is physiologically sequestered in the spleen, and spleen is also the site of platelet destruction, it is expected, that after their removal, thrombocytosis will develop. In contrast, this study demonstrates significant decrease of platelet number in splenectomized animals (Diagram 10.). As documented, total splenectomy leads to decreased number of T-lymphocytes (Smith et al., 1999; Westermann & Pabst, 1986) which are essential factors in the production of platelets (Mazur, 1987), so we can conclude that this could be the reason of thrombocytopenia that has shortly occurred in splenectomized group of this study.

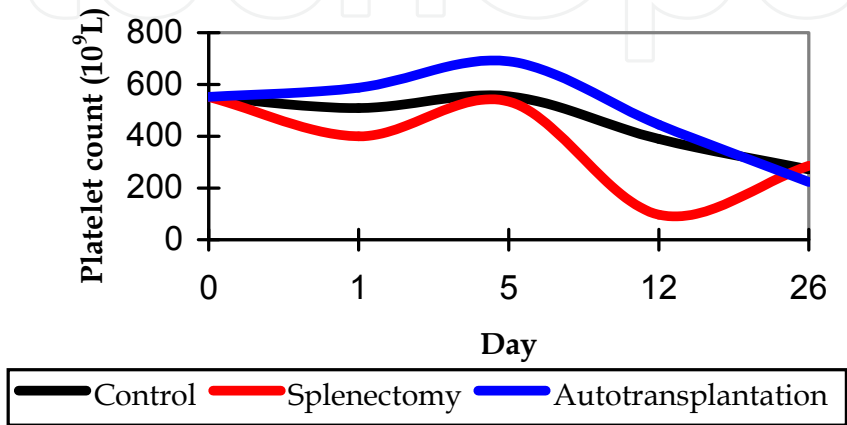


Diagram 10. Changes in platelet count in the blood of experimental pigs during the experiment

4.6 Reticulocytes

Usually, the degree of reticulocytosis is related to the magnitude of hemorrhage during the surgery. Also, many studies report that reticulocytosis following splenectomy is more significant than following other surgical procedures (Knežević et al., 2002; Miko et al., 2003). All experimental groups in this study had the same rate of reticulocytosis growth until twenty sixth day after surgeries when accelerated recovery from acute postoperative anemia was observed in the control group of pigs, suggesting that the least blood loss and surgical trauma occurred during the sham operation (Diagram 11.). In contrast, number of reticulocytes in the peripheral blood of splenectomized and autotransplanted pigs continued to grow on the twenty sixth and fortieth postoperative day as the result of inadequate blood filtration and prolonged life span of reticulocytes, as well as the loss of splenic humoral control mechanism responsible for releasing young red blood cells into the blood stream. Because of the different intensity of anemia determined in the experimental pig groups, the reticulocyte production index was calculated to avoid erroneously elevated reticulocyte count (Table 4.). On the first postoperative day reticulocyte production index 1 in all three groups of pigs showed insufficient response of bone marrow to compensate postoperative anemia. From fifth to twelfth postoperative day reticulocyte production index in splenectomized and autotransplanted pigs was increased, but still insufficient for compensation of anemia. At the same time, higher reticulocyte production index (over 3) in control group was matched with recovery of red blood cell count. On the twenty sixth

postoperative day, data indicates extremely high values of reticulocyte production index in splenectomized and autotransplanted pigs, but that was probably due to increased reticulocyte production in bone marrow, and inadequate blood filtration and prolonged life span of reticulocytes, as well as the loss of splenic humoral control, as mentioned before.

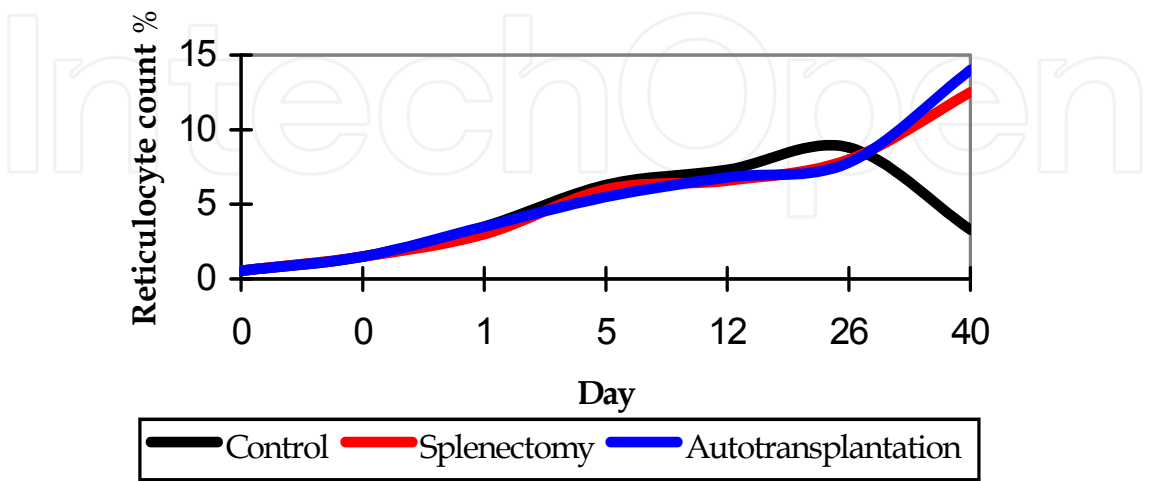


Diagram 11. Average percentage of reticulocytes in the peripheral blood of observed pigs during the experiment

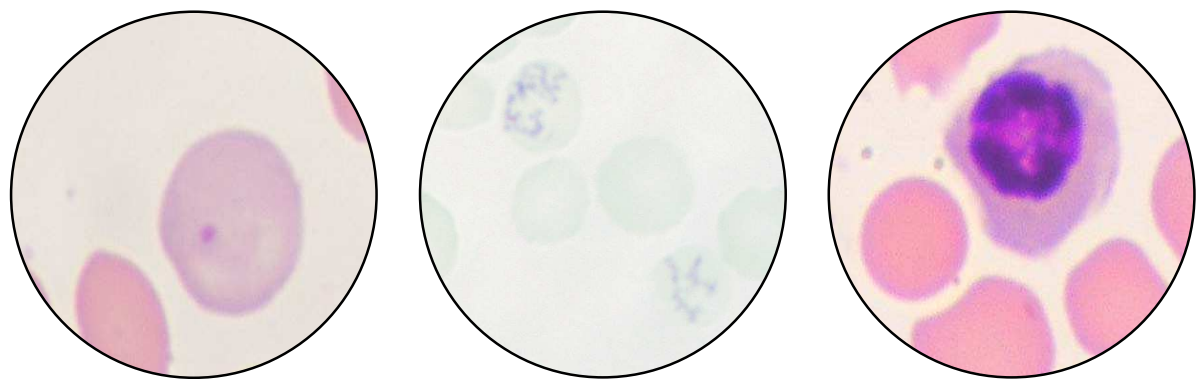
4.7 Erythrocyte morphology

The main developments found in this study were the creation of leptocytes and codocytes and increased number of Howell-Jolly bodies in the peripheral blood of splenectomized pigs (Picture 1.). This founding is in accordance with the results of previous studies on various species, so we can conclude these changes were specific for splenectomized patients. Some authors use the number of erythrocytes containing Howell-Jolly bodies to assess preservation of spleen’s blood filtering function (Patel et al., 1981; Resende & Petroianu, 2003; Resende et al., 2002), but number of oxidatively modified erythrocytes containing Heinz bodies can also be used for that purpose (Haklar et al., 1997). Polychromasia and increased number of circulating erythroblasts (Picture 2.) came as a side effect of significant reticulocytosis in all experimental groups. More frequent occurrence of morphologically abnormal red blood cells on the blood films of autotransplanted pigs when compared with sham-operated pigs suggests that the autologous splenic tissue was not able to filtrate the blood effectively.

There is still controversy about the effectiveness of regenerated splenic tissue, but the one conclusion of all researches done is common, that functionality and histological restitution of the transplanted splenic tissue depends on the amount of successfully transplanted mass of spleen (Haklar et al., 1997; Sipka, et al., 2006; Tang et al., 2003). In the present research the implant in overall amount of 20 % has not been enough for keeping filtration function of healthy spleen, which correlates with researches from Tang et al. (2003), who find that architecture of red and white pulp, as well as restitution of cardiovascular system is not sufficient for another seven months after transplantation.



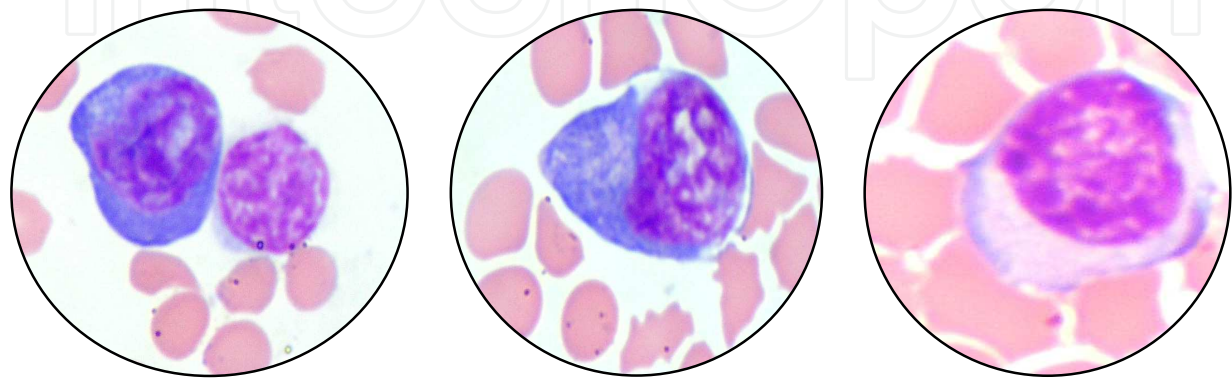
Picture 1. Erythrocyte containing Howell-Jolly bodies, leptocyte and codocytes



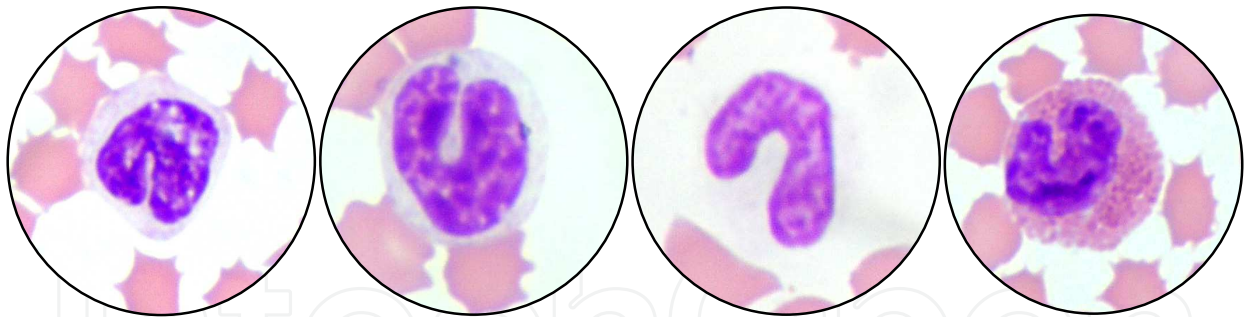
Picture 2. Polychromatophyl, reticulocyte and acydofile erythroblast

4.8 Morphological changes of white blood cells

On the first and fifth day of the experiment morphological evaluation of white blood cells revealed the presence of reactive, granulated and cytotoxic lymphocytes on the blood films of all experimental groups (Picture 3.). After this, morphologically altered lymphocytes appeared only on the twelfth postoperative day on the blood smears of pigs with autotransplanted splenic tissue. Most likely, in this case, inflammatory cascade and production of cytokines were triggered as a response to extensive tissue lesions following autotransplantation, thus leading to the greater number of morphologically altered lymphocytes in the peripheral blood. Metamyelocytes, and dividing cells appeared



Picture 3. Reactive lymphocytes



Picture 4. Metamyelocyte, band neutrophils and band eosynophil

sporadically in the peripheral blood of all experimental animals until the twelfth day, but they were most commonly found on the blood smears of splenectomized piglets, with the highest frequency on the fifth day of the experiment (Picture 4.). These results correspond with those reported in splenectomized human patients (Labar & Hauptman, 1998).

5. Conclusion

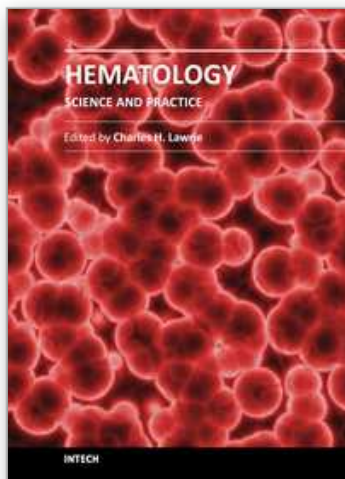
All groups showed leukocytosis following the operation but this was not regarded as a change specific for splenectomy or autotransplantation, rather than a post-injury inflammatory response due to tissue lesions during operation. Increased number of band neutrophils, subsequent with findings of granulocyte precursors and dividing cells, suggest an increased bone marrow activity and release of immature cells, as well as their mobilization from the marginal pool. Anemia and reticulocytosis found in blood samples of all three groups of pigs may have been physiological results showing the classical postoperative organism reaction to blood loss and surgical trauma. On the other side, frequenter appearance of variations in red blood cell morphology such as appearance of leptocytes, codocytes and Howell-Jolly bodies on the blood smears of splenectomized pigs when compared with other two experimental groups suggests that this was a change specific for splenectomy. More frequent occurrence of morphologically abnormal red blood cells on the blood films of autotransplanted pigs compared with sham-operated pigs suggests that the autologous spleen tissue was not able to filtrate the blood effectively. The mass of implant in overall amount of 20% has not been sufficient for keeping filtration function of healthy spleen, therefore should the amount of transplanted mass of spleen be increased.

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Hematology - Science and Practice

Edited by Dr. Charles Lawrie

ISBN 978-953-51-0174-1

Hard cover, 596 pages

Publisher InTech

Published online 02, March, 2012

Published in print edition March, 2012

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