Changes of red blood cell aggregation parameters in a long-term follow-up of splenectomy, spleen-autotransplantation and partial or subtotal spleen resections in a canine model

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Abstract

BACKGROUND: Decrease or loss in splenic filtration function may influence the hemorheological state.

OBJECTIVE: To follow-up the long-term effects of splenectomy, spleen autotransplantation and spleen resections on red blood cell aggregation in a canine model.

METHODS: Beagle dogs were subjected to control (n=6), splenectomy (SE, n=4), spleen autotransplantation (AU, Furka's spleen-chip method, n=8) or partial and subtotal spleen resection (n=4/each) groups, and followed-up for 18 postoperative (p.o.) months. Erythrocyte aggregation was determined in parallel by light-transmittance aggregometry (Myrenne MA-1 aggregometer) and syllectometry (LoRRca).

RESULTS: Erythrocyte aggregation decreased three months after splenectomy, with lower aggregation index and elongated aggregation time. It was more or less associated with relatively lower hematocrit and fibrinogen concentration. However, in autotransplantated animals a relatively higher fibrinogen did not increase the aggregation markedly. Spleen

resection resulted in the most controversial red blood cell aggregation findings, and it seems, that the degree of the resection is an influencing factor.

CONCLUSIONS: Splenectomy alters erythrocyte aggregation, spleen autotransplantation can be useful to preserve filtration function. However, the degree of restoration shows individual differences with a kind of 'functional periodicity'. Spleen resection controversially influences erythrocyte aggregation parameters. The subtotal resection is supposed to be worse than spleen autotransplantation.

Keywords: spleen filtration function, splenectomy, spleen autotransplantation, spleen partial or subtotal resection, red blood cell aggregation

1. Introduction

Filtration, immunological and storage function of the spleen has been revealed in the past decades. Loss of these functions may lead to the often fatal overwhelming postsplenectomy infection (OPSI) syndrome, and/or may increase the risk of thromboembolic complications [4,5,11,19,23]. Thus, in case of traumatic or intraoperative injury of the otherwise healthy spleen, the organ sparing surgical approach is highly recommended [6,10,21,24].

Decrease or loss in splenic filtration function may influence the hemorheological state, since the elimination of the damaged and/or elder red blood cells is altered, as it has been demonstrated by our previous studies as well [1-3,9,13,14,16,22]. Red blood cell aggregation is determined by several cellular (cell shape-morphology, membrane strain, deformability, cell surface glycocalyx structure) and plasmatic factors (macromolecules) [1,2]. These factors may alter after splenectomy (asplenic state) or in case of decreased splenic functions (hyposplenic state) [9,14,16]. It is believed that micro-rheological parameters, such as erythrocyte deformability and aggregation might serve as a good supplementary marker in screening and following-up splenic filtration function [13,14,16].

In recent study we aimed to follow-up the long-term effects of splenectomy, spleen autotransplantation (spleen ‘chips’ placed in between the sheets of the greater omentum [7,8]), and spleen resections at various degree [7], using complex investigative methods. From these results we show the red blood cell aggregation parameters in this paper.

2. Materials and methods

2.1. Experimental animals and operative techniques

The experiments were approved by the University of Debrecen Committee of Animal Welfare (permission Nr.: 26/2011/UDCAW) in accordance with the national regulations (Law XXVIII/1998) and EU directives (2010/63).

Twenty-six healthy male and female beagle dogs (age: 18-23 months, body weight: 12.98±1.1 kg) were involved in this study. All the operations were performed in sterile condition under general anesthesia with intramuscularly administered combination of ketamine and xylazine (10 mg/bwkg, CP-Ketamin, ketamine hydrochloride 10% + 1 mg/bwkg, CP-Xylazin, xylazine-hydrochloride 2%; Produlab Pharma BV, The Netherlands).

Animals were subjected to one of the following experimental groups:

I. Control group (C, n=6): This group contains the sham operated and healthy control animals as well. In sham operated control animals (n=3) median laparotomy was performed, while in healthy controls (n=3) there was no any surgical intervention.
II. Splenectomy group (SE, n=4): after median laparotomy the spleen was removed.
III. Spleen-autotransplantation group (AU, n=8): after median laparotomy and the consecutive splenectomy, ten pieces of splenic slices (20 mm x 50 mm x 1 mm) were placed between two layers of the greater omentum, close to a well-vascularized area according to the ‘Furka’s spleen chip’ method [7,8].

IV. One-third (partial resection) and two-third (subtotal) spleen resection groups (R1/3 and R2/3, n=4/each): After median laparotomy one-third or two-third part of the distal region of the spleen was resected) using 'Furka-type' embracing suture technique [7].

In every operated group the abdominal wall was closed in two layers. First the muscle and peritoneum were closed with 1-0 polyglycolic acid absorbable suture material (Optime®, Peters Surgical, France) using simple interrupted stitches, then to close the skin 2-0 polyglycolic acid absorbable suture material (Optime®, Peters Surgical, France) using Donati vertical mattress stitches were used.

2.2. Blood sampling protocol

Regarding the presented parameters in this paper, blood samples were taken before the operations (base) and in the 1st, 3rd, 6th, 9th and 18th postoperative months via puncturing the cephalic vein using closed blood sampling system with 21 G BD Eclipse™ needles (Becton, Dickinson and Company, USA). For hematological and red blood cell aggregation tests K_3-EDTA was used as anticoagulant (1.8 mg/ml, BD Vacutainer®), while fibrinogen concentration was determined in plasma from blood anticoagulated with sodium-citrate (0.129 M, BD Vacutainer® tubes).

2.3. Testing hematological parameters

Quantitative and qualitative hematological parameters were determined using an Advia 120 hematology automate (Siemens Healthcare Gmbh, Germany). The measurements require 175 µl of blood sample. In this study we focused on the hematocrit, as a major red blood cell aggregation determinant hematological parameter.

2.4. Measuring fibrinogen concentration

Fibrinogen concentration (Fbg [g/l]) of the plasma was determined using a Sysmex CA-500 coagulometer (TOA Medical Electronics Co., Ltd., Japan) based on the Clauss's method.

2.5. Determining red blood cell aggregation

2.5.1. Light-transmittance aggregometry

For testing the red blood cell aggregation a Myrenne MA-1 erythrocyte aggregometer (Myrenne GmbH, Germany) was used. The technique is based on light-transmittance photometric method. The test requires approximately 20 µl of blood. During the measurements the sample is disaggregated (at 600 s^{-1}) then the shear rate drops to zero (M mode) or to 3 s^{-1} (M1 mode). According to the changes in light-transmittance the instrument calculates the aggregation index values at the 5th or 10th second of the process. The indices (M 5s, M 1 5s, M10 s, M1 10s) increase with enhanced red blood cell aggregation [1,2].

2.5.2. Syllectometry

In parallel with the light-transmission technique, erythrocyte aggregation was also tested with a LoRRca MaxSis Osmoscan ektacytometer (Mechatronics BV, Hollandia) operating with laser-backscattering method. The tests require 1 ml of blood.

After disaggregating the blood sample with rotation in the Couette-system, the rotor stops and the changes in the intensity of the light reflected from the sample is measured. From the intensity-time curve the software calculates several parameters, including aggregation index (AI [%]), amplitude (Amp [au]) and aggregation half time (half-amplitude time, $t_{1/2}$ [s]), describing the rate, magnitude and the kinetics of the aggregation process [1,2].

2.6. Statistical analysis

Data are presented as mean values with standard deviation (means ± S.D.). Intra-group analyses were carried out with ANOVA tests (Bonferroni's or Dunn's post hoc tests). For inter-group comparisons at definitive time points of the follow-up period Student's t-test or Mann-Whitney rank sum tests were used, depending on the data distribution. A p value of <0.05 was considered to be significant.

3. Results

3.1. Changes of hematocrit and fibrinogen concentration

Absolute values of hematocrit (Htc [%]) and fibrinogen concentration (Fbg [g/dl]) are shown in Table 1, and their changes compared to base values are plotted in Figure 1.

In Control group *hematocrit* did not change markedly over the follow-up period. In the other groups values dropped by the 1st postoperative months, and with the exception of splenectomy group, hematocrit fluctuated at various degrees in the groups, and never reached the base values. In Splenectomy group Htc were significantly lower over the entire follow-up period compared to the base (relative values, 1st month: $p=0.009$; 3rd month: $p<0.001$; 6th month: $p<0.001$; 9th month: $p=0.002$; 18th month: $p=0.057$) or to Control (1st month: $p=0.042$; 3rd month: $p=0.013$; 6th month: $p=0.035$; 9th month: $p=0.019$). In Autotransplantation group although the values were higher, but still being significantly lower compared to base (relative values, 1st month: $p=0.01$; 3rd month: $p<0.001$; 6th month: $p<0.001$; 9th month: $p=0.016$; 18th month: $p=0.002$), and in the 1st and 3rd months versus the Control ($p=0024$ and $p=0.008$, respectively). Spleen resection groups' values started to 'normalize' after the 1st month, however, the subtotal resection (R2/3) resulted in lower values (versus base: $p=0.09$ in the 1st and 3rd months; versus Control: $p=0.052$ in the 9th month).

Fibrinogen concentration showed a slight and non-significant increase in Control group. In Splenectomy group values showed an increase in the 6th and by the 18th months (p=0.057 vs. base). In Autotransplantation group, except for two animals, values increased by the 1st month (relative values, p=0.01 vs. base, p=0.08 vs. Control), and expressed further elevated values from the 6th month compared to the base (6th month: p=0.021; 9th month: p=0.02; 18th month: p=0.002). The R1/3 group did not show important difference from the Control, except for a decrease by the end of the follow-up period. However, in subtotal resection (R2/3) group fibrinogen values were permanently lower than the control, reaching significant difference in the 3rd (p=0.09 vs. base; p=0.059 vs. AU), 6th (p=0.02 vs. base; p=0.016 vs. AU), 9th month (p=0.006 vs. base; p=0.048 vs. Control) and 18th month (p=0.026 vs. AU).

3.2. Red blood cell aggregation parameters by light-transmission aggregometry

Table 2 summarizes the aggregation index parameters tested by the Myrenne MA-1 aggregometer.

Parameters of the Control group showed a relative stability over the follow-up period, except for an increase in the 18th month (M 10 s: p<0.001; M1 5 s: p=0.014; M1 10 s: p=0.01 vs. base).

In Splenectomy group index values dropped by the 3rd postoperative month (M 5 s: p=0.053 vs. base, p<0.001 vs. Control; M 10 s: p=0.009 vs. base, p<0.001 vs. Control; M1 5 s: p<0.001 vs. base and Control; M1 10 s: p<0.001 vs. base and Control). The animal Nr. SE-3 showed the lowest M1 10 s index values. Afterwards the data started to ‘normalize’.

Autotransplantation group also expressed a decrease in values by the 3rd month, however, in smaller manner compared to the SE animals (M 10 s: p=0.012 vs. base, p=0.002 vs. Control and SE; M1 5 s: p=0.005 vs. base). By the 18th month index values increased (M 5

s: p=0.025 vs. base; M 10 s: p=0.005 vs. base; M1 5 s: p<0.001 vs. base, p=0.001 vs. SE; M1 10 s: p<0.001 vs. base and SE). The animal Nr. AU-4 expressed the highest aggregation index values amongst the others (M 5 s, M 10s and M1 5s).

Data of the resection groups changed irregularly. By the 3rd months M 10 s (p=0.003 vs. base; p<0.001 vs. Control) and M1 10 s values (p=0.01 vs. base; p<0.001 vs. Control) dropped in R1/3 group, while M 5 s increased (p=0.009 vs. base) and M1 5 s was similar to the base. Over the 6th-9th month values were relatively stable. By the 18th months M1 5 s (p<0.001 vs. base) and M1 10 s values increased (p=0.003 vs. base, p<0.001 vs. Control), and M 10 s were almost unchanged. In R2/3 (subtotal resection) group by the 18th month M 10 s data were higher (p<0.001 vs. base, p=0.009 vs. Control), M1 5 s and M1 10 s lower (p=0.005 vs. Control), and M 5 s values similar to the R1/3 group's data.

3.3. Red blood cell aggregation parameters by syllectometry

Absolute values are shown in Table 2, and their changes compared to base values are plotted in Figure 2.

Aggregation index (AI) tested by the LoRRca was almost unchanged in Control group, except for a slight but non-significant lowering in the 6th and 9th months. Values of the Splenectomy group rose by the 1st month and dropped in the 3rd (relative values, p=0.045). After a relative increase in the 6th month, we could see another decrease by the 9th months (p<0.001 vs. base). Autotransplanted animals showed increase by the 1st month, then the values were relative stable over the follow-up period. In resection groups values were suppressed between the 3rd and 9th month (R1/3 group, 3rd month: p=0.004, 6th month: p<0.001, 9th month: p=0.003 vs. base; R2/3 – subtotal resection group, 3rd month: p=0.067, 6th month: p=0.03).

Amplitude increased in Control groups over the follow-up period. In Splenectomy ($p=0.051$ vs. Control), Autotransplantation ($p=0.065$ vs. base) and R1/3 groups the values decreased by the 3rd month, then elevated again. In R2/3 (subtotal resection) group increased Amp data were obtained.

The aggregation half-time ($t_{1/2}$) rose in all groups, but most expressedly in the Splenectomy and the two Resection groups between the 3rd (SE group: $p=0.077$ vs. base; R2/3 – subtotal resection group: $p=0.03$ vs. base) and 9th months (SE group: $p=0.029$ vs. base; R1/3 resection group: $p=0.007$ vs. base).

4. Discussion

Red blood cell aggregation is determined by several plasmatic and cellular factors that might be modified in numerous pathophysiological conditions, such as metabolic, acid-base and oxygenization changes, free-radical effects, acute phase reactions, inflammation, sepsis [1,2]. Any conditions that influence cell shape, deformability, ultrastructure of the surface glycocalyx and the plasmatic microenvironment might have an effect on the aggregation process [1,2,17]. By *in vitro* studies the red blood cell aggregability can be well studied. However, *in vivo* conditions often provide controversial results. We have experienced that changes of red blood cell deformability and aggregation are not always correlated to each other. Usually the increased hematocrit and fibrinogen concentration result in enhanced red blood cell aggregation. On the other hand, increased aggregation can be even found when hematocrit or fibrinogen data does not support this finding. The *in vivo* changes and effects of red blood cell aggregation must be more complex than it has been thought. Methods that can test both ‘static’ and ‘dynamic’ parameters of the aggregation process give valuable information [1,2,18].

In this canine experimental series we have found that red blood cell aggregation in peripheral blood decreased three months after splenectomy, with low aggregation index and elongated aggregation time (Table 2, Figure 2). Generally, a decrease in aggregation can be explained by lowered hematocrit and/or fibrinogen concentration, as well as by morphological changes of the cells and/or altered deformability. We did not found deformability impairment at that time. The changes were more or less associated with a relatively lower hematocrit and fibrinogen concentration (Table 1, Figure 1). However, in autotransplantated animals a relatively higher fibrinogen did not increase aggregation markedly. Filtration function can be partly restored by the reticulo-endothelial system (liver, bone marrow), therefore histological examination of those organs may provide necessary information for the evaluation.

When splenic filtration function is decreased or disappears, the micro-rheological parameters of the peripheral blood can be impaired. Decreased deformability, altered (more often enhanced) red blood cell aggregation occurs, with higher amount of elder erythrocyte subpopulation in the circulation [1,2].

In 2010 we published red blood cell aggregation results in a 24-month follow-up series, highlighting the 20^{th} and 24^{th} months [12], but without any spleen resection groups. According to the main findings hematocrit decreased in SE, AU-5 (spleen autotransplantation with 5 ‘chips’) and AU-10 (spleen autotransplantation with 10 ‘chips’) groups, fibrinogen concentration did not change essentially, aggregation index values increased by the 12^{th} month in SE, AU-5 and AU-10 groups. Aggregation index values of various red blood cell populations (‘young’ versus ‘old’) showed the largest differences in SE group, while AU groups expressed lower values. As conclusion we could state that an increase in erythrocyte aggregation index may reflect the lost or decreased filtration function after splenectomy or autotransplantation, and by comparing aggregation properties of ‘young’ and ‘old’ red blood cells, further information can be gained about the splenic filtration function [12].

When analyzing the data obtained during the follow-up period it is also important to count with the ageing [2,12] and the seasonal effects [20]. These might explain the background of those slight changes in hematocrit, fibrinogen concentration and certain red blood cell aggregation parameters.

Spleen autotransplantation could partly restore the splenic filtration function. Also it is supposed that the amount of the autotransplanted splenic tissue is related with the restoration degree of the splenic function. In previous canine and murine studies we found that compared to control animals, significantly elongated relative cell transit times (RCTT; inversely proportional to red blood cell deformability) are existed following splenectomy, while autotransplantation seemed to improve these values and showing irregular alterations during the postoperative weeks and months. It is also important to note that time is needed for neovascularization and regeneration of the autotransplanted splenic chips. Approximately 4-6 months are necessary for regeneration, which process may also show a kind of 'functional periodicity' [9,13-16]. Current results enforce our previous findings with light- and electron microscopical [13,15] and laboratory methods [9,13-15]: *„These laboratory investigations of different surviving times also suggested that there may be certain functional periodicity of the spleen chips, showing hyposplenic and asplenic states"* [13].

Spleen resection resulted in the most controversial red blood cell aggregation findings, and it seems, that the degree of the resection is an influencing factor. The subtotal (two-third) spleen resection is supposed to be worse than spleen autotransplantation. However, further studies are necessary to clarify this issue.

It is also important to mention that besides the general statistical analysis of experimental groups, the individual evaluation of the cases is highly important: general condition of the animals, possible complications, symptoms. To better understand the background of the alterations, the individual analysis of other laboratory parameters (e.g.,

hematological, hemostaseological, enzymological, etc) and the complex histological examinations are necessary.

5. Conclusion

Splenectomy alters red blood cell aggregation, spleen autotransplantation can be useful to preserve filtration function. However, the degree of restoration shows individual differences and we have to face a kind of ‘functional periodicity’. Spleen resection controversially influences red blood cell aggregation parameters, more obviously in case of subtotal (two-third) resection. Individual analysis of hemorheological changes together with hematological, hemostaseological, enzmyological investigations, functional imaging techniques as well as complex histological examinations may provide more accurate information to screen the degree of restoration of the splenic filtration function in these organ preserving surgical operative techniques.

6. Acknowledgements

The authors are grateful for the technical staff of the Department of Operative Techniques and Surgical Research, Faculty of Medicine, University of Debrecen. Special thanks to Dr. Ferenc Kiss, Erzsebet Vanyolos, Viktoria Sogor and Bence Tanczos.

Grant sponsors: The Hungarian Research Funds (Grant Nr.: OTKA T-049331 and OTKA K-105618).

The authors comply with the Ethical Guidelines for Publication in *Clinical Hemorheology and Microcirculation* as published on the IOS Press website and in Volume 63, 2016, pp. 1-2. of this journal.

7. References

[1] Baskurt OK, Hardeman HR, Rampling MW, Meiselman HJ, editors. Handbook of Hemorheology and Hemodynamics. Amsterdam: IOS Press; 2007.

[2] Baskurt OK, Neu B, Mesielman. Red Blood Cell Aggregation. Boca Raton: CRC Press; 2011.

[3] Baskurt OK. The role of spleen in suppressing the rheological alterations in circulating blood. Clin Hemorheol Microcirc. 1999;20(3):181-8.

[4] Cadili A, de Gara C. Complications of splenectomy. Am J Med. 2008;121(5):371-5. doi: 10.1016/j.amjmed.2008.02.014.

[5] Chong J, Jones P, Spelman D, Leder K, Cheng AC. Overwhelming post-splenectomy sepsis in patients with asplenia and hyposplenia: a retrospective cohort study. Epidemiol Infect. 2017;145(2):397-400. doi: 10.1017/S0950268816002405

[6] Di Carlo I, Toro A. Splenic autotransplantation is always valid after splenectomy. J Invest Surg. 2017 [Epub ahead of print], doi: 10.1080/08941939.2016.1268656

[7] Furka I, Miko I, Papp L, Miko T. Salvaging the spleen by experimental resection or autotransplantation. In: Jubileuszowy Zjazd Towarzystwa Chirurgow Polskich Vol. 2. Krakow, 1989. p. 453-56.

[8] Furka I, Miko I, Serfozo J, Frendl I, Hauck M. Autotransplantation of the spleen, In: Second World Week of Professional Updating in Surgery and in Surgical and Oncological Disciplines of the University of Milan, Lecture Book Vol. II. Bologna: Monduzzi Editore; 1990. p. 767-9.

[9] Furka I, Miko I, Toth K, Furka A, Kappelmayer J, Szikszai Z, et al. Hematological, hemorheological and catalase level changes following splenectomy and spleen autotransplantation in experimental animals. Early results. Acta Chir Austr. 1997; 9(Suppl 137): 31-2.

[10] Grandic L, Pogorelic Z, Banovic J, Perko Z, Boschi V, Ilic N, et al. Advantages of the spared surgical treatment of the spleen injuries in the clinical conditions. Hepatogastroenterology. 2008;55(88):2256-8.

[11] Hansen K, Singer DB. Asplenic-hyposplenic overwhelming sepsis: postsplenectomy sepsis revisited. Pediatr Dev Pathol. 2001;4(2):105-21.

[12] Kiss F, Nemeth N, Sajtos E, Brath E, Peto K, Baskurt OK, et al. Examination of aggregation of various red blood cell populations can be informative in comparison of splenectomy and spleen autotransplantation in animal experiments. Clin Hemorheol Microcirc. 2010;45(2-4):273-80. doi: 10.3233/CH-2010-1304.

[13] Miko I, Brath E, Nemeth N, Furka A, Sipka SJ, Peto K, et al. Spleen autotransplantation. Morphological and functional follow-up after spleen autotransplantation in mice: A research summary. Microsurgery. 2007;27(4):312-6. doi: 10.1002/micr.20362

[14] Miko I, Furka A, Acs G, Nemeth N, Sipka S, Olah VA, et al. Laboratory follow-up of spleen autotransplants after experimental spleen injuries. Eur Surg. 2002;34(Suppl. 189):10-2.

[15] Miko I, Furka I, Serfozo J, Joos Gy, Telek B, Matesz K, et al. Comparative study of haematological and micro-morphological results in long-surviving spleen autotransplants. In: Uranus S, ed. Chirurgische Forschung. München, Bern, Wien, New York: W. Zuckschwerdt Verlag; 1994. p. 50-5.

[16] Miko I, Nemeth N, Sajtos E, Brath E, Peto K, Furka A, et al. Splenic function and red blood cell deformability: The beneficial effects of spleen autotransplantation in animal experiments. Clin Hemorheol Microcirc. 2010;45(2-4):281-8. doi: 10.3233/CH-2010-1307.

[17] Muravyov AV, Tikhomirova IA, Maimistova AA, Bulaeva SV. Extra- and intracellular signaling pathways under red blood cell aggregation and deformability changes. Clin Hemorheol Microcirc 2009;43(3):223-32. doi: 10.3233/CH-2009-1212

[18] Nam JH, Yang Y, Chung S, Shin S. Comparison of light-transmission and -backscattering methods in the measurement of red blood cell aggregation. J Biomed Optics 2010:15(2):027003. doi: 10.1117/1.3365951

[19] Theilacker C, Ludewig K, Serr A, Schimpf J, Held J, Bögelein M, et al. Overwhelming Postsplenectomy Infection: A prospective multicenter cohort study. Clin Infect Dis. 2016;62(7):871-8. doi: 10.1093/cid/civ1195

[20] Watters JM, O'Rourke K. Effects of age and gender. In: Souba WW, Wilmore DW, eds. Surgical Research. Amsterdam: Elsevier Academic Press; 2001. p. 167-174.

[21] Weledji EP. Benefits and risks of splenectomy. Int J Surg. 2014;12(2):113-9. doi: 10.1016/j.ijsu.2013.11.017

[22] Wernick B, Cipriano A, Odom SR, MacBean U, Mubang RN, Wojda TR, et al. Temporal changes in hematologic markers after splenectomy, splenic embolization, and observation for trauma. Eur J Trauma Emerg Surg. 2016 [Epub ahead of print], doi: 10.1007/s00068-016-0679-0

[23] William BM, Corazza GR. Hyposplenism: a comprehensive review. Part I: basic concepts and causes. Hematology. 2007;12(1): 1-13. doi: 10.1080/10245330600938422

[24] William BM, Thawani N, Sae-Tia S, Corazza GR. Hyposplenism: a comprehensive review. Part II: clinical manifestations, diagnosis, and management. Hematology. 2007;12(1):89-98. doi: 10.1080/10245330600938463

Table 1. Changes of hematocrit (Htc [%]) and fibrinogen concentration (Fbg [g/dl]) in control, splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3) during the follow-up period of 18 months.

Variable	Experimental group	Base	Postoperative month				
			1st	3rd	6th	9th	18th
Htc [%]	Control	43.24 ± 1.90	43.24 ± 1.70	44.66 ± 4.76	41.08 ± 4.76	41.86 ± 2.17	42.84 ± 3.84
	SE	45.8 ± 3.36	39.6 ± 5.37	37.5 ± 2.82 * #	37.95 ± 0.96 *	38.3 ± 1.08 * #	39.13 ± 1.16 *
	AU	46.95 ± 3.62	39.77 ± 3.65 *	39.7 ± 2.76 *	41.01 ± 1.88 * $^{+}$	41.25 ± 3.12 *	41.42 ± 2.28 *
	R1/3	44.35 ± 0.44	40.65 ± 2.56 *	44.4 ± 6.10 $^{+}$	42.65 ± 3.15	41.15 ± 5.57	39.2 ± 3.47 *
	R2/3	46.02 ± 4.89	41.87 ± 2.27	39.87 ± 2.13 *	41.7 ± 1.92 $^{+}$	39.1 ± 1.34 *	38.56 ± 2.85 *
Fbg [g/dl]	Control	2.3 ± 0.15	2.26 ± 0.41	2.44 ± 0.30	2.74 ± 0.52	2.45 ± 0.43	2.58 ± 1.25
	SE	1.98 ± 0.94	1.57 ± 0.60	1.59 ± 0.27 #	2.47 ± 0.35	1.96 ± 0.68	1.88 ± 0.34
	AU	1.5 ± 0.23	2.06 ± 0.47 *	1.77 ± 0.59 #	2.13 ± 0.56 #	1.9 ± 0.37 #	2.29 ± 0.25 *
	R1/3	2.04 ± 0.68	2.02 ± 0.56	1.76 ± 0.13 #	2.06 ± 0.29 #	1.73 ± 0.21 #	2.07 ± 0.09
	R2/3	2.62 ± 0.43	2.14 ± 0.71	1.94 ± 0.15 #	2.09 ± 0.40	2.01 ± 0.27	2.13 ± 0.03

SE – splenectomy group, AU – spleen autotransplantation group, R1/3 and R2/3 – one-third and two-third (subtotal) spleen resection groups
means ± S.D., * $p<0.05$ vs. Base, # $p<0.05$ vs. Control, $^{+}$ $p<0.05$ vs. SE

Table 2. Changes of red blood cell aggregation parameters in control, splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3) during the follow-up period of 18 months.

Variable	Experimental group	Base	Postoperative month				
			1st	3rd	6th	9th	18th
M 5 s	Control	4.29 ± 1.89	4.07 ± 0.93	3.56 ± 1.25	4.23 ± 0.86	3.28 ± 1.43	4.94 ± 1.56
	SE	2.96 ± 1.85	4.1 ± 1.01	1.95 ± 0.78 * #	3.19 ± 0.74	3.14 ± 0.85	2.8 ± 1.77
	AU	3.54 ± 1.95	3.57 ± 1.73	2.58 ± 1.34	3.85 ± 0.89	3.71 ± 1.24	6.31 ± 4.51 *
	R1/3	4.53 ± 1.16	3.76 ± 1.1	5.73 ± 3.49 # +	2.56 ± 0.67 *	2.79 ± 1.19 *	3.61 ± 1.86
	R2/3	2.96 ± 1.39	4.11 ± 1.5	3.21 ± 0.81	3.67 ± 1.62	2.68 ± 0.8	3.5 ± 1.72
M 10 s	Control	12.25 ± 3.96	13.66 ± 3.58	11.16 ± 2.63	11.58 ± 2.46	10.69 ± 3.43	16.68 ± 4.41 *
	SE	10.2 ± 4.64	10.04 ± 2.24	5.88 ± 2.23 * #	10.89 ± 1.95	9.52 ± 3.52	10.63 ± 4,66 #
	AU	11.38 ± 4.59	13.04 ± 5.59	8.66 ± 3.03 * # +	11.96 ± 2.62	10.15 ± 2.26	15.13 ± 4.26 *
	R1/3	10.93 ± 5.91	11.33 ± 3.48	4.8 ± 2.43 * #	8.64 ± 2.51	9.09 ± 2.84	10.18 ± 5.67 #
	R2/3	7.95 ± 3	11.66 ± 2.54 *	8.24 ± 2.35 # +	11.34 ± 1.81 *	10.13 ± 2.67	12.36 ± 4.38 * #
M1 5 s	Control	5.3 ± 1.6	4.8 ± 1.03	4.95 ± 1.14	5.11 ± 1.41	4.35 ± 1.35	6.64 ± 2.01 *
	SE	5 ± 0.96	4.15 ± 0.97	2.81 ± 0.74 * #	3.65 ± 0.9 *	3.52 ± 0.94 *	4.78 ± 0.86
	AU	4.83 ± 2.27	4.18 ± 1.25	3.46 ± 1.01 *	4.53 ± 1.52	3.94 ± 1.37	8.79 ± 4.74 * +
	R1/3	4.41 ± 1.92	4.66 ± 1.74	5.37 ± 1.6	4.76 ± 1.09	3.69 ± 1.96	7 ± 0.58 *
	R2/3	6.02 ± 2.73	3.98 ± 0.98 *	4.22 ± 0.82	4.6 ± 1.58	3.51 ± 1.2 *	5.62 ± 0.75
M1 10 s	Control	12.28 ± 3.44	11.56 ± 4.05	11.18 ± 2.15	12.76 ± 2.5	9.93 ± 4.21	16.01 ± 5.18 *
	SE	13.08 ± 3.31	9.38 ± 4.16 *	6.69 ± 2.49 * #	9.81 ± 3.38	8.62 ± 2.4	10.47 ± 3.17 #
	AU	10.53 ± 3.82	10.23 ± 5.23	8.91 ± 4.06 #	11.08 ± 3.73	8.69 ± 3.8	17.16 ± 3.34 * +
	R1/3	10.73 ± 4.58	9.3 ± 2.28	6.44 ± 4.12 * #	9.63 ± 3.14 #	9.97 ± 3.91	15.9 ± 2.21 * +
	R2/3	9.5 ± 2.74	9.38 ± 2.83	9.24 ± 1.79 #	10.48 ± 2.78	8.24 ± 1.89	10.68 ± 4.52 #
AI	Control	55.61 ± 5.42	53 ± 4.79	52.18 ± 4.17	51.72 ± 8.81	45.27 ± 5.45	56.38 ± 7.1
	SE	44.53 ± 14.3	48.26 ± 3.88	22.95 ± 9.89 * #	44.42 ± 5.15	33.16 ± 9.94 *	33.57 ± 7.09 #
	AU	43.86 ± 9.86	51.47 ± 11.6	42.16 ± 6.79 # +	44.19 ± 7.75	41.44 ± 7.75	47.51 ± 5.56 +
	R1/3	51.6 ± 9.55	46.53 ± 6.61	37.52 ± 5.04 * # +	38.61 ± 4.54	34.12 ± 7.5 *	44.18 ± 5.69 # +
	R2/3	51.27 ± 13.09	55.66 ± 4.94	40.32 ± 5.26 # +	39.33 ± 12.12	42.99 ± 5.46	44.86 ± 2.31 # +
Amp	Control	20.43 ± 2.66	23.97 ± 3.16 *	21.82 ± 4.88	25.15 ± 1.06 *	24.39 ± 0.84 *	23.09 ± 1.55 *
	SE	18.43 ± 3.97	20.35 ± 2.65	13.13 ± 3.29 * #	19.6 ± 5.06	18.8 ± 3.69	18.14 ± 0.69 #
	AU	20.7 ± 3.59	20.76 ± 5.79	16.81 ± 2.57 * # +	22.51 ± 2.13	21.93 ± 3.02	20.4 ± 4.43
	R1/3	22.05 ± 2.75	23.54 ± 2.8	17.26 ± 2.59 * # +	22.27 ± 1.04	21.61 ± 1.9	23.63 ± 2.71 +
	R2/3	16.29 ± 2.65	21.15 ± 3.09	17.9 ± 3.7 +	22.78 ± 4.11 *	22.16 ± 2.39 *	21.4 ± 3.43 * +
$t_{1/2}$ [s]	Control	3.25 ± 0.9	3.61 ± 0.79	3.68 ± 0.71	3.98 ± 1.72	5.06 ± 1.2 *	3.14 ± 0.79
	SE	5.87 ± 2.69	4.36 ± 0.65	14.88 ± 5.96 * #	5.2 ± 1.15	9.16 ± 3.48 * #	8.54 ± 2.87 #
	AU	5.6 ± 2.05	4.25 ± 2.26	5.84 ± 1.77	5.46 ± 2.04	6.49 ± 3.5	4.57 ± 1.11 +
	R1/3	4.02 ± 1.77	4.81 ± 1.19	6.96 ± 1.33 * +	6.63 ± 1.42	8.57 ± 2.52 * #	5.24 ± 1.36 +
	R2/3	4.28 ± 2.39	3.22 ± 0.72	6.19 ± 1.22	7.06 ± 3.16	5.54 ± 1.22 +	5.04 ± 0.46 +

SE – splenectomy group, AU – spleen autotransplantation group, R1/3 and R2/3 – one-third and two-third (subtotal) spleen resection groups
means ± S.D., * $p<0.05$ vs. Base, # $p<0.05$ vs. Control, + $p<0.05$ vs. SE

9. Figure legends and figures

Figure 1

Changes (relative to base) of hematocrit (rel. Htc) (A) and fibrinogen concentration (rel. Fbg) (B) in the sequence of control, splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3: one-third and two-third -subtotal- spleen resection groups) during the 18-month follow-up period.

means ± S.D., * $p<0.05$ vs. Base, # $p<0.05$ vs. Control, + $p<0.05$ vs. SE (splenectomy)

Figure 2

Changes (relative to base) of aggregation index parameters determined by the LoRRca device: aggregation index (rel. AI) (A), amplitude (rel. Amp) (B) and time at half-Amp (rel. $t_{1/2}$) in the sequence of control, splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3: one-third and two-third -subtotal- spleen resection groups) during the 18-month follow-up period.

means ± S.D., * $p<0.05$ vs. Base, # $p<0.05$ vs. Control, + $p<0.05$ vs. SE (splenectomy)

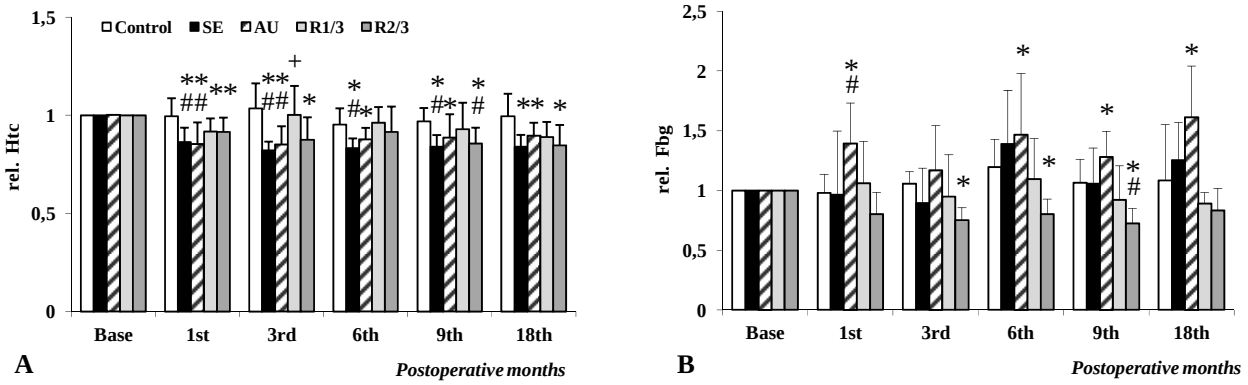

Figure 1

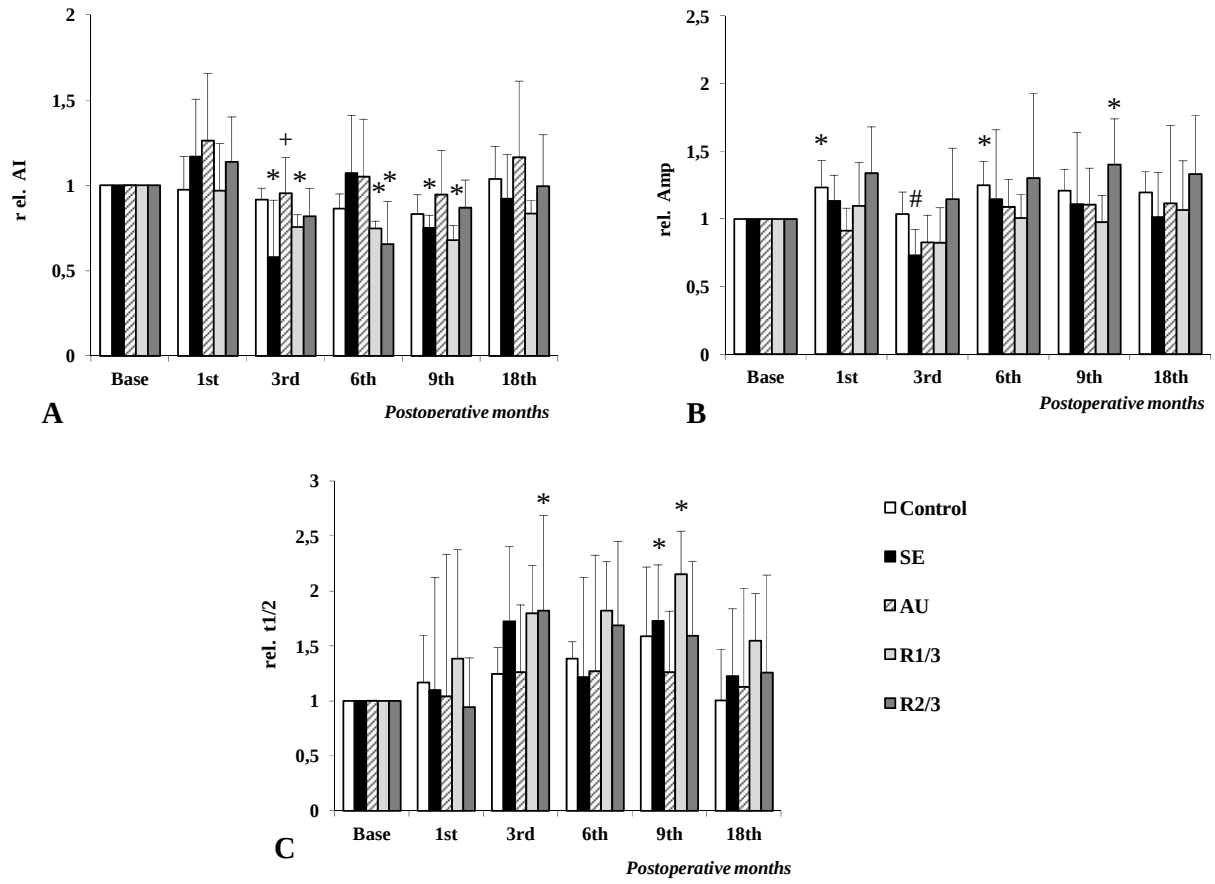

Figure 2