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Comparative erythrocyte deformability investigations by filtrometry, slit-flow and rotational ektacytometry in a long-term follow-up animal study on splenectomy and different spleen preserving operative resection and spleen autotransplantation

Iren Miko[a,1], Norbert Nemeth[a,1,*], Viktoria Sogor[a], Ferenc Kiss[a], Eniko Toth[a], Katalin Peto[a], Andrea Furka[b], Erzsebet Vanyolos[a], Laszlo Toth[c], Jozsef Varga[d], Krisztian Szigeti[e], Ilona Benkő[f], Anna V. Olah[f] and Istvan Furka[a]

[a]*Department of Operative Techniques and Surgical Research, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[b]*Department of Clinical Oncology, Division of Radiotherapy, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[c]*Department of Pathology, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[d]*Department of Nuclear Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[e]*Department of Biophysics and Radiation Biology, Faculty of Medicine, Semmelweis University, Budapest, Hungary*
[f]*Department of Pharmacology and Pharmacotherapy, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*
[g]*Department of Laboratory Medicine, Faculty of Medicine, University of Debrecen, Debrecen, Hungary*

Abstract.
BACKGROUND: Partial or subtotal spleen resection or spleen autotransplantation can partly preserve/restore the splenic filtration function, as previous studies demonstrated.
OBJECTIVE: For better evaluation and follow-up of the various spleen-preserving operative techniques' effectiveness versus splenectomy, a composite methodological approach was applied in a canine experimental model.
METHODS: Beagle dogs were subjected to control ($n=6$), splenectomy (SE, $n=4$), partial and subtotal spleen resection ($n=4$/each) or spleen autotransplantation groups (AU, Furka's spleen-chip method, $n=8$). The follow-up period was

[1]These authors contributed equally this work.

*Corresponding author: Norbert Nemeth, MD, PhD, Department of Operative Techniques and Surgical Research, Institute of Surgery, Faculty of Medicine, University of Debrecen, H-4032 Debrecen, Nagyerdei krt. 98., Hungary. Tel./Fax: +36 52 416 915, E-mail: nemeth@med.unideb.hu.

18 postoperative (p.o.) months. Erythrocyte deformability was determined in parallel by bulk filtrometry (Carat FT-1 filtrometer), slit-flow ektacytometry (RheoScan D-200) and rotational ektacytometry (LoRRca MaxSis Osmoscan).
RESULTS: By filtrometry, relative cell transit time increased in the SE group (mostly in animal Nr. SE-3), showing the highest values on the 3rd, 9th and in 18th p.o. months. Elongation index values decreased in this group (both by slit-flow and rotational ektacytometers). In general, AU and two resection groups' values were lower versus control and higher than in SE.
CONCLUSIONS: Forasmuch in the circulation both elongation by shear stress and filtration occur, these various erythrocyte deformability testing methods together may describe better the alterations. Considering the possible complications related to functional asplenic-hyposplenic conditions, individual analysis of cases is highly important.

Keywords: Spleen filtration function, splenectomy, spleen autotransplantation, spleen partial or subtotal resection, red blood cell deformability, bulk filtrometry, slit flow and rotational ektacytometry

1. Introduction

Spleen preserving surgical techniques have a great significance in the clinical practice, in order to prevent possible complications originated for functional asplenia or hyposplenia [7, 14, 15, 24, 28–30]. Since decades one of our main research focuses of the department is the spleen preserving surgical techniques, introducing and using various investigative methods for following-up the function of the remnant splenic tissue. The aim is the comparison of partial or subtotal resection and spleen autotransplantation, when after splenectomy at least 30% of splenic tissue was replanted into the double layer of the greater omentum versus splenectomy [9–12].

Spleen autotransplantation into the greater omentum after traumatic splenic injury or surgical origin caused splenectomy can partly preserve/restore the splenic filtration function by the regeneration of the splenic tissue, as our previous studies demonstrated on mongrel dog's experiments [13, 18].

Spleen has an important filtration function [8, 16] presenting an undoubted link to hemorheological approach in the research and the diagnosis [5, 6, 27].

On the basis of investigations it can be concluded that splenectomy causes significant changes in red blood cell functions, but one of the spleen preserving technique titled "Furka's spleen-chip" method, with other name "Furka's spleen-apron" technique can save the very important functions of the spleen, such as immunological and filtration function [13]. It is highly important to reveal in time if a possible functional asplenic or hyposplenic condition appeared. In our early papers we used bulk filtometry (Carat FT-1 filtrometer) [13, 29]. We could mesure this red blood cell deformability changing at first by Carat filtrometry method and it can be concluded that this relatively simple laboratory investigation is suitable to detect changes resulting from loss (asplenia) or decrease of splenic functions (functional hyposplenia) in both splenectomised and autotransplantad animals [13, 20, 29]. Related to hemorheological investigative methods, we have called the attention amongst the firsts that changes in erythrocyte deformability might reflect the impairment in functioning splenic tissue earlier than other rutinely used parameters in the clinical practice [6, 20].

Afterwards, we have adapted this splenic autotransplantation method for mice and got the same results [19, 20, 22]. We used this relatively simple measuring method on beagle dogs, as well. Later, by developing our laboratory equipments, we had the opportunity to test deformability by ektacytometry as well (RheoScan D-200) presenting novel data in a beagle animal model [21]. In the latest experimental series we could apply rotational ektacytometry measurement (LoRRca MaxSis Osmoscan).

The aim of recent study was to examine for better evaluation and follow-up of the effectiveness of various spleen preserving operative techniques and a composite methodological approach has been applied in a new serie of our beagle experimental model.

In this study we aimed to compare the results of changes in red blood cell deformability tested paralelly by bulk filtometry, slit flow- and rotational ectacytometry in a long-term follow-up experimental model.

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: 19.4 ± 1 months old, bodyweight: 12.98 ± 1.1 kg) were involved to in this study.

All of the operations were performed in sterile condition under general anaesthesia with intramuscular ketamine (10 mg/bwkg, CP-Ketamin – ketamine hydrochloride 10%, Produlab Pharma B.V., Netherlands) and xylazine (1 mg/bwkg, CP-Xylazin – xylazine-hydrochloride, 2%, Produlab Pharma B.V., Netherlands) combination.

Animals were subjected to the one of the following experimental groups:

I. Splenectomy group (SE, $n = 4$): after median laparotomy the spleen was removed.
II. Spleen-autotransplantation group (AU, $n = 8$): after median laparotomy and the consecutive splenectomy, ten pieces of splenic slices (20 mm × 50 mm × 1 mm) were placed between two layers of the greater omentum (Fig. 1A), close to a well-vascularized area according to the "Furka's spleen chip" method [9, 11, 12].
III. 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 (Fig. 2A and C) using "Furka-type" embracing suture technique [9, 10].
IV. 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.

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

2.2. Blood sampling protocol

To determine the blood parameters presented in this manuscript, blood samples were taken before the operations (base) and in the 3rd, 6th, 9th, 12th, 15th and 18th postoperative months via puncturing the cephalic vein, using closed blood samping system with 21G BD Eclipse™ needles (Becton, Dickinson and Company, USA) into Vacutainer tubes containing K_3-EDTA as anticoagulant (1.8 mg/ml, Becton, Dickinson and Company, USA).

2.3. Testing hematological parameters

The Advia 120 hematology system (Siemens Healthcare GmbH, Germany) was used to determine quantitative and qualitative hematological parameters. In this study the red blood cell count (RBC [T/l]), hemoglobin (Hgb [g/dl]), mean corpuscular volume (MCV [fl]) and mean corpuscular hemoglobin concentration (MCHC [g/dl]) were analyzed. Changes in these parameters may affect the assessment of changes in the red blood cell deformability. These measurements required blood sample voluem of 175 μl.

2.4. Determination of red blood cell deformability

The red blood cell deformability was tested paralelly by three different methods: bulk filtrometry, slit-flow and rotational ektacytometry.

2.4.1. Bulk filtrometry method

Carat FT-1 filtrometer (Carat Ltd., Hungary) was used for determining red blood cell deformability. The device is based on the St. George's blood filtrometer technique [2].

The tests need approximately 1.5 ml of blood. From the blood samples 5% red blood cell – phosphate buffered saline (PBS, osmolality: 295 ± 5 mOsm/kg, pH: 7.4) suspension aliquots were prepared and were being filtrated through a 5 μm pore-sized polycarbonate Nucleopore® filters (Whatman Co., UK) at constant filtration pressure (4 cmH_2O). The unit interfaced to a computer which automatically analyzes the sequential flow rates by fotodetectors and calculates the initial filtration rate (IRFR) and the relative cell transit time (RCTT), according to the following formula: $RCTT = [(IRFR^{-1} - 1)/Hct] + 1$, where Hct is the hematocrit of the suspension. The RCTT value increases with the decrease of red blood cell deformability [2].

2.4.2. Slit-flow and rotational ektacytometry

For the ektacytometrial measurement of red blood cell deformabilty a Rheoscan D-200 slit-flow ektacytometer (Sewon Meditech Inc., Korea) and a LoRRca MaxSis Osmoscan rotational ektacytometer (Mechatronics BV, The Netherlands) [2] was used.

For the measurements 5 μl of whole blood was taken into high-viscosity fluid suspension (polyvinylpyrrolidone, PVP 360 kDa, Sigma Aldrich, USA, dissolved in PBS. PVP-PBS suspension viscosity: 32.5–34.7 mPas, osmolality: 290–305 mOsm/kg, pH 7.3).

The measurement is based on the analysis of the diffracted laser images pattern from the elongated red blood cells against sher stress. By the method the elongation index (EI) is determined in the function of shear stress (SS [Pa]). For comparison EI-SS curves, EI values at 3 Pa, as well as maximal elongation index (EI_{max}) and the shear stress at half EI_{max} ($SS_{1/2}$ [Pa]) were used by Lineweaver-Burk analysis [3].

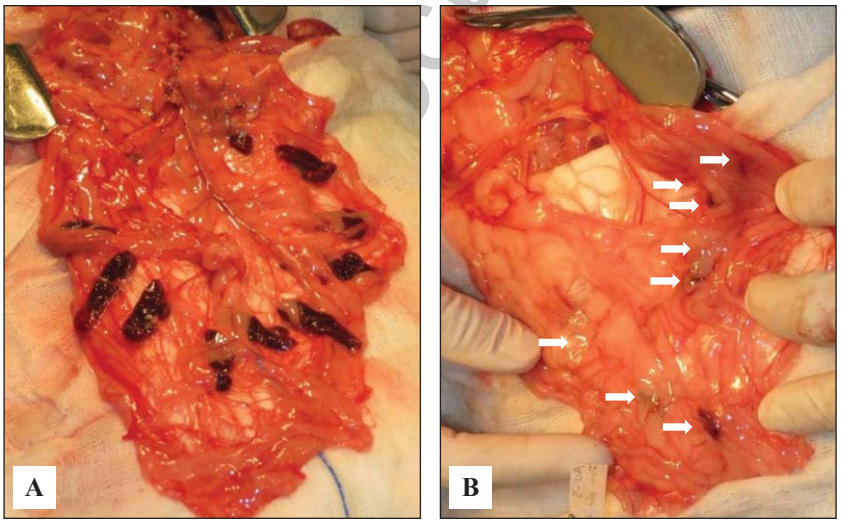

Fig. 1. Spleen-autotransplantation technique: 10 pieces of splenic slices were placed between two layers of the greater omentum by the "Furka's spleen chip" method (A), and the autotransplanted splenic tissue in the 18th postoperative month (animal Nr: AU-3) (B). The white arrows show the position of the existing spleen chips.

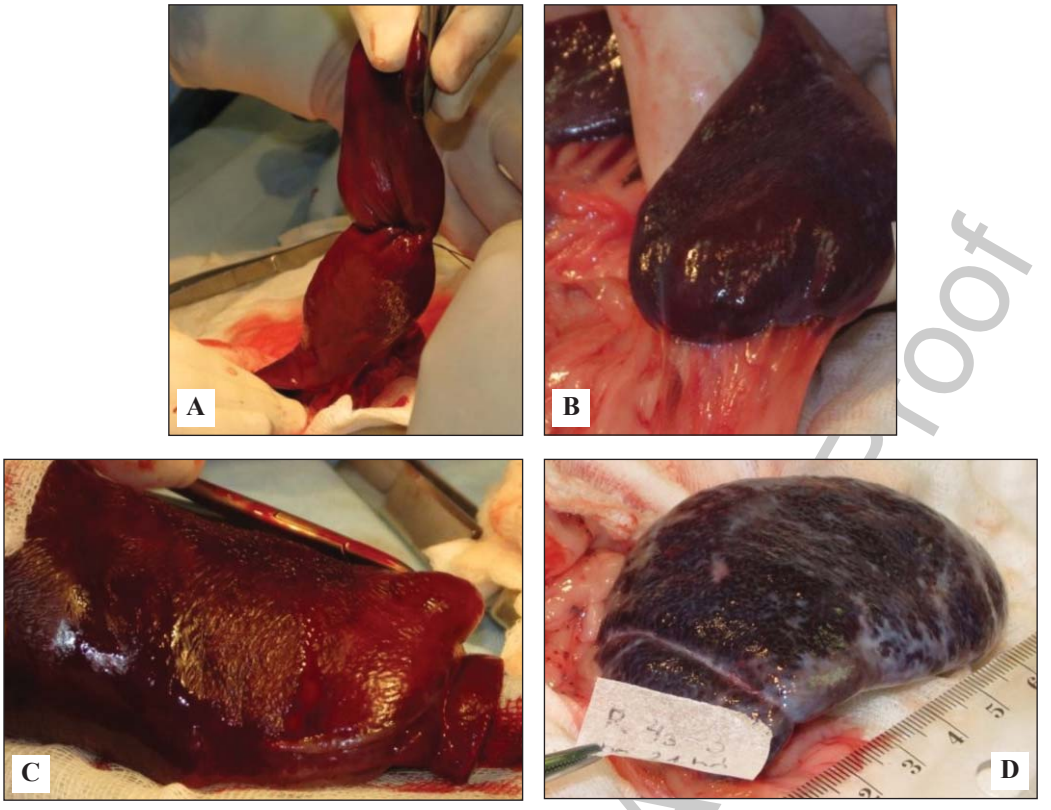

Fig. 2. Partial resection (one-third) spleen resection technique (A) and the resected splenic tissue in the 18th postoperative month (animal Nr: R 1/3-2) (B), subtotal (two-third) spleen resection technique using "Furka-type" embracing suture technique (C) and the resected splenic tissue in the 18th postoperative month (animal Nr: R 2/3-3) (D).

The EI at 3 Pa, EI_{max} values decreases and the $SS_{1/2}$ value increases with the impairment of cell deformability [2, 3].

2.5. *Statistical analysis*

Data are presented as mean values with standard deviation (means ± S.D.). Besides analysing individual animals' data, for general intra-group analysis we used ANOVA tests (Bonferroni's or Dunn's *post hoc* tests). For inter-group comparisons at definitive time points Student's *t*-test or Mann-Whitney rank sum tests were used, according to the normality of data distribution. A p value of < 0.05 was considered to be significant.

3. Results

There were no intraoperative or postoperative complications. The macroscopic investigation of the autotransplanted splenic tissue is showed on the Fig. 1B. The partial resected remnant spleen is seen on the Fig. 2B and D.

3.1. *Red blood cell related hematological parameters*

Table 1 shows red blood cell count, haemoglobin, mean corpuscular volume and mean corpuscular hemoglobin concentration data.

Table 1
Changes of red blood cell related hematological parameters in 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			
			3rd	6th	9th	18th
RBC [T/l]	Control	6.70 ± 0.39	6.98 ± 0.76	6.57 ± 0.62	6.72 ± 0.89	6.17 ± 1.92
	SE	7.33 ± 1.01	$5.56 \pm 0.56^{*\#}$	5.97 ± 0.18	$6.17 \pm 0.33^{*}$	$6.12 \pm 0.25^{*}$
	AU	7.21 ± 0.70	$6.14 \pm 0.40^{*\#}$	$6.45 \pm 0.29^{*+}$	$6.40 \pm 0.63^{*}$	6.68 ± 0.35
	R1/3	6.87 ± 0.08	6.83 ± 1.03	$6.75 \pm 0.58^{+}$	6.66 ± 1.19	6.53 ± 0.64
	R2/3	7.11 ± 0.71	6.21 ± 0.41	$6.62 \pm 0.43^{+}$	6.31 ± 0.37	6.49 ± 0.59
Hgb [g/dl]	Control	15.34 ± 1.00	15.52 ± 1.72	14.77 ± 1.06	14.77 ± 2.00	15.48 ± 1.42
	SE	16.70 ± 1.91	$12.50 \pm 0.92^{*,\#}$	$13.08 \pm 0.32^{*,\#}$	$13.75 \pm 0.54^{*}$	$13.8 \pm 0.4^{*}$
	AU	16.35 ± 1.37	$13.73 \pm 0.91^{*,\#}$	$13.96 \pm 0.77^{*}$	$13.99 \pm 1.03^{*}$	$14.94 \pm 0.80^{+}$
	R1/3	15.50 ± 0.14	$15.45 \pm 2.09^{+}$	$14.93 \pm 1.03^{+}$	14.95 ± 2.44	15.03 ± 1.45
	R2/3	16.33 ± 1.27	$13.90 \pm 0.64^{*,+}$	$14.65 \pm 0.51^{*,+}$	$13.95 \pm 0.64^{*}$	14.5 ± 0.91
MCV [fl]	Control	64.72 ± 4.06	64.00 ± 0.88	62.65 ± 1.01	$59.35 \pm 0.95^{*}$	61.41 ± 2.42
	SE	66.48 ± 2.16	$67.58 \pm 2.28^{\#}$	63.58 ± 0.85	$62.15 \pm 2.34^{*}$	63.93 ± 1.85
	AU	65.28 ± 3.20	$64.66 \pm 1.92^{+}$	63.56 ± 1.92	$64.64 \pm 2.88^{\#}$	61.98 ± 1.68
	R1/3	64.55 ± 1.77	65.08 ± 1.04	63.23 ± 0.98	$62.10 \pm 2.36^{\#}$	$60 \pm 1.11^{+}$
	R2/3	64.83 ± 4.31	$64.33 \pm 1.31^{+}$	63.18 ± 2.36	$61.90 \pm 1.71^{\#}$	$59.47 \pm 1.02^{+}$
MCHC [g/dl]	Control	35.52 ± 2.59	34.75 ± 0.48	36.02 ± 2.20	37.02 ± 0.28	35.9 ± 0.66
	SE	34.40 ± 1.12	$33.35 \pm 0.58^{\#}$	$34.50 \pm 0.44^{\#}$	$35.95 \pm 0.96^{\#}$	35.36 ± 0.11
	AU	34.80 ± 0.89	$34.55 \pm 0.49^{+}$	$34.08 \pm 0.71^{\#}$	$33.91 \pm 0.36^{*,\#,+}$	$36.06 \pm 0.62^{*}$
	R1/3	35.00 ± 0.28	$34.75 \pm 0.26^{+}$	34.98 ± 0.69	36.20 ± 1.56	$38.26 \pm 0.90^{\#+}$
	R2/3	35.58 ± 2.05	$34.90 \pm 0.41^{+}$	35.13 ± 0.33	$35.75 \pm 0.86^{\#}$	$37.6 \pm 0.46^{\#+}$

RBC – red blood cell count; Hgb – hemoglobin; MCV – mean corpuscular volume; MCHC – mean corpuscular hemoglobin concentration. SE – splenectomy group, AU – spleen autotransplantation group, R1/3 and R2/3 – one-third and two-third spleen resection groups. means $\pm$ S.D., $^{*}p < 0.05$ vs. Base, $^{\#}p < 0.05$ vs. Control, $^{+}p < 0.05$ vs. SE.

The *red blood cell count* of the Control and the R1/3 – partial resection groups did not change significantly during the follow-up period. In SE, AU and R2/3 – subtotal resection groups significantly lower values were determined over the follow-up period versus their base (SE: 3rd month $p = 0.007$, 9th month $p = 0.049$; AU: 3rd month $p = 0.003$, 6th month $p = 0.017$, 9th month $p = 0.045$). The largest decrease was found in the SE group. In the 3rd month red blood cell count of SE and AU groups were significantly lower than the Control ($p = 0.013$ and $p = 0.019$). In the 6th postoperative month we found that the values of SE group were significantly lower compared to the AU, R1/3 or R2/3 groups ($p = 0.013$, $p = 0.041$ and $p = 0.032$, respectively). By the 18th month SE group's data showed further lowering ($p = 0.05$ vs. base).

The values of *hemoglobin (Hgb)* in the SE group, similarly to the red blood cell count, was the highest, while AU and R2/3 groups expressed a decreasing tendency (SE: 3rd month $p = 0.004$, 6th month $p = 0.034$, 9th month $p = 0.024$; AU: 3rd month $p = 0.001$, 6th month $p = 0.001$, 9th month $p = 0.003$; R2/3 : 3rd month $p = 0.041$, 9th month $p = 0.01$). On the 3rd month the lowering in the SE and AU groups was significant compared to Control group ($p = 0.013$ and $p = 0.026$). SE group's data showed significant diofference versus the R1/3 and R2/3 groups as well ($p = 0.041$ and $p = 0.046$). By the 6th month only in the SE group we found significantly lower values compared to Control ($p = 0.01$), R1/3 ($p = 0.014$) and R2/3 groups ($p = 0.002$). By the 18th month AU data increased, showing significant difference versus the SE group ($p = 0.036$).

A slight decrease was experienced in *mean corpuscular volume (MCV)* values during the follow-up period. By the 9th postoperative month we found significant decrease in the Control and SE groups ($p = 0.017$ and $p = 0.025$ vs. base). In the 3rd postoperative month, there could be observed a significantly higher value versus the Control ($p = 0.007$), AU ($p = 0.041$) and R2/3 groups ($p = 0.048$). By the 9th month MCV decreased in Control group, which lowering was significant compared to the AU ($p = 0.001$), R1/3 ($p = 0.031$) and R2/3 group's values ($p = 0.016$). By the 18th month MCV showed further decrease in AU and resection groups (AU group: $p = 0.068$ vs. base, R1/3 group: $p = 0.034$ vs. SE, R2/3 group: $p = 0.057$ vs. base and $p = 0.022$ vs. SE).

Mean corpuscular hemoglobin concentration (MCHC) did not show characteristical changing, however, in AU group significantly lower values were tested compared to base values ($p = 0.024$) in the 9th postoperative month. In the 3rd month, the SE group showed significantly lower values versus the Control ($p = 0.003$), AU ($p = 0.004$), R1/3 ($p = 0.005$) and R2/3 groups ($p = 0.005$). In the 6th month, there was a slight increase in the Control group, while in the SE and AU groups the data showed siginificantly lower values ($p = 0.0019$ and $p < 0.001$). Also there was a significant difference between the Control group in the 9th month compared to SE ($p = 0.03$), AU ($p < 0.001$) and R2/3 groups ($p = 0.009$). At the same time AU values were closer to Control and significantly differed from SE group's data ($p < 0.001$). By the 18th month MCHC increased in AU and resection groups (AU group: $p = 0.027$ vs. base, R1/3 group: $p = 0.003$ vs. Control and $p = 0.005$ vs. SE, R2/3 group: $p = 0.001$ vs. Control and $p = 0.006$ vs. SE).

3.2. Red blood cell deformability

3.2.1. Bulk filtrometry

Figure 3 shows the changes of initial relative filtration rate (IRFR) and relative cell transit time (RCTT) values being tested during the follow-up period.

Based on the results, it could be seen that that the difference between the groups during follow-up period was not uniform. Control group did not express important changes nor in IRFR neither in RCTT values. Concerning the changes in initial relative filtration rate (IRFR), the SE, AU and R2/3 groups showed irregular decrease.

The base IRFR values (before operation) were significantly higher compared to Controls in AU and R2/3 groups (0.866 ± 0.048 and 0.871 ± 0.048 vs. 0.811 ± 0.04; $p = 0.02$ and 0.017). In parallel,, the RCTT in the SE, AU and R2/3 groups were significantly lower versus the Control group (4.5 ± 1.16, 4.36 ± 0.95, 4.01 ± 1.16 vs. 5.82 ± 0.89; $p = 0.039$, $p = 0.016$ and $p = 0.005$).

Six months later there was a decrease in IRFR and an increase in RCTT values of AU group compared to base and the Control group (IRFR: 0.785 ± 0.038 vs. 0.866 ± 0.048 and 0.841 ± 0.053; $p < 0.001$ and $p = 0.006$; RCTT: 6.53 ± 1.24 vs. 4.37 ± 0.95 and 5.82 ± 0.89; $p = 0.002$ and $p = 0.016$).

The results became probably more comparable over half a year after the surgery. Relative cell transit time increasing was seen in the SE group (mostly in animal nr. SE-3). The graph also shows that the values were the highest in the 9th postoperative month.

In the 9th month the IRFR values were significantly lower in SE, AU and R2/3 groups compared to the initial base values. Consequently we observed the increased RCTT values (SE group: 0.797 ± 0.015, 6.12 ± 0.49 vs. 0.853 ± 0.0465, 4.5 ± 1.16, $p = 0.019$ and $p = 0.014$; AU group: 0.814 ± 0.023, 5.59 ± 0.69 vs. 0.866 ± 0.048, 4.37 ± 0.5 $p < 0.001$ and $p = 0.004$; R2/3 group: 0.8 ± 0.042, 6.14 ± 1.43 vs. 0.871 ± 0.048, 4.01 ± 1.16 $p = 0.005$ and $p = 0.006$).

While in the 18th month the RCTT data of both resection groups were greater than their base values.

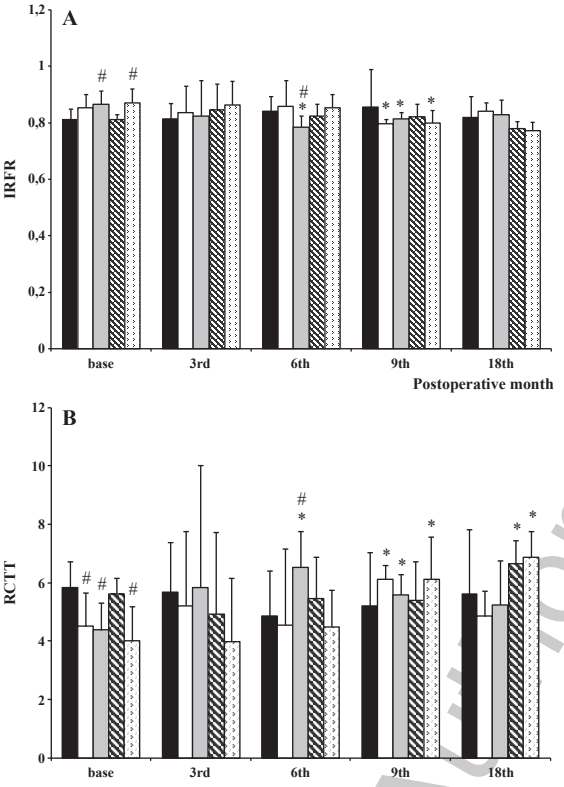

Fig. 3. Changes of initial relative filtration rate (IRFR) (A) and relative cell transit time (RCTT) (B) values tested by the Carat FT-1 filtrometer in splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3) during the 18-month follow-up period. means ± S.D., $^{*}p < 0.05$ vs. Base, $^{\#}p < 0.05$ vs. Control.

3.2.2. Slit-flow ektacytometry

Figure 4 presents the changes in erythrocyte deformability values determined by the RheoScan D200 device.

The *elongation index (EI) at 3 Pa* shear stress was significantly lower in R1/3 and SE compared to the Control groups (on the 3rd month: 0.267 ± 0.022 vs. 0.29 ± 0.011; $p = 0.011$). In the 9th month SE and R1/3 groups had significantly lower values compared to Control ($p < 0.05$). In parallel, AU and R2/3 data were higher versus the SE group. SE and R1/3 groups'values were significantly lower than the Control (0.262 ± 0.013 and 0.26 ± 0.018 vs. 0.276 ± 0.014; $p = 0.22$ and $p = 0.047$), while the AU and R2/3 were significantly higher (0.28 ± 0.016 and 0.28 ± 0.017 vs. 0.262 ± 0.013; $p = 0.004$ and $p = 0.015$).

The EI_{max} *values* were the lowest in SE group on the 3rd, 6th, 9th and 18th postoperative month compared to Control (6th month: $p = 0.049$) or to the R2/3 group ($p = 0.003$), and on the 9th month compared to R1/3 group ($p = 0.019$).

Interestingly the $SS_{1/2}$ *values* also showed a decreasing tendecy, probably due to the morphological changes of the EI-SS curves. Values ofthe AU and Control groups were significantly higher compared to the SE (3.3 ± 0.56 vs. 2.71 ± 0.61 and 2.73 ± 0.78 Pa; $p = 0.019$ and $p = 0.05$) in the 3rd month. In the 6th month, we measured significantly lower values in case of the SE group versus the Control (2.71 ± 0.28 vs. 3.33 ± 0.69 Pa; $p = 0.035$). In the 9th month the R1/3 group expressed significantly higher values compared both to the Control and the SE group (3.36 ± 0.36 vs. 2.7 ± 0.61 and 2.56 ± 0.64 Pa; $p = 0.029$ and $p = 0.018$). All operated groups showed low values on the 18th month ($p < 0.01$ vs. Control).

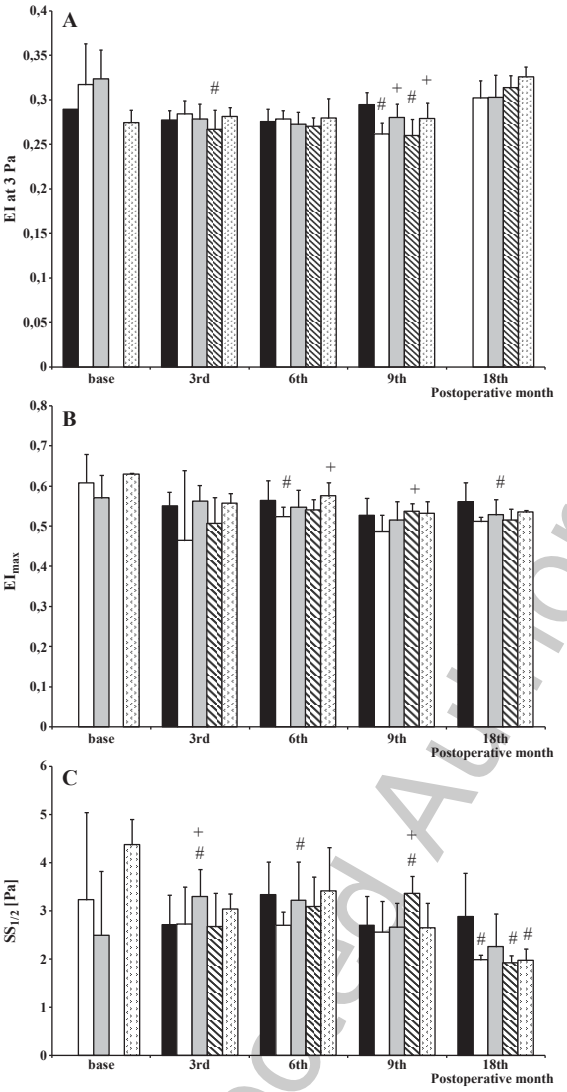

Fig. 4. Changes of the elongation index (EI) at 3 Pa shear stress (A), maximal elongation index (EI_{max}) (B) and shear stress values at half EI_{max} ($SS_{1/2}$ [Pa]) (C) measured by the Rheoscan D200 slit-flow ektacytometer in splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3) during the 18-month follow-up period. means ± S.D., $^{\#}p < 0.05$ vs. Control, $^{+}p < 0.05$ vs. SE.

3.2.3. Rotational ektacytometry

Figure 5 presents the changes in erythrocyte deformability values determined by the LoRRca device.

The *EI values at 3 Pa* in all groups showed an increasing tendency during the follow-up period. The increase, with a few exceptions (3rd month in SE, R1/3 and R2/3; 9th month in SE) were significant ($p < 0.01$). In the 3rd month, all data -expect for the AU group- showed significantly lower values compared to the Control (0.233 ± 0.016, 0.237 ± 0.017 and 0.245 ± 0.019 vs. 0.265 ± 0.019; $p = 0.011$, $p = 0.009$ and $p = 0.005$, respectively). In the 6th postoperative month there were no such discrepancy in the data, only the R1/3 group had significantly lower values compared to the Control (0.245 ± 0.024 vs. 0.269 ± 0.022; $p = 0.035$). In the 9th month the SE group's values were significantly lower compared to all the other groups (0.225 ± 0.021 vs. 0.275 ± 0.015, 0.263 ± 0.02, 0.249 ± 0.019 and 0.268 ± 0.021;

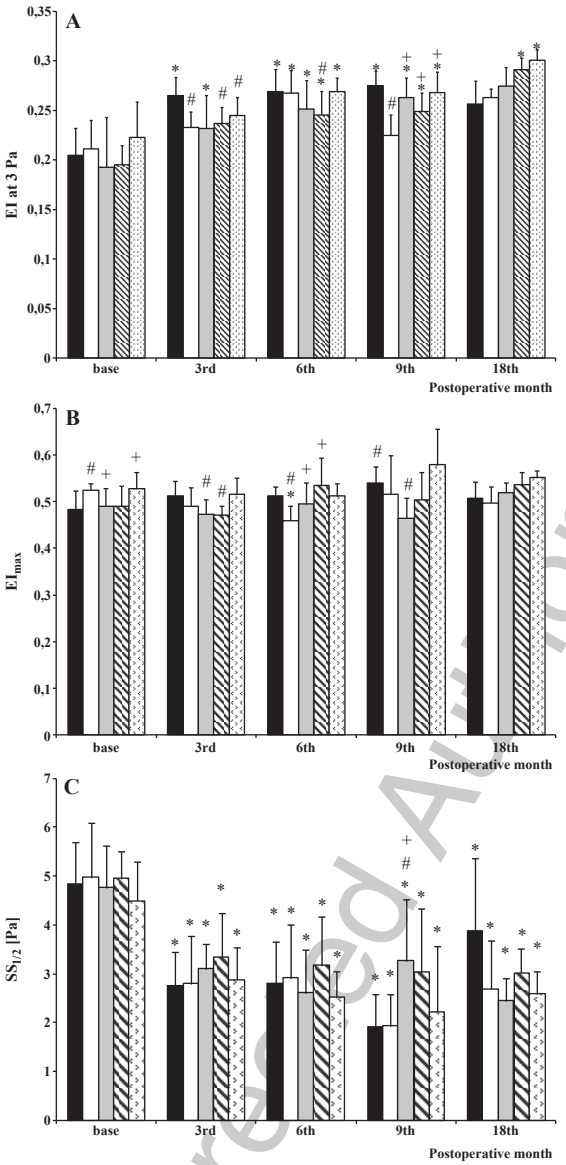

Fig. 5. Changes of the elongation index (EI) at 3 Pa shear stress (A), maximal elongation index (EI_{max}) (B) and shear stress values at half EI_{max} ($SS_{1/2}$ [Pa]) (C) measured by the LoRRca MaxSis Osmoscan rotational ektacytometer in splenectomy (SE), spleen autotransplantation (AU) and spleen resection groups (R1/3, R2/3) 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.

$p < 0.001$, $p < 0.001$, $p = 0.003$ and $p = 0.002$). Afterwards, both resection groups showed a significant increase compaired to their base values by the 18th postoperative month ($p < 0.001$).

The base *EI_{max} values* at the SE group were significantly higher compared to the Control, the AU or to the R1/3 group (0.525 ± 0.015 vs. 0.483 ± 0.041, 0.49 ± 0.038 and 0.489 ± 0.044; $p = 0.023$, $p = 0.023$ and $p = 0.049$, respectively). In the 3rd postoperative month we measured significantly lower values in case of the AU and R1/3 groups versus the Control (0.473 ± 0.033 and 0.471 ± 0.021 vs. 0.513 ± 0.031; $p = 0.003$ and $p = 0.004$). By the 6th month the SE group showed significantly lower EI_{max} values compared to the base, Control, R1/3 and R2/3 groups'values (0.46 ± 0.032 vs. 0.525 ± 0.015, 0.512 ± 0.02,

Table 2
Worsening red blood cell deformability parameters tested by bulk filtrometry, slit flow- and rotational ektacytometry in SE-3 animal (splenectomy) compared to its group means during the follow-up period of 18 months

Device		3rd postoperative month		9th postoperative month		18th postoperative month	
	Parameter	SE-3	SE group *means* (except SE-3)	SE-3	SE group *means* (except SE-3)	SE-3	SE group *means* (except SE-3)
Carat FT-1 filtrometer	RCTT	3.140	*5.669*	6.015	*6.155*	5.450	*4.535*
	IRFR	0.904	*0.816*	0.800	*0.796*	0.818	*0.851*
Rheoscan D-200 slit-flow ektacytometer	EI 3 Pa	0.271	*0.289*	0.272	*0.258*	0.312	*0.298*
	EI_{max}	0.544	*0.422*	0.499	*0.483*	0.511	*0.513*
	$SS_{1/2}$ [Pa]	2.893	*2.696*	2.489	*2.585*	1.958	*2.003*
LoRRca MaxSis Osmoscan ektacytometer	EI 3 Pa	0.245	*0.229*	0.232	*0.222*	0.256	*0.267*
	EI_{max}	0.475	*0.496*	0.490	*0.520*	0.462	*0.515*
	$SS_{1/2}$ [Pa]	3.805	*2.463*	1.140	*2.193*	3.785	*2.140*

0.534 ± 0.061 and 0.511 ± 0.028; $p = 0.001$, $p < 0.001$, $p = 0.009$ and $p = 0.004$, respectively). In the 9th month the Control group values were significanly higher compared to the base (0.54 ± 0.035 vs. 0.483 ± 0.041; $p < 0.001$). The lowest values were presented by the AU group, which decrease was significant compared to Control (0.464 ± 0.044 vs. 0.54 ± 0.035; $p < 0.001$). By the 18th month the lowest values were found in SE and Control group.

Similarly to the Rheoscan results measurement, the *$SS_{1/2}$ values* of the LoRRca measurements also showed a decreasing tendency in the follow-up period. The values decreased in a significant manner in all groups during the follow-up period. By the 9th month the $SS_{1/2}$ values were the highest in the AU group being significant compared to the Control and the SE groups (3.26 ± 1.26 vs. 1.9 ± 0.68 and 1.93 ± 0.64 Pa; $p = 0.005$ and $p = 0.042$, respectively). In the R1/3 group the values were significantly higher versus the Control (3.03 ± 1.31 vs. 1.9 ± 0.68 Pa; $p = 0.028$). By the 18th month $SS_{1/2}$ increased in Control group, while in operated groups it remained significantly lower compared to the base ($p < 0.001$).

3.3. Individual analysis and case example in splenectomy group

Table 2 also shows that the different measuring methods did not present the differences at the same level.

In SE group the animal Nr. 3 (code: SE-3) showed the worst deformability results in the 3rd postoperative month. Both ektacytometry methods could detect increase in $SS_{1/2}$ values. In parallel, in the filtration measurements we did not detect impairment. On the 9th month EI_{max} values measured by the LoRRca showed the remarkable differences. However, by the 18th month the filtrometrial values showed impairment which was enforced by the LoRRca data, while Rheoscan results did not supported it.

4. Discussion

Spleen preserving surgical techniques are important tools to prevent possible complications originated from the possible functional asplenic/hypospenic conditions [7, 14, 24, 30–32] in the management

of traumatized but healthy spleen [23, 24, 30]. The trauma remains the principal indication for the different types of the partial splenectomy in general in younger age [24–26]. The effectiveness of spleen preserving metods can be monitored by following-up the splenic functions. Besides immunological, certain hemopoietic and storage function, spleen is a major organ with filtration function [8, 16]. It is well known that rigid red blood cells besides other particles are removed from the circulation normally by the spleen. Consequently, decrease in splenic filtration function may results in hemorheological changes [2, 20, 21, 29]. Impaired rheological properties of the blood lead microcirculatory disturbances [2, 17, 28, 33]. Furthermore, postsplenectomy complications, such as septic conditions (e.g., Overwhelming Postsplenectomy Infection - OPSI - syndrome,) cause significant worsening in blood rheology and microcirculation [1, 2, 15, 32]. Thus, hemorheological, especially micro-rheological following-up of splenic function is considered to be highly important in spleen preserving surgical studies.

In our recent study we have found that deformability results showed irregular fluctuation over the follow-up period, almost similarly to the previous series of the research [20, 21, 29]. By filtrometry, relative cell transit time increasing was seen in the splenectomy group (mostly in animal Nr. SE-3), showing the highest values on the 3rd, 9th and in 18th postoperative months. Elongation index values decreased in the splenectomy group (tested both by slit-flow and rotational ektacytometers). In general, spleen autotransplantation and both spleen resection groups' values were lower versus control and higher than in splenectomy. However, by the analysis of the two resection groups's data, different tendencies were observed. These should be evaluated together with macroscopic autopsy and histological results in order to clarify the question whether spleen autotransplantation or subtotal resection is the better choice when performing spleen salvaging surgical technique in the case of traumatic spleen injury.

The methods of deformability measurement are different in sensitivity, measuring basic theory, techniques, sample preparation and measurement condtitions as well [2, 6]. For bulk filtrometry we have to prepare red blood cell – PBS suspension by multiple washing of cells, and providing a low hematocrit (e.g., 5%) [2, 6]. The suspension then is filtrated mechanically through a filter driven by hydrostatic pressure gradient [2]. In ekacytometry the whole blood samples is taken into a high-viscosity media resulting in a more lower final hematocrit. Then the cells are subjected to shearing force and the laser diffraction is detected from the border of the cell-suspension surfaces [2]. Besided further technical differences, the generation of shear stress is also different in slit-flow and in rotational ektacytometers in range and direction of shear force generating, as well as in reproducibility [2, 4]. Thus their comparison is difficult. However, all these devices are capable to detect deformability changes well, but from differenc point of view [4].

Loss of splenic function (asplenia by splenectomy or decrease in function (hyposplenia by partial or subtotal resection or autotransplantation) means a condition, but the magnitude of changes may also show individual alterations. We share an opinion with the recommendation of Morgenstern: "*Clinical judgment should dictate which procedure is of greatest benefit to the patient*" [24]. Additionally, the possible postoperative complications might appear, however, not in every case. Thus the individual analysis of the data within groups, together with other parameters is very important, not only simply compare the groups with regular statistical methods. In the future, during the micro-rheological analyses special attention is needed when comparing partial or subtotal resection groups and splenic autotransplantation groups. Thus, besides micro-rheological tests other investigations were also done in parallel, such as complex hematological, hemostaseological, stem-cell-, hybrid nuclear medicine imaging (e.g., SPECT/CT, microCT) and histological methods in a wide collaborative project for revealing the general and individual changes as well.

5. Conclusion

Forasmuch in the circulation both elongation by shear stress and filtration occur, these various erythrocyte deformability testing methods together may describe better the alterations. Considering the possible complications related to functional asplenic-hyposplenic conditions, individual analysis of cases is highly important.

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References

[1] O.K. Baskurt, D. Gelmont and H.J. Meiselman, Red blood cell deformability in sepsis, *Am J Respir Crit Care Med* **157**(2) (1998), 421–427. doi: 10.1164/ajrccm.157.2.9611103

[2] O.K. Baskurt, H.R. Hardeman, M.W. Rampling, H.J. Meiselman, editors., Handbook of Hemorheology and Hemodynamics. Amsterdam: IOS Press; 2007.

[3] O.K. Baskurt, M.R. Hardeman, M. Uyuklu, P. Ulker, M. Cengiz, N. Nemeth, et al., Parameterization of red blood cell elongation index – shear stress curves obtained by ektacytometry, *Scand J Clin Lab Invest* **69**(7) (2009), 777–788. doi: 10.3109/00365510903266069

[4] O.K. Baskurt, M.R. Hardeman, M. Uyuklu, P. Ulker, M. Cengiz, N. Nemeth, et al., Comparison of three commercially available ektacytometers with different shearing geometries, *Biorheology* **46**(3) (2009), 251–264. doi: 10.3233/BIR-2009-0536

[5] O.K. Baskurt, The role of spleen in suppressing the rheological alterations in circulating blood, *Clin Hemorheol Microcirc* **20(3)** (1999), 181–188.

[6] S.I. Bernat, L. Bogar, M. Csornai, S. Imre, I. Juricskay, L. Kollar, Z. Novak, Zs. Pecsvarady, E. Pongracz, I. Rozsos and K. Toth, Módszertani útmutató a haemorheologiai mérések végzéséhez, *Érbetegségek* **1** (2005), 27–33. [Article in Hungarian]

[7] A. Cadili and C. de Gara, Complications of splenectomy, *Am J Med* **121**(5) (2008), 371–375. doi: 10.1016/j.amjmed.2008.02.014

[8] W.H. Crosby, Splenic remodeling of red cell surfaces, *Blood* **50**(4) (1977), 643–645.

[9] I. Furka, I. Miko, L. Papp and T. Miko, Salvaging the spleen by experimental resection or autotransplantation. In: *Jubileuszowy Zjazd Towarzystwa Chirurgow Polskich Vol. 2*. Krakow, 1989. pp. 453–456.

[10] I. Furka, I. Mikó, T. Mikó and L. Papp, Partial splenectomy performed by a special technique in dogs, *Acta Chir Hung* **31** (1990), 317–323.

[11] I. Furka, I. Miko, J. Serfozo, I. Frendl and M. Hauck, 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. pp. 767–769.

[12] I. Furka, Z. Hajdu, T. Szendroi, I. Miko, A. Bokk and G. Barnak, Spleen autotransplantation. Experimental and clinical experiences. In: *23rd World Congress of the International College of Surgeons*, Cairo. Bologna: Monduzzi Editore; 1992. pp. 907–912.

[13] I. Furka, I. Miko, K. Toth, A. Furka, J. Kappelmayer, Z. Szikszai, et al., Haematological, hemorheological and catalase level changes following splenectomy and spleen autotransplantation in experimental animals. Early results, *Acta Chir Austr* **29**(Suppl.137) (1997), 31–32.

[14] L. Grandic, Z. Pogorelic, J. Banovic, Z. Perko, V. Boschi, N. Ilic, et al., Advantages of the spared surgical treatment of the spleen injuries in the clinical conditions, *Hepatogastroenterology* **55** (2008), 2256–2258.

[15] K. Hansen and D.B. Singer, Asplenic-hyposplenic overwhelming sepsis: Postsplenectomy sepsis revisited, *Pediatr Dev Pathol* **4**(2) (2001), 105–121.
[16] J.W. Harris and R.W. Kellermeyer, The Red Cell: Production, Metabolism, Destruction: Normal and Abnormal. Revised Edition. Cambridge, Massachusetts: Harvard University Press; 1970. p. 795.
[17] H.H. Lipowsky, Microvascular rheology and hemodynamics, *Microcirculation* **12**(1) (2005), 5–15. doi:10.1080/10739680590894966
[18] I. Miko, I. Furka, J. Serfozo, Gy. Joos, B. Telek, K. Matesz et al., Comparative study of haematological and micromorphological results in long-surviving spleen autotransplants, In: Uranus S, ed. Chirurgische Forschung, München, Bern, Wien, New York, W. Zuckschwerdt Verlag; 1994, pp. 50–55.
[19] I. Miko, E. Brath, I. Furka, J. Kovacs, D. Kelvin and R. Zhong, Spleen autotransplantation in mice: A novel experimental model for immunology study, *Microsurgery* **21**(4) (2001), 140–142.
[20] I. Miko, E. Brath, N. Nemeth, A. Furka, S. Sipka Jr, K. Peto, et al., Spleen autotransplantation. Morphological and functional follow-up after spleen autotransplantation in mice: A research summary, *Microsurgery* **27**(4) (2007), 312–316. doi: 10.1002/micr.20362
[21] I. Miko, N. Nemeth, E. Sajtos, E. Brath, K. Peto, A. Furka, et al., Splenic function and red blood cell deformability: The beneficial effects of spleen autotransplantation in animal experiments, *Clin Hemorheol Microcirc* **45**(2-4) (2010), 281–288. doi: 10.3233/CH-2010-1307
[22] I. Miko, N. Nemeth, S. Sipka Jr, E. Brath, K. Peto, A. Gulyas, et al., Hemorheological follow-up after splenectomy and spleen autotransplantation in mice, *Microsurgery* **26**(1) (2006), 38–42. doi: 10.1002/micr.20208
[23] E.E. Moore, T.H. Cogbill, G.J. Jurkovich, S.R. Shackford, M.A. Malangoni and H.R. Champion, Organ injury scaling: Spleen and liver (1994 revision), *J Trauma* **38**(3) (1995), 323–324.
[24] L. Morgenstern, Oartial Splenectomy In: Hiatt JR, Phillips EH, Morgenstern L, eds. *Surgical Disease of the Spleen*. Berlin, Heidelberg: Springer-Verlag; 1997. pp. 263–279.
[25] D.N.S. Paulo and M.S.L. Paulo, Subtotal splenectomy with inferior pole preservation (ESTPI), *Salus J Health Sci* **1** (2015), 72–81. doi.org/10.5935/2447-7826.201500009
[26] A. Petroianu, Conservative Surgical Procedures on the Spleen, In: Petroianu A, ed. *The Spleen*, Sharjah, Benthan Science Publishers Ltd; 2015. pp 217–249.
[27] D.A. Robertson, F.G. Simpson and M.S. Losowsky, Blood viscosity after splenectomy, *Br Med J (Clin Res Ed)* **283**(6291) (1981), 573–575.
[28] S. Simchon, K.M. Jan and S. Chien, Influence of reduced red cell deformability on regional blood flow, *Am J Physiol* **253**(4 Pt 2) (1987), H898–H903.
[29] G. Szabo, I. Miko, Z. Szikszai, S. Imre and I. Furka, The importance of red blood cells deformability measuring for following the function of autotransplanted spleen chips, *Acta Chir Austr* **31**(Suppl.159) (1999), 17–18.
[30] D.D. Trunkey, F. Hulka and R.J. Mullins, Splenic trauma. In: Hiatt JR, Phillips EH, Morgenstern L, eds. *Surgical Disease of the Spleen*. Berlin, Heidelberg: Springer-Verlag; 1997. pp. 233–261.
[31] B.M. William and G.R. Corazza, Hyposplenism: A comprehensive review. Part I: Basic concepts and causes, *Hematology* **12**(1) (2007), 1–13. doi: 10.1080/10245330600938422
[32] B.M. William, N. Thawani, S. Sae-Tia and G.R. Corazza, Hyposplenism: A comprehensive review. Part II: Clinical manifestations, diagnosis, and management, *Hematology* **12**(2) (2007), 89–98. doi: 10.1080/10245330600938463
[33] Z. Xu, Y. Zheng, X. Wang, N. Shehata, Z. Wang, S. Xie and Y. Sun, Stiffering of sickle cell trait red blood cells under simulated strenous exercise conditions, *Microsystems & Nanoengineering* **2** (2016), 16061. doi: 10.1038/micronano.2016.61