SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

FOLLOW-UP POSSIBILITIES WITH HEMORHEOLOGICAL MEASUREMENTS AND IMAGING TECHNIQUES IN ASPLENIC-HYPOSPLENIC CONDITIONS RELATED TO SPLEEN-PRESERVING SURGICAL TECHNIQUES IN ANIMAL EXPERIMENTAL MODEL

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The Examination takes place at the Library of the Department of Ophthalmology, Faculty of Medicine, University of Debrecen, 27th May 2014, 12:00.

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1. INTRODUCTION

During the past 100 years, significant changes have been observed in the management of spleen injuries. In the early 20th century, the most often way in which splenic injuries were managed was the splenectomy. Till the 1950's this strategy was not questioned, when the Overwhelming Postsplenectomy Infection (OPSI) syndrome was recognized. New investigations were started, which helped to identify the splenic functions, as the protection against the encapsulated bacterias.

This was followed by the paradigm shift resulting the introduction of splenic preservation -operative or/and non-operative techniques- as a main strategy, first in the pediatric medicine then generally. The -at least partially- preserved splenic functions could have a great importance to prevent the OPSI syndrome or other possible complications following splenectomy.

In the literature there is no consideration about the effectiveness of spleen preserving techniques. This difficulty exists because there's no elaborated diagnostic protocol for the investigation of splenic functions, caused by its complexity.

The Department of Operative Techniques and Surgical Research started the experimental research of spleen-preserving operative techniques in 1986. Initially mixed-breed dogs were examined, resulting the Furka's spleen chip technique to become known worldwide. The results suggested that the narrow spleen slices need 4-5 months to regenerate and the light- and electronmicroscopical data showed similar histological structure as the original spleen. The ultrasound and the scintigraphy were proved as a possible postoperative investigation method, too.

Further hematological, hemorheological and immunological investigations verified the viability of spleen autotransplants. Since, the average volume of erythrocytes, the number of platelets, red blood cell deformability defined with filtrometry method and peripheral phagocytic zimosan dependent activity in the autotransplanted group approached the levels of the control group, while the splenectomy group showed higher values. These results suggested partial recovery of the splenic functions.

These previous investigations of our department had also demonstrated the actuality of spleen preserving surgical techniques and asplenic-hyposplenic states research. Therefore, long-term follow-up for inbred large laboratory animal model raised the demand.

The aim of this study was to analyze and compare the possible methods, used for monitoring the splenic function following spleen preserving surgical techniques in beagle canine model.

2. AIMS

1./ Detection of the filtration functions of the spleen autotransplants with hemorheological measurements. Clarification of the applicability of two different measuring methods -filtrometry and ektacytometry- with the comparison of the obtained data.

2./ Examination of the leukocyte antisedimentation rate (LAR) applicability in animal experiments. Is it applicable to follow the possible asplenic-hyposplenic states following different spleen preserving surgical techniques?

3./ Demonstration of the viability of the spleen autotransplants with diagnostic laparoscopy and with the examinations of the applicability of the colloid scintigraphy performed in parallel.

4./ For the further researches, setting a new imaging method with the adaptation of the human spleen-specific scintigraphy's protocol, for periodic control the viability of the spleen autotransplants.

5./ Histological examinations for the verification of the viability of the spleen autotransplants at the end of follow-up.

3. MATERIALS AND METHODS

3.1. Laboratory animals, anesthesia

Our experiment was divided into two periods, and a total of 31 beagle dogs were involved between 2005 and 2006 (phase I.) and 2006 and 2008 (phase II.). The experiments were approved and registered by the University of Debrecen Committee of Animal Research (permission Nr.: 12/2003., 7/2006., 34/2007. UD CAR)

All the surgical interventions were performed in general narcosis with intramuscular ketamine and xylazine (SBH Ketamin, Produlab Pharma B.V., The Netherlands, 10 mg/kg; Primazin, Alfasan International B.V., The Netherlands, 1mg/kg) combination.

At the end of the follow-up, the anesthetized animals were euthanatized with intracardially administrated potassium-chloride solution (2 mmol/kg). Except 3 dogs, they

were kept alive due to the further follow-up of the splenic functions and the possible introduction of new measuring methods.

3.2. Experimental groups, surgical techniques

The surgical interventions of the involved animals were performed during two consecutive periods, because of the long-term follow-up, and the specific rules of the standard individual cages keeping.

In the I. phase, 15 beagle dogs (9.98±1.67 kg, n=3-3) were involved to the research, the II. phase was followed with 16 beagle dogs (9.41±1.49 kg, n=4-4), both of them with the use of the following experimental groups:

1. *group:* Non-operated control (C) - no surgical intervention.
2. *group:* Sham-operated control (SH) - performed median laparotomy and closure of abdominal cavity in two layers.
3. *group:* splenectomy (SE) - after the upper median laparotomy, the whole spleen was removed, the abdominal wall was closed in two layers.
4. *group:* spleen autotransplantation with 5 spleen-chips (AU5) - autotransplantation with 5 spleen-chips using "Furka's spleen-chip" technique following splenectomy and closure of abdominal cavity in two layers.
5. *group:* spleen autotransplantation with 10 spleen-chips (AU10) - as in the previous group, with 10 spleen-chips

Remark: the non-operated control animals of the I. phase served the values of the same postoperative periods in the II. phase.

The aim of the "Furka's spleen chip technique" is the following: from the removed and healthy spleen parenchyma 5 or 10 chips were made (thickness: 0.5 mm, length: 12-20 mm, width: 8-16 mm) . These chips were rinsed in room temperature physiological salt solution and then the chips were placed between the well vascularised layers of the greater omentum without any fixation, than the omentum was closed. 3/0 non-absorbable coated polyester (Ethibond, Ethicon, Inc., Germany) suture materials were used for the ligation of the vessels during the splenectomy and for the closure of the omentum. 0 nonabsorbable polyamide (Ethilon, Ethicon, Inc., Germany) was used for closing the muscle and peritoneum layers. To close the skin 3/0 absorbable polyglactin 910 (Vicryl, Ethicon, Inc., Germany) was used, to avoid causing additional discomfort for the animals during the procedure of stitch removing.

3.3. Protocol of the pre- and postoperative investigations

For the planning the investigative protocols, the complex sample used in the previous decades was applied. Of course, besides the traditional measurements (I. phase), the investigations were extended with the new available rheological measuring methods (II. phase).

In all cases blood samples were obtained one day prior the operations -as a base-, on the 1st postoperative week and monthly afterwards for 1 year. In the II. phase, during the 2nd postoperative year, the blood samples were collected bi-monthly.

Complex hematological, hemorheological examinations were carried out from the blood samples with supplementary hemostaseological parameters, for the comparison of asplenic-hyposplenic and normal states and to follow the functions of the spleen autotransplants.

For regular, yearly vaccination of the animals and for evaluating the immune response, one year after the operations killed and adjuvanted rabies vaccines (Rabigen mono, Virbac S.A.) and modified live canine distemper-adenovirus type 2-parainfluenza-parvovirus vaccine (Vanguard plus 5, Pfizer Animal Health S.A.) were given to all of the animals. Blood samples were taken before the vaccination (12th postoperative month) and after it on the 1st week from the cephalic vein.

In these points, the previously described protocol was completed with the measurement of leukocyte antisedimentation rate (LAR) too.

At the end of the 1st postoperative year, colloid scintigraphy were performed on some of the I. phase animals, to indentify the spleen autotransplants. The three surviving experimental animals were involved in order to set a new spleen-specific scintigraphy method, after the end of the 4th postoperative year.

At the end of the follow-up (I phase: 12th postoperative month, II. phase: 24th postoperative month) diagnostic laparoscopy was performed to clarify the viability of the spleen autotransplants. After the extermination, comparative histological examinations were performed, too.

3.3.1. Laboratory investigations

After overnight fasting, venous blood samples were obtained in the morning by veinpuncture of the cephalic vein in closed system, with minimal stasis, due to the hemorheological principles. Measurements were carried out at controlled room temperature ($22 \pm 1°C$), as soon as it was possible, within maximum 2 hours after blood collection.

3.3.1.1. Hematological investigations

For the hematological investigations the blood samples were anticoagulated with 1.5 M K_3-EDTA (BD Vacutainer®, Belliver Industrial Estate, UK). The parameters were determined with the Coulter-principles based Sysmex F-800 microcell counter (TOA Medical Electronics Co., Japan).

3.3.1.2. Hemorheological investigations

Among the hemorheological parameters, erythrocyte deformability was measured in two different ways and leukocyte antisedimentation rate was calculated and applied.

3.3.1.2.1. Determination of red blood cell deformability

At the time of the blood sampling, erythrocyte deformability was measured with the method of bulk filtration, in both phases. However, the ektacytometry method was applied only from the 20^{th} postoperativ month of the II. phase, because the required device was obtained at this time at our department.

3.3.1.2.1.1. Bulk filtration method

For filtrometry tests sodium-heparine (143 IU BD Vacutainer®, Belliver Industrial Estate, U.K.) was used as anticoagulant.

Measurements were carried out using Carat-FT1 filtrometer (Carat Ltd, Hungary) based on the St. George's filtration.

From the blood samples 5% red blood cell - PBS suspensions (osmolarity: 295 ± 5 mOsm/kg; pH: 7.4) were prepared and filtrated through 5 μm pore-sized polycarbonate filters (Nucleopore®, Whatman International Ltd., U.K.), at constant filtration pressure (4 cmH_2O). From the filtration profile the interfaced computer calculates the initial filtration rate (IRFR) and the relative cell transit time (RCTT) parameters according to the following formula: $RCTT = (IRFR^{-1} - 1) / Hct) + 1$, where Hct is the hematocrit of the suspension.

3.3.1.2.1.2. Ektacytometry

For the determination of the erythrocyte deformability by ektacytometry tests K_3-EDTA (7.5%, 0.04 ml, BD Vacutainer®, Belliver Industrial Estate, U.K.) was used as anticoagulant. The measurements did not require any sample preparation, such as filtrometry steps, the whole blood was used for the tests.

Rheoscan-D200 slit-flow ektacytometer (Sewon Meditech Inc., South Korea) was used

for the determination of the red blood cell deformability.

The ektacytometry test is based upon the analysis of red blood cell laser diffraction images at various levels of shear stress. Red blood cells -suspended at about 1% Hct in a viscous, isotonic solution of 360 kDa polyvinylpyrrolidone (viscosity=20 Pa.s)- deformation at shear stresses (SS) between 0.5 and 20 Pa was quantified by calculating an elongation index (EI) equal to (L - W)/(L + W) where L is the length and W is the width of the deformed cell, at a constant shear stress.

For the comparison of EI–SS curves Lineweaver-Burke analysis ($1/EI = SS_{1/2} / EI_{max} \times 1/SS + 1/EI_{max}$) was used to calculate the maximal elongation index (EI_{max}) and the shear stress at half maximal elongation index ($SS_{1/2}$).

3.3.1.2.2. Determination of leukocyte antisedimentation rate

For the sedimentation tests the blood samples were anticoagulated with 0.109 M sodium-citrate (BD Vacutainer®, Belliver Industrial Estate, U.K.).

To determine the LAR, the upper and the lower part of the sedimentation blood column were gently separated and the white blood cell count was measured in both column parts after one-hour gravity sedimentation, with Sysmex F-800 microcell counter.

LAR [%] was calculated after Bogar's formula: LAR = 100 × (WBC upper – WBC lower)/(WBC upper + WBC lower).

3.3.2. Imaging tests

All of the scintigraphic interventions were performed in general narcosis with intramuscular ketamine and xylazine (SBH Ketamin, Produlab Pharma B.V., The Netherlands, 10 mg/kg; Primazin, Alfasan International B.V., The Netherlands, 1mg/kg) combination, like the surgical investigations.

3.3.2.1. Colloid scintigraphy

In the 12th postoperative month colloid scintigraphy was performed on 3 animals: one in sham-operated and spleen autotransplanted animals with 5 or 10 spleen-chips. 80-110 MBq ^{99m}Tc labeled sodium phytate (FYTON®, Institute of Isotopes, Budapest, Hungary) was administrated via the cephalic vein under general narcosis. After 20 minutes, SPECT acquisition was started by a Cardio-C gamma camera (Mediso Ltd., Hungary) in "step and shoot" mode with 3 degrees steps.

The colloid was phagocytized by the cells of the reticuloendothelial system; therefore

increased activity was expected in the liver and spleen as well as in the spleen autotransplants. The reconstructed distribution was presented in "browser view": visualizing the transaxial, sagittal and coronal slices through a selected 3-D point. The investigations were performed at the Department of Nuclear Medicine.

3.3.2.2. Spleen-specific scintigraphy

The protocol is based on the human spleen-specific scintigraphy and was modified with the required changes in order of the application in canine model. The labeling process was performed in the Department of Nuclear Medicine.

Images by a two-headed gamma camera (AnyScanSC, Mediso Ltd, Hungary) were started with SPECT acquisition (detectors were set to 180°) with 5 degrees steps, 30 minutes after the injection of the labeled red blood suspension. The reconstructed distribution was presented in "browser view": visualizing the transaxial, sagittal and coronal slices through a selected 3-D point.

3.3.3. Laparoscopic investigations

Diagnostic laparoscopy was performed in narcosis at the 12^{th} postoperative month on one part of the experimental animals of the I. phase, and at the 24^{th} month on one part of the experimental animals of the II. phase. We observed the abdominal state to identify the spleen autotransplants and to check the possible adhesions.

3.3.4. Histological investigations

Comparative histological investigations were performed on the removed spleens of the splenectomy group and in the $12^{th}/24^{th}$ postoperative month -at the end of the investigations- on the spleen-autotransplants.

3.3.5. Statistical analysis

The numerical data of the laboratory tests was presented in the form mean ± S.D. Statistical analyses were carried out using Mann-Whitney rank sum tests and one-way ANOVA on ranks tests (Dunn's method) for inter- and intra-group comparisons.

Results of the leukocyte antisedimentation rate were presented as means ± standard error (S.E.). Concerning the low case number, as a limited statistical analysis Mann-Whitney rank sum test was used.

The significance level was set at $p < 0.05$

4. RESULTS

4.1. Results of the laboratory investigations

4.1.1. Hematological investigations

The planned hematological investigations were performed as part of the investigative protocol, but it is not intended to present the detailed analysis of the data separately. The parameters used to characterize the red blood cells in the chapter of the deformability, the white blood cells related parameters in the chapter of the LAR were presented with their correlations too.

4.1.2. Hemorheological investigations

4.1.2.1. Red blood cell deformability

The results of the red blood cell deformability were presented from both phase together. Filtrometry results of the 2^{nd}, 4^{th}, 6^{th}, 9^{th}, 12^{th}, 20^{th}, and 24^{th} postoperative months were presented, ektacytometry data were analyzed only at the 20^{th} and 24^{th} postoperative month, when the equipment was purchased.

The ektacytometry healthy control data were obtained from the department's database. This was collected from all incoming experimental animals (n=12) performed basic hematological and hemorheological tests.

4.1.2.1.1. Bulk filtration method

During the experiments, the changes of the relative cell transit time (RCTT) was the following:

On the 2^{nd} postoperative month RCTT values were similar in all groups, showing a slight increase, compared to the initial, base values.

On the 4^{th} postoperative month RCTT did not change in the sham-operated group compared to previous months, but markedly increased in the splenectomy and in both of the autotransplantation groups. This increase was significant compared to base and sham group's values.

On the 6^{th} postoperative month RCTT level of the sham-operated group decreased to the base level. In the splenectomy group hardly changed the previously observed high values, but a greater moderation was found, that in the case of the 10 spleen-chips autotransplantation group was most pronounced. Both of the autotransplantation groups presented lower values, than the splenectomy group.

On the 9th month the sham-operated control group showed no significant changes and minor decrease was seen in the splenectomy group compared to the previous month. A slight increase was observed in both of the autotransplantation groups. The values of the autotransplantation with 10 spleen chips group almost reached, and the autotransplantation with 5 spleen-chips group exceeded the level of the splenectomy group.

On the 12th postoperative month, the sham-operated group presented a significant rise. The value of the autotransplantation with 5 spleen-chips group did not change, but it was lower, than the value of splenectomy group. The autotransplantation with 10 spleen-chips group showed a greater decrease compared to the previous presented values, and reached the level of the sham-operated control group.

On the 20th postoperative month the sham-operated control group didn't show great changes and in the splenectomy group the RCTT was still significantly higher than the base level. The values of the autotransplantation groups were close to the sham operated control's level.

On the 24th postoperative month, these differences became more remarkable. The splenectomy groups didn't show any changes compared to the previous values, but a further decrease was observed in the autotransplantations and sham-operated groups. The values of this last three groups remained almost the same, contrary to the very high RCTT values of the splenectomy group.

Summarily, the splenectomy group presented high RCTT levels during the experiments, while in the autotransplantations groups a decreasing was observed with mold fluctuation. Furthermore, from the 6th postoperative month the values of the autotransplantation groups approached the level of the sham-operated control. These changes had resulted in, than on the 12th postoperative month the autotransplantation with 10 spleen-chips group reached level of the sham-operated group, and the autotransplantation with 5 spleen-chips group reached it on the 20th postoperative month. From the 12th postoperative month a significant difference was seen between the splenectomy and autotransplantation group: in the first group higher values were observed but the other two groups was closer to the sham-operated group.

4.1.2.1.2. Ektacytometry

The results of the ektacytometry measurements were carried out with the comparison of the maximal elongation index (EI_{max}) and the shear stress at half maximal deformation ($SS_{1/2}$), and the elongation index-shear stress curves were analyzed too.

On the 20th and 24th postoperative months, the elongation index-shear stress curves in splenectomy group showed unusual characteristic compared to autotransplantation groups. Although the slope of the curves was slightly higher in the splenectomy group compared to autotransplantations groups, over 5-6 Pa shear stress it was close or lower than the autotransplantation groups' curves. The elongation index at 3 Pa shear stress was significantly lower in splenectomy and in both autotransplantation groups compared to the sham-operated animals in both measuring months.

The calculated EI_{max} was the lowest in splenectomy group in both months. These differences were significant compared to the sham-operated and healthy control groups, and on the 20th postoperative month were significant compared to the autotransplantation with 10 spleen-chips group too. The values of the sham-operated control were similar to the healthy control group's level, the spleen autotransplantation groups showed a little lower than that values, but really higher than the splenectomy group's.

The results of the $SS_{1/2}$ values were also similar in both measuring time. The lowest level was observed in the splenectomy group, which difference was significant contrary to healthy control and autotransplantation with 10 spleen-chips group, in the 20th postoperative month. There was also a significant decrease between the two autotransplantation groups in the 24th postoperative month. In both measuring points, autotransplantation groups showed almost the same values as the control groups.

4.1.2.2. Leukocyte antisedimentation rate

There was no data in the literature about the normal range of the leukocyte antisedimentation rate. To determine the normal level, 10 healthy beagle dogs (12.1±1.243 kg) were involved to the measurements. The LAR was evaluated by the white blood cell count.

In the healthy control group the leukocyte count was found between 8.0 and 16.8 × 10^3/μl (average: 12.04±0.56×10^3/μl). The LAR values varied between -3.8% and 10.3% (average: 3.11±1.18%).

One year after surgery and just before vaccination the leukocyte count in the splenectomy group (10.9±0.74×10^3/μl) was close to the values of the sham-operated group (11.27±0.4×10^3/μl). Higher counts were found in the autotransplantation groups (AU5: 15.63±0.78×10^3/μl; AU10: 14.27±0.98×10^3/μl), but within the normal range.

After vaccination the sham-operated control (10.74±0.41×10^3/μl) and autotransplantation with 5 spleen chips (16.08±0.34×10^3/μl) groups showed no significant changes. The

splenectomy group showed a slightly increased, while the autotransplantation with 10 spleen-chips group showed a decreased leukocyte count (SE: 12.23±0.66×10^3/μl; AU10: 12.41±0.48×10^3/μl) compared to the state before vaccination.

Before vaccination, the lowest LAR value was found in the sham-operated group (–0.21±1.09%), The LAR values were a slightly higher, but nearly similar in the autotransplantation groups and the splenectomy group (SE: 7.06±2.43%; AU5: 7.05±2.85%; AU10: 7.31±2.93%). All of the values were in the normal range observed in the healthy control group.

After vaccination the LAR increased in each group but in different manner. Although SE group expressed the largest changes and the highest LAR values (16.56±6.4%), the total leukocyte count of native blood samples increased only slightly. In autotransplantation groups, a not significant increase was observed, the LAR values were smaller than in splenectomy group (AU5: 13.34±3.95%; AU10: 12.62±5.62%).

4.2. Results of the imaging investigations

4.2.1. Colloid scintigraphy

In the 12th postoperative month colloid scintigraphy was performed on 3 animals of the I. phase: one from sham-operated group and one-one from spleen autotransplanted with 5 or 10 spleen-chips groups.

The colloid scintigram of the sham-operated control animal (experimental animal: SH-3 [99]) showed the highest activity in the liver, in all three plane of the imaging slices. The spleen accumulated low activity in the usual anatomical location.

In the experimental animal (experimental animal: AU5-3 [110]), which underwent to spleen autotransplantation of 5 spleen-chips, there was no activity accumulation in the spleen transplants, only in the parenchyma of the liver.

The scintigram of the experimental animal with 10 autotransplanted spleen-chips (experimental animal: AU10-2 [128]) also showed the highest activity in the liver, in all of the imaging planes. However, focal increased activity accumulation could be visualized corresponding to the reticuloendothelial cells in the spleen autotransplants. The enhancements -defined as spleen autotransplants- were clearly observed on the scintigrams.

4.2.2. Spleen-specific scintigraphy

The spleen-specific scintigraphy was performed on the previously presented three survival experimental animals of the I. phase, at the 56th postoperative month. The

autotransplanted animals were the ones previously examined with colloid scintigraphy.

All scintigrams of the sham-operated control animal (experimental animal: SH-2 [123]) showed the highest activity in the liver in the normal anatomical location

In the experimental animal (experimental animal: AU5-3 [110]), which underwent to spleen autotransplantation of 5 spleen-chips, scans failed to identify relevant activity for the spleen autotransplants. Enhancements were observed in the region of the thoracic organs and large vessels.

The scintigrams of the experimental animal with 10 spleen-chips (experimental animal: AU10-2 [128]) showed a large, connected area with a high accumulation. Moreover, expressed enhancements suggested well-functioning spleen autotransplants in the abdominal region.

4.3. Results of the laparoscopic investigations

In the I. phase, on the 12th postoperative month diagnostic laparoscopy was performed on the same animals -sham-operated, autotransplantation with 5 or 10 spleen-chips-, which were underwent to the scintigraphic investigations.

The diagnostic laparoscopy in the sham-operated control animal (experimental animal: SH-3 [99]) showed well the spleen in average size, and being situated in the usual anatomical position. There was no considerable adhesion in the abdominal cavity.

In the 5 spleen-chips autotransplanted animal (experimental animal: AU5-3 [110]) the diagnostic laparoscopy found some of the spleen autotransplants between the layers of the omentum. In the abdominal cavity there were no several adhesions, like in the previous animal.

In the animal that underwent autotransplantation with 10 spleen chips (experimental animal: AU10-2 [128]), all of the replanted spleen chips were found during the diagnostic laparoscopy. The own blood supply of the autotransplants were observed. We found no significant adhesion in this case, too.

In the II. phase, diagnostic laparoscopy was performed in the 24th postoperative month on all of the autotransplanted experimental animals, and on 2-2 animals from the splenectomy and sham-operated control groups.

Except one animal, the diagnostic laparoscopy found all of the spleen autotransplants in the autotransplanted animals and there were no several adhesions in the abdominal cavity.

The investigations of the experimental animals' autotransplanted with 5 spleen-chips showed all of the spleen autotransplants, except that indexed case. Most of the autotransplants

were well regenerated, only in one experimental animal (experimental animal: AU5-3 [40]) 2 spleen autotransplants were mildly athrophized. However, close to these athrophized spleen autotransplants one specifically well regenerated spleen autotransplants was observed.

In three cases of the 10 spleen-chips autotransplanted animals all of the spleen autotransplants were found with advanced blood supply. In one experimental animal (experimental animal: AU10-4 [32]) only 8 spleen autotransplants were observed, and all of them showed a moderate atrophy.

Splenosis was identified in one-one experimental animal of both autotransplantation groups (experimental animals: AU5-1 [41], AU10-2 [35]).

4.4. Results of the histological investigations

The pictures of the histological investigations of removed and intact spleen with normal follicular and trabecular structure.

On the section of the autotransplants removed at the end of the 12 month follow-up we observed well formed follicules, slightly hemorrhaged red pulp and little disorganized trabecular structure were seen with the magnification 40X. The pictures made with higher magnification showed the similar organization.

The regenerated spleen autotransplants' histological structure was similar with the intact spleen's structure.

5. DISCUSSION AND CONSEQUENCES

Despite of the modern chemo- and immunoprophylaxis, the possible complications of splenectomy are still a great problem. Spleen preserving methods play an important role in preventing these complications.

This inbred canine model has allowed us to set a complex investigative protocol, which helps to examine the asplenic-hyposplenic states with the related changes and the possible late complications too.

Our results re-confirmed that red blood cell deformability was significantly impaired after splenectomy even 2 years after surgery, opposite with the states after spleen autotransplantation. The measurements were obtained efficiently by filtrometry and ektacytometry in parallel.

We found that after vaccination LAR increased variously in the different experimental groups. The results suggest that LAR can be applicable for the investigation of the immune response after vaccination in asplenic-hyposplenic states.

In the autotransplanted animal the colloid scintigraphy showed activity in the region of the greater omentum, which could be defined as spleen autotransplants.

The human spleen-specific scintigraphy method was successfully adopted in our beagle canine model. In the autotransplanted animal, the replanted spleen chips were identified clearly, confirming the phagocytic function of the autotransplants

During diagnostic laparoscopy no considerable adhesions and no abscess were found, most of the spleen autotransplants were observed.

The histological structure of the regenerated spleen autotransplant was similar to the intact spleen's structure.

6. SUMMARY OF THE NEW RESULTS

1./ Successfully used the filtrometry and the slit-flow ektacytometry simultaneously to follow the filtration functions of the spleen autotransplants. Our working group was the first, to use the slit-flow ektacytometry to examine the splenic functions. The parallel investigations with the two different method proved, that spleen autotransplantation could partly restore the lost of the splenic filtration function related to the splenectomy.

2./ Our results suggest that LAR can be applied in animal experiments too. It may serve as a supplementary parameter in hemorheological studies for investigation asplenic-hyposplenic states. There was no data or paper in the literature with these findings.

3./ The diagnostic laparoscopy confirmed the viability of the spleen autotransplants, and supported the results of the colloid scintigraphy.

4./ For the further experiments, the spleen-specific scintigraphy was adopted to experimental model in first time. With this, the functioning splenic tissue will identify more sophisticated, and the amount of it could be determined too.

5./ At the end of the follow-up the histological investigations confirmed the viability of the spleen autotransplants, because the histological structure of the "spleen pieces" were similar to the normal spleen's structure.

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Korea-Aust. Rheol. J. 21 (2), 127-133, 2009.
IF:0.965

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