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Results of experimental development of a laparoscopic method for auto transplantation of splenic tissue under a liver capsule method

[1]Anna G. Dynnik, [2]Igor I. Babich

ABSTRACT:

Despite the fact, spleen considers to be an important immunomodulating organ, splenectomy continues to be a primary tactic during extensive spleen injuries. Modern laparoscopic technologies force us to look for new low-invasive splenic autotransplantation methods. Meanwhile, the momentous aspect of such invasion is topographical and anatomical transplantation zone choosing, which allows maintaining the physiological connection between portal blood-flow and neo-splenic tissue. 47 experimental animals were included in exploration with the following steps of the study: experimental modeling laparoscopic splenic autotransplantation, dynamic clinical indicators monitoring of animals, experimental animal removal with further histological evaluation of neo-splenic tissue zone in the liver area. Clinical and histological data were evaluated on 7th, 15th, 30th and 60th days after surgery. Histological examination was provided by hematoxylin-eosin staining, magnification: eyepiece W-PL 10x / 23; A-Rlan 100x / 1.25 lens. Statistical data processing was performed using the program Statistica v.6.0. Clinical indicators normalization in the main group was observed by the 15th day. Absence of clinical blood parameters statistical difference between healthy animals and animals after surgery was observed by the 30th day ($P<0.05$). The histological structure of the neo-splenic tissue and spleen of intact rabbits is identical, by the 60th day. Our experiment showed the feasibility of further splenic autotransplantation investigation and implementation of this method in endoscopic treatment of abdominal organs traumatic injuries.

KEYWORDS: *spleen, splenectomy, splenic autotransplantation, laparoscopy, liver.*

I. INTRODUCTION:

Splenic autotransplantation (SAT) actuality:

The contemporary condition of spleen knowledge induces plenty of surgeons around the world to find ways of lien preservation in extensive damage cases instead of splenectomy[5,8,13]. Nevertheless, due to the portal blood flow isolation, existing splenic autotransplantation (SAT) techniques do not fully preserve the splenic

[1] Rostov Regional Children's Hospital, Doctor Pediatric Surgeon, Rostov-on-Don, 344015, Russian Federation.
[1] Rostov State Medical University, Doctor of Medical Sciences, Professor, Rostov-on-Don, Russian Federation

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tissue functional activity[3,15]. Appropriately, modern surgical tactics focus on non-surgical spleen damage treatment. Focused on minimal surgical trauma, laparoscopy and organ-preserving surgery become the surgery of the choice due to the high risk of the life-threatening condition developing during conservative treatment [1,4,6].

Splenectomy features:

The most serious complication after post-traumatic splenectomy is the development of an overwhelming post-splenectomy infection (OPSI), manifested by infection fulminant form and constant immunosuppression in the future. Compensatory lymphoid hyperplasia leads to different types of viral lymphadenopathy development. The average life expectancy of a patient who underwent splenectomy in childhood is 51 years[3,8].

Numerous methods of postoperative hyposplenism syndrome development prevention in compulsory splenectomy cases are described. All splenic autotransplantation methods have different indications, different efficacy, but all of them serve as iatrogenic splenosis optimization[2].

SAT techniques:

Most of such methods involve splenic tissue transplantation outside the portal bloodstream system. There are different recommendations of transplant attachment: greater or lesser omentum, greater curvature of the stomach, small and large intestine mesentery, fibers of the muscle, etc., which does not give information of neo-splenic tissue activity[4, 9, 11, 14, 15].

Neo-splenic tissue vs portal blood flow:

Some authors propose methods that include neo-splenic tissue in the portal blood flow system, which preserves the full value of the preserved organ[4]. After splenic autotransplantation into v.portae pool greater mortality in distant periods of experimental animals associated with embolization and thrombosis of liver vessels was observed. However, the structure of the newly formed splenoids was similar to the spleen structure [15]. Clinical and laboratory picture of experimental and control groups of rats had no difference which confirmed liver transplantation suitability assumption[11].The given transplantation method shows similarity to our research where the formation of splenoids happening directly in the liver. Still, this method is applicable only in experimental conditions due to high mortality and technical complexity. The difficulty of v.portae access will significantly complicate laparoscopy and lengthen surgery time which can negatively affect the patient's condition, as well as letal long-term outcomes, do not allow the method to be used in humans[2,4].

Liver techniques:

There is a technique of big-sized (1.0 * 5.0 cm) splenic fragments transplantation on the liver diaphragmatic surface with special glue. There is no adequate revascularization of the autograft in such conditions. There is no clinical efficiency of such a method due to almost complete transplant necrosis with subsequent regeneration ischemic tissues collateral formation[11].

Transplantation of spleen cells into a round liver ligament was examined in a pig experiment. In humans, this method requires a very small quantity of graft due to the small size of ligamentum. Taking into account complete obliteration of the umbilical vein during the first two months of life, the functioning of the

splenic autotransplant is completely turned off from the portal circulation. At the same time, adequate revascularization of the splenic tissue homogenate and the viability of all structural elements are doubtful[10].

Another transplantation way is proposed for combined liver and spleen damage. Liver wound tamponade by spleen tissue is happening with fixation by absorbable suture material. Among the method's shortcomings, necrosis of the graft's central part and wide laparotomic access can be noted. There is no necessity for liver wound simulation while spleen wound is isolated[7].

Laparoscopy interventions:

Currently, the list of surgical interventions performed using laparoscopic access is becoming wider. Nowadays, low-invasive technologies are using since the neonatal period and virtually have no contraindications. The advantages of laparoscopy compared with laparotomy approaches are well known around the world, also laparoscopy is justified both clinically and economically.

Laparoscopy method decreases surgical time intervention, access completely examine the abdominal cavity, and carries out thorough hemostasis with minimal organs contact. All of these advantages minimizes the number of complications[8,10,14].

We need to find more improved ways of organ-preserving during the transplantation which also will help to provide adequate conditions for further functioning.

II. MATERIALS & METHODS:

Method features:

We used 47 experimental animals- male albino rabbits, with 3500 ± 100g mass. Under general anesthesia, we developed a new method for splenic autotransplantation under a liver capsule.

Animals are divided into 2 groups. The first group (main group, n = 24) had surgery according to the current method, the second (control group, n = 23) had surgery according to the previously used technique of splenic autotransplantation by using of a large splenic tissue fragment with further fixture of it to a simulated liver wound with additional fixation with U-shaped sutures. For greater statistical validity, both methods were performed laparoscopically.

Received data were analyzed using descriptive statistics and presented as the mean and standard error of the mean; a significant difference was revealed by the Student's parametric criterion with Bonferroni correction. Differences between groups at $P < 0.05$ were regarded as significant.

Steps of the developed method in the main group of animals:

After anesthesia induction of main group animal (n=24), following fixation and disinfection of the anterior abdominal wall were performed with further working trocars installation.

In the place of the estimated navel, the video port was installed. After carboxyperitoneum application up to 4 mm. a ligature was introduced under the xiphoid process, where the spleen was visualized by traction

behind the stomachs' bottom. Immobilization, ligation of the spleen was performed, the organ was removed from the abdominal cavity. The stomach returned to its physiological position.

The spleen was shredded. A homogenate was prepared by adding an isotonic solution in a 1: 1 ratio and thorough mixing. 1/20 of the obtained material was taken into the injection system. Through an additional puncture in the right anterolateral surface of the anterior abdominal wall, the splenic homogenate was administrated under the capsule of the right lobe of the liver anterior surface (Figure 1). After the main process of the surgery ends, the abdominal cavity was examined for bleeding, desufflated, working trocars were removed, internal sutures for postoperative wound were implemented. The total surgery duration was 20 minutes.

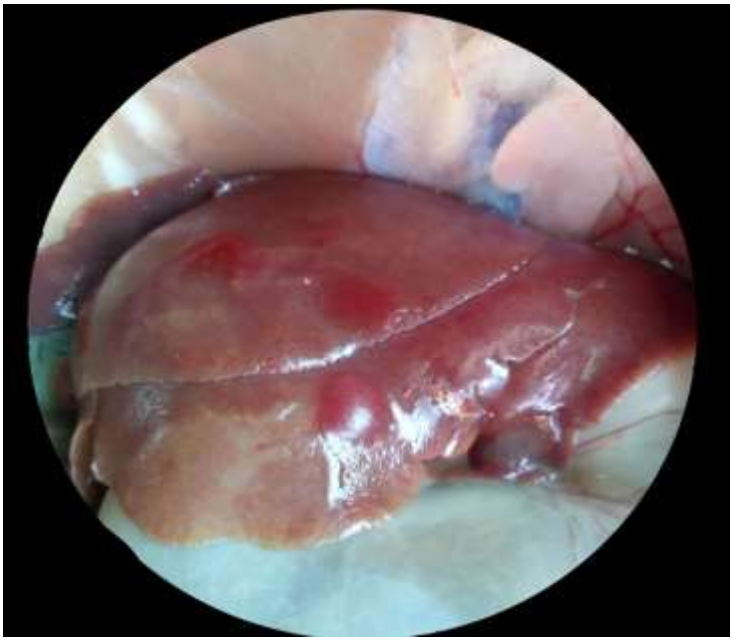

Fig. 1: liver after subcapsular splenic homogenate administration

Control group's steps:

The Control group of the animals (n = 23) received surgery from similar accesses. After splenectomy and liver linear wound modeling, wound tamponade was performed as an integral fragment of decapsulated splenic tissue with liver wound fixation by U-shaped sutures. The abdominal cavity is drained, desufflated with further trocar removal. Internal sutures on the wounds were applied. The total duration of the operation was 30 minutes.

Rabbits of both groups received Paracetamol suspension at a dose of 10 mg/kg, the after-surgery awakening of animals -independent, within 30 minutes after the operation; full water and food access were provided. Postoperative wound disinfection with antiseptic solutions in the postoperative period was performed for 3 days.

Further observation included animal behavior assessment, clinical and laboratory changes, sampling of liver areas with parts of splenic transplantation zone on the 7th, 15th, 30th, and 60th days after surgery by removing animals from the experiment in equal numbers from both groups.

III. RESULTS:

In the main group, on the 7th day, leukocytosis with a moderate shift to the right, hypoalbuminemia, bilirubin reached the upper limit of normal values were observed. Histologically, a slightly expressed exudative inflammation was observed, manifested by its infiltration by macrophages, lymphocytes, large mast cells without pronounced degranulation (Figure 2).

The control group showed more denominated leukocytosis and hyperbilirubinemia, a moderate hepatic transaminases elevation to 10% of the upper reference values in rabbits. The difference in the groups was P> 0.1, which was estimated as significant. During the histological evaluation, exudative inflammation of graft border in the control group was more expressed comparing with the main group represented by a large number of macrophages, leukocytes and mast cells with severe degranulation.

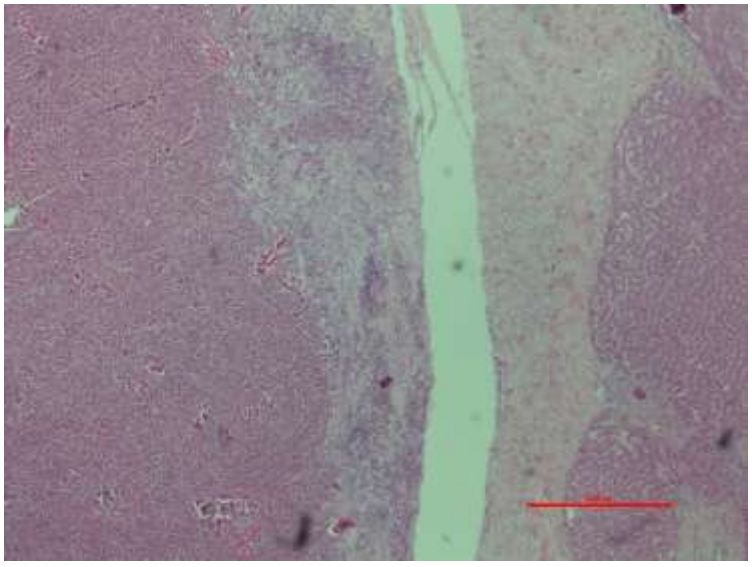

Fig. 2: A liver area with splenic autotransplantation zone on the 7th day

On the 15th day, general clinical indicators in the main group were within normal limits. For that moment, SAT zone was not determined with clear borders, between the sinuses of the red pulp filled with red blood cells and plasma, newly formed bile ducts were determined. Periarteriolar couplings were absent, small monomorphic ones appeared - immature, "embryonic" lymphoid follicles were detected. Control group blood parameters were within normal reference values; P≤0.05, which was regarded as an acceptable deviation. Micropreparations of this group showed necrosis of the central zone with a clear determination of capsule borders.

Absence of clinical blood parameters statistical difference between healthy animals and animals after surgery was observed by the 30th day (P<0.05). Histologically, closely located lymphoid sinuses consolidation tendency was confirmed, mainly of the white pulp, the leukocyte infiltration absence. During this period of the experiment, the SAT slightly increased in size, the red pulp with full-blooded sinuses was quite developed, with a small number of leukocytes addition; the white pulp was represented mainly by small, immature, rarely located lymphoid sinuses. Laboratory indicators showed no significantly important deviations (P<0.05). The histological picture had substantial differences- neo-splenic tissue 1.5-2 times smaller than the initial one; follicles of significant size without germinal centers appear; connective tissue trabeculae grow from the side of the capsule, center of the graft unpure (Figure 3).

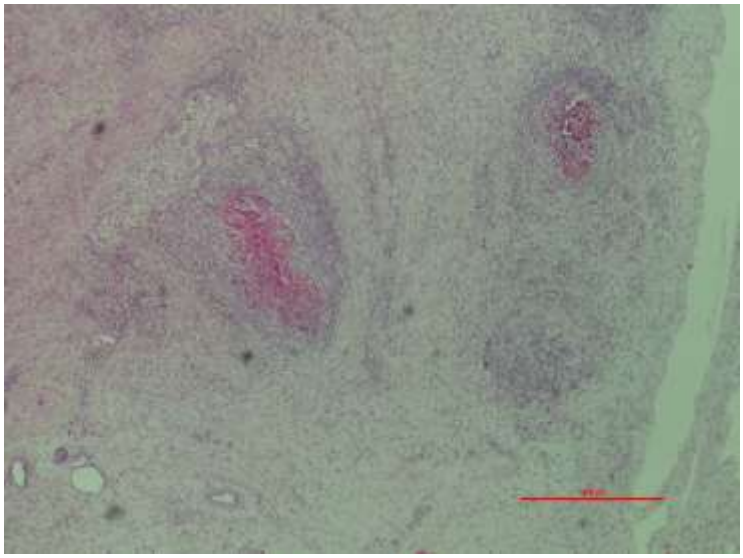

Fig. 3: SAT zone on the 30th day, the main group

On the 60th day, there were no statistically significant differences in the general clinical indices of the experimental animals of both groups (P <0.05). During this period the size of neo-splenic tissue compared with the SAT increased twice; large vessel germination from the liver side is observed. The histological structure of neo-splenic tissue is identical intact rabbit spleen. The neo-splenic tissue capsule is presented on the liver border only by the serous membrane; fibroblasts, collagen, and elastic fibers, a small number of smooth muscle cells were determined from the liver capsule side[16]. While the control group graft was surrounded by a mature connective tissue capsule, with moderate exudative inflammation; full-blooded sinuses containing unlysed red blood cells mixed with leukocytes were determined; fixed macrophages in the sinuses walls (Figure 4).

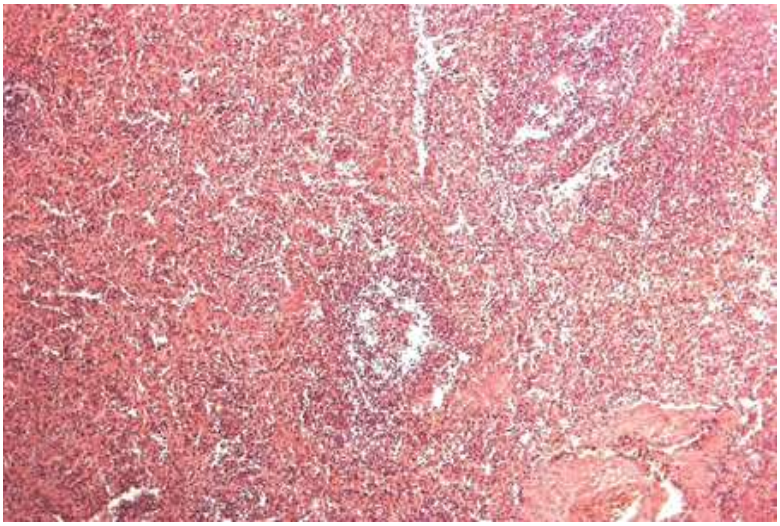

Fig. 4: Neo-splenic tissue on the 60th day, the main group

IV. DISCUSSION:

The modern surgical approach to organ-preserving technologies using necessitates the research for new ways of heterotopic autologous transplantation in cases of spleen injuries conservative treatment impossibility. Multiple SAT methods have been developed and applied. However, the emerging knowledge of the spleen functional activity forces us to reconsider the feasibility of many methods due to the exclusion of neo-splenic tissue from the portal blood flow system. Plenty of authors, during their experiments, proposed different spleen transplantation methods including transplantation into the liver, portal vein or round liver ligament. All of these methods have different insufficiency. In the generality of SAT cases, authors note the formation of neo-splenic tissue through the process of reverse development of necrosis, histological signs of inflammation persist for a long time, which significantly lengthens the process of graft recovery[6,7,10,12]. The methods of inflammation and necrosis morphological manifestations elimination, unfortunately, are feasible only in the experiment and cannot be applied in practical public health due to a large number of complications. The homogenate puncture into the round ligament of the liver splenic autotransplantation, developed in the experiment, is advisable only for patients with portal hypertension due to the umbilical cord vessels complete obliteration after birth in healthy patients. Existing methods of heterotopic splenic autotransplantation, preserving the connection between neo-spleen tissue and portal blood flow, imply wide laparotomic access, which further aggravates the patient's condition[13].

SAT and laparotomy combination:

Our study illustrates the absence of the main complications encountered wide laparotomy using, such as pyemic complications from postoperative wounds and the abdominal cavity. It should be noted the fast recovery period of experimental animals and the normalization of clinical blood parameters after splenic autotransplantation according to the developed technique[17].

We found that the proposed method allows creating conditions for the neo-splenic tissue regeneration without the formation of extensive necrosis zones, prolonged inflammation, and a thick capsule formation. Our method used to preserve spleen tissue elements and choice of graft localization was topographically and functionally justified as primary blood supply and the neo-splenic tissue full functionality resumption with further inclusion into the portal blood flow system.

Among the privileges of laparoscopic access: a relatively small surgical invasion, which favorably affects the early postoperative period course and prevents adhesive disease development.

V. CONCLUSION:

Thus, the experimental data quite convincingly indicate the possibility and necessity of using the proposed developed method of splenic autotransplantation under the capsule of the liver in conditions of endoscopic correction of abdominal traumatic injuries in compliance with all the technical characteristics of the developed method.

CONFLICTS OF INTEREST:

The authors declare no conflicts of interest.

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