JCTEI
JOURNAL OF CLINICAL TRIALS AND EXPERIMENTAL INVESTIGATIONS
Year: 2023 Volume: 2 Issue: 3

ORIGINAL ARTICLE

10.5281/zenodo.8342027

The morphological and functional rationale for the potential compensatory role of disseminated postsplenectomy splenosis in an experiment

Sergey Klyuev[1] - Sergey Gorodkov[1]

1. Department of Pediatric Surgery, Urology, and Andrology, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia.

Correspondence
Sergey Klyuev, Department of Pediatric Surgery, Urology, and Andrology, Saratov State Medical University named after V. I. Razumovsky, Saratov, Russia.

e-mail
proklyuev@gmail.com

Received: 10 June 2023
Revised: 29 June 2023
Accepted: 1 July 2023
Published: 12 September 2023

Keywords
- Asplenia
- Experiment
- Hyposplenism
- Postsplenectomy sepsis
- Splenosis

ORCID ID of the author(s):
SK: 0000-0002-3217-6874
SG: 0000-0001-9281-6872

Abstract

Objective: To investigate disseminated post-splenectomy splenosis (DPSS) by creating an experimental model and analysing the timing, frequency, morphological features, and functional capabilities of splenosis nodules.

Materials and methods: The experiment involved surgical modelling of DPSS in white laboratory rats and a sham operation group. The rats were euthanized at different time points after the surgery, and the DPSS foci were examined histologically. Functional assessments were conducted by evaluating phagocytic parameters and morphological examination of erythrocytes.

Results: DPSS foci were observed in the majority of rats (79%) at various time points after the surgery. The foci appeared as dark cherry-coloured round formations of different sizes and were commonly found on the greater omentum, stomach serous membrane, colon serous membrane, and root of the mesentery. Histological examination revealed a cell composition similar to the spleen, including white pulp components and a high number of plasma cells. However, the typical histological architecture of the spleen was not preserved in the DPSS nodules. The phagocytic index and phagocytic number were within normal range in rats with DPSS after 30 days, indicating normal phagocytic activity. However, after splenectomy, these parameters were lower compared to the DPSS group. The opsonic index was significantly below normal levels in the early stages after splenectomy but reached normal values later on. Morphological examination of erythrocytes showed poikilocytic deviations and increased Howell-Jolly bodies, indicating inadequate utilization of degenerative forms of erythrocytes by DPSS nodules.

Conclusion: The DPSS showed partial functional activity in experiment, including phagocytic capabilities, although the histological architecture of the spleen was not fully preserved. The study provides valuable insights into the characteristics of DPSS nodules and their potential functional role

Cite as: Klyuev S, Gorodkov S. The morphological and functional rationale for the potential compensatory role of disseminated postsplenectomy splenosis in an experiment. J Clin Trials Exp Investig. 2023;2(3):173-180.

Introduction

Disseminated post-splenectomy splenosis (DPSS) is characterized by the ectopic implantation of splenic tissue fragments at multiple sites throughout the body. It occurs as a result of spontaneous post-traumatic implantation of splenic tissue, which manifests in the form of nodules (1-8).

Numerous case reports and case series have documented the various locations where DPSS nodules can be found (3-6). However, there is still uncertainty regarding whether these implanted tissues retain the functional characteristics of the spleen, which is crucial to fully understand their potential for partially compensating for the loss of the organ. The significance of spontaneous DPSS for the body, as well as splenic autotransplantation, raises questions among many researchers and requires further investigation. In some cases, splenosis nodules can lead to acute intestinal obstruction and intra-abdominal bleeding (7). However, the optimal approach regarding the removal or preservation of splenosis nodules in such cases remains uncertain, as some authors believe that DPSS can potentially correct immune disorders following splenectomy (1,2,7). Due to contradictory findings in the literature, the creation of an experimental model was necessary to investigate the detailed characteristics of splenosis nodules.

The aim of this study was to develop model DPSS in an experiment, investigate the timing and frequency of occurrence, study the morphological features of disseminated splenosis nodules and its functional capability.

Materials and methods

The study covered multiple aspects, which included initial experimental surgical modelling of DPSS along with a sham operation group. Furthermore, it involved an investigation into the morphology of DPSS foci and conducting functional assessments. Specifically, the phagocytic indexes were analysed and abnormal forms of red blood cells were counted to evaluate the functional characteristics of splenosis nodules, comparing them with a control group. All experimental procedures involving animals were approved by the relevant institutional animal ethics committee (protocol No: 20092014 – 31) and performed in accordance with the principles set out in the "Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986).

Experimental model of DPSS and morphological study of splenosis foci

To create an experimental model of postsplenectomy splenosis, 36 white laboratory rats of the "Wistar" line, aged 1 month, were used. The sham surgery was performed on the other 10 rats of the control group. After giving inhalation fluorothane anesthesia and antiseptics wash all rats underwent a "cut-window" in the left hypochondrium, approximately 1 cm in length, then splenectomy was performed (**Figure 1**). Several deep incisions were made in the removed spleen using a scalpel in a Petri dish, and the blood, without obvious tissue elements, was drawn back into the free abdominal cavity using a syringe. The wound was sutured. Antibacterial therapy was not carried out in the postoperative period. Thus, the conditions and mechanisms for activating the growth of "extra-splenic" tissue of splenosis were most similar to those that occur in human spleen injury accompanied by bleeding from ruptured pulp and splenectomy.

To determine the initial moment of possible macroscopic visualization of DPSS foci, it was decided to withdraw animals (by using overdose of isoflurane) from the experiment at 25, 30, 47, 57, 63, 79, 85, 99, and 128 days after the operation, and then autopsied. The foci of the presumed splenosis were fixed in a 10% neutral formalin solution. Microtome sections were prepared by the standard method and stained with hematoxylin-eosin. When examining the resulting histological preparations, the following were evaluated: the correspondence of the data of the foci to the structure of splenic tissue, their structure, type of blood supply, severity of white pulp follicles and venous sinuses, cellular composition.

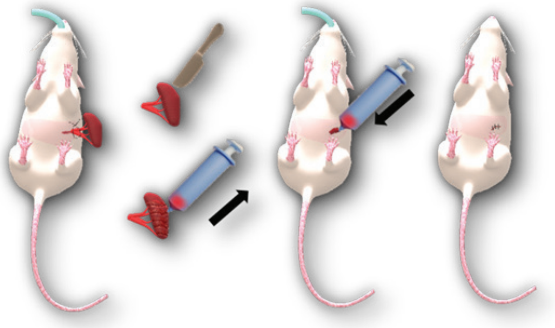

Figure 1: Modelling of DPSS in experiment

Experimental study of functional parameters of DPSS

To study the functional capabilities of focal DPSS lesions, another 30 white Wistar rats aged 3 months and weighing 150-200 g were used. Five groups of animals were distinguished (**Figure 2**): the first group (n=6) served as a control (sham surgery) for evaluating normal parameters; the second group (n=6) consisted of animals 30 days after DPSS modelling (according to the method described above); the third group (n=6) - animals 100 days after DPSS modelling (according to the method described above); the fourth group (n=6) - asplenic animals on day 30 after splenectomy; and the fifth group (n=6) - animals 100 days after splenectomy. On days 30 and 100 after the procedures, the II-V groups were euthanized and autopsied for DPSS assessment.

In all groups, phagocytic parameters of blood neutrophils were evaluated at the specified time points. For this purpose, a bacterial suspension was prepared from a 24-hour agar culture of a standard museum strain of E. coli and sterile physiological saline, according to the McFarland standard, at a concentration of 1 billion/ml. The bacterial suspension was then added to the native heparinized blood of the experimental animals at a 1:1 ratio. Then suspension was incubated at 37°C in a thermostat for one hour. The dried smears were fixed and stained with Leucodiff 200 solution. Microscopic preparations were evaluated using a Lomo microscope. The main parameters of phagocytic activity, including phagocytic index, phagocytic count, opsonic index, and locomotor activity of phagocytes were determined.

Morphological examination of erythrocytes was performed on all groups of rats at 30, 50, and 100 days after the operation. The smears for morphological examination were air-dried and stained with Romanowsky-Giemsa stain. The presence and quantity of degenerative erythrocytes, poikilocytic forms (codocytes, spherocytes, stomatocytes), and erythrocytes with Howell-Jolly bodies, which normally filter through the sinusoids of the red pulp of the spleen, were taken into account.

Results

Morphological features of "neosplenic" tissue

The first group of rats was autopsied after 25 days of the surgery, no DPSS was observed in their abdominal cavities. However, on day 30 after the operation, two out of the four rats showed two foci of DPSS each. These foci appeared as dark cherry-coloured round formations with a diameter of approximately 1.0 mm. Subsequently, in all the remaining time periods (47, 57, 63, 79, 85, 99, and 128 days after surgery), foci of post-splenectomy splenosis were found in all the experimental animals, except for one rat in the group at day 63.

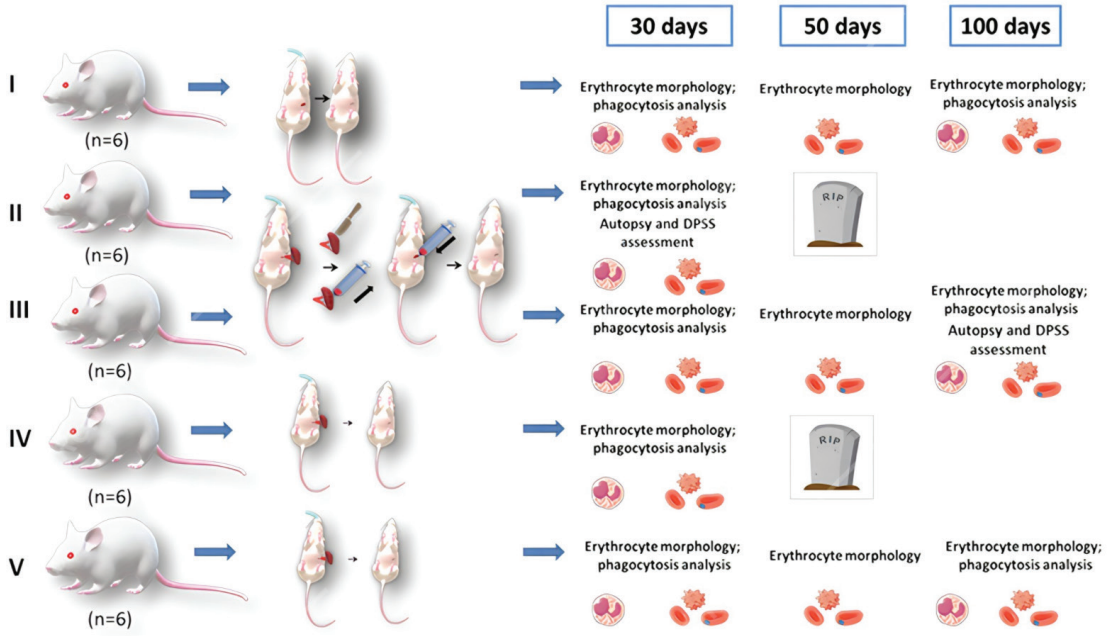

Figure 2: Design of the functional study of DPSS and it's comparison with asplenic animals

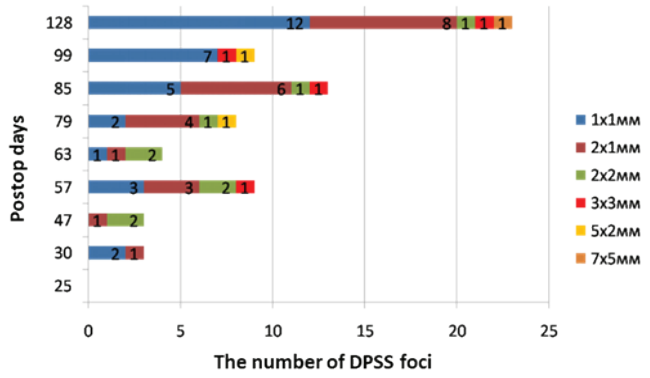

Figure 3: Number and size of DPSS foci in different terms after modelling

The number of splenic foci ranged from 1 to 12 (M=2.6) for every rat (**Figure 3**). The sizes of the foci varied from 1.0 mm to 7×5 mm. The average length of the splenic foci was 1.7 mm.

The majority of DPSS foci were commonly located on the greater omentum (47.2%), followed by the serous membrane of the stomach (19.4%), serous membrane of the colon (16.6%), and root of the mesentery (2.7%). Other less frequent locations included the parietal peritoneum, splenic bed, skin suture area, adhesions, and surface of the ovary (ranging from 1.3% to 6.9%) (**Figure 4**).

The histological examination of the splenic nodules (presumed DPSS foci) revealed the presence of "neosplenic" tissue with a cell composition characteristic of the spleen (**Figure 5**), hemosiderin granules, reticular-elastic stroma, and a clearly defined thin fibrous capsule of the nodules. However, the typical histological architecture of the spleen was not preserved in the DPSS nodules. Underdeveloped venous sinuses were commonly observed in the majority of the foci. Most of the nodules displayed a prominent presence of white pulp components, including a high number of plasma cells. These plasma cells are known to be active B-lymphocytes that produce antibodies, suggesting potential functional activity of the tissue. All splenic nodules exhibited a diffuse type of blood supply.

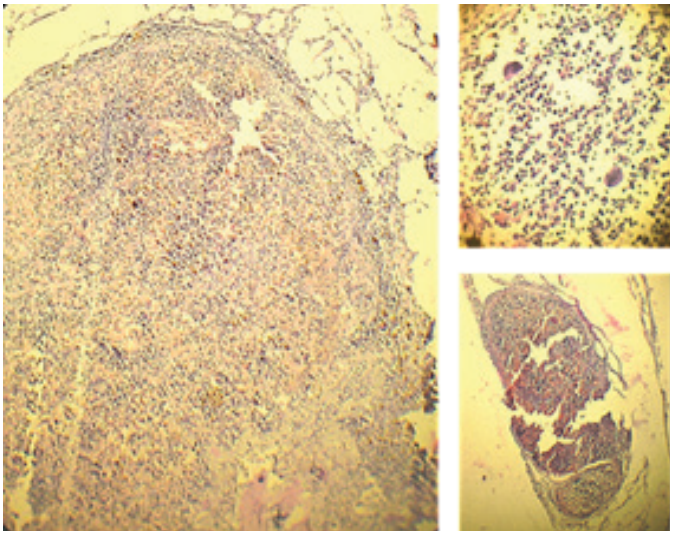

Figure 5: Cell composition of the DPSS foci, micro view

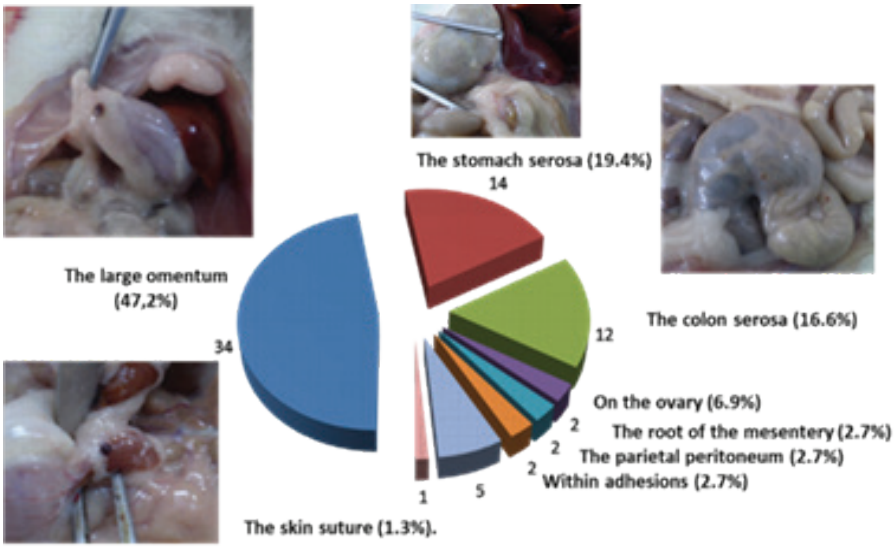

Figure 4: The distribution of DPSS location sites

Stages of development of foci of post-splenectomy splenosis in an experiment

Comparing the histological pattern of the splenic nodules at various time points following the operation, a sequential progression of changes was observed. At 30 days post-modelling of DPSS foci, the nodules exhibited a distinct thin fibrous capsule infiltrated by polymorphonuclear leukocytes and components of splenic tissue, along with a significant presence of hemosiderin granules (**Figure 5**). By day 47 after the operation, there was a notable reduction in the number of hemosiderin granules, accompanied by an increase in the accumulation of lymphoid cells at different stages of maturation. By day 57 after the operation, giant cells resembling megakaryocytes were evident in nearly all preparations, highlighting that hematopoiesis in the rat spleen occurs throughout their lifespan.

From 63 days after the surgical modelling, groups of lymphoid cells began to organize into white pulp follicles around small arteries within the nodules. However, there were no more than 2-3 clusters per nodule. Subsequently, the proliferation of lymphoid cells and the development of white pulp were observed. However, the formation of red pulp was not consistently evident throughout all stages of nodule formation. In 90% of the histological samples taken at 63 days, venous sinuses were irregularly distributed and occupied a maximum of 10-15% of the tissue area.

Functional indicators of "neosplenic tissue" activity

The immune function of the spleen can be assessed using various indicators, including the phagocytic index, phagocytic number, completeness index of phagocytosis, opsonic index, and the sizes of active phagocytes. The spleen plays a crucial role in regulating phagocytosis at different stages, and these indicators provide insights into its involvement in immune response and defense mechanisms.

The phagocytic index represents the ratio of leukocytes engaged in phagocytosis to the total number of leukocytes. Reference values typically fall within the range of 75-95%. In the study, the phagocytic index, which reflects the percentage of active phagocytes, was within the normal range (median 86%) in the group of rats with splenosis after 30 days. However, after splenectomy, the indicator did not reach normal values during the same timeframe (median 68%). After 100 days following the surgical intervention, the phagocytic index in both compared groups returned to normal levels (**Figure 6**).

The phagocytic number represents the ratio of ingested microorganisms to the number of leukocytes

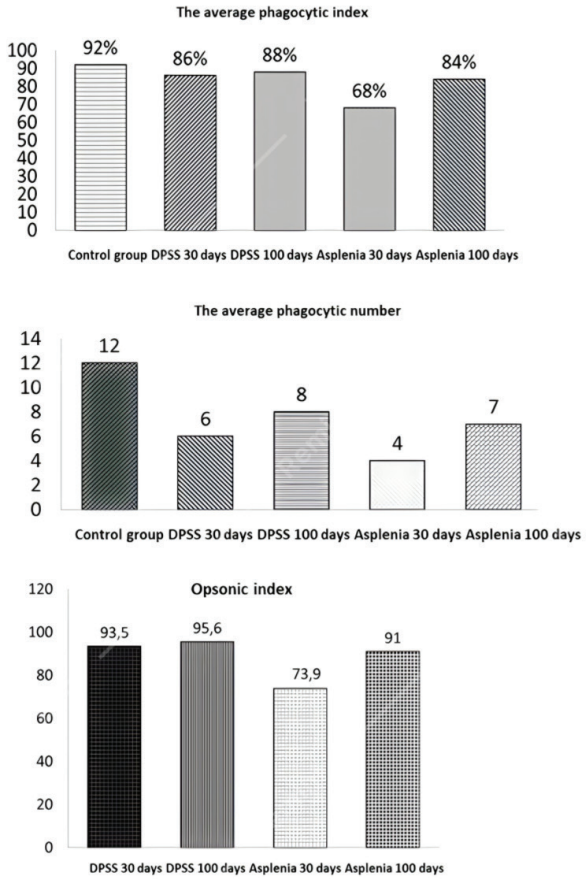

Figure 6: Phagocytic indices in experimental and control group of animals

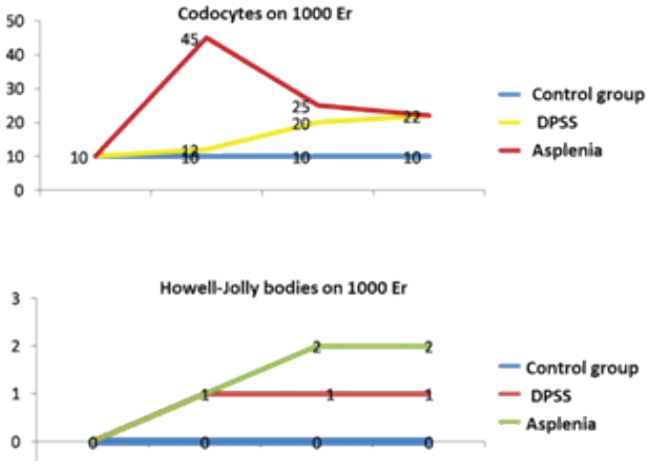

Figure 7: Morphological examination of erythrocytes in experimental and control groups

that have undergone phagocytosis, and the reference range is typically 5-10. In the group with modelled DPSS at 30 days, the average number of bacteria inside the phagocytes was normal, with 6 bacteria (±2). However, after 30 days of splenectomy, the phagocytic number decreased to 4 (±1). By the hundredth day, the indicators in both groups were almost equalized. Under microscopy, a noticeable difference was observed in the number of active phagocytes and their phagocytic capacity between the group after splenectomy and the group with modelled splenosis at 30 days (**Figure 6**).

Following the calculation of the main phagocytic indices, the opsonic index was determined. The opsonic index represents the ratio of the phagocytic index in the experimental group to that in the control group, indicating the level of humoral attack on bacterial cells that enhances subsequent phagocytosis. Normal values for this parameter typically exceed 90%. The study revealed that all groups with DPSS and 100 days after splenectomy exhibited normal values for the opsonic index. However, in the early stages after splenectomy, the index was significantly below normal levels (**Figure 6**).

The size of flattened active phagocytes provides an indirect indication of their chemotaxis degree. In the experimental groups, the highest average values were observed in rats with splenomegaly 100 days after surgery. The smallest range of sizes was observed in splenectomized animals in the early postoperative period. The sizes of rat phagocytes 30 days after splenectomy were comparable to those of non-stimulated cells, suggesting a lack of significant activation or changes in size during this period.

Morphological examination of erythrocytes in a peripheral blood smear, including the identification of degenerative forms (codocytes, spherocytes, stomatocytes) and erythrocytes with intracellular inclusions (Howell-Jolly bodies), provides insights into the filtration process within the red pulp sinuses of the spleen. According to the erythrocytogram findings, the most significant poikilocytic deviations were observed in codocytes (target-shaped erythrocytes), with occasional instances of acanthocytosis (one case after splenectomy). On the 30th day of the experiment, the number of codocytes in the group of rats without a spleen was four times higher than the normal range. By the 100th day, the average number of codocytes in the experimental groups reached 22 per 1000 erythrocytes (**Figure 7**).

In both analyzed groups, starting from 30 days after surgery, the number of Howell-Jolly bodies exceeded the normal range. However, in rats after splenectomy, this indicator was more than twice as high as the other group from day 50 onwards (**Figure 7**). These findings indicate the inadequate utilization of degenerative forms of erythrocytes by splenic nodules, likely due to insufficient amounts of red pulp in their composition and disrupted histological architecture.

Discussion

Based on the data obtained, the frequency of DPSS nodule development in "Wistar" rats during the experiment was 79%. Macroscopically visible splenic nodules were first observed 30 days after modeling. This model confirms previous clinical reports suggesting the possibility of autonomous implantation of splenic tissue components on various tissues throughout the body (3-6). However, the study revealed that in the majority of cases (47.2%), splenic nodules were localized on the greater omentum, likely due to its rich vascularity and high adhesive properties. The diffuse type of blood supply observed in these formations indicates center directed ischemia-driven angiogenesis and may not contribute to the development of the typical angioarchitecture seen in normal splenic tissue.

The stages of development of splenic foci have been identified, demonstrating morphological signs of functional activity at 47 days after surgery. This includes the highest number of plasma cells, active proliferation of various pools of lymphoid cells, and the presence of megakaryocytes. Red pulp did not occupy more than 15% of practically all splenic foci, despite the presence of all components of spleen tissue. The absence of typical histological architecture in DPSS foci is likely to affect the functional abilities of these "neosplenic" foci. The lack of well-developed venous sinuses in the foci suggests an altered and potentially disrupted morphological substrate for the contact and filtration of aging and damaged erythrocytes, platelets, bacteria, and immune complexes with macrophages. This may indicate inadequate clearance of these objects by the "neosplenic" tissue and the absence of mechanical blood filtration, which could explain the observed trends of aged and atypical forms of red

blood cells in the experimental groups.

However, despite these findings, the presence of a large number of plasma cells in splenic foci and the active division of lymphoid cells with the formation of white pulp follicles at later stages of foci development (identified at 47 days after modelling) suggests the possibility of humoral support of immunity. Further investigations should aim to track the origin of these cells and explore their potential use in understanding immune responses.

In the second stage of the experimental research, the functional significance of splenic foci in asplenic animals was determined. The study focused on the influence of spleen tissue on phagocyte activity in peripheral blood (particularly neutrophils) and tissue macrophages. It was hypothesized that despite the altered histoarchitecture of splenic foci, the presence of specific cell populations in "neosplenic tissue" would not affect the opsonizing function of the spleen. This assumption was based on previous literature indicating the significant humoral influence of the spleen on phagocytosis mediated through factors such as taftsin, complement system proteins, and macrophage-associated proteins (1,8).

Hypothetically, positive qualities of splenic nodules, such as supporting the phagocytic arm of immunity indicate the presence of humoral support from immunocompetent cells. It is most likely that immune cells proliferating in splenic nodules produce taftsin, gamma-interferon, and other opsonins. This fact explains the formation of white pulp follicles with characteristic zones around small arterial stems in splenic nodules on day 60 after modelling. Thus, paracrine interactions of immune cells in “neosplenic” tissue contribute to the reconstruction of the spatial structure of white pulp and potentially maintain the phagocytic arm of the immune response. Analysing the obtained data, it has been found that the presence of splenic nodules contributes to maintaining phagocytic indicators as early as 30 days after surgery. In contrast, a pronounced depression of phagocytosis is observed in the early postoperative period after splenectomy. Phagocytic insufficiency after splenectomy can be considered transient – after 100 days, phagocytic indicators are close to normal. According to some literature data, the highest rate of developing post-splenectomy sepsis occurs within the 1 - 3 years (1,2) after surgery although the risk persists throughout life (2). Also it is well known (1,2), that the most common causative agents of fulminant post-splenectomy sepsis are Streptococcus pneumoniae and Haemophilus influenzae, which are respiratory tract commensals. Typically, the lungs (pneumonia) or upper respiratory tract (haemophilus, meningococci – nasopharyngitis) serve as the primary focus of such sepsis. In our view, this is partially due to the loss of control over alveolar and other tissue macrophages over the opportunistic flora. Natural immune barriers weaken after splenectomy, leading to an increased degree of transient bacteremia. Since peripheral immune organs, particularly the spleen, act as filters for microorganisms, splenic insufficiency exacerbates the development of a systemic inflammatory response. This fact is evidence of the need for conservative organ-saving treatment of spleen ruptures and saving DPSS foci after splenectomy.

The morphological examination of erythrocytes in peripheral blood smears confirms the insufficient ability of neosplenic tissue to properly sort and utilize aging, degenerative, and erythrocytes with pathological inclusions, including nuclear remnants. The exact pathogenesis of this phenomenon is still under debate. It is possible that the development of this condition is related to impaired membrane clearance of these cells in the imperfectly restored red pulp or due to insufficient residual splenic tissue. It is noteworthy that the morphological study also revealed a reduction in the percentage of red pulp in most splenic nodules. According to literature data (9,10), the presence of Howell-Jolly bodies in peripheral blood smears is considered a reliable marker of hyposplenism. Occasional variations in the shape or size of erythrocytes in peripheral blood may be considered normal, but the presence of erythrocytes with inclusions indicates significant changes in the spleen. Therefore, our findings suggest that the neosplenic tissue in DPSS may not effectively perform the clearance function of abnormal erythrocytes, leading to their presence in the peripheral blood.

Conclusions

The findings of this study have implications that reach far beyond its immediate scope. It is crucial for future research to prioritize the tracking of these cells in order to uncover their origin and functional characteristics, as this knowledge can have significant implications for clinical applications. Understanding the role of these immune cells within the reconstructed white pulp follicles of DPSS is particularly important.

Such investigations can pave the way for innovative therapeutic interventions, especially for patients with hematological conditions related to the red pulp. Additionally, studying how splenic nodules support immunity and whether they can compensate for the loss of spleen functionality presents exciting prospects for clinical applications.

Conflict of interest:

The authors report no conflict of interest.

Funding source:

No funding was required.

Ethical approval:

This study was performed in accordance with the principles set out in the "Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes" (Strasbourg, 1986). Approval was granted by the Ethics Committee of Saratov State Medical University named after V. I. Razumovsky (protocol No: 20092014 – 31).

Acknowledgment:

No

Peer-review:

Externally. Evaluated by independent reviewers working in at least two different institutions appointed by the field editor.

Data availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Contributions

Research concept and design: **SK, SG**
Data analysis and interpretation: **SK, SG**
Collection and/or assembly of data: **SK**
Writing the article: **SK**
Critical revision of the article: **SG**
Final approval of the article: **SK, SG**

References

1. Tahir F, Ahmed J, Malik F. Post-splenectomy Sepsis: A Review of the Literature. Cureus. 2020;12(2):e6898.
2. Kanhutu K, Jones P, Cheng AC, Grannell L, Best E, Spelman D. Spleen Australia guidelines for the prevention of sepsis in patients with asplenia and hyposplenism in Australia and New Zealand. Intern Med J. 2017;47(8):848-55.
3. Liu C, Liu J, Wang F. Intrahepatic splenosis mimicking liver cancer: report of a case and review of literature. Int J Clin Exp Pathol. 2015;8(1):1031-5.
4. Pumberger W, Wiesbauer P, Leitha T. Splenosis mimicking tumor recurrence in renal cell carcinoma: detection on selective spleen scintigraphy. J Pediatr Surg. 2001;36(7):1089-91.
5. Rudenko MS, Motus IY, Saloutin MV. Post-traumatic thoracic splenosis. Khirurgiia (Mosk). 2021;2021(9):100-2.
6. Wang Y, Shen H. Pelvic Splenosis. N Engl J Med. 2021;384(3):e7.
7. Moralejo Lozano Ó, Aparicio Cabezudo M, Cruz Aparicio M, Caso Viesca A, Colmenares Bulgheroni M, Barrajón Masa AJ, et al. Splenosis: An underappreciated cause of gastrointestinal bleeding in splenectomized patients. Case report and literature review. Gastroenterol Hepatol. 2021;44(5):369-73.
8. Hijazi LS, Zahra F, Yarrarapu SNS, Mead T. Functional Asplenism. 2022 Sep 23. In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 29763124.
9. Nakagami Y, Uchino K, Okada H, Suzuki K, Enomoto M, Mizuno S, et al. Potential role of Howell-Jolly bodies in identifying functional hyposplenism: a prospective single-institute study. Int J Hematol. 2020;112(4):544-52.
10. Alhyari A, Geisler L, Eilsberger F, Dietrich CF, Findeisen H, Trenker C, et al. „Hyposplenie", eine weitgehend unerkannte Immunschwäche: Ist die Sonografie hilfreich? (Hyposplenism, an underrecognised immune diffeciency: Is sonography helpful?). Z Gastroenterol. 2023;61(07):852-61.