Vascular Remodeling And Endothelial Dysfunction After Experimental Splenectomy

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ABSTRACT

Objective: To investigate morphofunctional changes in blood vessels after splenectomy in an experimental model.
Materials and Methods: The experiment was conducted on adult white male rats divided into a control group and two experimental groups examined on days 14 and 30 after splenectomy. Histological and morphometric methods were applied to assess endothelial integrity, vascular wall structure, and microcirculatory parameters.
Results: Splenectomy resulted in endothelial dysfunction, arterial wall remodeling, and significant microcirculatory disturbances. Early changes were predominantly compensatory-adaptive, whereas later stages were characterized by persistent structural alterations, capillary rarefaction, and signs of chronic tissue hypoxia.
Conclusion: The findings confirm the systemic nature of vascular remodeling following splenectomy and provide a morphological basis for understanding post-splenectomy vascular complications.

Keywords: splenectomy, endothelium, vascular remodeling, microcirculation, morphometry

Introduction

The spleen is a central organ of the immune and hematopoietic systems, performing filtration of circulating blood elements, removal of aged erythrocytes, platelet sequestration, and regulation of immune homeostasis. Owing to its complex immunoregulatory and hemorheological functions, splenectomy cannot be considered a purely local surgical intervention. Instead, it induces systemic adaptive and pathological responses affecting vascular, hematologic, and inflammatory pathways.

One of the most clinically significant consequences of splenectomy is the development of a prothrombotic state. Postoperative splenic and portal vein thrombosis remains a well-documented complication, described both in early classical studies and contemporary systematic reviews [6,10]. Recent meta-analyses confirm that portal venous system thrombosis after splenectomy is not a rare phenomenon and may be influenced by preoperative splenomegaly, altered portal hemodynamics, and hematologic disorders [7,8,15]. Modern risk stratification models further emphasize the importance of individualized thrombosis prevention strategies in postsplenectomy patients [4].

Reactive thrombocytosis is considered one of the earliest and most consistent hematologic findings after splenectomy [5]. Predictive factors influencing platelet count dynamics have been described in both traumatic and non-traumatic splenectomy settings [2]. However, quantitative platelet elevation alone does not fully explain the complexity of vascular complications. Several

authors have stressed that postoperative splenic/portal vein thrombosis remains an unresolved clinical issue, suggesting the involvement of additional pathogenic mechanisms beyond platelet count increase [6,11].

Increasing attention has been directed toward endothelial dysfunction as a key mediator of postsplenectomy vascular remodeling. Experimental data demonstrate that splenectomy modifies thrombus architecture and vascular wall remodeling processes [3]. Elevated levels of platelet-derived microparticles and enhanced leukocyte–platelet aggregates contribute to thrombus stabilization and vascular wall alteration, thereby promoting persistent procoagulant conditions [3]. Furthermore, systemic vascular consequences of splenectomy extend beyond the portal system, as evidenced by associations with pulmonary hypertension and long-term vascular complications [9].

From a morphological standpoint, the vascular wall undergoes multi-layered remodeling involving the intima, media, and adventitia. Endothelial integrity plays a central regulatory role in maintaining vascular tone, permeability, and antithrombotic balance. Structural changes in the endothelium initiate cascades leading to smooth muscle cell hypertrophy, extracellular matrix accumulation, and luminal narrowing. Experimental morphologic investigations have confirmed that splenectomy induces significant microcirculatory and vascular alterations in different organs [12,13]. These findings underscore the necessity of quantitative morphometric assessment in order to objectively characterize the phases of vascular adaptation and remodeling.

Early vascular responses after splenectomy are often compensatory, including vasodilation, endothelial activation, and reactive wall thickening. However, in later stages, persistent structural remodeling may occur, characterized by intimal hyperplasia, medial hypertrophy, connective tissue proliferation, and reduction of capillary density. Such changes form the morphological basis for chronic tissue hypoxia and progressive vascular dysfunction. Contemporary reviews and risk prediction models further support the concept of systemic vascular remodeling following splenectomy [1,15].

Despite substantial clinical evidence regarding thrombotic risk and hemodynamic alterations after splenectomy, the integrated morphologic analysis of the “endothelium – vascular wall – microcirculation” complex remains insufficiently explored within a unified experimental morphometric framework. Most studies focus on clinical thrombosis outcomes or hematologic parameters, whereas detailed structural evaluation of vascular remodeling at different postoperative time points is limited.

Therefore, comprehensive experimental morphometric investigation is warranted to clarify sequential vascular alterations following splenectomy and to establish the structural substrate underlying postsplenectomy vascular complications.

Objective: To investigate morphofunctional changes of blood vessels after splenectomy in an experimental model with quantitative assessment of endothelial integrity, vascular wall remodeling, and microcirculatory alterations at different postoperative stages.

Materials and Methods

The experimental study was conducted on 36 adult male outbred white rats weighing 200–230 g and aged 4–5 months. Animals were obtained from a certified breeding facility and housed in a standard vivarium environment under controlled laboratory conditions (temperature 22 ± 2°C, relative humidity 50–60%, 12-hour light/dark cycle). Rats had free access to standard laboratory chow and water ad libitum. Prior to the experimental procedures, all animals underwent an adaptation period of 7 days.

The animals were randomly divided into three equal groups (n = 12 in each group):

- Control group – intact animals without surgical intervention;
- Experimental group I – animals examined on the 14th postoperative day after splenectomy;

- Experimental group II – animals examined on the 30th postoperative day after splenectomy.

All experimental procedures were performed in accordance with international guidelines for the care and use of laboratory animals and complied with institutional ethical standards.

Surgical Procedure

Splenectomy was carried out under general ether anesthesia. Following induction of surgical anesthesia, a left subcostal laparotomy was performed under strict aseptic conditions. The spleen was carefully mobilized, and the vascular pedicle was ligated using atraumatic sutures. The organ was completely excised, and hemostasis was ensured. The abdominal wall was closed in layers using absorbable sutures. Postoperative animals were monitored daily for general condition, wound healing, and signs of infection or distress.

No postoperative mortality or severe complications were observed.

Tissue Sampling

At the designated time points (14 and 30 days after splenectomy), animals were euthanized by overdose of anesthesia. Fragments of the thoracic aorta, femoral artery, and veins of the microcirculatory bed were carefully excised. Particular attention was paid to preserving vascular integrity and avoiding mechanical deformation during dissection.

Histological Processing

Collected tissue samples were immediately fixed in 10% neutral buffered formalin for 24–48 hours. After fixation, specimens were processed through graded alcohol dehydration, cleared in xylene, and embedded in paraffin according to standard histological protocols.

Paraffin sections 5–7 μm thick were prepared using a rotary microtome. Sections were mounted on glass slides and stained using the following methods:

- Hematoxylin and eosin (H&E) for general morphological assessment;
- Van Gieson staining for evaluation of connective tissue components;
- Orcein staining for visualization of elastic fibers within the vascular wall.

Microscopic examination was performed using a light microscope at magnifications ×100, ×200, and ×400.

Morphometric Analysis

Quantitative morphometric evaluation was conducted using a digital image analysis system integrated with calibrated measurement software. Measurements were performed in at least 10 randomly selected fields of view for each specimen to ensure representativeness.

The following parameters were assessed:

- Thickness of the intima, media, and adventitia (μm);
- Diameter of the vascular lumen (μm);
- Height of endothelial cells (μm);
- Number of endothelial cells per 1 mm of vascular lining;
- Percentage of endothelial desquamation foci;
- Diameter and density of capillaries within the microcirculatory bed;
- Degree of perivascular edema (percentage area analysis).

All measurements were averaged for each animal and subsequently for each experimental group.

Results

Morphometric Parameters of Arterial Vessels

Quantitative morphometric analysis revealed statistically significant structural remodeling of arterial vessels at both postoperative time points (Table 1).

On day 14 after splenectomy, intimal thickness increased by approximately 25% compared with control ($p < 0.05$), indicating early subendothelial and intimal hyperplastic response. Medial thickness also increased significantly, reflecting smooth muscle cell hypertrophy and adaptive vasomotor activation. Adventitial expansion suggested activation of connective tissue components.

By day 30, vascular remodeling became more pronounced and structurally stable. Intimal and medial layers continued to thicken, while lumen diameter showed statistically significant narrowing ($p < 0.05$), indicating

progressive vascular wall restructuring and increased peripheral resistance.

Table 1

Morphometric parameters of arterial vessels after splenectomy (M ± m)

Parameter	Control	Day 14	Day 30
Intimal thickness (μm)	6.2 ± 0.3	7.8 ± 0.4*	8.9 ± 0.5*
Medial thickness (μm)	42.5 ± 1.6	47.3 ± 1.8*	50.6 ± 2.1*
Adventitial thickness (μm)	18.1 ± 0.7	20.4 ± 0.9*	22.8 ± 1.0*
Lumen diameter (μm)	310 ± 12	295 ± 10	278 ± 11*

** p < 0.05 vs control*

Microcirculatory Changes

The microcirculatory bed demonstrated a biphasic pattern of alteration (Table 2). At day 14, capillary dilation was observed, representing compensatory vasodilatory mechanisms. However, capillary density decreased significantly, indicating partial functional exclusion of microvessels from effective perfusion. Perivascular edema increased markedly, reflecting endothelial permeability disruption.

By day 30, capillary diameter decreased below control values, suggesting vasoconstrictive remodeling. Capillary density showed further significant reduction, corresponding to structural rarefaction of the microvascular network. Persistent and increasing perivascular edema indicated sustained endothelial dysfunction and chronic microcirculatory disturbance.

Table 2

Microcirculatory parameters after splenectomy (M ± m)

Parameter	Control	Day 14	Day 30
Capillary diameter (μm)	6.8 ± 0.2	7.6 ± 0.3*	6.1 ± 0.2*
Capillary density (per 1 mm^2)	132 ± 5	118 ± 4*	96 ± 3*
Perivascular edema (%)	4.2 ± 0.6	11.5 ± 1.1*	14.3 ± 1.4*

** p < 0.05 vs control*

Endothelial Morphometric Characteristics

Endothelial analysis demonstrated progressive endothelial dysfunction (Table 3). By day 14, endothelial cell height significantly decreased, while the number of endothelial cells per unit length increased, reflecting compensatory proliferative response. The percentage of desquamation foci increased markedly, indicating disruption of endothelial integrity.

At day 30, endothelial flattening became more pronounced, and desquamation further increased. The coexistence of hyperplasia and injury suggests dysregulated endothelial turnover and prothrombotic transformation of the vascular lining.

Table 3

Endothelial morphometric characteristics (M ± m)

Parameter	Control	Day 14	Day 30
Endothelial cell height (μm)	3.9 ± 0.2	3.4 ± 0.2*	3.1 ± 0.1*
Endothelial cells per 1 mm	112 ± 4	125 ± 5*	138 ± 6*
Desquamation foci (%)	1.8 ± 0.3	6.7 ± 0.8*	9.5 ± 1.0*

** p < 0.05 vs control*

Summary of Morphological Dynamics

The morphometric findings indicate a time-dependent progression of vascular alterations following splenectomy. Early changes are characterized by adaptive

endothelial activation and vasomotor remodeling. Later stages demonstrate fixed structural thickening, lumen narrowing, capillary rarefaction, and sustained endothelial injury, forming the morphological basis of chronic vascular dysfunction.

Discussion

The present experimental study demonstrates that splenectomy initiates a complex, multi-stage pathogenetic cascade involving endothelial dysfunction, structural vascular remodeling, and progressive microcirculatory impairment. The morphometric data obtained at days 14 and 30 allow a time-dependent interpretation of these processes and provide morphological substantiation for the systemic vascular consequences of splenic removal.

Endothelial Dysfunction as the Primary Trigger

The earliest and most fundamental alteration appears to be endothelial dysfunction. The observed decrease in endothelial cell height combined with increased endothelial cell density and elevated desquamation foci indicates both structural injury and compensatory proliferative activation. Such a pattern reflects functional stress of the endothelial layer under altered hemodynamic and hemorheological conditions following splenectomy.

The spleen plays a significant role in blood filtration, immune regulation, and platelet sequestration. Its removal leads to reactive thrombocytosis, altered platelet–leukocyte interaction, and changes in circulating microparticles. These systemic changes increase shear stress and inflammatory signaling within the vascular lumen, promoting endothelial activation. Morphologically, this state manifests as endothelial flattening, focal desquamation, and unstable cell turnover.

Endothelial dysfunction disrupts nitric oxide–dependent vasoregulation and shifts the vascular balance toward vasoconstrictive and procoagulant dominance. This disturbance forms the initial mechanistic link between splenectomy and vascular structural changes.

Vascular Wall Remodeling

Following endothelial injury, secondary remodeling of the vascular wall becomes evident. Progressive thickening of the intima and media suggests smooth muscle cell hypertrophy and enhanced extracellular matrix deposition. Adventitial expansion further indicates activation of fibroblasts and perivascular connective tissue proliferation.

By day 30, these changes become structurally consolidated. The narrowing of the vascular lumen reflects reduced arterial compliance and increased peripheral vascular resistance. From a hemodynamic perspective, such remodeling predisposes to impaired tissue perfusion and elevated vascular tone.

The transition from early adaptive thickening (day 14) to sustained structural remodeling (day 30) demonstrates that initial compensatory mechanisms gradually transform into fixed architectural alterations. This shift marks the transition from reversible functional response to persistent vascular pathology.

Microcirculatory Transformation

The microcirculatory bed exhibits a biphasic pattern. Early dilation of capillaries and pronounced perivascular edema likely represent compensatory mechanisms aimed at maintaining tissue perfusion under altered systemic conditions. However, reduced capillary density at this stage already indicates functional exclusion of microvessels from effective circulation.

By day 30, capillary rarefaction becomes more pronounced, accompanied by reduced capillary diameter and persistent edema. This indicates structural regression of the microvascular network and progressive impairment of oxygen diffusion. Such rarefaction is a recognized morphological substrate of chronic tissue hypoxia.

The coexistence of arterial remodeling and microvascular rarefaction forms a hemodynamically unfavorable environment characterized by increased resistance, reduced perfusion reserve, and impaired oxygen delivery.

Integrated Pathogenetic Cascade

Taken together, the results allow the following pathogenetic sequence to be proposed:

Endothelial dysfunction → Vascular wall remodeling → Microcirculatory impairment → Chronic tissue hypoxia

Each stage potentiates the next:

- Endothelial injury disrupts vascular homeostasis.
- Remodeling increases vascular stiffness and narrows lumen diameter.
- Microcirculatory regression reduces effective perfusion area.
- Chronic hypoxia further aggravates endothelial dysfunction, forming a self-sustaining pathological loop.

This cascade explains the progressive nature of post-splenectomy vascular complications and provides a morphological framework for understanding increased thrombotic and ischemic risk observed clinically.

Systemic Implications

The findings confirm that splenectomy should not be considered merely as removal of an isolated organ. Instead, it induces systemic vascular adaptation with potential long-term consequences. The spleen appears to contribute indirectly to vascular homeostasis through regulation of blood elements, immune mediators, and endothelial interaction.

The progressive endothelial instability observed in this study may represent the morphological basis for the prothrombotic phenotype described in clinical literature. Moreover, capillary rarefaction and chronic tissue hypoxia may contribute to long-term organ dysfunction beyond the vascular compartment itself.

Conclusions

The present experimental study demonstrates that splenectomy induces systemic morphofunctional alterations within the vascular system, encompassing endothelial structures, arterial wall architecture, and the microcirculatory network. The removal of the spleen does not remain a localized surgical intervention but triggers a generalized vascular response characterized by sequential structural and functional transformations.

Endothelial dysfunction appears to represent the initiating and central mechanism of post-splenectomy vascular pathology. Morphometric evidence of endothelial flattening, increased cellular density, and elevated desquamation confirms the destabilization of the vascular lining. These alterations indicate impairment of the endothelial barrier and regulatory functions, including disturbances in vasomotor balance, anticoagulant properties, and microvascular permeability.

Subsequent to endothelial impairment, progressive remodeling of the arterial wall develops. Thickening of the intima, media, and adventitia layers reflects adaptive hypertrophic and fibrotic responses that gradually become structurally consolidated. The resulting reduction in vascular lumen diameter contributes to increased peripheral vascular resistance and diminished arterial compliance, creating unfavorable hemodynamic conditions.

Microcirculatory disturbances exhibit a phased evolution. Early compensatory capillary dilation and perivascular edema are replaced in later stages by capillary rarefaction, structural regression of the microvascular network, and reduced tissue perfusion capacity. These changes constitute a morphological substrate for chronic tissue hypoxia and may perpetuate endothelial injury, reinforcing the pathological cycle.

Collectively, the identified morphometric alterations support the existence of a pathogenetic cascade: endothelial dysfunction → vascular remodeling → microcirculatory impairment → chronic tissue hypoxia. This cascade explains the progressive and systemic nature of vascular complications observed after splenectomy.

The findings provide a solid morphological foundation for understanding post-splenectomy vascular pathology and highlight the importance of preventive and therapeutic strategies aimed at preserving endothelial integrity, modulating vascular remodeling, and protecting microcirculatory function.

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