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# CONTENTS

## ORIGINAL ARTICLES

- Comparison of the ONSTEP and Lichtenstein techniques for inguinal hernia repair—early results of a prospective study**  
Konrad Pielaciński, Katarzyna Pruszczyk-Matusiak, Jan Pielaciński, Agata Bosak Pielacińska, Andrzej B. Szczepanik 437
- Digital differential diagnostics of thyroid pathology by interference scanning of ellipticity polarization maps of microscopic images of native histological sections**  
Oleksandr V. Bilookyi, Yurii Ye. Rohovyi, Yurii A. Uschenko, Oksana V. Kinzerska, Valeriy M. Sklyarchuk, Viacheslav V. Bilookyi 444
- Evaluation of Vitamin D3 as a diagnostic marker in hypothyroidism**  
Karar Nadhm Obaid Aljabry, Yasseen Abdulurda Yasseen, Nibras Hussein Abdulsada Al-Ghuraibawi, Ali A. Al-Fahham 452
- The peculiarities of biochemical and morphological changes in the heart of the rats under chronic hypodynamia in the development of adrenalin damage of heart**  
Olha V. Denefil, Roman B. Druziuk, Volodymyr Ye. Pelykh, Olena O. Kulianda, Larysa Ya. Fedoniuk, Zoya M. Nebesna, Oleh B. Yasinovskyi 458
- Evaluation of some immune mediators (IL-16, IgE and eosinophils) as diagnostic markers for COVID-19**  
Sarah Kassab Shandaway Al-Zamali, Ruqaya Yahya abd AL-Shaheed, Hawraa S. AL-Jobory, Ali A. Al-fahham 467
- Prevention of pseudoexfoliation glaucoma in patients with age-related cataract in the background of pseudoexfoliation syndrome**  
Volodymyr O. Melnyk, Anastasiia O. Likhatska, Liudmyla I. Haliienko, Borys I. Palamar 473
- The screening and analysis of the response questionnaire in the care of temporomandibular disorders and psychoemotional state diagnosis in the Polish population**  
Justyna Grochala, Małgorzata Pihut, Jolanta E. Loster 478
- Coronavirus disease 2019 (COVID-19) during pregnancy: Pathomorphological changes in the terminal villi of the placenta**  
Tetiana V. Savchuk, Ivan V. Leshchenko, Viktoriya V. Vaslovykh, Oksana H. Chernenko, Tetiana A. Malysheva 485
- Analysis of the work of the inpatient military hospital during the Russian-Ukrainian war**  
Oleksandr M. Korneta, Iryna A. Holovanova, Maksym V. Khorosh 495
- Rational nutrition as a factor of healthy lifestyle and prevention of chronic non-communicable diseases**  
Grygoriy P. Griban, Olha S. Zablotska, Olena O. Mitova, Soslan G. Adyrkhaiev, Ludmyla V. Adyrkhaieva, Yuliia V. Paryshkura, Alimia M. Osmanova 502
- Morphological features of the great saphenous vein in patients with chronic venous disease of the lower extremities undergoing the most common endovenous treatment techniques**  
Olena O. Dyadyk, Valentyn A. Khodos, Hlib O. Melnychuk, Mykhailo S. Myroshnychenko, Kateryna I. Popova 509

## REVIEW ARTICLES

- Pedeutology of the profession of an academic teacher in the field of medical studies**  
Tadeusz Pietras, Karol Batko, Aleksander Stefanik, Kasper Sipowicz, Anna Mosiołek, Ignacy Stefańczyk, Magdalena Dutch-Wicherek 515
- Practical application of motivation theories for engaging and retaining medical staff**  
Angelika O. Keretsman, Valeriya V. Brych, Emiliia M. Shykula 522
- Protection of the rights of drug addicts and the right to a fair trial: Practice of the European Court of Human Rights**  
Oleksandr M. Shevchuk, Oleksandr M. Drozdov, Oleksandra V. Babaieva, Inna L. Bepalko, Alisa V. Panova 529

# Morphological features of the great saphenous vein in patients with chronic venous disease of the lower extremities undergoing the most common endovenous treatment techniques

Olena O. Dyadyk<sup>1</sup>, Valentyn A. Khodos<sup>1</sup>, Hlib O. Melnychuk<sup>1</sup>, Mykhailo S. Myroshnychenko<sup>2</sup>,  
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## ABSTRACT

**Aim:** The purpose was to identify the morphological features of the great saphenous vein in patients with chronic venous disease of the lower extremities undergoing treatment with endovenous high-frequency electric welding in automatic mode, endovenous laser ablation, and ultrasound-guided microfoam sclerotherapy.

**Materials and Methods:** The material for the comprehensive morphological study consisted of fragments of the great saphenous vein obtained from 32 patients with chronic venous disease of the lower extremities. The material was divided into three groups according to the endovenous treatment techniques applied. Group 1 included vein fragments from 12 patients who underwent endovenous high-frequency electric welding in automatic mode. Group 2 comprised material from 9 patients treated with endovenous laser ablation. Group 3 included material from 11 patients who underwent ultrasound-guided microfoam sclerotherapy using a 3% polidocanol solution. Histological and immunohistochemical research methods were used.

**Results:** The great saphenous vein in patients with chronic venous disease of the lower extremities undergoing treatment with endovenous high-frequency electric welding in automatic mode and endovenous laser ablation was characterized by similarly pronounced diffuse alterative and desquamative changes in endothelial cells, disruption of the structural organization of the venous wall layers accompanied by edema, and alterative changes in both muscular and connective tissue fibers. In cases where ultrasound-guided microfoam sclerotherapy was applied, the great saphenous vein showed focal alterative and desquamative changes in endothelial cells, preservation of the layered structure, edematous changes in the media and adventitia, focal areas of dysmucoidosis, and regions of angiomas.

**Conclusions:** The severity of structural changes in the great saphenous vein in patients with chronic venous disease of the lower extremities following ultrasound-guided microfoam sclerotherapy was significantly lower compared to endovenous high-frequency electric welding in automatic mode and endovenous laser ablation, resulting in less favorable conditions for the qualitative development of the fibrous process.

**KEY WORDS:** morphology, great saphenous vein, chronic venous disease of the lower extremities, endovenous high-frequency electric welding in automatic mode, endovenous laser ablation, ultrasound-guided microfoam sclerotherapy

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## INTRODUCTION

Chronic venous disease of the lower extremities is a common condition, with an estimated global prevalence ranging from 62.5% to 83.6% [1]. It is a polyetiological disease involving genetic, proteomic, and cellular mechanisms that lead to alterations in the morphofunctional state of the venous wall [2]. From a mechanical standpoint, chronic venous disease may be associated with venous outflow obstruction, reflux, or a combination of both [3].

Chronic venous disease presents with a broad clinical spectrum, ranging from heaviness, pain, nocturnal cramps, itching, telangiectasias, varicosities, and edema to skin hyperpigmentation, lipodermatosclerosis, and venous ulceration. This condition may significantly impair a patient's

ability to engage in occupational and social activities, thereby reducing quality of life and contributing to economic decline [4, 5].

The 21<sup>st</sup> century has witnessed revolutionary advances in the treatment of patients with chronic venous disease of the lower extremities, owing to the expansion of therapeutic interventions [6]. One of the novel approaches to eliminating vertical reflux in the great and small saphenous veins in the treatment of chronic venous disease of the lower extremities is endovenous laser ablation [7, 8]. The outcome of treatment is influenced by the choice of laser wavelength, the type of optical fiber, and the amount of energy to be delivered, which must be carefully determined [7]. According to various authors, when the above-mentioned parameters are optimally

aligned, stable vein occlusion can be achieved in 94.3% to 99.2% of cases during a follow-up period of 12 months or more [8, 9]. Among the complications of endovenous laser ablation, researchers report pain along the treated vein (21.1%), hyperpigmentation (9.8%), thrombophlebitis (5%), and paresthesia (2.5-7.3%) [7, 9].

Another endovenous technique is ultrasound-guided microfoam sclerotherapy [10, 11]. According to various authors, the reported success rates following the use of this treatment method range from 63.6% to 95% during a follow-up period of 12 months or longer [10-12].

In recent years, a novel method known as high-frequency electric welding has been introduced as an endovenous technique that involves targeted thermal fusion of the venous wall using high-frequency current and includes automatic adjustment of energy parameters to ensure effective and safe vein closure [13-16].

Our review of the available literature has revealed the presence of numerous unresolved issues related to the use of the aforementioned endovenous techniques, as well as the lack of morphological justification for their clinical effectiveness. This fact underscores the relevance of conducting the present study.

## AIM

The purpose was to identify the morphological features of the great saphenous vein in patients with chronic venous disease of the lower extremities undergoing treatment with endovenous high-frequency electric welding in automatic mode, endovenous laser ablation, and ultrasound-guided microfoam sclerotherapy.

## MATERIALS AND METHODS

The material for the comprehensive morphological study consisted of fragments of the great saphenous vein obtained from 32 patients with chronic venous disease of the lower extremities. The material was divided into three groups according to the endovenous treatment techniques applied. Group 1 included vein fragments from 12 patients who underwent endovenous high-frequency electric welding in automatic mode. Group 2 comprised material from 9 patients treated with endovenous laser ablation. Group 3 included material from 11 patients who underwent ultrasound-guided microfoam sclerotherapy using a 3% polidocanol solution.

The surgical specimens were fixed in 10% neutral buffered formalin (pH 7.4) for 24-36 hours. After fixation, tissue processing was performed using the Excelsior AS apparatus (Thermo Fisher Scientific, UK). Embedding in paraffin blocks was done on a HistoStar apparatus (Thermo Fisher Scientific, United Kingdom). Serial sections 2-3  $\mu$ m thick were prepared from paraffin blocks using the HM 325 rotary microtome (Thermo Shandon, UK). Histological slides were stained with hematoxylin and eosin, picrofuchsin according to van Gieson.

During immunohistochemical analysis, tissue sections were mounted on Super Frost Plus adhesive slides (Menzel, Germany). Heat-induced epitope retrieval was performed

using citrate buffer (pH 6) and EDTA buffer (pH 8). Detection was carried out with the Vitro Master Polymer Plus Detection System (Peroxidase) including the DAB Quanto chromogen (Master Diagnostica, Spain). Mouse monoclonal antibodies against CD34 (clone QB-End/10) and alpha-smooth muscle actin ( $\alpha$ -SMA) (clone 1A4 (asm-1), as well as rabbit monoclonal antibody against vimentin Ab-2 (clone SP20), were used.

Immunohistochemical reactions using monoclonal antibodies against CD34,  $\alpha$ -SMA, and vimentin were evaluated based on the intensity and extent of marker expression. The intensity of expression was assessed using a semi-quantitative scoring system, where 0 indicated no staining, 1 indicated weak («+») staining intensity, 2 indicated moderate («++») intensity, and 3 indicated strong («+++») intensity. The prevalence of expression was analyzed by calculating the ratio of the area of positively stained cells/tissues to the total tissue area within the visual field (in 5 fields of view at  $\times 200$  magnification in each case) with subsequent conversion into the scores (0 – no staining, 1 – staining covering less than one-third of the tissue section, 2 – staining covering one-third to two-thirds of the section, 3 – staining covering more than two-thirds of the section).

Microscopic examination and photo archiving were performed using ZEISS light-optical microscope (Germany) equipped with the Axiomager.A2 data processing system at objective magnifications of 5 $\times$ , 10 $\times$ , 20 $\times$ , and 40 $\times$ , with a 1.5 $\times$  binocular tube and 10 $\times$  eyepieces, and an ERc 5s camera. Additionally, a ZEISS Primo Star microscope (Germany) equipped with an Axiocam 105 color camera was used.

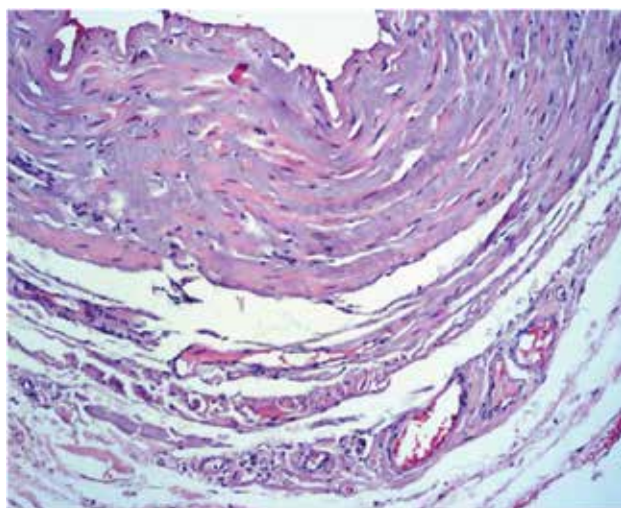
## RESULTS

In Group 1, the endothelium, media, and adventitia of the venous wall exhibited edema, dysmucoidosis, disrupted architectonics, and alterative changes. These changes were manifested by homogenization of collagen fibers and smooth muscle structures (Fig. 1). At the same time, preservation of perivascular structures and marked hyperemia of small-caliber vessels were observed.

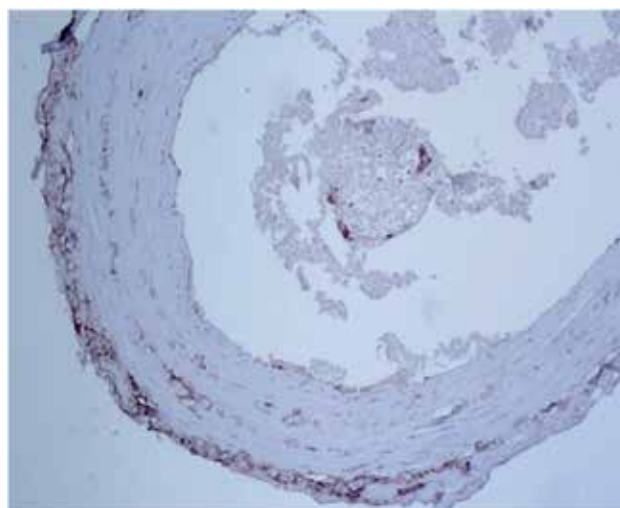
In all cases, immunohistochemical staining with monoclonal antibody against CD34 revealed pronounced endothelial damage, with a complete absence of expression in endothelial cells («-», score 0). In some vessels, positive CD34 expression was observed in the perivascular structures, indicating that the automatically regulated energy parameters acted selectively within the boundaries of the venous wall tissue (Fig. 2).

Immunohistochemical reaction with monoclonal antibody against  $\alpha$ -SMA revealed uneven expression in smooth muscle structures of the media and in some adventitial cells. Positive expression was observed only in 2 cases (16.7%) and corresponded to «++» (score 2) (Fig. 3). Morphologically, the smooth muscle cells were predominantly elongated in shape and arranged parallel to the orientation of the venous wall layers.

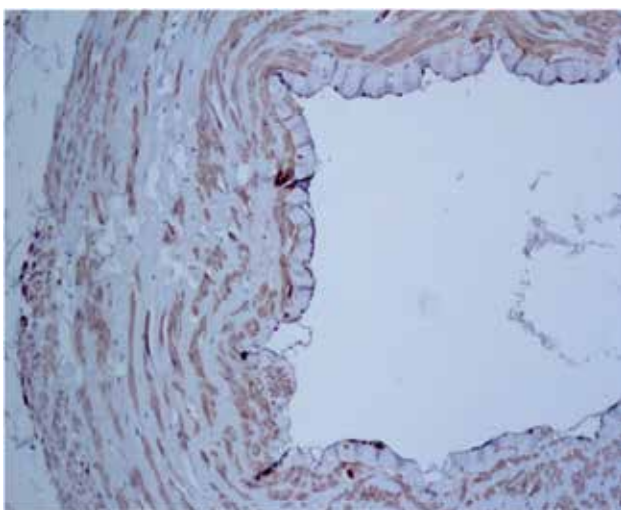
In immunohistochemical reaction with monoclonal antibody against vimentin, only one case (8.3%) showed moderately pronounced positive staining («+», score 1)



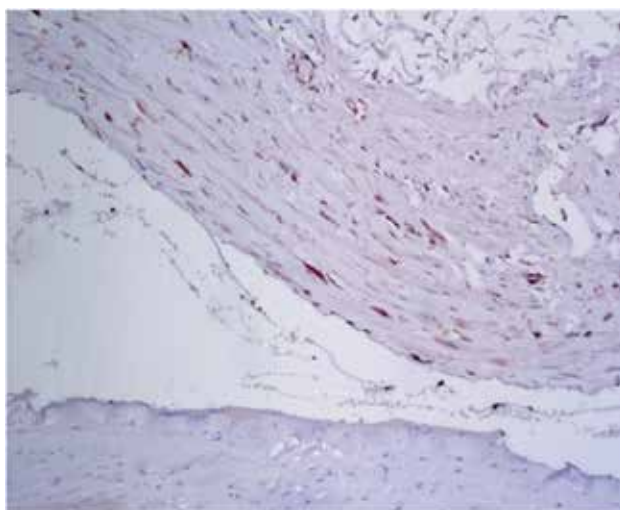
**Fig. 1.** Group 1. Uneven disruption of the architectonics in all layers of the venous wall, edema, dysmucoidosis, endothelial damage, and homogenization of collagen fibers in the intimal and medial layers. Hematoxylin and eosin staining,  $\times 100$ .



**Fig. 2.** Group 1. Absence of CD34 expression in the endothelium of the venous wall; positive expression in vessels of the perivascular structures. Immunohistochemical reaction with monoclonal antibody against CD34,  $\times 50$ .



**Fig. 3.** Group 1. Positive  $\alpha$ -SMA expression in some smooth muscle cells of the medial layer and in a portion of adventitial cells in the vessel wall, in perivascular structures. Immunohistochemical reaction with monoclonal antibody against  $\alpha$ -SMA,  $\times 100$ .



**Fig. 4.** Group 1. Positive moderately pronounced expression of vimentin by fibroblastic cells in all layers of the vein wall. Immunohistochemical reaction with monoclonal antibody against vimentin,  $\times 100$ .

of the indicated antibody by fibroblastic cells in the vein wall (Fig. 4).

In Group 2, edema, disruption of the wall architectonics, alternative changes, homogenization of all layers, dysmucoidosis, endothelial damage, and fibrous tissue proliferation were observed in the areas affected by laser ablation (Fig. 5).

Immunohistochemical reaction with monoclonal antibody against CD34 showed a complete absence of expression («-», score 0) in all cases. Immunohistochemical reaction with monoclonal antibody against  $\alpha$ -SMA revealed moderate expression («++», score 2) in 2 cases (22.2%).

Vimentin expression was detected as weak («+», score 1) in 3 cases (33.3%). Additionally, we observed that during immunohistochemical reaction with monoclonal antibody against  $\alpha$ -SMA, smooth muscle cells appeared fragmented, mostly lacked an elongated shape, and were often arranged perpendicularly to the orientation of the venous wall layers (Fig. 6).

In group 3, in all studied cases, the vein wall retained its layering. The changes were registered mainly in the endothelium. The latter were manifested by focal dystrophic-necrotic and desquamative changes. In the media and adventitia, edema, focal areas of dysmucoidosis, intact

smooth muscle cells and collagen fibers, as well as regions of angiomatosis, were identified (Fig. 7). Fragments of thrombotic masses were observed in the vein lumen.

Immunohistochemical reaction with monoclonal antibody against CD34 revealed positive, uneven expression of this marker by vascular endothelial cells in 4 cases (36.4%), with an intensity score of «+» (score 1) (Fig. 8).

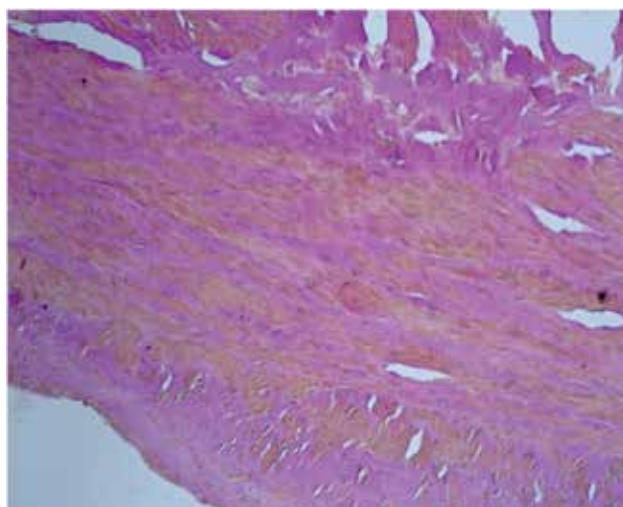
Immunohistochemical reaction with monoclonal antibody against  $\alpha$ -SMA revealed moderately positive expression («++», score 2) of this marker by smooth muscle cells in 6 cases (54.5%). The expression pattern of  $\alpha$ -SMA indicated damage to a portion of smooth muscle cells, manifested as their homogenization, predominantly in the medial layer of the venous wall. Immunohistochemical reaction

with monoclonal antibody against vimentin showed in 6 cases (54.5%) weakly positive expression («+», score 1) of this marker by single cells of the fibroblastic series.

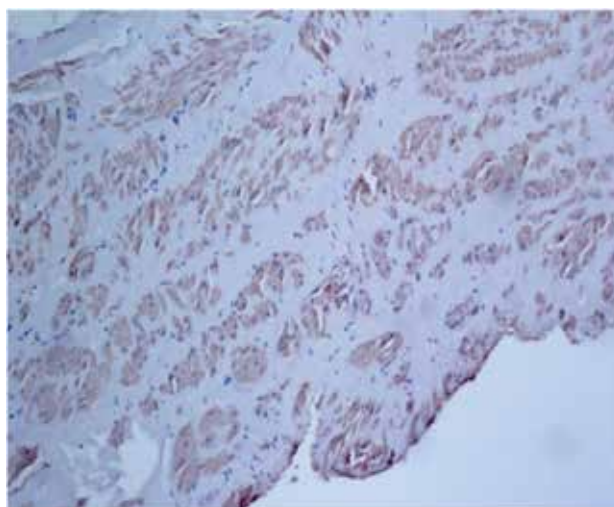
## DISCUSSION

The authors conducted a comprehensive morphological study of great saphenous vein fragments obtained from patients with chronic venous disease of the lower extremities who underwent treatment with the most commonly used endovenous techniques, including high-frequency electric welding in automatic mode, endovenous laser ablation, and ultrasound-guided microfoam sclerotherapy.

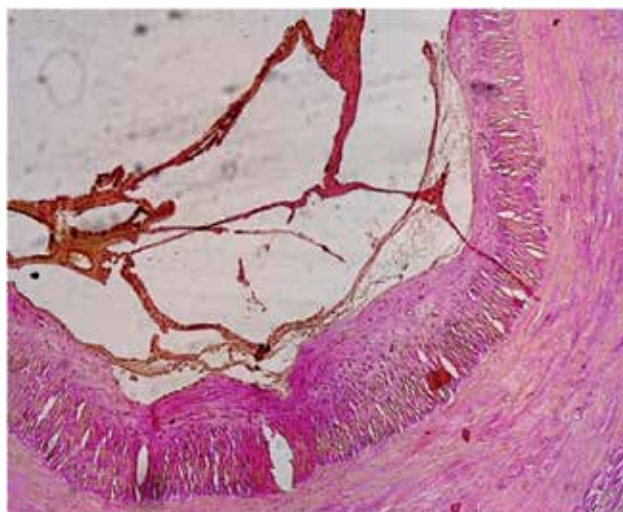
During microscope slides examination in cases treated with endovenous high-frequency electric welding in automatic



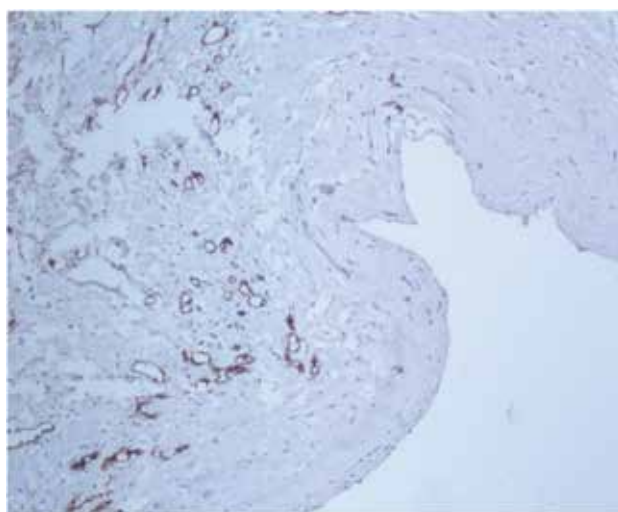
**Fig. 5.** Group 2. Destruction of layers structures, complete homogenization. Staining with picrofuchsin according to van Gieson,  $\times 100$ .



**Fig. 6.** Group 2. Moderately positive expression of  $\alpha$ -SMA by some fragmented smooth muscle cells. Immunohistochemical reaction with monoclonal antibody against  $\alpha$ -SMA,  $\times 100$ .



**Fig. 7.** Group 3. Preserved layered structure of the venous wall, partially intact endothelium, and thrombus fragments in the vessel lumen. Hematoxylin and eosin staining,  $\times 50$ .



**Fig. 8.** Group 3. Positive CD34 expression in venous endothelial cells and in vessels of the perivascular structures. Immunohistochemical reaction with monoclonal antibody against CD34,  $\times 50$ .

mode and endovenous laser ablation, the authors identified similarly pronounced and uniform structural changes across all layers of the venous wall. The endothelial layer was characterized predominantly by diffuse dystrophic and necrotic changes. Damaged endothelial cells were detached from the basement membrane and displaced into the vascular lumen. The dystrophic-necrotic and desquamative changes in endothelial cells resulted in a negative immunohistochemical reaction with monoclonal antibody against CD34 in all cases. The observed morphological disruption of endothelial integrity, as noted by the authors, indicates the presence of endothelial dysfunction. According to numerous studies, endothelial dysfunction is considered a key factor in the pathogenesis and progression of chronic venous disease of the lower extremities [17].

In cases where endovenous high-frequency electric welding in automatic mode and endovenous laser ablation were applied, the authors observed equally pronounced violations of the architectonics of the layers, edematous changes, and alterative changes in muscle and connective tissue fibers. These alterations, as revealed by immunohistochemical analysis, were manifested by decreased expression of vimentin and  $\alpha$ -SMA. The identified changes in the wall of the great saphenous vein will subsequently lead to fibrous transformation of the vein.

The morphological changes in the venous wall observed by the authors in this study following the use of endovenous high-frequency electric welding in automatic mode were consistent with previously reported findings in the literature [1-3].

In patients with chronic venous disease of the lower extremities who underwent ultrasound-guided microfoam sclerotherapy, focal alterative and desquamative changes in endothelial cells of the great saphenous vein were observed, accompanied by reduced CD34 expression by these cells. In the media and adventitia, the layered structure was

preserved with edema, focal areas of dysmucoidosis, regions of angiomatosis, and decreased expression of vimentin and  $\alpha$ -SMA.

A subsequent comparative analysis conducted by the authors demonstrated that the severity of structural changes in the venous wall layers following ultrasound-guided microfoam sclerotherapy was significantly lower compared to endovenous high-frequency electric welding in automatic mode and endovenous laser ablation, which to a lesser extent provides conditions for the qualitative development of the fibrotic process. This difference indicates the effectiveness of the effects of endovenous high-frequency electric welding in automatic mode and endovenous laser ablation.

## CONCLUSIONS

The great saphenous vein in patients with chronic venous disease of the lower extremities undergoing treatment with endovenous high-frequency electric welding in automatic mode and endovenous laser ablation was characterized by similarly pronounced diffuse alterative and desquamative changes in endothelial cells, disruption of the structural organization of the venous wall layers accompanied by edema, and alterative changes in both muscular and connective tissue fibers. In cases where ultrasound-guided microfoam sclerotherapy was applied, the great saphenous vein showed focal alterative and desquamative changes in endothelial cells, preservation of the layered structure, edematous changes in the media and adventitia, focal areas of dysmucoidosis, and regions of angiomatosis. The severity of structural changes in the venous wall following ultrasound-guided microfoam sclerotherapy was significantly lower compared to endovenous high-frequency electric welding in automatic mode and endovenous laser ablation, resulting in less favorable conditions for the qualitative development of the fibrous process.

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### CONFLICT OF INTEREST

The Authors declare no conflict of interest

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**A** – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article

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# Wielka Księga Balneologii, Medycyny Fizykalnej i Uzdrowiskowej

**Tom I**  
Część  
ogólna

**Tom II**  
Część  
kliniczna

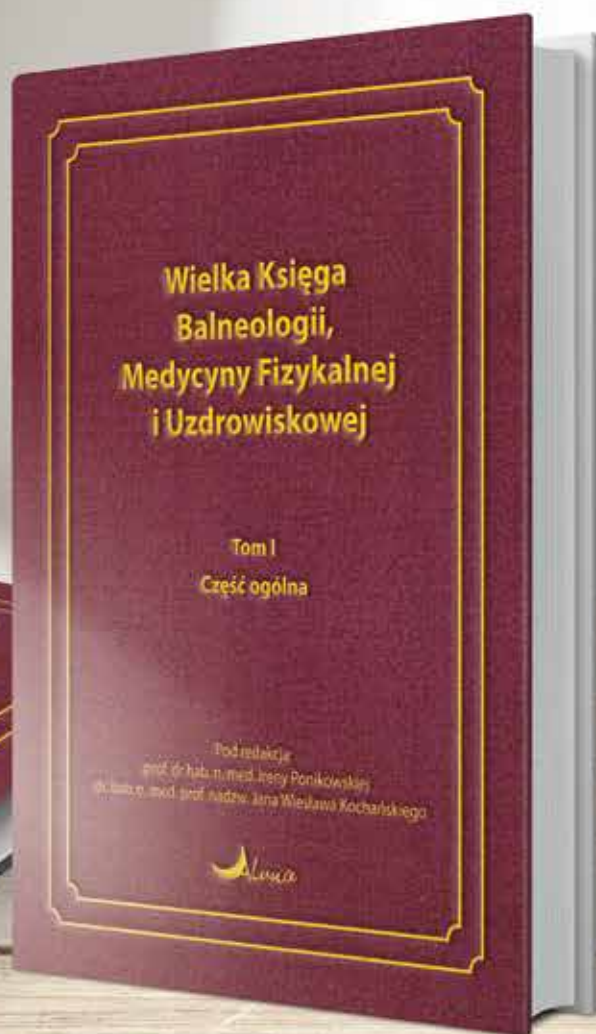
Pod redakcją:  
prof. dr hab. n. med. Ireny Ponikowskiej  
dr. hab. n. med. prof. nadzw. Jana Wiesława Kochańskiego

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