

THE UNIVERSITY OF MEDICINE AND PHARMACY
“VICTOR BABEȘ” FROM TIMIȘOARA
Doctoral School of Medicine-Pharmacy/
Doctoral School of Medicine

Andrei Florin Părau



PhD THESIS ABSTRACT

Histological and morphological aspects regarding the impact of
cryostripping on the venous wall

PhD Supervisor: **M.D. Ph.D. Prof. Habil. Sorin Olariu**

Timișoara

2024

Table of content

I. Introduction	1
II. General part	5
II.1. Chronic venous disease	5
II.1.1. Definition	5
II.1.2. Anatomical and morphological aspects of the superficial venous system of lower limbs	5
II.1.3. Epidemiology and risk factors	7
II.1.4. Signs and symptoms	7
II.1.5. Diagnosis	8
II.1.6. CEAP classification	9
II.1.7. Monitoring and prognosis.	11
II.1.8. Management of comorbidities associated with chronic venous disease.....	11
II.2. Therapeutic principles and modalities	15
II.2.1. Preventive	15
II.2.2. Curative	16
II.2.2.1. Classic	16
II.2.2.2. Modern	16
II.3. Cryostripping as minimally invasive, radical and effective procedure in the treatment of BVC	21
II.3.1. Principle	21
II.3.2. Operative and instrumental device.....	21
II.3.3. Technique	21
II.3.4. Postoperative conduct	22

III. Special part	25
III.1. Scope	25
III.2. Objectives	25
III.2.1. Etiological particularities in chronic venous disease - vein compression by intra-abdominal tumor masses. Gastrointestinal stromal tumor.-5 years follow up	25
III.2.1.1. Methodology	25
III.2.1.1.1. Case report	26
III 2.1.1.2. Therapeutical intervention and follow-up	26
III.2.1.1.3. Discussions	29
III.2.1.1.4. Conclusions	30
III.2.2. Hypothesis of etiology and treatment in primary venous aneurysms	31
III.2.2.1. Methodology	31
III.2.2.1.1. Patients and methods	31
III.2.2.1.2. Results and discussion.....	31
III.2.2.1.3. Conclusions	33
III.2.3. A comparative morphological analysis of healthy and insufficient veins related to the possibility of using them as a graft	33
III.2.3.1. Materials and methods	35
III.2.3.2. Statistical methods	36
III.2.3.2.3. Results and discussion.....	36
III.2.3.2.4. Conclusions	42
III.2.4. Morphological and immunohistochemical analysis	43
III.2.4.1. The particularities of connective fibers from the wall of varicose veins extirpated by cryostripping	74

III.2.4.2. Methodology	75
III.2.4.3. Materials and methods	75
III.2.4.4. Results and discussions	76
III.2.4.5. Conclusions	82
III.2.5. Cryosurgery harvested veins used as grafts? - an immunohistochemical analysis of the morphology and viability of venous samples obtained by cryostripping....	82
III.2.5.1. Methodology	82
III.2.5.1.1. Materials and methods	83
III.2.5.1.2. Statistical analysis.	83
III.2.5.1.3. Results and discussions	84
III.2.5.1.4. Conclusions	89
III.3. General conclusions	90
IV. Bibliography	93
ARTICLES PUBLISHED IN EXTENSO	I

ABSTRACT

The presented work is a scientific research project aimed at discussing, on one hand, the current stage regarding chronic venous disease, its implications, and on the other hand, the possibility of using varicose veins as viable materials in cardiovascular pathologies.

The thesis is divided in 2 distinct parts; the general part, which describes the present stage of knowledge about venous chronic disease, notions of anatomy, specific signs of CVD.

There are listed and explained the main causes leading to venous chronic disease, every individualization of treatment regarding the stages of the disease(CVD management includes lifestyle modifications, elevate lower limbs, avoiding prolonged standing, and maintain a balanced diet), pharmacological treatment, with great benefit in all stages(C0-C6) of CVD, pharmacotherapy include medications which improve symptoms such as pain, swelling, and promoting venous circulation, compression therapy, and interventional procedures,, different classic and modern investigations and procedure which are used in prognosis ,preventive and curative treatment in this current and widespread disease.

The entire research process starts from the idea of harvesting the great saphenous vein obtained through cryostripping following saphenectomy for chronic venous disease. It will then be stored in serum and formalin for various periods of time to observe, based on histological and immunohistochemical studies, their viability.

Firstly, I chose to initiate the research project by presenting some lesser-described and understood pathologies in chronic venous insufficiency, highlighting the importance of interdisciplinary collaboration in the treatment of complex cases.

Study 1-entitled **Etiological particularities in chronic venous disease - vein compression by intra-abdominal tumor masses.Gastrointestinal stromal tumor.-5 years follow up**, present a 65-year-old patient with a giant gastrointestinal stromal tumor (GIST) causing venous compression and CVD. The study highlights the importance of interdisciplinary treatment approaches.

Therapeutical Intervention and Follow-Up: The patient underwent multiple surgeries and treatments, including chemotherapy with Imatinib and Sunitinib, resulting in varying degrees of tumor reduction and recurrence.

Discussions: GISTs, though rare, can have significant implications on venous health due to their compressive nature. The study emphasizes the need for comprehensive diagnostic and therapeutic approaches in managing such complex case.

Study 2 Hypothesis of etiology and treatment in primary venous aneurysms.

Primary venous aneurysms are rare clinical-pathological entities. Also, there are relatively few literature data referring to the etiological hypotheses of this condition. This paper presents our results regarding the histopathological appearance highlighted in surgically resected venous aneurysm specimens that reveal the potential causes that lead to this condition onset, as well as a brief review the literature data. At the same time, current therapeutic options are discussed.

Conclusions: Due to the rarity of venous aneurysms, larger studies are needed to establish standardized treatment protocols. For patients who are not in the first decades of life, we consider that the presence of aneurysms is due to the processes of endofleboscclerosis.

The destruction of tunica media produced by the separation of the smooth muscle bundles and the significant deposits of fibrous tissue, which weaken the structure of the venous wall, allows aneurysmal dilatation.

Multicentric studies which analyze more data about the VA etiology would be useful to reach a consensus. Regarding the treatment, most data, including our clinical experience, recommend venous aneurysm resection performed through different procedures as the first therapeutic option

The main objectives, are reflected in each of the subsequent studies , represented by morphological and immunohistochemical analyses aiming to demonstrate the possibility of using veins obtained through cryostripping from the perspective of their viability as grafts.

Study 3 -. A comparative morphological analysis of healthy and insufficient veins related to the possibility of using them as a graft

The purpose was to evaluate the possibility of using varicose veins as grafts for coronary artery bypass grafting (CABG).

Materials and Methods: Morphological comparisons between healthy veins and varicose veins, including statistical analyses. This prospective study included patients evaluated in public health services in Timisoara, Romania, between September 2022 and September 2023, with healthy or varicose lower limb veins, which underwent different surgeries in which at least one venous specimen was harvested

Results and Discussions:The study found significant morphological differences, suggesting that while varicose veins have potential as grafts, further research on larger patient groups is necessary to determine their long-term viability.

Conclusions: More extensive studies are needed to conclude definitively on the use of varicose veins as grafts in CABG.

Study 4- The particularities of connective fibers from the wall of varicose veins extirpated by cryostripping

This study analyzed the structural features of collagen, elastic, and reticulin fibers in 109 samples collected via cryostripping. Samples were processed using standard histochemical staining techniques to evaluate the distribution and density of connective fibers. Scores were assigned based on fiber appearance, ranging from 0 (normal) to 3 (severe depletion).

Results

The study identified varying degrees of fibrosis and fiber alterations in the varicose vein samples:

- Collagen Fibers: Collagenization ranged from minor disorganization to extensive fibrosis affecting the entire venous wall.
- Elastic Fibers: Changes included partial depletion to complete absence, with some specimens showing internal elastic lamina multiplication.
- Reticulin Fibers: These were the most resilient, with fewer cases of severe depletion compared to collagen and elastic fibers.

Discussion

Varicose veins exhibit significant morphological changes, including endothelial hypertrophy, collagenosis, and muscle cell hypertrophy. Chronic venous disease progression, classified using the CEAP system, highlights the increase in symptoms from simple varicosities to severe ulcerations. Histological studies reveal substantial depletion of elastic fibers and increased collagen deposition in affected veins. These changes are believed to result from the chronic nature of the disease rather than the surgical method.

Conclusion

The study underscores the extensive structural alterations in varicose veins, characterized by increased collagen and decreased reticulin fibers. The introduction of orcein staining could enhance the investigation of venous vessels, providing better insights into the pathological changes in varicose veins. Cryostripping remains a viable and effective treatment option, demonstrating comparable outcomes to more popular methods like endovenous laser ablation.

Study 5- Cryosurgery harvested veins used as grafts? - an immunohistochemical analysis of the morphology and viability of venous samples obtained by cryostripping

This study focuses on evaluating the viability of venous fragments harvested by cryostripping, utilizing markers CD34 and Ki67 to assess the endothelial integrity and cell proliferation potential of these specimens.

CD34: A marker primarily for hematopoietic stem and progenitor cells, and also for endothelial cell progenitors and mature endothelial cells. CD34 is involved in endothelial sprouting and angiogenesis, making it crucial for evaluating the viability of harvested veins.

Ki67: A marker for cell division and the immediate postmitotic period. While not specific to endothelial cells, Ki67 helps determine cell proliferation, providing insights into whether the cells in the harvested veins are actively dividing.

109 great saphenous vein samples from patients who underwent cryostripping for varicose veins. Each specimen was divided into six samples, with one fixed in formalin immediately and the others maintained in saline for varying durations (2, 4, 12, 24, and 48 hours) before fixation.

Immunohistochemistry: Performed using the Leica Bond-Max autostainer for CD34 and Ki67. CD34 localization and continuity, along with Ki67-positive cell density, were analyzed microscopically.

Initial (Zero-hour): CD34 was intensely positive in the intima of 89 (81.61%) samples, indicating endothelial viability.

2 to 12 Hours: No significant changes in CD34 expression. 24 to 48 Hours: Increased lacunar expressions in the intima, with 37 (33.94%) specimens showing negative CD34 reactions after 48 hours, indicating endothelial damage due to prolonged storage.

The findings emphasize the importance of CD34 in testing the viability of cryostripped venous specimens and indicate that endothelial cells begin to show damage after 12 hours in saline, with significant damage observed after 48 hours. Ki67 analysis showed that cell proliferation is significantly reduced after 12 hours and non-existent after 48 hours, questioning its utility for assessing vein graft viability after extended storage.

Conclusions

CD34: Effective for evaluating the morphological and molecular integrity of venous specimens, with viability best preserved within the first 24 hours of harvesting.

Ki67: Not recommended for determining vein graft viability due to the rapid decrease in expression post-harvesting.

Cryostripping is viable for harvesting veins for grafting if used within 24 hours post-harvesting to ensure endothelial integrity and viability.

Further research is necessary to evaluate clinical validity and explore additional markers for a comprehensive assessment of vein graft viability.

Starting from chronic venous disease, its implications and complications, in the presented study we tried to find usefulness in terms of the use of varicose veins as potential grafts, and the results obtained open new perspectives in this regard.