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THE EXPRESSION PROFILE OF INTRINSIC APOPTOTIC PATHWAY GENES IN VARICOSE VEINS AND HEALTHY VEINS

MASTER THESIS

2023-2024

NEAR EAST UNIVERSITY

INSTITUTE OF GRADUATE STUDIES

DEPARTMENT OF MEDICAL GENETICS

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APPROVAL

We certify that we have read the thesis submitted by Fatima Ageed titled "**The expression profile of intrinsic apoptotic pathway genes in varicose veins and healthy veins**" and that in our combined opinion it is fully adequate, in scope and quality, as a thesis for the degree of Master of Medical Biology and Genetics.

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Declaration

I hereby declare that all information, documents, analysis and results in this thesis have been collected and presented according to the academic rules and ethical guidelines of Institute of Graduate Studies, Near East University. I also declare that as required by these rules and conduct, I have fully cited and referenced information and data that are not original to this study.

Fatima AGEED

05/02/2024

Acknowledgment

This work stands as a testament to the collective effort and encouragement of many individuals, for which I am profoundly thankful. I extend my deepest gratitude to all those whose unwavering support and guidance made this research possible.

To my supervisor, Assoc. Prof. Dr. Mahmut Çerkez ERGÖREN, your exemplary support, immense knowledge, and extensive experience have inspired me to explore unfamiliar terrains and pursue novelty in research. Your tremendous understanding and encouragement were invaluable; without them, I could not have completed my studies.

I am indebted to my esteemed professors and their assistants who engaged with my questions and opinions with curiosity, provided support, tutelage, and were instrumental in shaping my academic career, preparing me for the challenges ahead. I am grateful to those who believed in my potential, sparked my interest in medicine and research, and challenged me to excel in my field. Thank you.

I am profoundly grateful to the members of my research team Prof. Dr. Barçın ÖZCEM, Dr. Uğurcan OLMAZ and Aya Badeea Ismael for their dedication and collaborative spirit. Each contribution has woven a tapestry of knowledge and perseverance. I extend my gratitude to the individuals who graciously participated in this study, enriching the quality of this work, and making it relevant and meaningful.

Heartfelt appreciation goes to my family for their love, understanding, and encouragement. Your unwavering support sustained me through the highs and lows of this endeavor. I thank my elder sister, Moshira, for being my role model, my best friend, and my biggest cheerleader.

Lastly, to all those not explicitly mentioned but who contributed in various ways, your support did not go unnoticed and is deeply appreciated.

Fatima AGEED

Abstract

The Expression Profile of Intrinsic Apoptotic Pathway Genes in Varicose veins and Healthy Veins

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February, 2024, 138 Pages

Varicose veins (VVs) constitute a chronic vascular condition impairing lower extremity blood circulation, often leading to debilitating symptoms such as pain, oedema, and skin ulcers. While the molecular underpinnings of VVs remain elusive, emerging evidence suggests a potential involvement of apoptosis. Central to this process are mitochondria, pivotal organelles orchestrating cellular energy metabolism and apoptosis regulation. This study aimed to probe the intrinsic pathway of apoptosis by investigating the expression profiles of key genes—*Cytochrome-c*, and *Caspase-3* in biopsies from individuals afflicted with VVs compared to healthy controls.

Through meticulous RNA extraction from vein biopsies followed by cDNA synthesis, q-PCR was employed to decipher the relative expression levels of these target genes. A comprehensive analysis involving 89 cases was conducted. The obtained p-value, surpassing the threshold of 0.0001, signifies statistical significance. These findings collectively suggest a strong substantial correlation between the expression levels of these key genes related to the intrinsic pathway of apoptosis and the studied vascular condition, Varicose Veins (VVs). Patients diagnosed with Varicose Veins (VVs) exhibited markedly elevated expression levels of *Cytochrome-c* and *Caspase-3* when compared to the control group. These observed alterations in expression profiles indicate a distinct association with dysregulated apoptosis-related genes. Such an imbalance within the intrinsic pathway of apoptosis suggests a potential contributory factor to the tissue damage observed in Varicose Veins. This statistical significance reinforces the putative involvement of these proteins in the molecular mechanisms underlying varicose veins, paving the way for further exploration into their roles as potential biomarkers or therapeutic targets.

Keywords: varicose veins, apoptosis, BCL-2 proteins, gene expression, biomarker.

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List of Abbreviations

aFGF:	Acidic Fibroblast Growth Factor
ATP:	Adenosine Triphosphate
Apaf-1:	Apoptotic Protease Activating Factor 1
Atg:	Autophagy-Related Gene
BMI:	Body Mass Index
Ca^{2+}:	Calcium Ion
CASP:	Caspase
CAD:	Caspase Activated DNAse
CARD:	Caspase Recruitment Domain
CASZ1:	Castor Zinc Finger 1
CVD:	Chronic Venous Disease
CVI:	Chronic Venous Insufficiency
CEAP:	Clinical Etiology Anatomy Pathophysiology
C:	Clinical Manifestations
CFV:	Common Femoral Vein
cDNA:	Complementary DNA
CLEAR:	Coordinated Lysosomal Expression and Regulation
cAMP:	Cyclic Adenosine Monophosphate
CTLs:	Cytotoxic T Lymphocytes
DR:	Death Receptor
DFV:	Deep Femoral Vein
DVT:	Deep Venous Thrombosis
DNAse:	Deoxy Ribonuclease
dNTPs:	Deoxy Ribonucleotide Triphosphates
EBF1:	Early B Cell Factor 1
ESCRT:	Endosomal Sorting Complex Required for Transport
ECs:	Endothelial Cells
ELA:	Endovenous Laser Ablation
EGFL7:	Epidermal Growth Factor-Like Domain 7
EDTA:	Ethylene-Diamine-Tetra Acetic Acid
ECM:	Extracellular Matrix
FIP:	Family Interacting Protein
FV:	Femoral Vein
GPCRs:	G Protein-Coupled Receptors
GATA2:	GATA-Binding Protein 2
GWAS:	Genome-Wide Association Studies
GSV:	Great Saphenous Vein
bHLH-Zip:	Helix-Loop-Helix Leucine-Zipper
LC3:	Hight Chain 3
HDAC5:	Histone Deacetylase
HIR:	Homeostatic Iron Regulator
IAP:	Inhibitor of Apoptosis Proteins
IGF-1:	Insulin-Like Growth Factor 1
IGF-1R:	Insulin-Like Growth Factor 1 Receptor

IL:	Interleukin
lncRNA:	Long Non-Coding RNA
MR:	Magnetic Resonance
MRV:	Magnetic Resonance Venography
mTORC1:	Mammalian Target of the Rapamycin Complex 1
MGP:	Matrix Gla Protein
MMPs:	Matrix Metalloproteinases
mRNA:	Messenger RNA
MFAP5:	Microfibril-Associated Protein 5
µl:	Microliter
µM:	Micromole
MPFF:	Micronized Purified Flavonoid Fraction
MiT:	Microphthalmia-Associated Transcription Factor
miRNA:	Micro-RNAs
mL:	Milliliter
MAPKs:	Mitogen-Activated Protein Kinases
MCP:	Monocyte Chemotactic Protein
ng/µL	Nanogram Per Microliter
NFAT:	Nuclear Factor of Activated T Cells
NFATC2:	Nuclear Factor of Activated T Cells 2
PPARs:	Peroxisome Proliferator-Activated Receptors
PE:	Phosphatidyl Ethanolamine
PI3K:	Phosphatidylinositol 3-Kinase
PI3P:	Phosphatidylinositol-3-Phosphate
PAI-1:	Plasminogen Activator Inhibitor 1
PDGF:	Platelet-Derived Growth Factor
PCR:	Polymerase Chain Reaction
PV:	Popliteal Vein
K^+:	Potassium
ProC:	ProCaspaes
PKA:	Protein Kinase
PPP3R1:	Protein Phosphatase 3 Regulatory Subunit B, Alpha
QOL:	Quality of Life
qPCR:	Quantitative Polymerase Chain Reaction
RF:	Radiofrequency
RFA:	Radiofrequency Ablation
RBCs:	Red Blood Cells
RT:	Reverse Transcriptase
RNase:	Ribonuclease
RNA:	Ribonucleic Acid
SFJ:	Saphenofemoral Junction
siRNA:	Short-Interfering RNA
SNPs:	Single Nucleotide Polymorphisms
SSV:	Small Saphenous Vein
SMCs:	Smooth Muscle Cells
Na^+:	Sodium
SNARE:	Soluble N-Ethyl Maleimide-Sensitive Protein Attachment Receptor

SOX9: SRY-Box Transcription Factor 9
STIM2: Stromal Interaction Molecule 2
SNAP: Synaptosomal-Associated Protein
Treg: T Regulatory
TFEB: Tanscription Factor EB
Th: T-Helper Cells
TIMPs: Tissue Inhibitors of Metalloproteinases
TGF-β1: Transforming Growth Factor Beta 1
TBE: Tris-Borate EDTA
TNF: Tumour Necrosis Factor
US: Ultrasound
ULK1: Unc-51-Like Autophagy-Activating Kinase 1
VPS: Vacuolar Protein Sorting
VV: Varicose Veins
VEGF: Vascular Endothelial Growth Factor
VAMPs: Vesicle-Associated Membrane Proteins
WIPI: WD Repeat Domain Phosphoinositide-Interacting Protein
WBCs: White Blood Cells

CHAPTER I
Introduction

Statement of the Problem

Chronic venous diseases (CVDs) are a medical condition characterised by impaired venous return from the lower extremities to the heart, resulting in inadequate blood pumping functionality inside the leg veins. They are characterised by varicose veins (VVs), venous insufficiency (VI), venous ulcers, deep vein thrombosis (DVT), or pulmonary embolism. The aetiology is multifactorial, and the exact cause remains poorly elucidated. A dearth of efficacious therapeutic interventions may be observed in the management of this condition. Its diagnosis often entails a thorough physical exam and imaging tests. The physical examination examines the lower limbs for symptoms such as varicosity, ulceration, and skin colour changes. Imaging examinations determine the veins' anatomical distribution related to the disease. This involves distinguishing between deep and superficial veins and even infrainguinal and proximal venous diseases. These examinations also detect pathophysiology, such as reflux, obstruction, or both.

To correlate the patient's symptoms and signs, imaging and diagnostic tests are used. However, a patient may show signs of venous reflux or obstruction during a duplex ultrasonography evaluation yet remain asymptomatic if the condition is mild. On the other hand, it is possible for a patient to have symptoms resembling venous illness yet provide negative test results, as these symptoms may be attributed to another cause. Venograms and duplex ultrasonography are often used to diagnose varicosities. However, these methods have limitations that may affect their accuracy. Weight, pregnancy, DVT, image quality, and compression are among the constraints. If the preceding criteria are satisfied, duplex ultrasonography and venograms may be able to identify VVs; conversely, they may reduce test accuracy and need further testing to confirm the diagnosis. To diagnose and monitor impairment, a reliable, non-intrusive diagnostic method is needed.

Purpose of the Study

This research compares the expression of intrinsic apoptotic proteins in healthy participants versus people who have been diagnosed with varicose veins. It aims to outline the crucial role that these proteins play in the development of varicose veins.

Research Hypotheses and Questions. The expression of apoptosis proteins may undergo changes in people with varicose veins as compared to those who are in good health. An inequilibrium in the levels of pro- and anti-apoptotic proteins, for instance, Bcl-2 and Bax, may have an impact on the development of varicose veins. Furthermore, Cytochrome-c being released from the mitochondria and subsequent initiation of the Caspase-3 cascade may potentially contribute to the development of varicosities. These pathways have the potential to result in varicose veins (VVs) by triggering the death of venous endothelial cells.

What is the apoptotic protein expression profile in individuals diagnosed with varicose veins?

Is there a correlation between the expression of apoptotic proteins and the severity of clinical symptoms in individuals diagnosed with varicose veins?

Significance of the Study. There are currently a few investigations on the participation of the intrinsic apoptotic proteins, particularly Bax, Bcl-2, Cytochrome-c, and Caspase-3, in varicosities. These proteins are crucial in the regulation of the critical equilibrium between cellular survival and death. Consequently, a notable disparity exists in this comprehension of the potential role of these proteins in the development of varicose veins. Therefore, the significance of this study lies in shedding light on the underlying mechanisms contributing to the pathophysiology of this common vascular condition. Understanding the intricate interplay and expression of apoptotic proteins within the vein wall could offer crucial insights into the cellular processes leading to the development and progression of varicose veins. Also, uncover potential biomarkers or therapeutic targets associated with varicose veins. Such findings could pave the way for

the development of novel diagnostic tools and innovative treatment strategies tailored to intervene at the molecular level, thereby offering more effective and targeted therapies for individuals affected by varicose veins.

This study holds substantial promise for expanding the existing scientific knowledge within the realm of vascular biology.

Limitations

The current investigation possesses certain constraints that necessitate consideration when interpreting the findings. Initially, it should be noted that the sample size of 89 may not possess the statistical power to establish results that are statistically significant. Furthermore, it is important to consider the possible influence of selection bias on the generalizability of the study outcomes. The sample for this study has been collected from a single medical facility, which might potentially affect the applicability of the results to a wider population. Likewise, it is important to consider the potential impact of variables, including age, gender, lifestyle factors, and comorbidities, on the onset and advancement of varicose veins. Despite attempts to consider these variables in the study's design and statistical analysis, the presence of residual confounding cannot be ruled out. Furthermore, it is important to acknowledge that ethical issues, such as obtaining informed permission from participants and ensuring their privacy, might have potentially influenced the implementation of the study protocols. Although it has obtained ethical approval from the relevant authorities, it is possible that some patients may have had limited comprehension of some aspects of their condition. Additionally, time limitations may have been a contributing factor.

Definition of Terms

CVD, particularly varicose vein, is a medical condition characterised by impaired venous return of blood from the lower extremities to the heart. This condition is defined by the inability of the venous valves to effectively prevent the retrograde flow of blood, resulting in the accumulation of blood within the veins and elevated venous pressure.

Apoptosis is a highly regulated process that begins with the production of Cytochrome-c from the mitochondria and ends with its migration to the cytosol. Then, it interacts with Apaf-1, triggering the Caspase-9 stimulation process. Caspase-9 then triggers the activation of downstream effector Caspases, including Caspase-3.

Bax is a pro-apoptosis protein known for its involvement in inducing apoptosis. This protein promotes apoptosis via stimulating the release of Cytochrome-c from mitochondria. It is triggered by responding to a variety of cellular stressors, notably oxidative stress, growth factor shortages, and damaged DNA. When activated, Bax moves from the cytosol to the outer mitochondrial membrane, where it oligomerizes, generating holes that enable Cytochrome-c to pass from the mitochondria to the cytosol.

Bcl-2 is an anti-apoptotic protein that regulates the cell death process. The primary mechanism of action of this compound involves controlling the permeability of the membranes surrounding the mitochondria, thereby preventing the release of Cytochrome-c.

Cytochrome-c is characterised by the presence of a heme group and is of great importance in the processes of oxidative phosphorylation and adenosine triphosphate (ATP) generation within mitochondria.

Caspase-3 is classified as a cysteine protease and is one of the caspase protein family members. Its primary function is to participate in the execution stage of apoptosis. It exhibits proteolytic activity, leading to the activation of several caspases and other proteins implicated in the process of programmed cell death.

CHAPTER II
Literature Review

Introduction

Chronic venous insufficiency (CVI) refers to the most serious presentations of venous illness, comprising symptoms such as oedema, alterations in the skin, and leg ulcers (Hyder & Soukas, 2017), which are frequently associated with a major reduction in the quality of life for those with this condition (Moura et al., 2010). Chronic venous disease (CVD) is characterised as an enduring, progressive condition that is often underestimated and is widespread in the general population, resulting in significant socio-economic, psychological, and physical consequences (Nicolaides & Labropoulos, 2019; Davies, 2019). A complicated interaction between hereditary and environmental variables is responsible for increasing ambulatory venous pressure, ultimately culminating in significant changes to the whole functioning and structure of the venous system (Ligi et al., 2018). It is critical to distinguish chronic venous disorder, which refers solely to the complete functional and morphological abnormalities of the venous system, excluding consideration of clinical manifestations and other significant issues pertaining to the patient (Eklof et al., 2009). Reticular and telangiectasias veins are additional venous symptoms. Even though clinical symptoms can occur in any vein, the venous system in the lower limbs exhibits increased susceptibility to varicose veins (Piazza, 2014). This sensitivity stems mostly from the increased resistance experienced when resisting the force of gravity, which is noticeably greater in the lower limbs than in other parts of the body.

Histology reveals that there are three distinct layers of veins. The tunica intima, or inner layer, is predominantly made up of endothelial cells (ECs). The media layer, also known as the tunica media, is distinguished by its abundance of vascular smooth muscle cells (SMCs) along with just enough elastic fibre. The tunica adventitia, or outside layer, is mostly formed of connective tissue with a significant proliferation of elastic fibres, providing the vessel with flexibility (Tucker et al., 2023). In the case of varicose veins (VVs), however, there are major modifications in the usual structure of the vein, resulting in substantial alterations in both the thickness and nature of the

venous wall (Jacobs et al., 2017). Furthermore, veins have venous valves, and these are bicuspid extensions of venous cells that serve an important function in maintaining the appropriate direction of the circulatory system and thereby preventing venous reflux (Tansey et al., 2019). Likewise, several muscle pumps collaborate with these valves to ensure one-directional blood flow. Particularly, the calf muscle has been identified as a crucial contribution to improving the circulation of blood back to the heart (Recek, 2013). It is critical to recognise that malfunctions in both muscle pumps and valves are critical for understanding the pathophysiology and progression of this condition, culminating in reflux and stasis of the venous system (Uhl & Gillot, 2015; Raetz et al., 2019; Sukhovatykh & Sukhovatykh, 2015; Nicolaides et al., 2018).

Figure 1

Anatomical Depiction of Vein Layers, Venous System Dynamics, and Varicose Vein. Figures modified with text, markings (line), and annotation after adaptation of "Veins figures A and C, Blood figure B" from Servier Medical Art by Servier, licensed under a Creative Commons Attribution 3.0 Unported License

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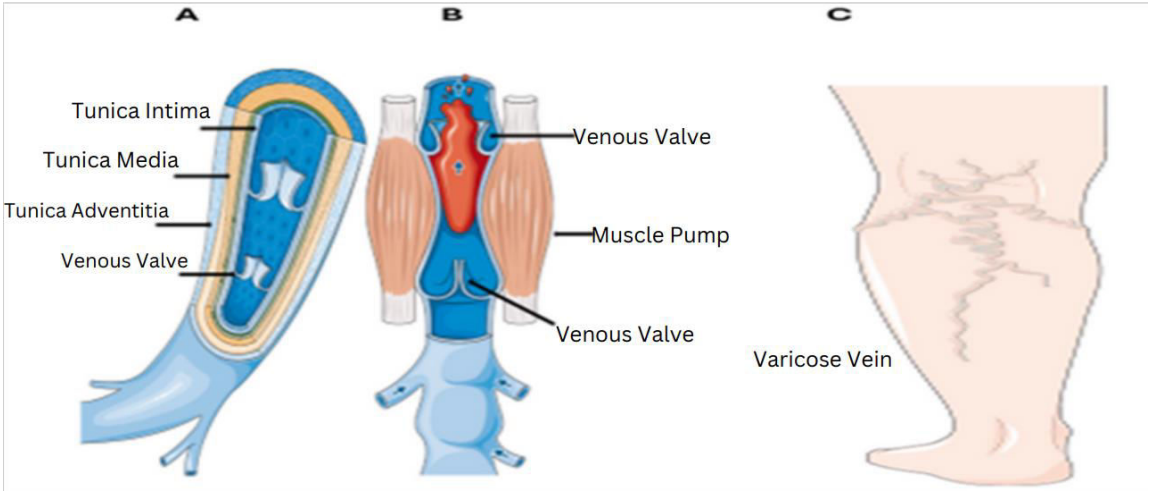


(A) Layers of vein structure (B) Venous valves and muscle pump collaboration and (C) Varicose vein.

Clinical Etiology Anatomy Pathophysiology Classification

Various criteria are currently in place to differentiate different presentations of CVD. The CEAP (Clinical Etiology Anatomy Pathophysiology) classification has become a widely recognised approach for conducting a comprehensive evaluation of venous disorders on a global scale. It is founded on distinct medical features that facilitate a precise assessment of vein status (Carman & Al-Omari, 2019). Clinical manifestations (abbreviated 'C') are frequently utilised to classify specific CVD manifestations. VVs, for example, may be classified as C2, whereas CVIs are classified as C3 to C6 (Santler & Goerge, 2017). Patients can be classified along a continuum from C0, signifying the absence of observable or palpable disease, through C1, indicating the presence of telangiectasias or reticular veins, while C2 signifies VVs, C3 indicates the occurrence of oedema, and C4A and C4B represent skin pigmentation and lipodermatosclerosis or atrophie blanche, respectively. Additionally, the CEAP scale allows for the classification of patients into C5 if they have a healed ulcer or C6 in cases of an active ulcer. To further distinguish between asymptomatic and symptomatic patients, an A or S signifier is affixed (Eberhardt & Raffetto, 2014; Grosse et al., 2016; Lurie et al., 2020). The majority of clinical studies on chronic venous insufficiency (CVI) employ the CEAP scale to evaluate and report the severity of the disease among patients.

Incidence and Contributing Factors in Disease Patterns

CVD is a prevalent condition that affects a substantial proportion of the global population. In broad terms, its estimated occurrence spans from 60% to 80% (Zolotukhin et al., 2017; Beebe-Dimmer et al., 2005; Rabe et al., 2012). Nevertheless, the percentages can vary significantly in the scientific literature due to differences in the populations under examination, the methodologies employed, and the definitions of the ailment (Salim et al., 2021). In the Rabe et al. (2016) study, most of the cases are classified into C0 and C1 categories, while around a quarter of patients have been diagnosed with venous varicosities (C2). CVI (C3–C6) is relevant for a smaller fraction of individuals with CVD, comprising up to 5% of patients (Rabe et al., 2016). According to the Framingham study, the occurrence of venous varicosities stands at about 2.6% among females and 1.9% among males (Brand et al., 1988). The risk increases with age, which explains the high frequency and growing incidence associated with these conditions (Vuylsteke et al., 2015). Furthermore, the diagnosis of varicose veins can significantly affect the overall quality of life (QOL) experienced by individuals. Even those with uncomplicated venous varicosities have a significant reduction in their overall QOL, whereas if abandoned without treatment, they are likely to develop CVI (Pannier & Rabe, 2012). Consequentially, varicose veins are undeniably considered among the most frequent vascular diseases worldwide, imposing a substantial burden not only on individuals but also on healthcare systems. As previously mentioned, advanced age represents a prominent and influential risk factor for varicosities. The findings of Edinburgh's study provide credence to this argument, which revealed that approximately one in three patients eventually developed CVI after having been diagnosed with venous insufficiency, especially as the years passed. This risk exhibited a consistent upward trend with increasing age (Lee et al., 2015). Furthermore, Vuylsteke et al. (2018) observed that individuals aged 65 years and older displayed heightened susceptibility to various CVD risk factors, with variations observed across different geographical regions. As a result, elderly individuals are more prone to both the initiation and progression of CVD, especially when additional risk factors like body mass index (BMI) (Musil et al., 2011) and female gender (Vuylsteke et al., 2016) are present. The higher prevalence of venous disease in women can be attributed to a combination of

physiological factors and hormones, making them more predisposed to the condition (Lohr & Bush, 2013). Pregnant women are notably susceptible to CVD, with roughly 40% experiencing this condition (Ortega et al., 2021). Furthermore, the parity of pregnancies seems to be a crucial determinant in the progression of this ailment. It is anticipated that 20% of women over the age of 40 who have never given birth will develop venous insufficiency. However, for women who have had one to four pregnancies, this ratio ascends to 40%, and for those who have had five or more pregnancies, it elevates to 65% (Cornu-Thenard & Boivin, 2014). Aside from gender and increasing age, numerous factors have been demonstrated in the progression of varicose veins. Obesity stands out among proven indicators of risk that are independently and directly associated with the CEAP classifications (Vlajinac et al., 2013). This association might be related to a variety of plausible and combinatorial factors, which include the pro-inflammatory state commonly associated with obesity. Moreover, higher abdominal pressure can lead to worsening venous reflux, expanded vein diameter, and raised pressure in the veins (Patel et al., 2021). A sedentary lifestyle is a further substantial contributory risk for the onset and development of CVD symptoms (Cavezzi, 2020). On the contrary, physical activity has not been associated with an increased incidence of this disease; rather, it is considered a vital approach for minimising the condition's progression (Jones et al., 2018). Sitting for an extended period and, to a lesser extent, standing for long periods have also been identified as contributing factors for CVD, both circumstances being tied to professional activities. Varicosities in the family have been identified as a further indicator of risk for the eventual occurrence of this health problem (Sharma et al., 2017). Plus, having a history of blood clots, especially deep vein thrombosis (DVT) (Vlajinac et al., 2012), besides habitual smoking (Kondo et al., 2019) have been considered to be possible risk variables. However, there is still some uncertainty about a prospective link between VVs and height. Even though a few investigations have proposed a causal association (Fukaya et al., 2018), evidence suggests that a person's height has no effect on the course of the condition (Spáčil, 2015). Fukaya et al. (2018) observed a correlation between varicose veins and height in their analyses. They concluded their findings by utilizing statistical methods including linkage disequilibrium score regression and mendelian

randomization, which demonstrated a common genetic cause and validated a significant causal association between taller stature and the development of varicose veins. In conclusion, a multitude of risk factors are potentially linked to the initial appearance and progression of varicose veins since they might influence multiple pathophysiological systems implicated in the disease's onset and progression.

Anatomy of the Lower Extremities

Three distinct systems can be identified within the lower extremities: the superficial veins, which primarily consist of the great and small saphenous veins; the saphenous vein, along with its tributaries, is anatomically connected to the anterior aspect; the deep veins, which are responsible for primary circulation and blood; and the perforating veins, which serve as connectors between both of these systems (Youn & Lee, 2019).

Superficial Veins

A multitude of minuscule venules within the subcutaneous tissues combine, forming smaller veins, which subsequently join with further smaller veins, ultimately converging into the primary superficial veins located in the lower limbs, the great and small saphenous veins (GSV) and (SSV), respectively. The initial branching of the GSV often takes place in the medial region of the dorsal foot, progressing in an anterior direction towards the medial malleolus. It then follows a posterior-medial path around the knee joint and expands up to the medial part of the thigh. The point at which the GSV converges onto the common femoral vein (CFV) typically occurs inside the inguinal crease, particularly above or adjacent to the juncture where the deep femoral vein (DFV) merges with the femoral vein. Although there are certain anatomical differences in the structure of the GSV, many of these can be more accurately described as significant tributaries or auxiliary veins. The phenomenon of a complete replication of the GSV is a rare event, with an approximate incidence rate of only 1% among the population (Lee et al., 2017; Chen & Prasad, 2009).

Perforator Veins

A multitude of small and diverse perforator veins are commonly involved in facilitating the flow of fluid into the deep venous system from superficial veins. The identification of those veins is accomplished by utilising anatomical designations such as thigh, knee, leg, or ankle in conjunction with positional descriptors such as anterior, medial, posterior, or lateral. This approach is particularly useful when the anatomical reference lacks precision in terms of location. Among them, the medial perforators situated in the thigh and leg have significant importance because of their frequent involvement in facilitating the flow of the GSV into the deep venous system (Lee et al., 2017). The inguinal perforator vein and femoral canal perforator vein are the most prominent instances of these medial perforators in the thigh. Those perforators are distinguished by their cranio-caudal positioning along the medial portion of the thigh. The calf region contains the posterior tibial and paratibial veins, which are considered to be the medial perforators. Perforator veins, although devoid of any clinical problems, often exhibit diminutive dimensions and need thorough inspection during ultrasound (US) imaging since they might be difficult to identify without attentive investigation (Lee et al., 2017).

Deep Veins

The deep veins in the lower limbs have names similar to those of the main arteries. The peroneal vein, in addition to the posterior and anterior tibial veins, usually occurs in pairs in the calf region, forming the three deep veins. The peroneal vein is accountable for the drainage of the posteromedial lower leg into the tibioperoneal trunk. Concurrently, the posterior tibial vein, highlighted in the Lee et al. (2017) study, facilitates the flow of fluid from the plantar surface of the foot and the posterior compartment of the leg into the tibioperoneal trunk. The deep veins in the thigh comprise the popliteal vein (PV), femoral vein (FV), DFV, and CFV. The posterior tibial vein goes in a posterior direction and is located medially to the artery below the knee. It runs superficially to the artery at knee level, then moves laterally with respect to the artery above the knee. The conversion of the popliteal vein (PV) to the femoral vein (FV) takes place at the adductor hiatus. The femoral vein (FV) courses down the

anteromedial aspect of the thigh, situated in a medial position relative to the artery. The superficial femoral vein, owing to its anatomical proximity to the superficial femoral artery, is now recommended to be described without the adjective "superficial" in order to prevent any ambiguity regarding its location inside the deep thigh. The deep femoral vein has its origin in the deep areas of the thigh, and its lower segments provide difficulties in terms of visualisation using ultrasonic techniques. It eventually joins the femoral vein to create the CFV, which then becomes the external iliac vein after passing through the inguinal ligament (Lee et al., 2017).

Pathophysiology

Chronic venous disease is a multifaceted condition characterised by numerous pathophysiological pathways. An increase in ambulatory pressure and the expansion of veins in the lower extremities initiate a complex vascular response, resulting in a subsequent inflammatory reaction, stasis, and alterations in shear stress. Blockage and reflux are two main pathomechanisms that contribute to notable alterations in the structure of venous walls, leading to the development of aberrant venous reflux. These, in turn, are pivotal factors in the advancement of venous disease (Labropoulos, 2019). Moreover, these alterations lead to the creation of a hypoxic milieu, which has been postulated to exert a significant influence on the pathogenesis of the illness. Likewise, specific genetic mutations or variants have been associated with the aetiology of the condition. Additionally, it has been shown that patients with chronic venous disease exhibit genetic modifications, and further substantiation exists to suggest a connection between the advancement of this condition and a deterioration in systemic function (Cavezzi, 2020; Raffetto, 2018; Raffetto & Mannello, 2014; Ortega et al., 2019).

Variations in Circulatory and Microcirculatory Dynamics

The return of venous blood to the heart presents more challenges compared to arterial blood flow due to the resistance posed by gravity. The primary purpose of venous valves is to prevent the retrograde flow and accumulation of blood. However, the efficacy of these valves in performing their role is compromised by mechanical stress, leading to their diminished functionality. The assessment of obstruction portions and

reflux may provide valuable insights for predicting the severity of their venous clinical condition (Nicolaides et al., 2014). Therefore, several morpho-functional diagnostic strategies have been utilised for the evaluation, which involve the use of plethysmographic methods in combination with ultrasound-based equipment. However, there is still a lack of standardisation in approaches. The results are dependent on fluctuations in volume inside the veins, which are later used to calculate the venous filling index (Meissner et al., 2007; Raju et al., 2019). The occurrence of venous dilatation and hypertension results in a reduction in shear stress, correlating with the force of friction imposed on the endothelial cells (ECs). Endothelial cells are capable of perceiving physical cues originating from fluid shear stress and then converting them into modified biomolecular signalling. This mechanism triggers a recurring reaction within veins, leading to an increase in blood pressure, remodelling of the venous walls, and inflammation (Ligi et al., 2018; Baeyens et al., 2016). Typically, the ankle venous ambulatory pressure in the lower leg falls to around 35 mm Hg under typical conditions (Recek, 2010). Nevertheless, this value exhibits an upward trend in individuals with chronic venous disease, as shown by the CEAP classification (Raju et al., 2019).

During the course of varicose vein treatment, it has been shown that the fluctuating pressure gradient has the potential to induce the return of reflux and neovascularization (the development of new blood vessels) (Recek & Pojer, 2000). In addition, the presence of persistent fluctuations in venous pressure, accompanied by symptoms such as oedema and hypoxemia, leads to a diminished microcirculatory response and ultimately the development of leg ulcerations (Senra Barros et al., 2019). Microangiopathic changes lead to the development of chronic venous insufficiency (CVI) by augmentation of artery permeability, reduction in capillary count, and impairment of lymphatic microcirculation. These modifications facilitate the extravasation of proteins, fluid, and blood cells. These modifications also take into consideration the reduced blood flow to the skin through capillaries and the propensity for the development of venous ulcers (Raffetto, 2016). Similarly, elevated permeability results in inflammation as a consequence of white blood cells (WBCs) infiltration, the release of cytokines, platelet activating factor, and stimulation of proteolytic enzymes (Sapelkin et al., 2017). Consequently, the impact of shear stress on ECs has a significant function in modulating

permeability, thereby regulating the functionality of leukocytes. In cases of severe chronic venous insufficiency (CVI), it is common to observe incompetence in the deep veins and perforators, which leads to hypoxia and the development of skin ulcers. There is conflicting data pertaining to the involvement of perforating veins in the onset and advancement of chronic venous insufficiency (CVI), which casts doubt on the necessity of perforator therapy (Cavezzi, 2020). Moreover, the deep vein system is surrounded by muscular structures that, upon contraction, exert pressure on the veins, facilitating the return of blood. Consequently, it is vital to examine peripheral muscle pumps in the lower extremities, particularly the calf muscle pump, to gain a comprehensive understanding of the hemodynamic alterations. Physiologically, the mechanism functions as a pump for the deep veins located in the soleus and gastrocnemius muscles. Extended durations of standing, together with factors such as joint, tendon, muscle, or nerve illnesses, as well as having been overweight, all contribute to a reduction in the effectiveness of the calf pump. This diminished function leads to the occurrence of venous reflux, oedema, and, in severe cases, deep vein thrombosis (DVT). The impairment of the calf muscle pump has a prominent pathologic element, indicating that persons with severe chronic venous insufficiency (CVI) experience leg ulcers that heal poorly and have a larger size, leading to a worse prognosis (Simka, 2004). Therefore, the fluctuation in venous pressure, alterations in hemodynamics in the lower extremities, and the subsequent development of chronic venous illnesses or disorders are influenced by factors such as the functionality of calf muscle pumps, the flexibility of blood vessels, the competence of valves, and the integrity of the venous wall (Reček, 2006). Typically, veins demonstrate a greater thickness of the adventitia layer and a lesser thickness of the media layer in comparison to arteries. Veins possess a greater amount of collagen in comparison to smooth muscle cells. The differentiation in structure confers less elasticity but increased extensibility to venous tissue. Therefore, the circulation of blood is contingent upon the existence of accompanying valves and the proper functioning of muscles.

Inflammation and the Contribution of Endothelial Impairment

Inflammation has a significant impact on the onset and advancement of venous disease (Labropoulos, 2019; Coleridge Smith, 2001; Danziger, 2007). Vascular inflammation is a multifaceted phenomenon characterised by complicated interactions involving SMCs, extracellular matrix (ECM), ECs, and WBCs. The abnormal connection mentioned above is also orchestrated by the uncontrolled generation of cytokines, which then elicit pathogenic reactions in the vascular system (Sprague & Khalil, 2009). There are many primary factors that contribute to the elevated response to inflammation. These factors encompass microcirculatory changes and hemodynamic abnormalities, both of which can cause an immune response that lasts longer (Zamboni et al., 2008). Immune cells' creation of an inflammatory environment is essential to pathogenesis and contributes to the impairment of endothelial function. However, it is still unknown how immunological alterations may contribute to venous stasis in individuals with varicosities, which may instead be the result of venous stasis. In order to elucidate the role of the endothelium, it is imperative to get a thorough understanding of the implications of inflammation in varicosities. Endothelial cells (ECs) may show significant phenotypic differences because of the shear stress and changes in hemodynamics that happen. These modifications have been recognised as key factors contributing to a range of vascular diseases (Davies, 2009). Prior study has indicated that individuals with chronic venous disease have endothelial cells (ECs) that display a pro-inflammatory phenotype, leading to increased recruitment of leukocytes into damaged blood vessels (Tisato et al., 2012). It is important to underscore the importance of glycocalyx. The glycocalyx within endothelial cells undergoes significant alterations with the progression of vascular disease, leading to a modification of endothelial mechano-transduction. This phenomenon leads to the disruption of the permeability barrier, thus facilitating the infiltration of WBCs and promoting the initiation of the response to inflammation (Tarbell & Cancel, 2016). Therefore, the appropriate progression of CVI will result in gradual alterations in the endothelial cells (ECs) and glycocalyx, ultimately resulting in impairment of endothelial function. Consequently, there exists a correlation between the degree of endothelial dysfunction and the observed clinical symptoms of CVD (Komarów et al., 2015). Moreover, it is probable that

endothelial dysfunction plays a crucial role as a pathophysiological link between deep vein thrombosis (DVT) and CVD (Carrasco et al., 2009). This underscores the significance of damaged endothelial cells in the exacerbation of inflammation and the progression of this specific condition. When it comes to immunity, the immune system responds to damaged endothelium, leading to the recruitment and infiltration of immune cells. Therefore, those with varicose veins exhibited a greater infiltration of innate or adaptive leukocytes in comparison to healthy individuals (Castro-Ferreira et al., 2018; Mosmiller et al., 2017). Leukocytes interact with endothelial cells (ECs) in a biphasic manner. Phase 1 refers to a rapid stimulation phase known as I-type stimulation, which is characterised by the secretion of endothelial cell vasoconstriction, von Willebrand factor, and the production of selectins. Phase 2, also known as II-type activation, involves the activation of cytokines, tissue factors, and adhesion molecules (Ojdana et al., 2009). Macrophages and monocytes are recognised as pivotal inflammatory mediators. According to Ono et al. (1998), there was a correlation between defective venous valves and an increase in the infiltration of macrophages and monocytes. According to Powell et al. (1999), the presence of CVI, with or without ulceration, was found to be correlated with increased activation and aggregation of platelets and monocytes. Furthermore, macrophages have a significant role in both the initiation and advancement of the condition. The extravasation of red blood cells (RBCs) into the adjacent tissues occurs due to the presence of venous stasis. In this process, interstitial macrophages undertake the digestion of these substances, while the proportion of iron in RBCs is retained in the ferritin state for further conversion into hemosiderin. This process of transformation has been seen to result in limb discoloration (Ferris & Harding, 2019). The presence of iron accumulation in the tissue might have substantial implications for people suffering from venous diseases. This phenomenon has the potential to initiate and sustain inflammation and oxidative stress; hence, this ultimately results in the manifestation of leg ulcers and the onset of chronic venous insufficiency (CVI) (Zamboni, 2006; Ferris & Harding, 2020; Caggiati et al., 2010). Thus, a prospective treatment approach for the management of venous ulcerations and CVI involves the specific targeting of pro-inflammatory M1 macrophages that are activated by iron (Sindrilaru et al., 2011). Mast cells and neutrophils are the initial cellular entities

that engage with the compromised endothelium and trigger the inflammatory reaction in individuals with CVD (Nicolaides, 2005). The presence of abnormal neutrophil function has been associated with elevated levels of lysosomal enzymes, adhesion molecules, and superoxide production, therefore exacerbating the progression of CVI (Stvrtinova et al., 2001; Bogachev et al., 2011; Whiston et al., 1994). Researchers have indicated a lower level of active neutrophils in the bloodstream of individuals with VVs compared to those who are in good health (Takase et al., 1999). The observed phenomenon, known as leukocyte trap, is characterised by the infiltration of leukocytes, particularly neutrophils, into the tissue via small blood vessels. This occurrence is believed to be triggered by conditions such as stasis, hypoxia, and venous hypertension (Grudzińska & Czuba, 2014). The utilisation of an elevated neutrophil/lymphocyte proportion as a marker for the severity of chronic venous insufficiency has been observed in recent study (Engin & Göncü, 2020). In contrast, the involvement of mast cells in pathogenesis remains unknown. While several studies have shown an observed elevation in mast cell presence inside the wall of VVs (Sayer & Smith, 2004), contrasting findings have indicated no notable alterations in mast cell population between the non-varicose wall and varicosities (Haviarová et al., 2002). In addition, Kakkos et al. (2003) observed a greater presence of mast cells in individuals with a familial predisposition to varicose veins in comparison to patients lacking such a familial history. This finding implies that the infiltration of mast cells is not attributable to venous hypertension. In a similar vein, a recent investigation (Chu et al., 2013) reported the presence of mast cell infiltration in thrombotic VVs. The valves and venous wall of individuals with varicose veins are subject to an inflammatory response (Boisseau, 2007), where T lymphocytes play a crucial role. Numerous studies have been undertaken to investigate the involvement of T lymphocytes in vascular inflammation (Lintermans et al., 2014). T cells are generally categorised into helper and cytotoxic T cells. T-helper cells (Th) are of utmost importance in the regulation of immunity in accordance with the specific threat encountered. The subsets of these cells may be classified into distinct categories, including Th1, Th2, Th17, and T regulatory (Treg) (Gagliani & Huber, 2017). Contrastingly, cytotoxic T lymphocytes (CTLs) are responsible for executing effector cytotoxic functions (Kumar et al., 2018). Studies have been conducted on the

involvement of T cell subsets in arterial illnesses, for instance, atherosclerosis (Saigusa et al., 2020). The presence of memory T helper (Th) cells and CD8+ cells was shown to influence the progression of venous disease, as demonstrated by Ojdana et al. (2009). Additionally, the varicose veins exhibited the presence of B lymphocytes; nevertheless, their precise role remains unclear (Buján et al., 2008). In their study, Grudzińska et al. (2018) observed elevated levels of inflammatory cytokines secreted by lymphocytes in GSVs with reflux that exhibited decreased functionality as compared to healthy veins. Numerous investigations have highlighted the importance of examining the cytokine profiles of individuals affected by chronic venous diseases. According to Lattimer et al. (2016), the investigation revealed that people who were diagnosed with varicosities had higher expression of certain proinflammatory cytokines, which include interleukin (IL) and monocyte chemotactic protein (MCP). Specifically, raised levels were observed in MCP-1, IL-6, and IL-8. Patients diagnosed with chronic venous insufficiency (CVI) exhibit heightened levels of inflammation. However, Guss et al. (2018) conducted a study that revealed that severe instances of CVI are associated with a reduced concentration of inflammatory cytokines. The researchers postulated that the presence of inflammatory cytokines might potentially promote the process of tissue regeneration as opposed to inducing detrimental effects. In the study conducted by Howlader and Smith (2003), no association was seen between the complaints of patients and inflammatory markers, suggesting that the role of cytokines in the development of chronic venous diseases is complex. One such instance involves the significant contribution of transforming growth factor beta 1 (*TGF-β1*) in multiple pathways, as it is activated and excessively generated within the inflammatory milieu associated with venous disease. This regulatory mechanism governs many targets involved in extracellular matrix (ECM) remodelling, resulting in the development of fibrosis in the vascular wall. The aforementioned phenomenon results in an elevation of *TGF-β1* activity, hence exacerbating inflammatory reactions, causing damage to endothelial cells, and inducing hypoxia in the venous wall (Serralheiro et al., 2017). On the other hand, it has been shown that the signalling of *TGF-β1* appears to be impaired during the latter stages, as evidenced by studies demonstrating a noteworthy downregulation in the expression of *TGF-β* receptors (Serralheiro et al., 2017; Pastar et al., 2010). On the contrary,

Kowalewski et al. (2010) investigated the expression of *TGF-β* receptor II, revealing higher levels in varicose veins compared to healthy veins. This suggests that *TGF-β* and its receptors are believed to play a significant role during the early stages. However, their influence may wane in subsequent phases of the pathological condition. The observed signalling has the potential to significantly impact future therapeutic approaches since it underscores the connection between extracellular matrix remodelling and inflammation in chronic venous diseases.

The Oxygen-Deprived Environment

In order to comprehensively investigate the initiation and progression of vascular disease, it is imperative to have an intimate understanding of the prolonged impact of hypoxia (Lim et al., 2013). In this context, the vascular wall experiences a reduction in oxygen supply during incidents of hypoxia. The oxygen delivery in the veins of the lower limbs is facilitated by the vasa vasorum, specialised vessels located within the adventitia and tunica medium layers (Kachlík et al., 2002). Hypoxia in vein diseases can arise from two distinct processes associated with blood stagnation and venous hypertension. The first, known as endoluminal hypoxia, arises when reduced blood flow, caused by blood stasis, leads to diminished oxygen detection within the vein wall, particularly by the endothelium and its inner layers. There are two types of hypoxia that can develop in this context. Adventitial hypoxia and medial hypoxia: the latter arises due to the compression of the vasa vasorum caused by the dilatation and elevated pressure of the veins. This mostly affects the outer and medial layers (Lim et al., 2011). At the molecular level, the activation of hypoxia-inducible factors (*HIF*) is initiated by hypoxia. The findings of the Lim et al. (2012) study demonstrate a greater level of activation in the transcription factors and their associated genes among individuals with varicose veins (VVs) compared to those without the condition. This suggests that the signalling pathway connected to hypoxia is vital for the development and progression of varicose veins. The presence of reduced oxygen levels inside the lumen has the potential to elicit two distinct physiological responses. Initially, the occurrence of acute hypoxia elicits the secretion of inflammation-related substances and growth factors,

thereby commencing the mobilisation of immune cells. Undoubtedly, it has been observed that in situations of reduced oxygen levels, there is an increased tendency for white blood cells to stick to endothelial cells (Takase et al., 1999). Then, prolonged hypoxia induces the activation of *HIF*, which subsequently upregulates the production of important genes, including platelet-derived growth factor (*PDGF*), proinflammatory cytokines, and vascular endothelial growth factor (*VEGF*). This process ultimately enhances immune adherence and recruitment in varicosities (Michiels et al., 2000). When hypoxia signalling is activated within this specific layer, the intimal layer thickens, and the inner wall undergoes degenerative alterations. As a compensatory mechanism, there is a significant enlargement of the vasa vasorum to provide sufficient oxygen delivery to the tunica media (Kachlík et al., 2008). Nevertheless, the evolution of chronic venous diseases entails the occurrence of an increased hypoxic environment, which has a detrimental effect on smooth muscle cells. Xu et al. (2017) observed significant changes in the morphological features of smooth muscle cells (SMCs) obtained from people with varicose veins in comparison to those with healthy veins. It was shown that these cells had elevated rates of proliferation and synthesis potential in comparison to smooth muscle cells derived from healthy veins. Additionally, these cells displayed a lower degree of differentiation. Furthermore, these cells experience significant degeneration, resulting in the loss of their structural characteristics while simultaneously increasing their ability to engulf elastic fibres, collagen, and even other smooth muscle cells (SMCs) (Wali & Eid, 2001). Similarly, endothelial stimulation has also been shown to cause SMCs to move from the medial layer to the intimal layer (Somers & Knaapen, 2006; Xiao et al., 2009). The presence of a hypoxic environment is a significant contributing factor to the manifestation of this aberrant behaviour. When cultured SMCs from individuals with venous insufficiency were subjected to low-oxygen conditions, there were significant alterations in the expression of hypoxia markers like *HIF-1α*, *TGF-β1*, *VEGF*, and eNOS. These findings suggest that the cells undergo epigenetic and genetic reprogramming during the development of chronic venous diseases (Ortega et al., 2018). Furthermore, it was observed that when these cells were subjected to prolonged and sustained hypoxic conditions, there was a decrease in the expression of adaptive responses to hypoxia. This decrease in adaptive mechanisms

could potentially lead to various consequences, such as an increased cell count and enlarged areas, dysregulated cell death processes, and enhanced collagen production, which are commonly observed in the vascular walls of individuals with varicose veins (Buján et al., 2000; Lim & Davies, 2009).

The Remodelling of the Venous Wall at the Molecular Level

The venous wall possesses a diverse array of mechanoreceptors responsible for regulating blood circulation, including integrins, flow-sensitive ion channels, and G-protein-coupled receptors (*GPCRs*). Nonetheless, the glycocalyx and the complex of platelet endothelial cell adhesion molecule-1, cadherin, and *VEGF* receptor-2 may potentially be involved in the mechano-transduction process (Atta, 2012). Consequently, the occurrence of venous hypertension and blood stagnation in varicose veins activate these receptors, leading to various molecular responses in endothelial cells (ECs), vascular fibroblasts, or smooth muscle cells (SMCs) (Bergan, 2007; Saberianpour et al., 2021). As previously shown, the endothelium will experience notable alterations as a result of shear stress, leading to compromised functionality. Simultaneously, the various layers will undergo notable modifications in response to the altered environment. Peptide growth factors, which include *VEGF*, insulin-like growth factor 1 receptor (*IGF-1R*), *IGF1*, and acidic fibroblast growth factor (aFGF) (Bruczko-Goralewska et al., 2019), have been shown to alter the vein wall's structure and composition. The aFGF pathway appears to hold particular significance, as recent studies have demonstrated a disturbed axis involving *IGF-1*, *PAPPA*, and *STC-2* (Ortega et al., 2020). Pascual et al. (2007) associated transforming growth factor beta 1 with the development of varicose veins. This factor's dysregulation has a vital role in the pathogenesis, particularly in the fibrotic remodelling within the venous wall. TGF-β1 exerts its influence via receptor II, which has been shown to be increased in varicose veins, whereas it has been seen to be decreased in venous ulcers (Kowalewski et al., 2010; Kim et al., 2003). Serralheiro et al. (2017) observed a reduction in *TGF-β1* receptor expression throughout the latter phases of venous disease. *TGF-β1* expression has been attributed to the dysregulation of numerous ECM components that impact EC and SMC function, including plasminogen activator inhibitor one (PAI-1), collagen, fibronectin, MMPs, TIMPs, and lysyl oxidase-

like 4 (Serralheiro et al., 2017). Significantly, this particular molecule and its subsequent signalling pathways appear to have a crucial role in remodelling of the venous wall, vascular damage, and the interconnection of inflammatory processes. The upregulated expression of TGF has the potential to interfere with several cellular processes implicated in the pathogenesis of CVD, including the PI3K/Akt pathway. Under pathological circumstances, the vital pathway PI3K/Akt is overactivated, which significantly alters cell growth, survival, motility, metabolism, and proliferation (Ortega et al., 2020). Ortega et al. (2018) explored the involvement of the PI3K/Akt pathway in a cohort of 110 individuals diagnosed with varicose veins, stratified according to valve reflux. The findings of this study indicate that individuals afflicted with venous reflux exhibit heightened activation of these components, suggesting that this pathway has a significant role in the advancement of this ailment. The venous wall of individuals exhibiting reflux had a notable degree of hyperactivation in the MAP kinases (MAPKs) pathway, indicating a potential collaborative interaction between both pathways (Ortega et al., 2019). It is noteworthy that individuals below the age of 50 exhibited significantly elevated levels of PI3K/Akt, or MAPKs, compared to older individuals. This observation suggests a potential association between PI3K/Akt and the acceleration of venous wall ageing. The process of ageing is further exacerbated in the venous wall due to the loss of certain homeostatic processes. For instance, CVD induces alterations in the homeostatic processes, including aberrant lysogenesis and senescence, which are associated with a modified functioning of peroxisome proliferator-activated receptors (*PPARs*) (Ortega et al., 2021). Additionally, chronic venous insufficiency accelerates osteogenesis through the activation of JNK signalling (Ortega et al., 2021). Calcium-dependent signalling pathways are one of the several components that contribute to the pathogenesis network in varicosities. SMCs derived from varicose saphenous veins exhibit a decreased ability to mobilise calcium ions (Ca^{2+}), resulting in impaired contractility (Schuller-Petrovic et al., 2002). The RhoA/Rho kinase signalling pathway, which plays a role in regulating the sensitivity of calcium ions to the actin cytoskeleton and the assembly of extracellular matrix fibronectin in SMCs, is also shown to be dysfunctional in varicose veins. This dysfunction leads to reduced activity and the deposition of both calcium ions and fibronectin in VVs (Cario-Toumaniantz et al.,

2002). According to Charpentier et al. (2013), CASZ-mediated *EGFL7* regulation affects both the function and shape of endothelial cells by regulating RhoA expression. The process is also linked to changes in the nuclear factor of activated T cells (NFAT) family. These changes depend on calcium ion (Ca^{2+}) signals to control T cell growth and function (Oh-hora & Rao, 2009). *NFATC4*, *NFATC3*, and other isoforms of the *NFATC* gene family could contribute to the regulation of tissue-vessel interactions during vascular formation (Graef et al., 2001). Furthermore, the induction of nitric oxide synthase results in the accumulation of the *NFATC3* isoform in the nucleus of smooth muscle cells (SMCs) (Gonzalez Bosc et al., 2004), which has been seen in patients with chronic venous insufficiency (CVI) (Feldo et al., 2018). In brief, the venous wall experiences notable alterations as a result of cellular reprogramming induced by shear stress and environmental stressors associated with varicose veins, including modified cell senescence and extracellular matrix (ECM) remodelling. The increased involvement of Matrix Gla protein (MGP) is what makes mineralization better in varicose veins' SMCs (Cario-Toumaniantz et al., 2007). The prevention of vascular calcification is attributed to the carboxylation of this protein by vitamin K; however, the precise process remains uncertain (Jaminon et al., 2020). MGP is widely regarded as a reliable indicator of vascular valve calcification state, and its functionality encompasses several mechanisms. These mechanisms include inhibiting the formation of calcium phosphate, facilitating the generation of matrix vesicles and apoptotic bodies, and inducing alterations in vascular smooth muscle cells (SMCs) (Bjørklund et al., 2020). MGP has been seen to upregulate the production of *TGFβ* and *VEGFs*, hence promoting lymphangiogenesis, angiogenesis, and vascular permeability. Several studies have demonstrated that the impact of MGP on *VEGF* is impeded by the presence of antibodies targeting *TGFβ* (Boström et al., 2004). As previously stated, many components, such as MGP, *VEGF*, and cell-cell junctions, rely on Ca^{2+} signalling, which has a pivotal role not only in controlling vascular function but also in the body's reaction to hydrostatic pressure. The modulation of voltage dependent Ca^{2+} channels undergoes alterations during the ageing process, hence impacting vascular function (Harraz & Jensen, 2020). Furthermore, some studies have shown an excessive release of vascular endothelial growth factor (*VEGF*) in varicose veins. This aberrant release, in conjunction

with other molecular alterations, has the potential to increase the permeability of the venous wall. Consequently, this may facilitate the entry of indicators of inflammation, as observed in the study (Horecka et al., 2021). The rheological characteristics of the venous wall are influenced by the histological disparities observed between normal veins and varicose veins, which are indicative of alterations in the extracellular matrix (ECM) composition that occur throughout the process of venous remodelling. Fibroblasts have the ability to release matrix metalloproteinases (MMPs) that, upon activation, can destroy collagen and elastin. This enzymatic activity has significant implications for the structural integrity and functional properties of other components, including the migration of SMCs. The invasion of white blood cells (WBCs) leads to a disruption in the equilibrium between MMPs and TIMPs, leading to the development of regions characterised by either hypertrophy (excessive extracellular matrix) or atrophy (insufficient extracellular matrix). The variation in the activity and degree of expression of MMPs is responsible for the development of varicose veins (VVs) characterised by dilatation and tortuosity (Chen et al., 2017; MacColl & Khalil, 2015). According to Raffetto et al. (2007), it has been proposed that the mechanism by which MMP-2 induces vein dilation involves the hyperpolarization of potassium (K^+) channels. The binding of collagen breakdown products to integrin receptors on SMCs has been shown to be a consequence of the activation of potassium (K^+) channels (Raffetto et al., 2007). The stiffening of arteries is attributed to modifications in elastin, fibronectin, collagen, and calcium deposits (Dorland & Huveneers, 2017). The thickening of the intimal and middle layers is attributed to the deposition of several subtypes of collagen, causing a reduction in elastic fibres and a consequent rise in collagen fibres. These changes have an impact on the typical alignment of SMCs (Faringthon Reyes & Sosa Veras, 2019). In the context of varicose veins, it has been shown that the abundance of type 1 collagen surpasses that of type 3 collagen (Boisseau, 2007). The latter is linked with the elastic properties of tissues, and the relatively large proportion of type 1 to type 3 collagen is responsible for the limited extensibility observed in VVs (Sansilvestri-Morel et al., 2003). Moreover, the process of elastin breakdown, which is a significant factor associated with accelerated ageing, leads to the production of chemicals that have cytokine-like properties. These molecules have the ability to attract leukocytes,

particularly Th1 subtypes (Antonicelli et al., 2007). The degradation rate of fibronectin exceeds its rate of deposition and synthesis. The reduced storage of fibronectin, which is generated by skin fibroblasts, might potentially contribute to the delayed healing of leg ulcers associated with CVD (Kanta & Zavadakova, 2020). The involvement of dermatopontin and tenascin C in patients with varicose veins (VVs) has been elucidated by proteomics investigations, demonstrating their increased degradation in these individuals (Barallobre-Barreiro et al., 2016). Dermatopontin is mostly located among dermal fibroblasts, specifically on collagen fibres, indicating its potential involvement in the process of wound healing (Okamoto & Fujiwara, 2006). Additionally, it has been seen to augment the cellular response to *TGFβ* (Shibuya et al., 2006). Tenascin C, an extracellular glycoprotein, has significant expression throughout the process of injury healing. It is synthesised by SMCs in retaliation to cellular proliferation (Imanaka-Yoshida et al., 2014). In addition, vinculin, a mechanosensory protein that participates in the remodelling of endothelial cell-cell adhesion (Huveneers et al., 2012), may also contribute to the intricate network of multifactorial varicosities. Nevertheless, there is a dearth of understanding regarding these proteins and their role in the advancement of varicosities. The glycome, including glycoconjugates that are linked to lipids and proteins, exhibits distinct glycosylation mechanisms in both normal and diseased states, hence presenting a potential avenue for novel therapeutic advancements (Reily et al., 2019). The process of glycosylation has a significant impact on several aspects of extracellular matrix (ECM) proteins (Barallobre-Barreiro et al., 2017), including their binding, folding, solubility, and degradation. Furthermore, the buildup of glycosylation-final products leads to an elevation in collagen crosslinking. The presence of hydroxyproline amino acid is essential for the establishment of hydrogen bond-mediated crosslinking inside collagen molecules. This post-translational alteration, which impacts the proline residue, is often observed in varicosities. Its occurrence significantly contributes to the stiffness of the venous wall as well as processes associated with ageing and inflammation.

Genetic and Epigenetic Mechanisms Underlying Varicose Veins

Genetics

The investigation elucidates that a multitude of genes are implicated in the aetiology and pathophysiology of varicose veins. Certain genes exhibit variances within introns or single nucleotides, commonly referred to as single nucleotide polymorphisms (SNPs). Additionally, certain genes undergo mutations, while others are exclusively elevated and associated with systemic alterations. These genes exert an influence on several facets of varicosities, including but not limited to blood pressure regulation, arterial wall composition and functionality (called elasticity and tension), as well as inflammatory processes. Genome-wide association studies (GWAS) have successfully found a greater multitude of variations within risk loci that have a substantial connection with varicose veins. Nevertheless, the current body of data pertaining to genetic inheritance remains constrained and warrants more scrutiny. Recent years have witnessed the identification of previously unknown genetic risk factors for varicose veins (VVs) using GWAS. SNPs have been found to affect the *KCNH8* (rs727139) and *EFEMP1* (rs17278665) genes. The potassium ion channel protein is encoded by the *KCNH8* gene. It has an essential function in controlling the flow of potassium ions across cellular membranes. This gene has been associated with the control of venous dilatation and the pathogenesis of varicose veins through its involvement in smooth muscle contraction and nerve stimulation (Ellinghaus et al., 2017). The *EFEMP1* gene is responsible for encoding a protein that serves as a constituent of the extracellular matrix (ECM) glycoprotein. Alterations in this specific gene have the potential to impact the synthesis of MMPs and the production of tissue inhibitors of metalloproteinases (TIMPs). TIMPs and MMPs are enzymes that control the ECM's structure and the flexibility of blood vessels (Serra et al., 2020). A further investigation documented a favourable correlation between venous insufficiency and genetic changes in the promoter regions of MMP-9 and TIMP-2 that are involved in the regulation of the ECM function equilibrium (Xu et al., 2011). Genetic investigations have successfully discovered several genes associated with varicose veins, particularly those that influence blood pressure regulation and blood vessel development. The genes encompassed by this set are *NFATC2*, *HFE*, *CASZ1*, *GATA2*, *PPP3R1*, *STIM2*, *EBF1*, *SOX9*, and *PIEZO1*

(Shadrina et al., 2019). The presence of a genetic mutation, such as the rs11121615 SNP variation within the castor zinc finger 1 (*CASZ1*) gene—responsible for encoding a protein with zinc finger domains—has been observed to significantly correlate with the onset of severe CVD, specifically designated by VVs (varicose veins). Nonetheless, the effects of the rs11121615 SNP variation do not demonstrate a similar impact on other forms of CVD (Jones et al., 2019). The gene specified earlier serves as a transcriptional factor that fulfils a critical role in the control of epidermal growth factor-like domain 7 (*EGFL7*), a protein that has been implicated in the acceleration of angiogenesis and the formation of blood vessels. The presence of this genetic variation of *CASZ1* has been found to influence the susceptibility to developing VVs (Jones et al., 2019). *PIEZO1*, an additional gene, functions as a cationic channel responsible for detecting shear stress through the facilitation of Ca^{2+} influx into endothelial cells (ECs), hence playing a crucial role in maintaining vascular integrity. Additionally, it facilitates the passage of sodium (Na^{+}) and potassium (K^{+}) ions while also serving as a baroreceptor and contributing to the regulation of vascular tone and valve morphogenesis (Douguet et al., 2019). Furthermore, it has an impact on the circulation of lymph (Nonomura et al., 2018). Research conducted on mice has shown that *PIEZO1* has the capacity to regulate blood flow and endothelial cell rearrangement (Li et al., 2014). Mutations resulting in the lack of function of that gene have been associated with a congenital condition known as autosomal recessive lymphatic dysplasia. This disorder mostly affects the lymphatic system and manifests as swelling in the lower limbs (Alper, 2017). Certain genetic variations, including rs2911463 (Fukaya et al., 2018), have been found to be linked to the recurrence of VVs. In their study, Shadrina et al. (2019) identified four genes, in particular *NFATC2*, *PPP3R1*, *GATA2*, and *EBF1*, that have been found to be related to immune response and inflammation in the context of vascular remodelling. This process has a vital contribution to the progression of varicose veins (VVs). The impact of two genetic variants, rs2241173 and rs2861819, on protein phosphatase 3 regulatory subunit B, alpha (*PPP3R1*), has been observed to influence vascular integrity and serve as determinants in the development of CVD (Ortega et al., 2021). The transcription factor Early B Cell Factor 1 (*EBF1*) plays a crucial role in B lymphopoiesis by regulating cell migration and adhesion. Notably, the variation rs11135046 of *EBF1* has been associated

with an elevated susceptibility to VVs (Vilagos et al., 2012; Ahmed et al., 2020). GATA-binding protein 2 (*GATA2*) is a transcription factor found in endothelial cells. The activation of genes involved in the formation of lymphatic vascular valves is significantly influenced by its pivotal role. The dysfunction of these valves is a characteristic feature of lymphedema (Kazenwadel et al., 2015). GWAS found that the gene variation rs9880192 was strongly linked with the risk of VVs (Shadrina et al., 2019). The impact of nuclear factor of activated T cells 2 (*NFATC2*) on the occurrence of VVs remains uncertain. Nevertheless, several genetic variations, including rs3787184 and rs12625547, have been identified as SNPs that have been identified as being correlated with an elevated likelihood of VVs (Ahmed et al., 2020; Smetanina et al., 2021). The signalling pathway of *NFATC* is reliant on the presence of calcineurin and collaborates with the transcription factor *FOXC2* to provide regulations for *GJC2*, an assortment of gap junction proteins mostly prevalent in endothelial tissue. Venous valve malfunction can arise as a result of mutations occurring in the *FOXC2* and *GJC2* genes. Ulceration and hypertension may arise as consequences of inadequate regulation of valve function associated with the aforementioned genes (Lyons et al., 2017). As stated before, there is an association between *FOXC2* mutations and lymphedema-distichiasis syndrome. The issue at hand has been examined in animal models, which have demonstrated that the inactivation of *FOXC2* leads to impairments in cell-cell junctions and valves. Consequently, this disruption results in the aberrant perception of shear stress and the disassembly of junctions (Sabine et al., 2015). One other potential target is stromal interaction molecule 2 (*STIM2*), an intracellular protein located within the endoplasmic reticulum that has an essential contribution in regulating the levels of calcium ions in the cytosol (Berna-Erro et al., 2017). The protein in question has been identified to possess risky single nucleotide polymorphisms (SNPs), in particular rs28558138, as reported in genome-wide association studies (GWAS) (Smetanina et al., 2021). The homeostatic iron regulator (*HIR*) has been shown to impact the ageing process. The previously described protein performs a crucial function in controlling iron absorption through its ability to modulate the synthesis of hepcidin. Hemochromatosis, a disorder characterised by excessive iron accumulation, can be attributed to recessive mutations in this protein (Barton et al., 2015). Elevated concentrations of iron have been

seen to have detrimental effects on endothelial function in individuals with hereditary hemochromatosis (Gaenzer et al., 2002). Additionally, iron deposits and the leakage of RBCs contribute to the heightened activity of matrix metalloproteinases (MMPs) or the inhibition of their regulators while also generating free radicals, hence exacerbating venous diseases. The potential association between iron overload and the occurrence of venous leg ulcers is supported by observational research, which revealed that individuals carrying the H63D variation (rs1799945) of the *HFE* gene experienced an earlier start of ulcers (Zamboni et al., 2006). The Genome-Wide Association Study (GWAS) has further found the variation rs7773004 to be significantly linked with the process of ageing. The SRY-box transcription factor 9 (*SOX9*) gene possesses the capacity to modulate the extracellular matrix, a process that is associated with elevated calcium deposition. The overexpression of *TGF-β1* (Augstein et al., 2018) can be enhanced. GWAS identified a variation, more precisely rs2241173, that is associated with susceptibility to varicosity. The gene *COL2A1*, specifically the variation rs73107980, is responsible for the synthesis of collagen type 2 alpha 1 chain, which is an integral component of the ECM. The ectopic expression of *SOX9* has been found to influence the process of extracellular matrix modelling, potentially leading to aberrant outcomes (Shadrina et al., 2019; Hanley et al., 2008). The isotype *COL1A2*, which is implicated in the dysregulation of collagen, has been subject to a more comprehensive investigation. The presence of genetic variants, specifically the insertion/deletion rs3917 polymorphism, has been associated with an elevated susceptibility to CVI (Jin et al., 2013). Ultimately, the two genes, *THBD* and *MTHFR*, serve as a pivotal connection between CVD and thromboembolic illness. Research findings have indicated that both of these illnesses exhibit a comparable hereditary tendency (Zöller et al., 2014), implying the existence of shared genetic processes contributing to their co-occurrence. Mutations in the aforementioned pair of genes have been linked to the occurrence of both varicosities and deep vein thrombosis (DVT) (Fukaya et al., 2018). The blood obtained from varicose veins exhibited heightened levels of inflammatory and prothrombotic biomarkers, along with indications of endothelial impairment, in contrast to the peripheral blood samples (Poredos et al., 2015). The structure of the venous wall and the physiological reaction within the vein might undergo modifications in atypical

circumstances, possibly associated with the influence of these two genetic factors. To a certain degree, there is a connection between the pathophysiological processes of deep vein thrombosis (DVT) and varicose veins.

Epigenetics

Epigenetics is an academic discipline that investigates the mechanisms by which various influences may induce modifications in gene expression patterns while leaving the underlying DNA sequence unaltered. These variables have the potential to be heritable through both mitotic (cell division in somatic cells) and meiotic (cell division in germ cells leading to gametes) processes. Epigenetic mechanisms encompass various regulatory processes, including DNA methylation and histone modifications, in addition to different types of non-coding RNA. The latter involves long non-coding RNA (lncRNA) and micro-RNAs (miRNA), as well as short-interfering RNA (siRNA), which play a key role (Moosavi & Ardekani, 2016; Al Aboud & Jialal, 2018). Epigenetic pathways can be influenced by a range of external variables, including but not limited to age, food, occupational practices, behaviour, circadian rhythms, stress levels, physical activity, tobacco use, and alcohol intake (Cavezzi, 2020; Alegría-Torres et al., 2011; Pal & Tyler, 2016; Sahar & Sassone-Corsi, 2013). These variables have the potential to trigger an epigenetic change, which might ultimately contribute to the onset and progression of the illness, in conjunction with other specific pathophysiological mechanisms implicated (Ordovás & Smith, 2010). Shear stress, a significant modulatory factor in endothelial cells (ECs) (Ku et al., 2019), triggers a diverse range of epigenetic responses. There is evidence to suggest that the development of atherosclerosis is associated with alterations in shear stress (Dunn et al., 2015). Likewise, it has been observed that epigenetic modifications occurring in vascular cells as a result of stimuli such as inflammation or hypoxia have the potential to enhance hypoxic and inflammatory reactions (Shanmugam & Sethi, 2013; Nakamura et al., 2021). There are two main pathways that have substantial influence on the regulation of genes related to vascular disease via epigenetic processes. The mechanisms encompassed within this context consist of DNA methylation and non-coding RNAs. DNA methylation is a biological process where methyl groups are enzymatically added to CG islands with the

mediation of methyltransferase enzymes (Edwards et al., 2017). DNA can undergo either hypermethylation or hypomethylation, depending on the enzymatic activity of methyltransferases. The gene undergoes transcriptional repression when subjected to hypermethylation, while it experiences transcriptional activation when subjected to hypomethylation (Sallustio et al., 2019). In the context of clinical situations, methylation patterns have been identified in many loci (Smyth et al., 2013). As previously established, the *EBF1* and *MTHFR* genes play significant roles in the modulation of varicose veins. *EBF1* is becoming acknowledged as an epigenetic regulator because of its potential to facilitate nucleosome modelling, DNA demethylation, and modify active chromatin within the B cell gene pathway (Derecka et al., 2020). A study conducted on *MTHFR* revealed that some polymorphic polymorphisms have a tendency to facilitate DNA hypomethylation, leading to abnormal synthesis of structural proteins and matrix. This has an influence on the integrity of DNA and accelerates the ageing process of venous tissue (Wilmanns et al., 2015). Furthermore, previous research has confirmed that mutations in the *MTHFR* gene are linked to genetic hypercoagulability, in addition to an elevated vulnerability to DVT and CVD (Xu et al., 2019). Through transcriptomic and DNA-methylomic analyses, it has been observed that the microfibril-associated protein 5 (*MFAP5*) gene exhibits overexpression in individuals with varicose veins, which can be attributed to the expansion of hypomethylation (Smetanina et al., 2018). Moreover, a number of genes, including integrin 3, *DPEP2*, *CCN5*, *WISP2*, *PLXNB1*, *ADCY3*, *HRC*, and osteopontin, exhibit considerable dysregulation in varicose veins (VVs) due to abnormal methylation patterns. The discussed patterns have also been observed to have an association with the varicose vein signalling pathway (Kazenwadel et al., 2015; Jiang et al., 2014). To ascertain the underlying reasons for these methylation abnormalities, more research in this area is needed. The significance of noncoding RNAs in the development of varicosities has also been prominent. Although ncRNAs lack the ability to encode proteins, they are a family of RNA molecules that are important in a number of biological and clinical processes (Beermann et al., 2016). miRNAs are essential regulators of protein synthesis during the post-transcriptional phase. Additionally, according to Barwari et al. (2016), the average ability of a single miRNA to regulate transcripts is around 200. However, a single transcript might

undergo regulation by multiple miRNAs. Furthermore, miRNAs have the potential to be utilised as valuable biomarkers for many diseases, hence having substantial implications for the accurate identification and prediction of patient conditions (Condrat et al., 2020). Jiang et al. (2012) identified a total of 14 microRNAs (miRNAs) with aberrant expression in the GSV of patients with varicosities. The primary proteins seen in these subjects were miR-202, miR-34a, and miR-155. The primary targets of these miRNAs encompassed many cellular components and processes, such as mitogen-activated protein kinases (MAPKs), the p53 signalling pathways, apoptotic genes, hyperproliferative targets, and cell cycle regulation. In a similar vein, Anwar et al. (2017) observed significant variations in the levels of expression of microRNA-216a-5p, both 3p miR-135a and 642a, and miR-4459-5p within the vascular tissue of people who have been clinically diagnosed with varicosities. These alterations were shown to be associated with modifications in the metabolomic profile, heightened cellular proliferation, and increased inflammatory responses. MiR-382 has been identified as a significant regulator of the *COL1A2* gene. Nevertheless, in individuals harbouring the polymorphism variant rs3917, there is evidently a decrease in the binding affinity between the microRNA and the target gene, leading to an upregulation of *COL1A2* expression (Pal & Tyler, 2016). In their study, Zalewski et al. (2020) discovered a total of 31 microRNAs (miRNAs) and 62 genes that have the potential to serve as biomarkers for CVD. The miRNAs have an intricate network that leads to the upregulation of select genes, including *CDS2*, histone deacetylase (*HDAC5*), *PRRC2B*, and *WNK1*. Simultaneously, they downregulated the expression of other genes, for example, *PABPC3*. Prior research has established the notable participation of various non-coding RNAs, such as lncRNAs, throughout both physiological and pathological circumstances (Schmitz et al., 2016). Long non-coding RNAs, akin to miRNAs, have been shown to regulate protein synthesis through their involvement in several intracellular processes within the cytoplasm or in the nucleus. The studies conducted by Li et al. (2014) and Biranvand et al. (2018) have demonstrated that the lncRNA effect is exerted through the modulation of many genes involved in varicosity development. This modulation mostly occurs in structural, metabolic, and inflammatory processes. Despite the considerable importance of non-coding RNAs in pathophysiology, further investigations are required

to attain a thorough comprehension of their involvement in the progression of this ailment. Furthermore, it is important to highlight that there is a dearth of research investigating the potential utilisation of non-coding miRNAs for diagnosis or prognostic biomarkers for varicose veins.

Apoptosis

Apoptosis is a biological process that can manifest at various developmental stages or in reaction to cellular damage. This process is characterised by a number of notable attributes, such as the cleavage of proteins, which is predominantly helped by the activation of caspases, which are a specific type of cysteine protease. Furthermore, according to O'Brien and Kirby (2008), this process includes the degradation of the nuclear genome along with the identification of apoptotic cells by phagocytic cells. These processes activate in response to extrinsic pathways, death receptor (DR)-derived external stimuli, or internal stimuli originating from the intrinsic or mitochondrial pathways. These pathways ultimately converge in what is commonly referred to as the apoptotic execution phase.

The Mitochondrial Pathway

The intrinsic process of apoptosis, which is sometimes referred to as the mitochondrial pathway, is initiated in reaction to inner cellular stress (Elmore, 2007). The initiation of BH3-only proteins, like HRK, BID, BMF, BAD, Noxa, and BIM, is induced by intrinsic factors that include DNA damage and endoplasmic reticulum stress. The research of Lomonosova and Chinnadurai (2008) indicated that the interplay between BH3 pro-survival and BH3 proteins, or pro-apoptotic proteins, may hinder or promote cell death. The proteins that play a crucial role in promoting cell survival, for example, BCL-2 and BCL-X, are the predominant factors responsible for effectively inhibiting cellular apoptosis at that specific point (Li & Dewson, 2015). On the other hand, the activation of pro-apoptotic proteins, specifically BAK and BAX, triggers MOMP. This has significant importance as it represents a pivotal juncture whereby the release of proteins accountable for caspase activation within the cytoplasm. Following that, a progressive decrease in the pH of the mitochondrial environment occurs (Tait &

Green, 2010; Galluzzi et al., 2012). The Cytochrome-c protein is released as a consequence of MOMP. Cytochrome-c serves a pivotal function in the assembly and activation of apoptosomes. Pro-apoptotic proteins like Smac/DIABLO and Omi/HtrA2 have also been shown to be active and to interact with inhibitory proteins, therefore initiating the activation of procaspases (ProC), including ProC3 and ProC7 (Cheng et al., 2016). The main objective of the release of pro-apoptotic proteins is to counterbalance the inhibitory influence imposed by inhibitor of apoptosis proteins (IAP) on procaspases (Yuan & Akey, 2013). The process of apoptosome formation in the cytoplasm is initiated by the liberation of Cytochrome-c. The apoptosome is a multiprotein complex located in the cytosol, encompassing apoptotic protease activating factor 1 (Apaf-1), Cytochrome-c and ProC9 (Gortat et al., 2015). The interaction between Cytochrome-c and the monomeric cytoplasmic form of Apaf-1 initiates the formation of this complex. Consequently, the generation of a single adenosine triphosphate (ATP) molecule leads to the development of the heptameric apoptosome. The apoptosome complex formation takes place following the binding and subsequent activation of ProC9. This activation mechanism is initiated by the interaction between ProC9 and Apaf-1. CASP9 has to remain bound to the apoptosome to sustain a significant degree of catalytic activity following a transition to its active state. Following this, the initiator CASP9 engages in proteolysis, a process that entails the cleavage and subsequent activation of execution caspases, including caspase-3 (CASP3) and caspase-7 (CASP7). The proteolytic process described in the text leads to the reorganisation of essential protein loops, ultimately leading to the creation of active sites (Shi, 2004; Yuan et al., 2011). According to McIlwain et al. (2013), cleaving execution caspases during activation might result in the activation of additional caspases, creating a feedback loop during the execution phase. It is essential to emphasise that, alongside the apoptosome, several proteins have been identified to participate in initiating reactions within both pathways of apoptosis. These protein platforms are associated with diverse initiator caspases (Mace & Riedl, 2010). Tinel and Tschopp's study (2004) was the first to describe the PIDDosome complex. The initial association between the PIDDosome and p53-mediated apoptosis has been demonstrated in response to DNA damage and genotoxic stress. Sladky et al. (2017)

research has shown its participation in non-apoptotic mechanisms, including centrosome monitoring during cellular differentiation.

BCL-2 (B-Cell Lymphoma Protein-2)

It is a gene that belongs to the BCL-2 family and codes for the apoptosis regulator B-cell lymphoma protein 2 (Bcl-2) (Li & Dewson, 2015). It comprises a group of proteins that can be categorised into three primary groups: anti-apoptotic proteins (including Bcl-2, Bfl-1/A1, and Bcl-xl), pro-apoptotic proteins (such as Bcl-b, Bok, and Bax), as well as four additional proteins that exhibit close relations (BCL2L13, BCL2L14, BCL2L15, and Bid) (Hardwick & Soane, 2013; Chao & Korsmeyer, 1998). Having four Bcl-2 homologous domains (BH) distinguishes Bcl-B, a member of this protein family. BH3-only proteins, particularly the pro-apoptotic Bcl-xs, Blk, Bad, and Bld, are derived from the domains that were indicated previously (Ke, Godzik & Reed, 2001). The cellular susceptibility to apoptotic stimuli is significantly influenced by the equilibrium between apoptotic proteins (Siddiqui et al., 2015). An illustration of this phenomenon involves the elevation of mitochondrial cyclic adenosine monophosphate (cAMP) concentrations, which initiates the process of phosphorylating BAD via cAMP-dependent protein kinase (PKA). As a result, the phosphorylation process impairs Bcl-2, BAD, and Bcl-X interactions. Consequently, this process of phosphorylation facilitates the promotion of cell survival (Lizcano et al., 2000; Harada et al., 1999). However, during the process of dephosphorylation, Bcl-2 translocates to the mitochondria, where its interaction initiates the release of Cytochrome-c into the cytoplasm, eventually resulting in cell death (Martinou & Youle, 2011; Bergmann, 2002; Wang et al., 1999).

Death Receptor Pathway

The initiation of this apoptotic pathway is prompted by external stimuli that exert their influence on the cellular system. This process is initiated not only through the process of transmembrane protein oligomerization belonging to the death receptor superfamily but also whenever specific extracellular environment variables surpass a predefined threshold. Consequently, this process initiates the transmission of signals that eventually dictate the cellular outcome (Galluzzi et al., 2012). In the context of the

extrinsic route, it has been observed that mitochondria play a role in amplifying caspase activation. However, it is important to note that they are not considered essential for this particular kind of cellular death (Galluzzi et al., 2012). Activation of death receptors (DR) by receptor binding or aggregation, on the other hand, starts the extrinsic signalling cascade. The tumour necrosis factor (TNF) receptor superfamily, which includes these DRs, is distinguished by extracellular domains rich in cysteines and cytoplasmic death domains (Ashkenazi & Dixit, 1998). Significant members of this domain encompass DR6, Fas, DR5, TNFR1, DR4, and DR3 (Elrod & Sun, 2008). Different proteins, such as MYC and p53, can activate distinct death receptors, such as DR5 and Fas. The protein MYC interacts with and activates the death receptors Fas and DR5/TRAIL. In addition to stimulating DR transcription and enhancing the levels of DR5 and Fas in the cytosol through non-transcriptional processes, this finding is supported by the studies conducted by Haupt et al. (2003), Amanullah et al. (2002), and Wang et al. (2004).

The Execution Pathway

This apoptotic pathway encompasses a multitude of molecules, whereby caspases assume a pivotal function. Caspases are proteases that belong to the cysteine-aspartic protease family. They function as endo-proteases, meaning they catalyse the breakdown of peptide bonds within proteins. This catalytic activity is dependent on the presence of cysteine residues within the active site of the enzyme. Upon activation, caspases initiate the process of cleaving a distinct subset of target proteins along their main sequence. The process of cleavage leads to a range of consequences, such as the inactivation of proteins. This can occur through two mechanisms: direct activation, where a negative regulatory domain may be eliminated, or alternatively, indirect activation can occur through the inactivation of the regulator's subunit (Hengartner, 2000). A wide variety of caspases could have been found, including caspase activated DNase (CAD), caspases -2, -3, and CASP6 to CASP10 (Shalini et al., 2015; Enari et al., 1998). Caspases are categorised into two distinct groups: initiators, which include CASP2, CASP8, to CASP10; and effectors or executioners, which consist of CASP6, CASP7, and CASP3. This categorization is based on their respective functions within apoptotic pathways (Parrish et al., 2013). The initiators initiate the activation of

executioners, triggering the subsequent activation of other executioners in a feedback loop. This process has an impact on essential structural proteins and a variety of enzymes, consequently facilitating the fundamental attributes associated with cellular demise (McIlwain et al., 2013). According to Elmore (2007), the activation of cytoplasmic endonucleases, which are enzymes that break down nuclear components, and proteases, which break down both cytoskeletal and core proteins, is initiated by the executioner caspases. These processes result in changes in cell morphology, including cellular shrinkage, condensation of chromatin, the appearance of cytoplasmic vesicles, and the production of apoptotic bodies. Following this, nearby macrophages or neoplastic cells engulf these entities.

Autophagy

Autophagy, a cellular phenomenon, is triggered by various physiological signals in the body, like amino acid deficits, reduced insulin levels, reduced ATP synthesis, and hypoxia conditions. The initiation of autophagy entails the activation of two intricate complexes: the Unc-51-like autophagy-activating kinase 1 (*ULK1*) complex and the autophagy-related gene (*Atg*)1 complex. These complexes comprise numerous constituents associated with autophagy. The commencement of this process results in the formation of a membrane-bound vesicle known as the phagophore. Afterwards, the phagophore passes through a sequence of maturation phases, eventually undergoing a transition into a spherical vesicle made up of two layers of lipids known as the autophagosome. After its formation, it proceeds to merge with either a vacuole or a lysosome, a crucial step for degrading the engulfed cytoplasmic components (Mizushima & Komatsu, 2011). The transcription factor EB (TFEB) is a member of the microphthalmia-associated transcription factor (MiT) family. It encompasses basic helix-loop-helix leucine-zipper (bHLH-Zip) transcriptional factors linked with microphthalmia. TFEB has a crucial function in regulating the autophagy-lysosomal pathway. During periods of nutritional scarcity, TFEB regulates the transcription of autophagy-related genes, thereby orchestrating the expression of genes associated with lysosomal and autophagy functions (Settembre et al., 2011). When the mammalian target of rapamycin complex 1 (mTORC1) becomes inactive, as seen during famine

conditions, the phosphorylation process of TFEB is inhibited, leading to TFEB's translocation to the nucleus. Within the nucleus, TFEB binds to coordinated lysosomal expression and regulation (*CLEAR*) gene elements, thereby activating genes involved in critical biological processes like autophagy and lysosomal exocytosis (Palmieri et al., 2011; Settembre et al., 2012; Napolitano et al., 2018). Functioning as a serine/threonine protein kinase within the class III phosphatidylinositol 3-kinase (PI3K)-related kinase family, mTORC1 acts to suppress autophagy (Saxton & Sabatini, 2017). Specifically, it exerts a negative regulatory effect on the ULK1 complex, consisting of focal adhesion kinase family interacting protein (FIP), particularly FIP200, ULK1, Atg13, and Atg101. This regulatory action occurs via mTORC1's phosphorylation of ULK1 and Atg13 (Kim et al., 2011). mTORC1 regulates autophagy by modulating TFEB's intracellular positioning and directing it towards lysosomes (Roczniak-Ferguson et al., 2012). The activation of mTORC1 takes place at lysosomal surfaces in the presence of the GTP-bound Ras homolog (Rheb), an abundant low-molecular-weight G protein in the brain (Yang et al., 2017). The spatial arrangement of mTORC1 is controlled by the low-molecular-weight G protein Rag, comprising RagA/B and RagC/D heterodimers. This protein influences mTORC1 activity by altering its structure upon binding GDP/GTP under conditions of amino acid scarcity (Takahara et al., 2020). mTORC1 inhibition triggers the formation of a phosphoinositide 3-kinase (PI3K) complex subsequent to ULK1, initiating the creation of the phagophore. This PI3K complex, comprising various components such as vacuolar protein sorting (VPS), which includes VPS34, VPS15, Beclin-1, and Atg14, engages in the initial stages of phagophore formation. The action of VPS34 within these complexes facilitates phosphatidylinositol-3-phosphate (PI3P) synthesis, closely associated with omegasome formation (Boukhalfa et al., 2020). Moreover, WD repeat domain phosphoinositide-interacting protein (WIPI), acting as a PI3P effector, recruits WIPI2, which specifically influences Atg2. This latter is crucial in promoting the linkage between the ER membrane and the phagophore. It is also involved in lipid transport, which is an essential supplement for the autophagosome (Osawa & Noda, 2019). As shown by Lystad et al. (2019) and Cadwell et al. (2008), the process of autophagosome biogenesis encompasses two distinct systems that bind proteins similar to ubiquitin: Atg12-Atg5 and the microtubule-associated protein light

chain 3 (LC3). LC3's involvement in autophagosome membrane elongation and closure occurs through its interaction with phosphatidylethanolamine (PE) within the LC3-binding system. Enzymes Atg7 and Atg10 facilitate the covalent linkage between Atg12 and Atg5. The resultant Atg12-Atg5 complex interacts with Atg16L1. The previously mentioned complex is enlisted in the phagophore with the purpose of conferring E3-like functionality upon the LC3-PE complex. The lipid-modified LC3 demonstrates analogous behaviour to that witnessed during E3-like activity. This behaviour entails the deliberate breakdown of certain substances by interacting with various selective autophagy receptors, as described by Brier et al. (2019) and Galluzzi and Green (2019). The final step of autophagosome formation entails the extension and encapsulation of the phagophore. The closure process is aided by the participation of the endosomal sorting complex required for transport (ESCRT) complex. Additionally, during this process, two distinct categories of soluble N-ethyl maleimide-sensitive protein attachment receptor (SNARE) complexes interact synergistically. These complexes involve synaptosomal-associated protein (SNAP), notably SNAP29 and SNARE conjugates, and lysosomal vesicle-associated membrane proteins (VAMPs) such as VAMP7 and VAMP8. The cooperation between these distinct complexes facilitates the fusion between autophagosomes and lysosomes. The fusion mechanism described here is crucial for the formation of autolysosomes, which ultimately leads to the breakdown of cellular components. Investigations carried out by Takáts et al. (2013) and Matsui et al. (2018) have underscored the significance of this fusion between lysosomes and autophagosomes in cellular degradation processes.

Imaging Modalities in Venous Evaluation

Handheld Doppler

A handheld doppler is a compact device equipped with a pencil probe that is used on the skin subsequent to the application of gel. The signal is generated by the circulation of blood, particularly the red blood cells within veins or arteries. The reflecting beam exhibits distinct characteristics in comparison to the projected beam; this machine is appropriately tuned to detect and capture this alteration. While this methodology possesses practical use, its results lack reliability. The given source lacks anatomical information. Due to the widespread availability of duplex ultrasound scanning, the use of handheld doppler has become infrequent, as noted by Campbell et al. (2005).

Duplex Ultrasound

A duplex scan is utilised to assess the competency of the saphenopopliteal and saphenofemoral junctions, as well as to determine the severity and extent of reflux, the dimensions of the veins, the presence of non-specific reflux, incompetence perforators, and any abnormalities in the veins. According to Yan et al. (2019), it is recommended to conduct duplex examinations with the patient in a standing position, ensuring optimal accessibility to the lower limbs. The process involves the integration of several postures, including sitting, standing, and active positions, to demarcate the anatomical structures. B-mode ultrasound (US) is employed to evaluate anatomical features and structures. Subsequently, doppler imaging is included to investigate physiological and pathological aspects, particularly pertaining to blood flow. When conducting research, it is crucial to examine the vein both without and with compression. The use of a 3 to 5 MHz probe is necessary for examining cases involving obese people when the 5 to 11 MHz probe could fail to yield sufficient results. The examination of the vasculature necessitates an analysis conducted in black and white, colour, and spectral modalities. The utilisation of pictures proves to be highly advantageous in challenging anatomical regions, which include the femoral vein within the adductor canal, and additionally in cases of pelvic venous illness (Lockhart et al., 2005). The use of duplex ultrasonography is effective in identifying several venous irregularities, including venous system duplication and the

existence of accessory veins. These findings have significant importance in the strategic formulation of therapeutic plans.

Magnetic Resonance Venography (MRV)

The MRV technique demonstrates superior efficacy in visualising thigh and pelvic veins as contrasted with calf veins. The use of MRV is also advantageous in the imaging of suspicious central thoracic venous thrombosis. The utilisation of MRV as an approach to solving issues is particularly relevant in the context of pelvic imaging during pregnancy. A combined magnetic resonance (MR) pulmonary angiography and MRV can be done on patients who are not allowed to have iodinated contrast but are allowed to have gadolinium (Tamura & Nakahara, 2014).

Treatment

Conservative Management

The conservative treatment options for this condition involve a range of strategies. These include the use of external compression, making lifestyle adjustments such as avoiding excessive straining and prolonged periods of standing, wearing non-restrictive clothing, engaging in physical exercise, addressing risk factors, elevating the affected leg, implementing interventions to reduce peripheral oedema, pursuing weight loss, and using phlebotonics. These approaches are recommended for persons who are not eligible for endovenous or surgical operations, those who choose not to undergo such procedures, or individuals who are pregnant (Gloviczki et al., 2011; Napolitano et al., 2018). Compression has long been touted as the predominant method for controlling varicose veins. However, it should be noted that there is a lack of substantial data to conclusively determine the effectiveness of compression stockings in treating varicose veins in cases where venous ulcers are neither active nor healed (Gloviczki et al., 2011; O'Meara et al., 2012; Shingler et al., 2013; Nelson & Bell-Syer, 2014).

Pharmaceutical Treatment

Venoactive drugs are commonly administered to people who are presenting symptoms associated with varicose veins, including ankle oedema and venous ulcers. The purpose of these medications is to enhance venous tone and increase the permeability of capillaries. Pharmaceuticals that are often employed encompass various substances such as horse chestnut seed extract, flavonoids, and micronized purified flavonoid fraction (MPFF) (Gohel & Davies, 2009; Ulloa, 2019; Akhmetzianov & Bredikhin, 2021). In contrast, it has been shown that pentoxifylline has a specific affinity for modulating inflammatory cytokine secretion, stimulating the activation of leukocytes, and facilitating the aggregation of platelets within the microcirculatory system. When the administration of pentoxifylline or MPFF is combined with compression treatment, there is evidence suggesting that it may have a positive impact on the healing process of venous ulcers as compared to the use of compression therapy alone or a placebo (Ulloa, 2019). Based on a comprehensive meta-analysis conducted by Cochrane, it has been shown that vasoactive medications have the capacity to mitigate the symptoms of pain and oedema associated with chronic venous insufficiency. However, the exact mechanism by which these treatments exert their effects remains uncertain (Gohel & Davies, 2009; Ulloa, 2019; Akhmetzianov & Bredikhin, 2021; Krasinski et al., 2021). Phlebotonics refers to therapeutic methods, both oral and topical, that aim to improve capillary hyperpermeability, potentially boost venous tone, and decrease blood viscosity. The main goal of these treatments is to alleviate symptoms related to chronic venous insufficiency (Bush et al., 2017). These therapeutic interventions commonly incorporate flavonoids and other bioactive chemicals, which are usually derived from botanical sources. The ingredients that may be included include diosmin, disodium flavodate, rutin, hidrosmin, pycnogenol, grape seed, and horse chestnut seed extract. It is worth noting that in the United States, Diosmiplex is the only available prescription formulation. Diosmiplex, a substance obtained from the peels of oranges, is categorised as a medicinal food rather than a pharmaceutical medicine. The standard suggested daily intake of diosmiplex is 630 mg (Bush et al., 2017).

Surgical

Surgical intervention has traditionally been the preferred method for controlling varicose veins, often involving the saphenopopliteal junction or the high ligation of the saphenofemoral junction (SFJ), commonly followed by vein stripping (HL/S) (Medical Advisory Secretariat, 2011). In a more precise manner, the operation involves the implementation of an incision either at the groyne or upper leg area subsequent to the administration of general or lumbar anaesthesia. Following this, the identification and incision of the great saphenous vein (GSV) takes place, accompanied by the closure of its proximal end, positioned immediately below the saphenofemoral junction (SFJ). A wire used for stripping, in conjunction with a probe, is introduced into the GSV (great saphenous vein) and then progresses in a distal direction. Then, the proximal section of the great saphenous vein (GSV) is securely affixed to the wire and then extracted. Nevertheless, new research has raised concerns over the efficacy of surgery as the predominant therapeutic option, primarily due to the probable occurrence of problems following the procedure. According to the clinical guidelines set forth by the national institute for health and care excellence in 2013, surgical intervention is recommended as a third-line therapeutic option following endovenous laser ablation, radiofrequency ablation, and sclerotherapy (Epstein et al., 2022; Farah et al., 2022; Snyder et al., 2020; Mazzei et al., 2020).

Radiofrequency Ablation (RFA)

RFA is a minimally invasive technique that utilises ultrasonography guidance. It involves the use of a radiofrequency catheter to transmit heat energy, which is used to ablate the portion of the vein that is experiencing reflux. Segmental approaches are utilised in radiofrequency ablation (RFA) procedures to elevate the temperature to 120 °C. To achieve accuracy, ultrasonography is employed for the purpose of guiding the placement of a guidewire into the desired vein. Following this, a sheath for introduction is progressed along the guidewire and subsequently withdrawn, facilitating the placement of the RFA catheter at the intended site. To optimise patient comfort and ensure their safety, a tumescent anaesthetic solution is administered in close proximity to the GSV. This proposed approach aims to mitigate discomfort, facilitate efficient blood

clotting, and mitigate the risk of burns and nerve injury. Following the administration of the tumescent solution, the radiofrequency (RF) generator is initiated, and the catheter is gradually withdrawn down the whole of the vein. Compression treatment is used as a means of mitigating the potential occurrence of venous thrombosis as well as minimising postoperative bruising and pain. It is recommended that patients initiate ambulation promptly following radiofrequency ablation (RFA) procedures (Somasundaram et al., 2019). There exists a positive correlation between RFA (radiofrequency ablation) and a significantly elevated degree of patient satisfaction, as well as an extraordinary rating of quality of life. Although the duration of the operational procedure for radiofrequency ablation (RFA) was longer compared to surgical treatments, the recovery time following RFA showed a notable acceleration in terms of returning to routine activities and resuming work within one week. Moreover, the use of RFA has been shown to be correlated with a reduced incidence of severe adverse effects. In a research study including 135 patients and a total of 164 limbs, the great saphenous vein was completely obliterated in an amazing 98.2% of instances. The median follow-up period for this study was 11 months (Borghese et al., 2021). Moreover, the study found that radiofrequency ablation (RFA) showed comparable effectiveness to other treatments in terms of reducing the recurrence of chronic hepatic venous insufficiency and hepatocellular carcinoma (HL/S) two years after the initial therapy. Significantly, there were no significant discrepancies observed in postoperative complications or pain levels when comparing the techniques of HL/S, RFA, and CHIVA (González Cañas et al., 2021).

Minimally Invasive Treatment

Endovenous laser ablation (ELA) is a medical procedure that utilises the insertion of a laser fibre via a vein to deliver heat energy to a specific target vein, often known as the great saphenous vein (GSV). The aforementioned procedure yields enduring eradication by inflicting harm to the endothelium lining, subsequently resulting in the development of fibrosis and scarring. In the realm of medicine, the process commonly referred to as "endovascular heat-induced thrombosis" is widely accepted as acceptable terminology. This method is usually considered the initial treatment of choice

for varicose veins, where applicable (American College of Radiology, 2012; Vuylsteke & Mordon, 2012). Using a catheter, the practitioner places a laser fibre in a peripheral vein that is affected to execute endovenous laser ablation (ELA). Subsequently, the operator advances the tip of the fibre towards the vein's point of drainage. The proximal portion of the calf is often where the catheter is inserted in the context of the GSV. Following this, the laser fibre's tip is carefully moved to the saphenofemoral junction, which is situated at the periphery of the inferior epigastric vein insertion site. The catheter is strategically positioned to exploit the preserved blood circulation in the inferior epigastric vein, therefore impeding the advancement of thrombosis into the CFV. Upon placement, the laser is initiated and subsequently retracted in a methodical manner down the vein. Following the completion of the treatment, doppler ultrasonography is utilised to verify the existence of thrombosis inside the treated vein, therefore guaranteeing that it does not propagate into the CFV. After the onset of fibrosis, the blocked great saphenous vein (GSV) experiences a reduction in diameter. The occlusion rates over a prolonged duration have been documented to potentially reach a maximum of 100% during a three-year timeframe (Mundy et al., 2005). However, previous research has indicated a higher risk of failure, as validated by doppler ultrasonography, with a 30% failure rate observed at the 6-year mark. Additionally, within the same group, a clinical failure rate of 12% was recorded. Also, it is worth noting that there is a higher likelihood of failure when the vein diameter is 8 mm or exceeds it, since the radiation from the laser might not sufficiently impact both walls of the vein (Spreafico et al., 2013). In instances of this nature, the use of tumescent anaesthesia may be applied as a strategy to induce vein constriction, aiming to enhance the overall results. It is crucial to acknowledge that electrocautery (ELA) has the capacity to induce skin burns, especially when the targeted vein is situated in close proximity to the dermal layer or when a reflux of blood occurs within a varicosity that lies in nearness to the skin (Sichlau & Ryu, 2004).

Sclerotherapy

Sclerosing therapy constitutes a viable treatment option for individuals afflicted with varicose veins in the lower extremities. This therapeutic approach entails the intravenous administration of chemical agents with the explicit objective of inducing inflammatory occlusion. It is particularly well-suited for the management of capillary dilation, small saphenous veins, superficial varicose veins, and large saphenous veins. Within the realm of this treatment modality, sclerosing foam, a composite comprising gaseous and liquid sclerosing solutions, is commonly employed as an efficacious means to address varicose veins (Nesbitt et al., 2014; Kotb et al., 2013). Promoting thrombosis within intravascular regions is achieved through the direct vascular endothelial damage inflicted by the injected sclerosing agent. Subsequently, aseptic inflammatory lesions are induced, leading to tissue fibrosis. This process results in the permanent occlusion of pathological blood vessels, thereby attaining the desired therapeutic outcomes. Foam sclerotherapy represents a modification of the conventional sclerotherapy technique, wherein bubbles of sclerosant, generated using either carbon dioxide or air, are introduced into the affected vein under sonographic guidance (Sharma et al., 2016). It is widely acknowledged that the effectiveness of sclerosing therapy relies on the drug concentration within the vein rather than the concentration in the syringe (Cavezzi et al., 2002).

Ambulatory Phlebectomy

Ambulatory phlebectomy is a suitable approach for addressing more extensive superficial varicosities that are observable during physical examination. It is crucial to consider that this approach requires the making of cutaneous incisions at the sites of varicosity targeted for treatment, where small hook instruments are then employed. Following this, the varicosity is tightly ligated and subsequently removed. Nonetheless, it is important to underscore that while this method can successfully address specific varicosities, it does not resolve the underlying cause (Kabnick & Ombrellino, 2005).

Invasive Treatment

Vein stripping represents a highly invasive approach for addressing great saphenous vein (GSV) insufficiency and is linked to heightened morbidity when compared to less invasive alternatives (Jaworucka-Kaczorowska et al., 2015). Typically, this method is reserved for situations where minimally invasive techniques are deemed unsuitable. The technique for vein stripping exhibits variability, influenced in part by the stripping device employed. In a broad sense, the process begins with the dissection of the target vein at its proximal end, followed by the division of its superior branches. Subsequently, the vein is then accessible and isolated from various tributaries. Subsequently, stripping or wire equipment is inserted into the intended vein, extending through the lower portion to the previously separated higher region, and securely affixed at both ends. The device, along with the vein, is subsequently extracted from the patient (Jaworucka-Kaczorowska et al., 2015; "MedlinePlus," n.d.; Gordon & Payne, 1953).

Clinical Presentation

The clinical manifestation of varicose veins exhibits considerable variation, with some patients remaining asymptomatic (Teruya & Ballard, 2004). Localised symptoms can manifest bilaterally or unilaterally and encompass pain, itching, burning sensations, and tingling localised at the site of the varicose veins. Generalised symptoms encompass aching, throbbing, heaviness, restlessness, cramping, and leg swelling (Gloviczki et al., 2011; Langer et al., 2005). These symptoms typically intensify towards the end of the day, especially following prolonged periods of standing, and are often alleviated when patients assume a seated position and elevate their legs. It is noteworthy that females are notably more likely than males to report lower limb symptoms (Bradbury et al., 1999). Furthermore, the presence and severity of symptoms tend to increase in correlation with the CEAP clinical class, progressing from C0 to C6 (Bergan et al., 2006). The diagnosis of CVI can be established through a thorough patient history and physical examination, in conjunction with various imaging techniques. Evaluation of the lower extremities should encompass an assessment for superficial signs of venous disease, such as telangiectasia or varicose veins, along with skin alterations indicative of venous stasis and edema. To distinguish between deep and superficial reflux, the Brodie-Trendelenburg test can be conducted. In this test, the patient is positioned supine, and pressure is applied to the superficial veins using either a tourniquet or manual compression. When the patient stands up, individuals with superficial reflux will exhibit a delayed refill of varicose veins, typically exceeding 20 seconds, while those with deep reflux will experience quicker refilling (Eberhardt & Raffetto, 2014; Youn & Lee, 2019). The primary imaging method for assessing CVI is duplex ultrasound, often supplemented with colour doppler (Youn & Lee, 2019). This technique enables the visualisation of both reflux and obstruction. Additional approaches, such as plethysmography or intravascular ultrasound, can offer more comprehensive insights into the venous system of the lower limb. Subsequently, following all examinations, patients should be diagnosed based on their findings and categorised according to the CEAP classification, which serves as the gold-standard classification system for CVI (Lurie et al., 2020).

CHAPTER III
Methodology

Research Design

The study employed a retrospective cohort design, which is an observational research approach that retrospectively analyses previously collected information to explore the relationship between exposure to certain risk factors and the occurrence of an outcome. The study design entails the identification of a cohort, including persons who have received a diagnosis of varicose vein, in conjunction with a control group consisting of individuals who have not received a diagnosis of the aforementioned ailment. Through a comparative analysis of both groups, this study aims to ascertain the presence of a potential association across the expression of *cytochrome-c*, *caspase-3*, and the occurrence of varicose veins.

The World Medical Association Declaration of Helsinki's guidelines and the ethical frameworks that govern medical investigations involving human subjects were both followed during the study's execution. Furthermore, it obtained approval from the Near East University Hospital (NEUH) and the ethical review board.

Participants

The individuals involved in this research are those receiving medical care at the NEUH. The study collected data from the hospital system to identify individuals who had been exposed to the factor of interest, venous insufficiency. The patients' group was made up of 47 people who were confirmed to have problems with their great saphenous vein (GSV) through a doppler ultrasonography test. The presence of reflux at the saphenofemoral junction, with a reflux time exceeding 0.5 seconds, was observed in all of the patients when in a standing posture. The assessment of blood flow was conducted with the use of physical compression. 42 participants made up the control group, and doppler ultrasonography confirmed that they each had a healthy great saphenous vein. The study subsequently addresses the incidence of the results, particularly the emergence of varicose veins, within both of those groups to ascertain any potential correlation between experiencing varicose vein and its subsequent progression.

Data Collection Tools

The patient records were retrieved from the hospital information system, spanning the time span from 2021 to 2023. Certain keywords were used in the search, including "varicose veins," "venous reflux," "chronic venous insufficiency," "chronic venous disease," and "mini-phlebectomy." Patients who satisfied the requirements for a diagnosis of venous insufficiency were chosen, while patients who showed no venous abnormalities made up the negative control group.

Materials

Kits

The study employed kits that included an RNA isolation kit (Hibrigen, Turkey), a cDNA synthesis kit (Hibrigen, Turkey), and 2X SYBR Green qPCR Mix (Hibrigen, Turkey).

Table 1.

Demonstrates the Components in the cDNA Kit

Component	Volume
Enzyme mix	50 µL
Reaction buffer	250 µL
Nuclease-free water	1 mL

Table 2.

Presents the Components in the 2X SYBR Green qPCR Mix

Concentration	Component
100µM	KCl
4µM	$MgCl_2$
400µM	dNTPs
0.1u/ µL	Taq DNA Polymerases
1x	SYBR Green

Oligonucleotides

The primer pairs utilised in this investigation have been obtained from Turkey (Oligomer).

Table 3.

Specifies the Primers Pairs' Target Base Sequences 5'-3'

Cytochrome-c	NM_018947.6	Forward	CGCCAATAAGAACAAAGGCATCA
		Reverse	TAAGGCAGTGGCCAATTATTACTC
Caspase-3	NM_004346.4	Forward	ATTTGGAACCAAAGATCATACATGG
		Reverse	TTCCCTGAGGTTTGCTGCAT

Methods

Doppler Ultrasound

The Siemens ACUSON S2000 and GE LOGIQ S6 Doppler ultrasound machines are significant technical tools in the evaluation of veins, playing a crucial role in vascular examinations. Both pieces of equipment consist of a console that integrates a monitor capable of displaying real-time imaging and Doppler waveforms. It provides a variety of control panels and settings that allow for precise customisation of imaging parameters. The doppler probe is a portable device that is connected to an ultrasound machine. It integrates standard ultrasound components for imaging purposes with specialised doppler technology, enabling the evaluation of blood flow within veins. Crafted with a smooth, rounded surface, the probe is delicately positioned on the skin's surface, primarily targeting the lower extremities during the venous assessment. The design of this technique enables optimal skin contact, hence facilitating the smooth transmission and reception of high-frequency sound waves. This is crucial for the purposes of imaging and doppler analysis. The examination protocol was conducted in the Radiology Department of Near East University Hospital. The examination approach starts with patient orientation and a thorough elucidation of the process to guarantee

patient comfort and comprehension. Following this, the patient is placed in a comfortable posture, often with the lower limbs exposed for assessment. In the context of the examination, hydrogel is carefully administered to optimise the transmission of sound waves and establish optimal contact between the skin and the doppler probe. The aquasonic ultrasound gel is used as a conductive medium to enhance the efficacy of ultrasound waves as they permeate the skin and engage with the underlying tissues. The doppler probe is carefully positioned across the specified region, with specific attention given to the great and small saphenous veins. The first stage of the procedure entails acquiring fundamental ultrasound pictures of the veins in the leg to detect any possible structural irregularities. By enabling the doppler feature, the probe emits high-frequency sound waves that are capable of detecting motion inside the veins. This enables real-time visualisation of the direction of blood flow. Concurrently, healthcare professionals diligently evaluate the direction of blood flow, with a special focus on identifying signs of reflux, which is a characteristic feature of venous insufficiency. Reflux is the term for the retrograde movement of blood that results from either venous system blockages or dysfunctional valves. To assess blood flow dynamics during certain patient motions or in an upright position, several specialised procedures, such as compression and release, can be executed. Thorough documentation of pertinent discoveries, including visual representations, Doppler waveforms, and measurements, is meticulously preserved for further examination. Proficient radiologists with expertise in ultrasound imaging analyse the data, therefore assisting in the diagnosis of venous insufficiency. The findings, in conjunction with diagnostic assessments derived from the CEAP classification system, are sent to both the patient and the healthcare professionals who made the referral. This allows additional examination and the development of customised strategies for managing the condition.

Mini-Phlebectomy

The procedure was conducted in the Surgery Department of Near East University Hospital. After undergoing a thorough doppler assessment to evaluate the venous system, patients underwent detailed consultations with vascular experts or surgeons to discuss the mini-phlebectomy surgery. This technique primarily focuses on the

extraction of superficial varicose veins. Prior to the operation, patients were provided with informed consent, which included comprehensive information regarding the potential risks, benefits, and alternatives, including the possibility of biopsy collection. The pre-procedural preparations were made in accordance with established protocols, including modifications in medication administration, adherence to fasting requirements, and wearing suitable apparel. The mini-phlebectomy is a surgical procedure characterised by its minimally invasive nature. It entails creating incisions of one to two millimetres in size and selectively extracting varicose veins using specialised equipment, such as hooks or micro-incision tools. The outpatient treatment was performed with local anaesthesia to optimise patient comfort. Following the removal procedure, the incision commonly underwent healing without necessitating the use of sutures. To facilitate the healing process and mitigate postoperative oedema, compression bandages or stockings were utilised. Following the treatment, patients were carefully observed in a designated recovery area to promptly identify and address any possible issues. Complete post-procedure care instructions were given out, including information on how to use compression garments, how long to elevate the legs, which activities to avoid, and how to take medicine as prescribed. Follow-up consultations were scheduled to monitor the course of healing and evaluate the effectiveness of the treatment, with the aim of promoting optimal patient recovery and satisfaction. There were no observed problems throughout the post-operation monitoring period, confirming the safety and effective implementation of the technique.

Biopsy Samples

Specimens were acquired from the cardiovascular surgery department of the Near East University Hospital. Following the mini-phlebectomy procedure, biopsies of the saphenous veins were extracted and placed into a sterile tube. That tube contained an RNA later reagent and has been kept at a temperature of -80° C until further processing for gene expression investigation.

RNA Extraction

Mechanically crushing the tissue samples was the first step in the RNA isolation process, which was then homogenization in a solution containing 1 ml of TRIZOL reagent for every 50 to 100 mg of tissue. Afterwards, 0.2 ml of chloroform was poured into each 1 ml of TRIZOL reagent, and then the sample tubes were tightly capped. After vigorous vortexing for 15 seconds, the samples underwent a 2–3-minute incubation period at room temperature. Following the incubation period, the samples underwent centrifugation. The procedure was conducted within a temperature range spanning 2 to 8°C. with a maximum force of 12,000 ×g for 15 minutes. The utilisation of centrifugation resulted in the partitioning of the mixture into discrete phases, including a lower phase characterised by a red hue consisting of phenol-chloroform, an intermediate phase, and an upper phase composed of an aqueous solution devoid of colour. The RNA, which was found just in the upper aqueous phase, was meticulously moved to new tubes, taking care to avoid any disruption to the interphase. To induce the separation of RNA from the aqueous phase, the RNA was mixed with isopropyl alcohol. 0.5 millilitres of that mixture were then added to each 1 millilitre of the already-used TRIZOL reagent. After that, the combination was allowed to incubate for ten minutes at a temperature range of fifteen and thirty degrees Celsius. Following centrifugation with a maximum force of 12,000 x g for 5 minutes at a temperature range of 2 to 4°C, a gel-like pellet composed of RNA was observed. This pellet was not visible prior to centrifugation and was found to accumulate along the side and bottom of the centrifuge tube. Following the full removal of the supernatant, a wash was conducted on the RNA pellet utilising 75% ethanol, with a minimum volume of 1 ml of 75% ethanol for 1 ml of the initially employed TRIZOL reagent. The process of vortexing and subsequent centrifugation was performed twice at a maximum speed of 7,500 x g for a duration of 5 minutes at a temperature range of 2 to 8°C. This was done to ensure the complete elimination of any remaining ethanol. Consequently, the RNA pellet was set aside to dry in the air for an approximate duration of up to ten minutes, intentionally avoiding the use of vacuum centrifugation to prevent excessive drying. To elute the RNA, 50 µl of DNase-RNase-free water was poured onto the pellet.

RNA Ratio

A nanodrop spectrometer (Thermo-Scientific, Pittsburgh, USA) was chosen as a method for assessing the purity and concentration of RNA due to its advantageous characteristics, including its minimal sample volume requirement, cost-effectiveness, wide commercial availability, and ease. The Nanodrop apparatus is a spectrophotometer designed for microvolume analysis, utilising UV-Vis spectroscopy techniques to determine the concentration and purity of nucleic acid (NA) and protein samples. The approach has the capability to accurately measure NA concentration at levels as low as 1 ng/l. Although concentration and purity measurements are typically regarded as reliable, a more comprehensive understanding of the results can yield significant insights on the quality of the sample and its appropriateness for further applications. Ultraviolet (UV) spectroscopy is employed to quantify the quantity of RNA by measuring the absorbance of an RNA sample at certain wavelengths, notably 260 nm and 280 nm. The Beer-Lambert law is employed for the determination of nucleic acid concentration, whereby it predicts a proportional relationship between absorbance and concentration. The A260/A280 ratio offers valuable information on the general assessment of its purity; 1.8 to 2.0 is considered ideal. A higher value in this ratio often signifies the existence of protein contamination, whereas a lower value suggests the existence of RNA contamination with other substances such as phenol and salt. The RNA concentration and purity were determined to be optimal and appropriate for the study's aims.

Complementary DNA (cDNA) Synthesis

The cDNA synthesis was conducted using Hibrigen cDNA synthesis kits, which consist of many essential components. The enzyme mix solution, an essential component, was a combination of reverse transcriptase and RNase inhibitors. The reaction solution, meticulously formulated for superior efficiency, included dNTPs, $MgCl_2$, random 6-mer primers, and oligo-dTs, all inside a well-calibrated buffer environment. The anchored oligo (dT) primer was one of these elements that was essential to molecular biology research. The purpose of this 18-nucleotide primer is to bind to the poly(A) tail's 5' end, which will make certain that complementary DNA (cDNA) synthesis occurs effectively. The Random Hexamer Primer is a widely used primer in molecular biology for the random priming of RNA molecules. The last element used in the synthesis process is nuclear-free water, which improved the reaction's overall accuracy. The whole process of cDNA synthesis lasted for one hour and ten minutes, as shown in Table 6.

Table 4.

Describes the Protocol for cDNA Synthesis

Component	**1x**
Enzyme Mix	1 µL
Reaction Buffer	4 µL
Nuclease-Free dH_20	10 µL
Total RNA	5 µL

Table 5.

Showcases the Polymerase Chain Reaction Condition for cDNA Synthesis

Step	Temperature	Time
cDNA Synthesis	42°C	60 Minutes
Inactivation of Kit	80°C	10 Minutes

Initial PCR

A first polymerase chain reaction (PCR) test was performed using the primers given, together with positive and negative controls, while precisely following the recommended primer concentration outlined in the manufacturer's instructions (10 micromolar). The objective of this approach was to directly evaluate the specificity of the primers towards the target sequence and measure their effectiveness in amplifying the targeted DNA fragments. The positive control consisted of the template DNA, including the target sequence, which served as a benchmark for successful amplification. Conversely, the negative control did not include the template DNA, allowing for the assessment of potential contamination or unintentional amplification. By carrying out the PCR under predetermined conditions, which included elements like annealing temperature, cycle numbers, and others, it was possible to ensure the precise and reliable amplification of targeted DNA sequences. This approach permitted the evaluation of primer performance.

Table 6.

Demonstrates Initial PCR, Showing Primer Dimer: 35x Cycles

Component	1x
2x Taq Master Mix	12.5 µL
Forward Primer (10µM)	0.5 µM
Reverse Primer (10µM)	0.5 µM
dH_2O	5 µL
cDNA (10 ng/µL)	5 µL

Gradient PCR

This study consisted of conducting a gradient PCR to investigate a variety of annealing temperatures. To determine the most optimal temperature for the primers, multiple annealing temperatures were assessed employing a temperature gradient ranging from 56°C to 62°C.

This procedure facilitates the determination of the optimal temperature at which the amplification of the specific target sequence is most efficient. This ensures the selection of an annealing temperature that maximises the efficacy of the PCR under the experimental conditions.

Table 7.

Specifies PCR Cycling Conditions: 35x Cycles

Stage	Temperature	Time
Initial Denaturation	96°C	1:30 Minutes
Denaturation	96°C	20 Seconds
Annealing	56°C, 59°C and 62°C	30 Seconds
Extension	72°C	45 Seconds
Termination	72°C	10 Minutes

Primer Volume Optimization Experiment

Volume reductions were performed to determine the optimum amount for reliable amplification. This approach enables a methodical assessment of several primer volumes to identify the most effective volume, resulting in efficient and accurate amplification of the target sequence. Initially, the primer stock with a concentration of 100 µM was diluted to get working primers with a concentration of 10 µM. This has been accomplished by combining 10 µL of the primer with 90 µL of dH_2O. In both the cytochrome-c and caspase-3 experiments, primer dimers were seen when 0.5 µM of the 10 µM concentration was used. Using 0.2 µM of cytochrome-c primers yielded the anticipated length of the PCR product, with no detection of primer dimers. Later on, the

initial volume of the primer was decreased even further to 0.08 µM, specifically for caspase-3. Through careful examination of the PCR products obtained using different quantities of primers, an ideal volume that promotes efficient amplification while maintaining specificity was determined.

Table 8.

Showcases the Final Amount of Forward and Reverse Primer of Each Gene

Primer	1x
Cytochrome-c (10µM)	0.2 µM
Caspase-3 (10µM)	0.08 µM
ACTB (10µM)	0.4 µM

Table 9.

Demonstrates PCR Component Quantities for Cytochrome-c Amplification: 35x Cycles

Component	1x
2x Taq Master Mix	12.5 µL
Forward Primer (10µM)	0.2 µM
Reverse Primer (10µM)	0.2 µM
dH_2O	6.5 µL
cDNA (10 ng/µL)	5 µL

Table 10.

Demonstrates PCR Component Quantities for Caspase-3 Amplification: 35x Cycles

Component	1x
2x Taq Master Mix	12.5 µL
Forward Primer (10µM)	0.08 µM
Reverse Primer (10µM)	0.08 µM
dH_2O	7.1 µL
cDNA (10 ng/µL)	5 µL

Table 11.

Specifies PCR Conditions: Annealing

Primer	Temperature	Time
Cytochrome-c	62°C	30 Seconds
Caspase-3	56°C	30 Seconds
ACTB	62°C	30 Seconds

PCR Product Analysis

A PCR product analysis was performed to evaluate the amplified products. The use of gel electrophoresis was implemented to analyse the PCR products. The procedure entails the separation of the amplified DNA fragments by size using an agarose gel. The amplified product's anticipated size is confirmed by comparing the fragmented samples to a molecular weight marker. Concurrently, this study allows us to identify and validate the lack of non-specific bands or artefacts, confirming the specificity of the amplification process. By doing a thorough analysis, the capability to determine the accuracy of the PCR amplification process was achieved. This involves verifying that the resultant products are consistent with the expected size and do not contain any unwanted or non-specific amplification products. The inclusion of this crucial phase ensures the dependability and precision of these optimised primer conditions, therefore confirming their appropriateness for subsequent experimental applications.

Gel Electrophoresis

The procedure involves several pivotal steps. A 1x tris-borate-EDTA (TBE) solution is used to dissolve agarose powder for the purpose of making the gel. then applying heat until full dissolution is accomplished. After the solution has cooled to about 56°C, ethidium bromide (EtBr) is added to facilitate staining and enhance the visibility of the bands. Subsequently, the liquid agarose with a concentration of 3% is cautiously transferred into the tray. The gel is poured into a tray with the insertion of a comb to form wells for the sample and a ladder loading.

Gel electrophoresis is performed by placing the gel in an electrophoresis chamber that is filled with 1x TBE buffer. 3 µL of 50 base pair ladder, followed by conjoining the sample with dye and thereafter introducing 10 microliters of the amalgamation onto the gel. This colour improves the visibility of the material during electrophoresis and adds density for loading into the wells. With precision, the pre-prepared samples were placed into the specified wells using a micropipette, being cautious to avoid leaking by refraining from excessive filling. Attach the electrodes to the power source, being sure to align the negative and positive terminals correctly. The gel is taken out of the chamber after the electrophoresis run is over. Ultraviolet (UV) light is used for observing bands that have separated.

Table 12.

Specifies Gel Preparation

Component	Working Concentration	For 350 ml 3% Agarose Gel
10x TBE Buffer	1x	100ml TBE (10x) Added to 900ml Distilled Water
Agarose Powder	3%	0.03x 350ml (1x) TBE=10.5 Gram
EtBr	0.625 mg/ml	1.75 µL
6x Blue Gel Loading Dye	1x	6 µL Dye Added to 30 µL Distilled Water

Table 13.

Specifies Agarose Gel and Electric Current to the Gel at a Voltage and Duration that are Suitable for the Specific Kind and Size of Molecules being Studied

Agarose Concentration	Ladder	Voltage	Time
3%	50 Base Pair DNA Ladder	105	30 Minutes

Replication and Validation

Following the establishment of optimised conditions, which encompassed primer concentrations and volume, annealing temperatures, and other pertinent elements, these optimised conditions were duplicated in a few independent experiments. The inclusion of this pivotal step within the procedure assures the consistency and reproducibility of these findings, thereby reinforcing the trustworthiness of the optimised primer conditions. Employing a qPCR experiment using 2x SYBR Green, six samples were run in duplicate, and two negative control samples were examined. The negative control samples exhibited no reported signals, suggesting a lack of primer dimers. The fluorescent emission recorded in all double strands was certainly derived from the intended sequence.

Real Time Polymerase Chain Reaction (Quantitative Polymerase Chain Reaction qPCR)

The selection of this technique was based on its notable sensitivity, specificity, and accuracy in accurately measuring gene expression levels. The process of amplification of cDNA is conducted using certain primers beyond the basic components, such as template cDNA, Taq DNA polymerases, dNTPs, potassium chloride (KCl), magnesium chloride ($MgCl_2$), and 1x SYBR Green. For real-time monitoring of polymerase chain reaction (PCR), a 2x SYBR Green probe is used. During the progression of the PCR, the fluorescence signal demonstrates a positive association with the amount of amplified DNA. Quantitative methods for figuring out gene expression levels involve comparing fluorescence signals with reference genes to find out how much RNA was in the sample to begin with.

Table 14.

Demonstrates the Protocol of the qPCR Experiment: 35x Cycles

Component	Cytochrome-c (1x)	Caspase-3 (1x)
2x SYBR Green Probe	12.5 µL	12.5 µL
Forward Primer (10µM)	0.2 µM	0.08 µM
Reverse Primer (10µM)	0.2 µM	0.08 µM
dH_2O	6.5 µL	7.1 µL
cDNA (10 ng/µL)	5 µL	5 µL

Table 15.

Specifies Components for Preparing the Housekeeping Gene (ACTB Gene*): 35x Cycles*

Component	1x
2x SYBR Green Probe	12.5 µL
Forward Primer (10µM)	0.4 µM
Reverse Primer (10µM)	0.4 µM
dH_20	5.5 µL
cDNA (10 ng/µL)	5 µL

Software System

The software system utilised in this research is Nucleus version 9.37.77, which is specifically designed for managing patient records. The GelCapture software version 4.25 has been employed for the goal of visualising and analysing gel pictures. The data passed through statistical analysis using GraphPad Prism 10 version 10.1.2(324).

CHAPTER IV

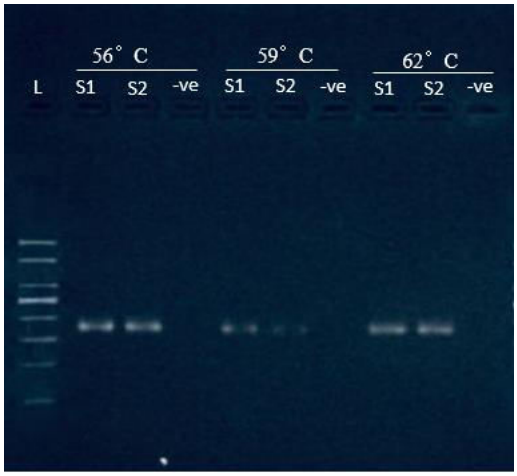
Results

The population being studied included patients who had been diagnosed with varicose veins, along with a control group composed of persons who did not have the disease. Varicose veins (VVs) may occur during any phase of an individual's lifespan. The rate of incidence of this condition varies across individuals, with a more noticeable occurrence reported in the retired population compared to younger demographic.

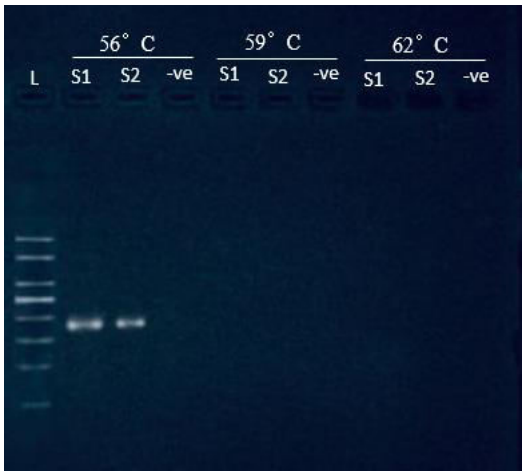
Figure 2

Specifies PCR Product Analysis Using Gel Electrophoresis: (A)Cytochrome-c (B) Caspase-3

A.



B.

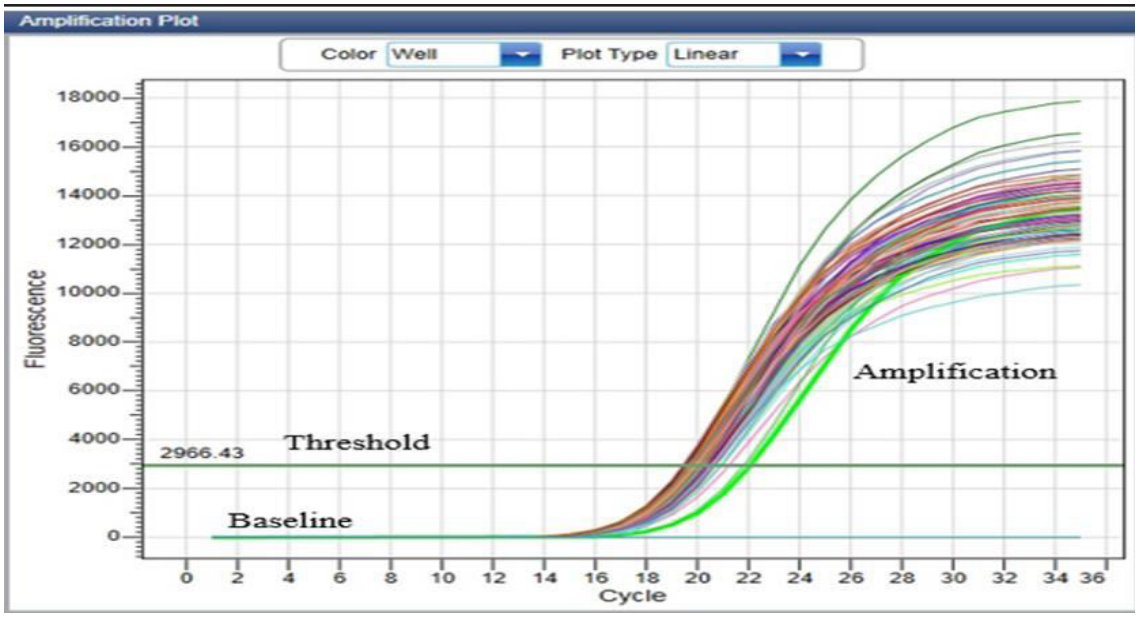


L: Ladder, S1: Sample 1, S2: Sample 2 and -ve: Negative Control

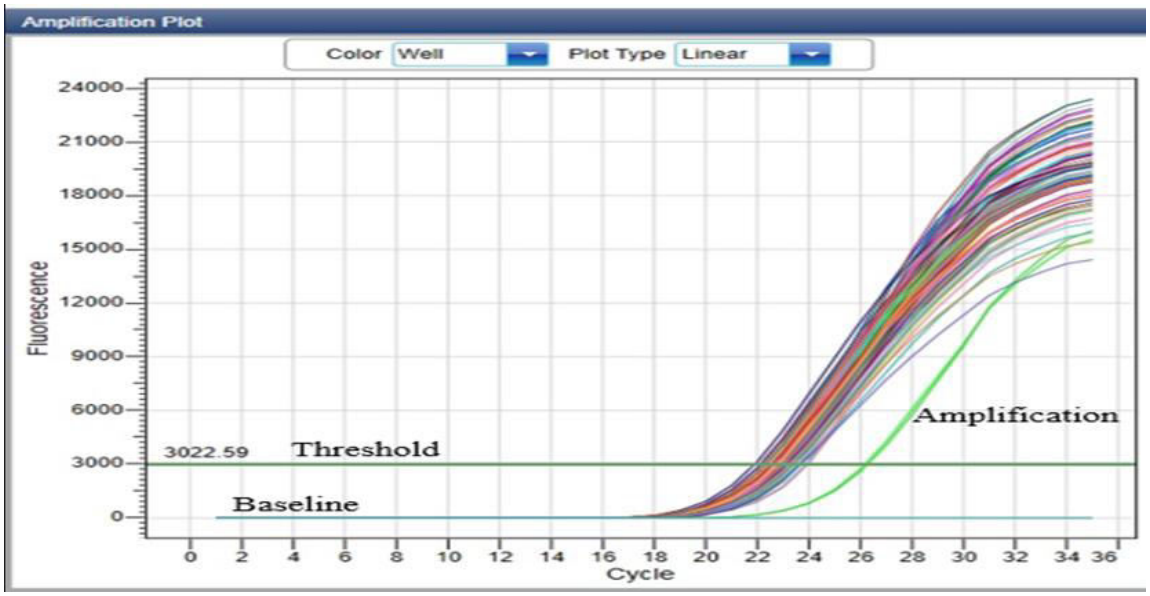
Figure 3

A and B Specifies the Amplification Plot of Cytochrome-c and Caspase-3, Respectively

A. *Cytochrome-c: Amplification Plot of 47 Patients, Each Run in Duplicate, Followed by One Negative Control*



Caspase-3: Amplification Plot of 47 Patients, Each Run in Duplicate, Followed by One Negative Control Also Run in Duplicate



Statistical Analysis

The correlation analyses explore the relationships between the housekeeping gene (*ACTB*) and the studied genes (*Cytochrome-c* and *Caspase-3*) within both control and patient groups across 89 samples. Table 16 showcases the correlation study of *ACTB* and *Cytochrome-c* in control cases. With a Spearman correlation coefficient (r) of 0.08915, indicating a very weak positive link, and a non-significant p-value of 0.5745, the association lacks statistical significance.

In Table 17, focusing on patients, the correlation between *ACTB* and *Cytochrome-c* reveals a moderate positive association (r = 0.2668). However, the two-tailed p-value of 0.0699 deems the correlation non-significant.

Moving to Table 20, the correlation analysis between *ACTB* and *Caspase-3* within the control group exhibits a very weak positive link (r = 0.04506). The non-significant p-value of 0.7769 suggests a lack of statistical significance.

Table 21 presents the correlation study between *ACTB* and *Caspase-3* within the patient group. With an exceptionally small positive connection (r = 0.01067) and a non-significant p-value of 0.9433, the association is considered statistically insignificant. In summary, these correlation analyses, along with their graphical representation in Figures 4, 5, 9, and 10, provide a comprehensive overview of the associations between the housekeeping gene and the studied genes in both control and patient groups.

Tables 19 and 23 provide a comprehensive statistical analysis of the average cycle threshold (Ct) values for *Cytochrome-c* and *Caspase-3*, respectively, across control participants and patients with varicose veins (VVs). The data includes the number of observations (N), median, mean, and standard deviation, offering insights into the central tendency and variability of gene expression.

Table 16.

Correlation Analysis of Control Cases and Housekeeping Gene: Cytochrome-c

ACTB (Control Participants) vs. *Cytochrome-c* (Control Participants)	
Spearman r	
R	0.08915
95% confidence interval	-0.2296 to 0.3906
P value	
P (two-tailed)	0.5745
P value summary	ns
Exact or approximate P value	Approximate
Significant (alpha = 0.05)	No
Number	42

Figure 4

Correlation Analysis: Housekeeping Gene vs. Cytochrome-c Expression in Control Participants

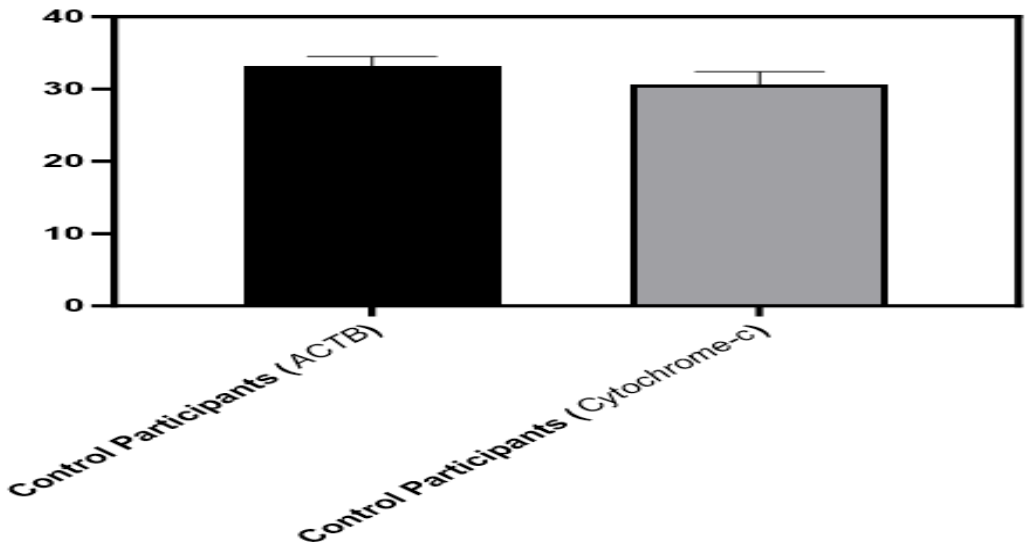


Table 17.

Correlation Between Cytochrome-c and Housekeeping Gene Among Patients

ACTB (Patients) vs. *Cytochrome-c* (Patients)	
Spearman r	
r	0.2668
95% confidence interval	-0.03082 to 0.5209
P value	
P (two-tailed)	0.0699
P value summary	ns
Exact or approximate P value	Approximate
Significant (alpha = 0.05)	No
Number	47

Figure 5

Correlation Analysis: Housekeeping Gene vs. Cytochrome-c Expression in Patients

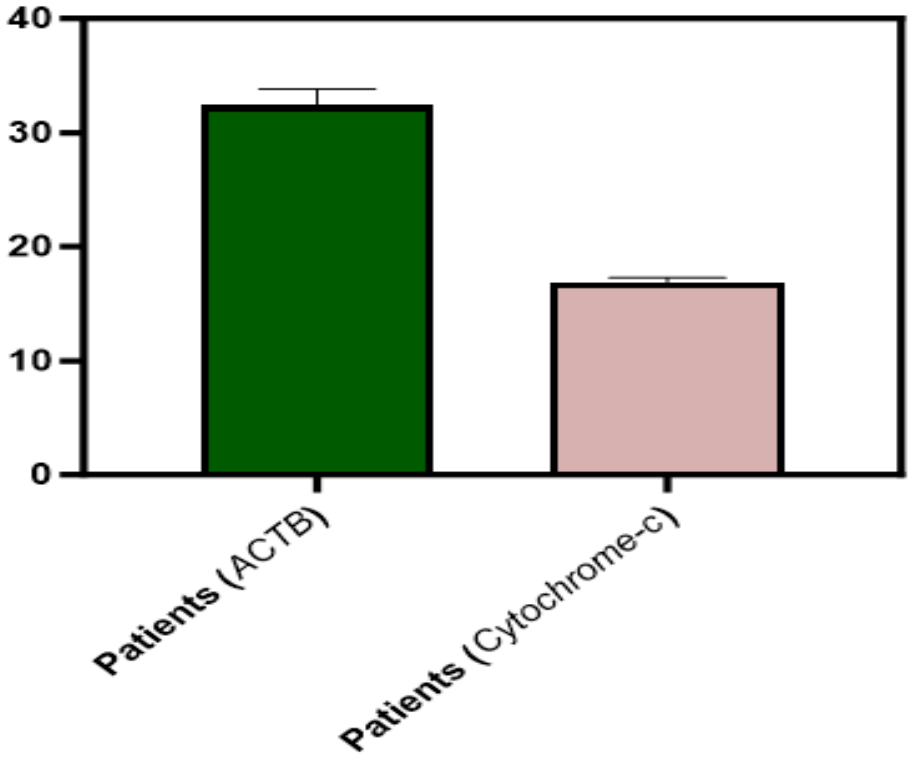


Table 18.

Average Ct Values

	Housekeeping (Control)	*Cytochrome-c* Control	Housekeeping (VVs Patients)	*Cytochrome-c* Patients
N	42	42	47	47
Median	33.24	30.68	32.27	16.87
Mean	33.22	30.61	32.38	16.91
Std. Deviation	1.303	1.790	1.466	0.3754

Figure 6

Compares the Expression Levels of ACTB and Cytochrome-c Between Control Participants and Patients

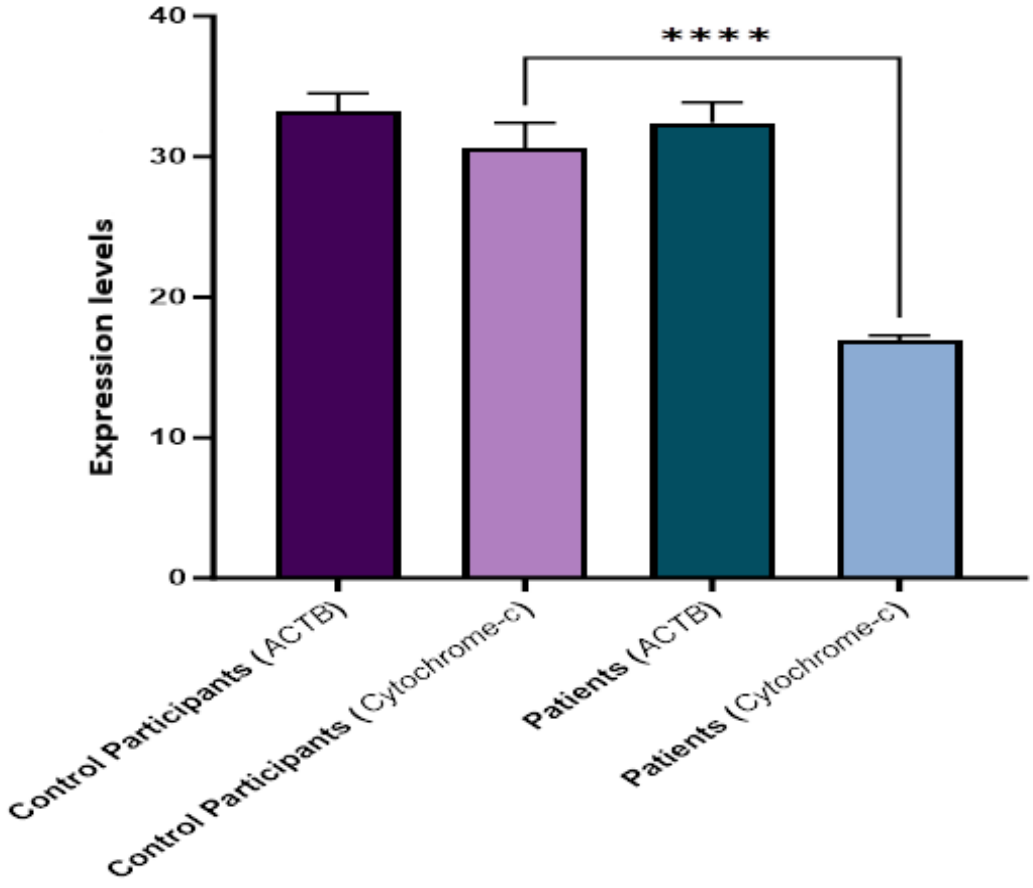


Table 19 reveals a substantial elevation in *cytochrome-c* expression among individuals with varicose veins compared to those without the condition, accompanied by graphical representations in Figures 7 and 8. In terms of central tendencies, both the median and mean values highlight a significant increase in *cytochrome-c* expression in patients (median: 7007, mean: 11584) in contrast to controls (median: 1.531, mean: 2.305).

Notably, patients exhibit considerable variability in the data (standard deviation: 11002), while controls show lower variability (standard deviation: 3.400). Confidence intervals ensure precision in estimating the median and mean values. Interestingly, controls display a higher coefficient of variation (147.5%) compared to patients (94.97%). This summary provides crucial insights into the differential expression and characteristics of cytochrome-c in both groups.

Table 19.

Fold Change in Expression of Cytochrome-c

Fold Change Data	Control	Patient
Number of values	42	47
Median	1.531	7007
Mean	2.305	11584
Std. Deviation	3.400	11002
Coefficient of variation	147.5%	94.97%

Figure 7

Fold Change in Expression of Cytochrome-c

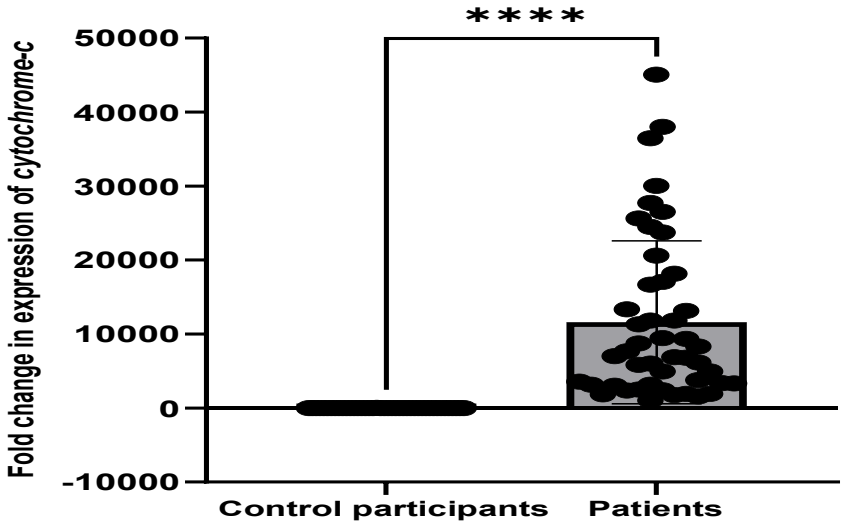


Figure 8

Difference Between Means

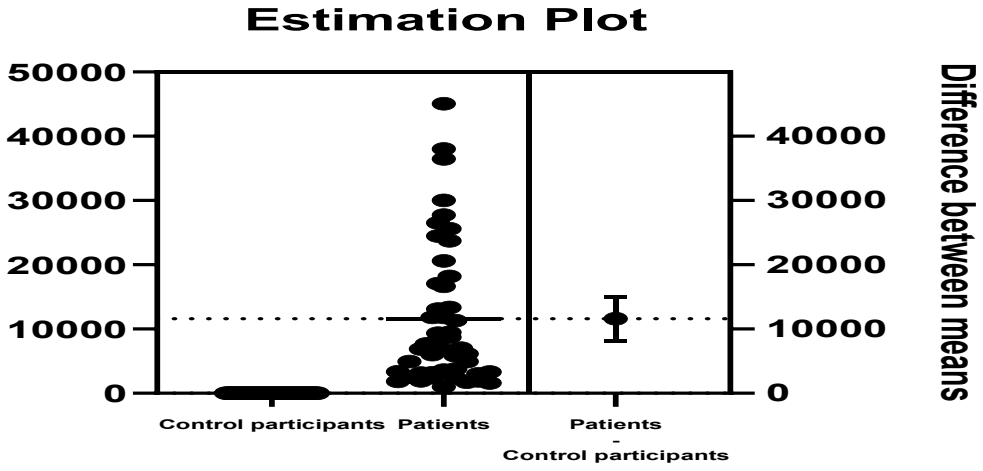


Table 20.

Correlation Analysis of Control Cases and Housekeeping Gene: Caspase-3

ACTB (Control Participants) vs. *Caspase-3* (Control Participants)	
Spearman r	
r	0.04506
95% confidence interval	-0.2711 to 0.3524
P value	
P (two-tailed)	0.7769
P value summary	ns
Exact or approximate P value	Approximate
Significant (alpha = 0.05)	No
Number	42

Figure 9

Correlation Analysis: Housekeeping Gene vs. Caspase-3 Expression in Control Participants

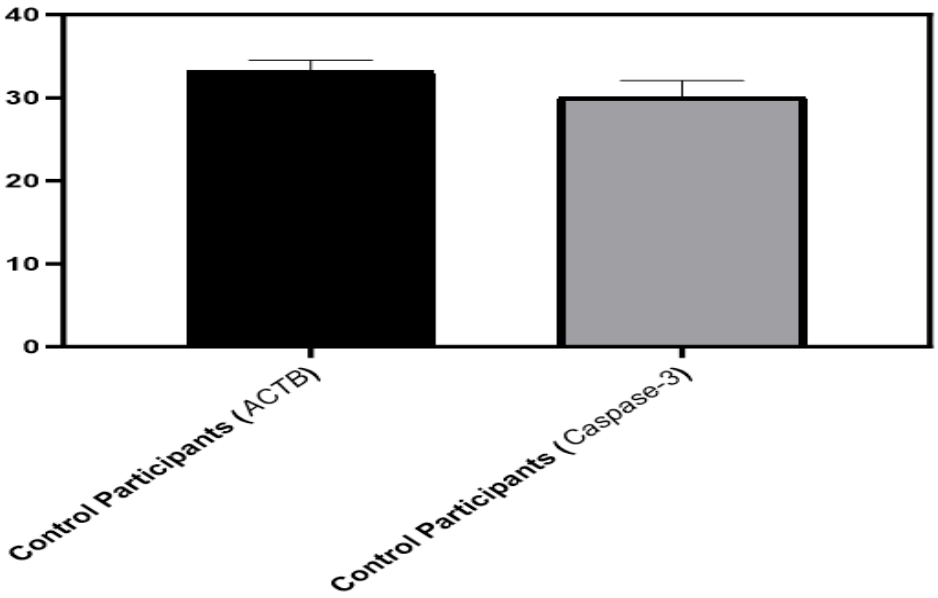


Table 21.

Correlation Between Caspasse-3 and Housekeeping Gene Among Patients

ACTB (Patients) vs. *Caspase-3* (Patients)	
Spearman r	
r	0.01067
95% confidence interval	-0.2854 to 0.3049
P value	
P (two-tailed)	0.9433
P value summary	ns
Exact or approximate P value	Approximate
Significant (alpha = 0.05)	No
Number	47

Figure 10

Correlation Analysis: Housekeeping Gene vs. Caspase-3 Expression in Patients

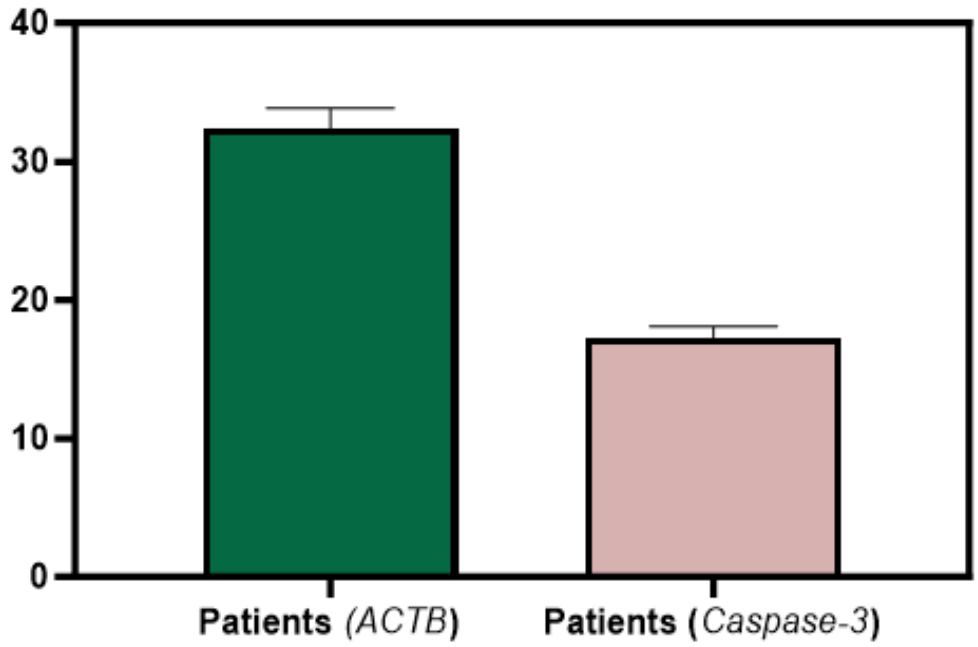


Table 22.

Average Ct Values

	Housekeeping (Control)	*Caspase-3* Control	Housekeeping (VVs Patients)	*Caspase-3* Patients
N	42	42	47	47
Median	33.24	30.26	32.27	17.21
Mean	33.22	30.12	32.38	17.32
Std. Deviation	1.303	1.949	1.466	0.7900

Figure 11

Compares the Expression Levels of ACTB and Caspase-3 Between Control Participants and Patients

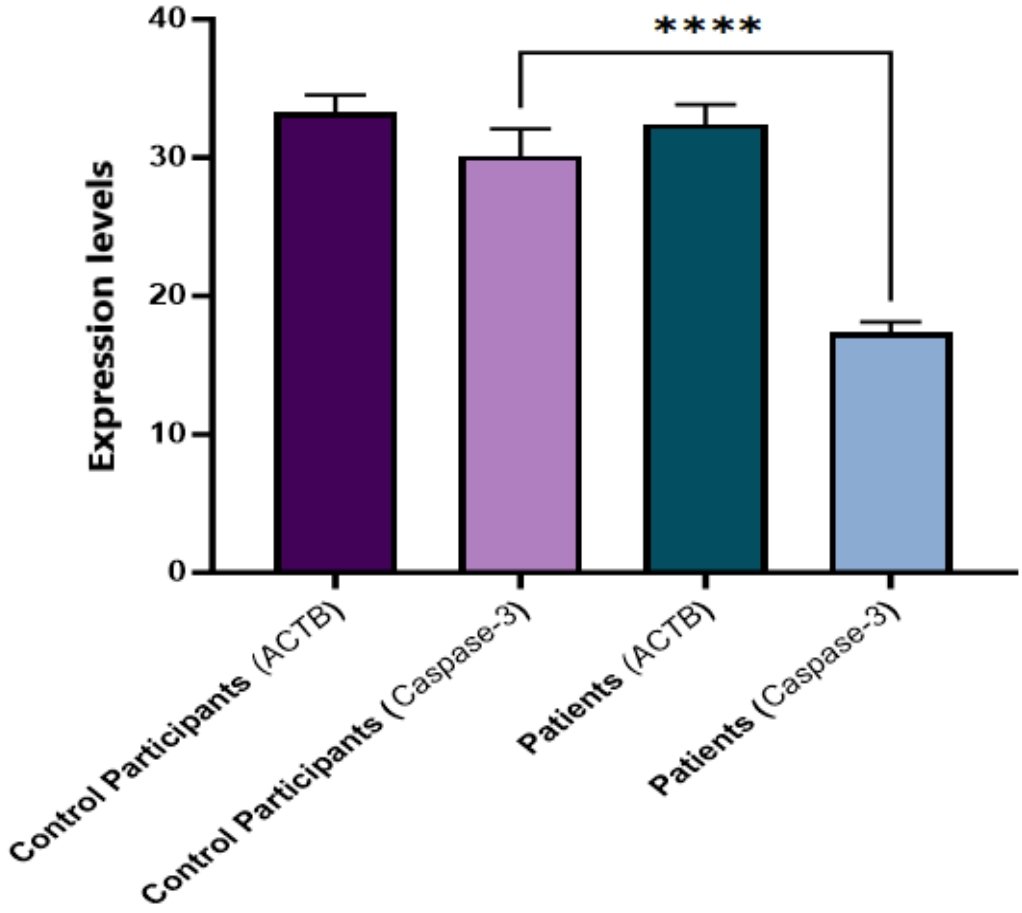


Table 23 illustrates a noteworthy elevation in caspase-3 expression among individuals with varicose veins compared to those without the condition, accompanied by graphical representations in Figures 12 and 13. In terms of central tendencies, both the median and mean values reveal a substantial increase in caspase-3 expression in patients (median: 3407, mean: 7424) as opposed to controls (median: 0.8154, mean: 2.871). Patients demonstrate significant data variability (standard deviation: 8746), contrasting with controls (standard deviation: 4.554). Confidence intervals ensure precision in estimating the median and mean. Notably, controls exhibit a higher coefficient of variation (158.6%) than patients (117.8%). This summary provides crucial insights into the differential expression and characteristics of caspase-3 in both groups.

Table 23.

Fold Change in Expression of Caspase-3

Fold Change Data	Control	Patient
Number of values	42	47
Median	0.8154	3407
Mean	2.871	7424
Std. Deviation	4.554	8746
Coefficient of variation	158.6%	117.8%

Figure 12

Fold Change in Expression of Caspase-3

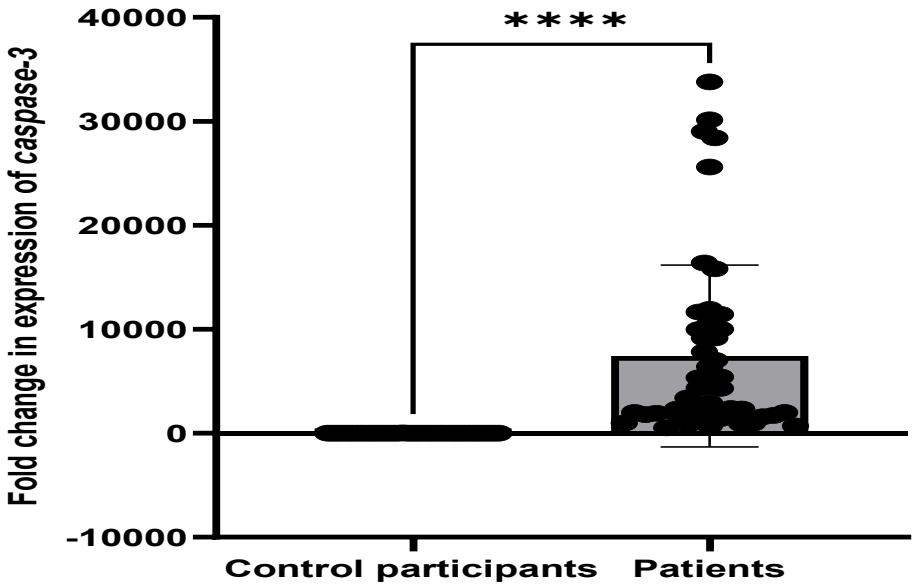
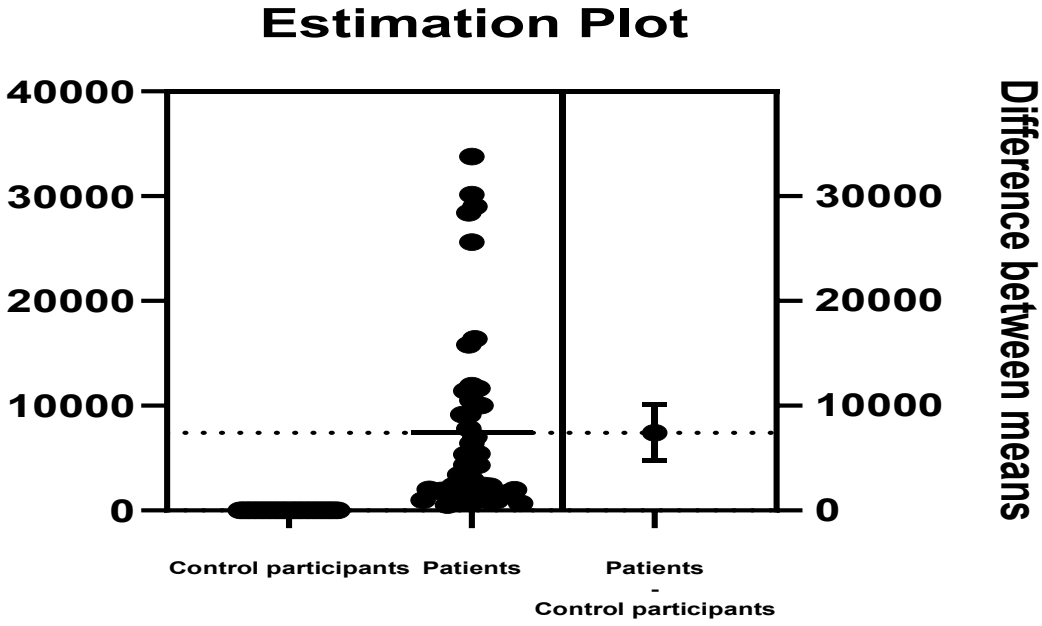


Figure 13

Difference Between Means



Results Interpretation

The qPCR analysis of the gene expression patterns linked to the intrinsic apoptotic pathway—specifically, *cytochrome-c* and *caspase-3*—in varicose veins and normal veins showed significant differences. Varicose veins exhibited substantial alterations in genetic expression levels, reflecting a marked tendency towards the initiation of apoptosis in contrast to healthy veins.

Varicose veins showed a substantial increase in the expression of the *cytochrome-c* gene at the genetic level compared to normal veins p-value <0.0001. This indicates a higher likelihood of initiating apoptosis (Figure 6). The genetic overexpression that has been seen in varicose veins raises the possibility of mitochondrial function that are associated with the initiation of apoptosis.

Meanwhile, varicose veins exhibited a notable upregulation of *caspase-3* gene expression in comparison to normal veins, p-value <0.0001, providing further evidence of the initiation of subsequent apoptotic pathways (Figure 11).

In addition to the genetic results, the investigation uncovered similar changes in the patterns of gene expression in varicose veins. Thus, pro-apoptotic inclinations will be reinforced by increased cytochrome-c and caspase-3 protein production in varicose veins, which will occur in tandem with the genetic upregulation, which strengthens the tendency towards programmed cell death.

These results indicate that the genes responsible for the intrinsic apoptotic pathway in varicose veins have an altered expression profile in comparison with normal veins. This suggests that there is a tendency for apoptosis (cell death) to occur more often in varicose vein tissue.

CHAPTER IV
Discussion

Varicose veins represent a complex vascular condition with multiple proposed etiological factors. When exploring the intricacies of varicose veins, various theories have emerged, attributing the condition to factors such as abnormal connective tissue, smooth muscle dysfunction, endothelial incompetence, valve abnormalities, and increased blood flow stress (Badier-Commander et al., 2001).

Despite various theories, the exact pathophysiological cause of varicose veins remains debated. Genetic predispositions, valve abnormalities, and blood vessel wall deterioration are believed to be associated with this condition. It is hypothesized that an excessive production of pro-apoptotic proteins is implicated. Hence, this study explores a less-explored facet of varicose vein development, focusing on the role of apoptosis-related proteins in the context of the turnover of cells within normal veins and varicosities.

The findings significantly contribute to the existing literature on gene expression in varicose veins, aligning with previous studies while introducing unique insights. Importantly, this study stands out by specifically investigating the gene profile of *cytochrome-c*, addressing a notable gap in the current literature. While the methodologies align with the overarching goals of existing studies, the nuances in this approach emphasise the need for a diverse array of research strategies in the exploration of varicose vein pathophysiology.

Contrary to conclusions drawn by Ascher et al. (2000), who determined, in accordance with their data, that programmed cell death is suppressed in varicose veins, this study yields contrasting results. Significant differences in the approach and groups of people studied might help explain the observed differences or similarities with previous research.

The statistical analysis of the expression levels of *cytochrome-c* and *caspase-3* in the study cohort of 47 varicose vein patients and 42 healthy controls yielded compelling insights into the molecular underpinnings of this vascular condition. A p-value ($P < 0.05$)

demonstrates statistical significance with p-values <0.0001 in both studied genes, indicating significantly different gene expression and fold change.

For *cytochrome-c*, a substantial upregulation in expression was observed in individuals with varicose veins compared to healthy controls. The mean expression level in varicose vein patients was 16.91 in average Ct values (Table 18), and a higher mean was found to be 11584 in fold change (Table 19). In contrast, the healthy control group exhibited a significantly higher mean expression of 30.61 in average Ct values (Table 18), and the lower mean fold change was found to be 2.305 (Table 19). The robustness of these findings is underscored by a p-value of < 0.0001 (Figures 6 and 7), suggesting a potential involvement of *cytochrome-c* in the pathogenesis of varicose veins.

Similarly, the analysis of *caspase-3* expression revealed a significant discrepancy between varicose veins and healthy controls. The mean expression level in varicose vein patients was 17.32 in average Ct values (Table 22) and 7424 in fold change (Table 23), notably lower average Ct values than the mean expression level in healthy controls, which stood at 30.12 in average Ct values (Table 22). While the mean expression level in healthy controls in fold change was 2.871, it was markedly lower than the mean expression level in patients (Table 23). The associated p-value of < 0.0001 (Figures 11 and 12) emphasises the statistical significance of this disparity, hinting at the potential contribution of *caspase-3* to the molecular mechanisms underlying varicose veins.

Given the pivotal role of activated *caspase-3* in initiating apoptotic processes, the activation process is crucially regulated for the purpose of preventing unwanted cell death. These results unequivocally demonstrate a significant elevation in *caspase-3* expression in patients with varicose veins compared to veins obtained from control participants. Filis et al. (2011) reported heightened apoptotic expression in varicose veins as opposed to normal veins, using immunohistochemical analysis. The outcomes of Leci-Tahiri et al. (2016), which were obtained utilising various histological analyses, are consistent with these current findings. They reported substantial variations in the expression of *caspase-3* across the participants in the research (patients vs. control groups).

Using TUNEL to measure cell death, Buján et al. (2000) found that there had been a five-fold rise in cell death in the veins that were varicose when compared to the

control. The changes in the abundances of *cytochrome-c* and *caspase-3* expression in the GSVs of individuals with varicose veins, compared with healthy GSVs, highlight the possible role of apoptosis in triggering the formation of varicose veins. Nevertheless, it is necessary to definitively identify apoptotic dysregulation as the primary causal component of the illness. These discoveries provide a valuable understanding of the molecular pathways that cause venous insufficiency. Yet Leu (1980) and Porto et al. (1995) reported similar observations.

This investigation into the expression profiles of *cytochrome-c* and *caspase-3* in varicose vein patients and healthy controls has revealed significant disparities in gene regulation.

In conclusion, these findings collectively suggest a plausible association between aberrant *cytochrome-c* and *caspase-3* expression and the development of varicose veins. The observed disparities in gene regulation, supported by p-values < 0.0001, suggest a potential association between these proteins and varicose vein development. The statistical robustness of these results enhances the credibility of these associations, inviting further investigation into the mechanistic roles of *cytochrome-c* and *caspase-3* in varicose vein pathophysiology. This newfound understanding may catalyse advancements in diagnostic approaches and therapeutic interventions, ultimately benefiting individuals affected by varicose veins. Future research endeavours should delve into the clinical relevance of these findings and explore the potential utility of altered gene expression patterns as biomarkers for diagnosis and prognosis in varicose vein patients.

CHAPTER IV
Recommendation

Recommendation Based on the Results

The research has yielded a significant understanding of the expression profile of apoptotic proteins in patients with varicose veins. Elevated expression levels of *caspase-3* and *cytochrome-c* suggest a potential role for the apoptotic pathway in the pathophysiology of this vascular disease. These results are important for both clinical practice and continuing research efforts.

While the existing study demonstrates a strong correlation between the housekeeping gene, *cytochrome-c*, and *caspase-3*, it is important to highlight that these proteins have biological significance in relation to varicose veins. Further research is required to thoroughly examine the association between *cytochrome-c* and *caspase-3* expression and the advancement of varicose veins.

The correlational assessment in this study yielded outcomes that were statistically significant. When combined with later studies, these discoveries possess the power to provide novel perspectives in the realm of vascular biology research. The findings from this research may provide similarities or add to a more comprehensive understanding of apoptosis in relation to coronary artery disease. Given the involvement of the apoptotic route in varicose veins, it is plausible that comparable pathways are also implicated in other vascular conditions, such as those necessitating bypass surgery.

The discovery of increased apoptotic expression in varicose veins in individuals following bypass surgery for cardiac repair has significant implications for cardiovascular therapies. Gaining a comprehensive understanding of the molecular complexities of varicose veins is crucial, since it may have a substantial impact on the effectiveness of bypass procedures. The observed rise in apoptosis implies a possible link between vascular health and cardiac outcomes, leading medical practitioners to take into account the state of the lower limb veins when planning cardiovascular therapies.

Providing patients with information and knowledge is crucial in the process of making decisions. Therefore, it is essential for surgeons to have thorough conversations with patients, elucidating the importance of varicose veins and heightened apoptosis in

the context of bypass surgery. Offering insights on the possible influence of varicose veins on the success of grafts.

A meticulous preoperative assessment of lower limb veins should be integral to the surgical planning process for bypass surgery. This entails not only the identification of varicose veins but also the assessment of the genetic and protein expression patterns. Given these discoveries, it is strongly advised to carefully deliberate on the choice of veins during the planning phase of bypass surgery. To get optimal surgical results, surgeons should choose veins with normal gene expression patterns, especially those that are located farther away from varicose veins. The objective of this strategic approach is to reduce any difficulties related to apoptosis by selecting vessels that have a more advantageous molecular profile along with optimal functional integrity. This proposal is in line with the overarching objective of maximising patient outcomes and promoting the progress of precision medicine in cardiovascular surgery.

The observed associations between the expression of apoptotic proteins and the degree of varicosity highlight the importance of comprehending apoptosis in the context of varicose veins from a therapeutic perspective. This association implies that apoptotic markers might be used as indications of the severity of an illness, which could be useful in clinical evaluations. Moreover, the theory proposes that the liberation of cytochrome-c and the subsequent initiation of the caspase-3 cascade align with the observed heightened expression levels in patients as compared to control persons. This not only confirms the original concept but also emphasises the possible mechanistic function of these apoptotic proteins in the pathogenesis of varicose veins.

To sum up, this work not only enhances the comprehension of the pathophysiology of varicose veins but also sets the stage for future research focused on uncovering the complex molecular pathways that cause this vascular illness.

Potential Avenues for Further Exploration

In order to expand on these discoveries, future studies should give priority to thorough investigations into the wider apoptotic pathway. An in-depth investigation of supplementary apoptotic markers, their interactions, and subsequent consequences might

provide a more intricate comprehension of the molecular processes that contribute to the development of varicose veins. Subsequent inquiries might investigate the involvement of apoptosis in the genesis and advancement of coronary artery disease, perhaps resulting in advances in surgical and medicinal therapies.

Furthermore, expanding the research to include a broader and more heterogeneous patient cohort might augment the applicability of the findings. Longitudinal studies with repeated assessments may effectively capture the dynamic changes in apoptotic protein expression over time, providing a more thorough understanding of the temporal elements of varicose vein formation. Moreover, it is essential to collaborate with physicians and specialists in vascular medicine in order to effectively use these molecular discoveries in real-world clinical settings. Creating focused therapies based on the discovered apoptotic targets may provide new opportunities to manipulate these pathways in order to prevent or alleviate vascular damage and enhance outcomes for people with varicose veins.

Conclusion

In this comprehensive investigation, researchers delved into the intricate molecular landscape associated with varicose veins by scrutinizing the expression patterns of two pivotal proteins: cytochrome-c and caspase-3. A cohort comprising 47 patients afflicted with varicose veins was meticulously compared with a demographically matched healthy control group consisting of 42 individuals. The focal point of this study lay in elucidating the expression of *cytochrome-c* and *caspase-3* in varicose and normal veins.

The statistical analyses of these experimental results revealed a striking and statistically significant distinction in the expression levels of *cytochrome-c* and *caspase-3* between the varicose veins patient group and the healthy control cohort. Notably, the p-values obtained for both genes were found to be less than 0.0001, underscoring the robustness and reliability of these observations. This statistical significance reinforces the putative involvement of these proteins in the molecular mechanisms underlying varicose veins, paving the way for further exploration into their roles as potential biomarkers or therapeutic targets.

REFERENCES

Ahmed, W. U. R., Wiberg, A., Ng, M., Wang, W., Auton, A., 23andMe Research Team, ... & Furniss, D. (2020). Genome-wide association analysis and replication in 810,625 individuals identifies novel therapeutic targets for varicose veins. *BioRxiv*, 2020-05.

Akhmetzianov, R. V., & Bredikhin, R. A. (2021). Clinical Efficacy of Conservative Treatment with Micronized Purified Flavonoid Fraction in Female Patients with Pelvic Congestion Syndrome. *Pain and therapy*, *10*(2), 1567–1578. https://doi.org/10.1007/s40122-021-00312-6

Alegría-Torres, J. A., Baccarelli, A., & Bollati, V. (2011). Epigenetics and lifestyle. *Epigenomics*, *3*(3), 267–277. https://doi.org/10.2217/epi.11.22

Alper, S. L. (2017). Genetic diseases of PIEZO1 and PIEZO2 dysfunction. *Current topics in membranes*, *79*, 97-134.

Amanullah, A., Liebermann, D. A., & Hoffman, B. (2002). Deregulated c-Myc prematurely recruits both Type I and II CD95/Fas apoptotic pathways associated with terminal myeloid differentiation. *Oncogene*, *21*(10), 1600-1610.

American College of Radiology. (2012). ACR Appropriateness Criteria: Radiologic Management of Lower-Extremity Venous Insufficiency. Retrieved from https://acsearch.acr.org/docs/69507/Narrative/

Antonicelli, F., Bellon, G., Debelle, L., & Hornebeck, W. (2007). Elastin-elastases and inflamm-aging. *Current topics in developmental biology*, *79*, 99-155.

Anwar, M. A., Adesina-Georgiadis, K. N., Spagou, K., Vorkas, P. A., Li, J. V., Shalhoub, J., Holmes, E., & Davies, A. H. (2017). A comprehensive characterisation of the metabolic profile of varicose veins; implications in elaborating plausible cellular pathways for disease pathogenesis. *Scientific reports*, *7*(1), 2989. https://doi.org/10.1038/s41598-017-02529-y

Ascher, E., Jacob, T., Hingorani, A., Gunduz, Y., Mazzariol, F., & Kallakuri, S. (2000). Programmed cell death (Apoptosis) and its role in the pathogenesis of lower extremity varicose veins. *Annals of Vascular Surgery*, *14*(1), 24–30. https://doi.org/10.1007/s100169910005

Ashkenazi, A., & Dixit, V. M. (1998). Death receptors: signaling and modulation. *science*, *281*(5381), 1305-1308.

Atta, H. M. (2012). Varicose veins: Role of mechanotransduction of venous hypertension. *International Journal of Vascular Medicine*, *2012*, 1–13. https://doi.org/10.1155/2012/538627

Augstein, A., Mierke, J., Poitz, D. M., & Strasser, R. H. (2018). Sox9 is increased in arterial plaque and stenosis, associated with synthetic phenotype of vascular smooth muscle cells and causes alterations in extracellular matrix and calcification. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1864*(8), 2526-2537.

Badier-Commander, C., Couvelard, A., Henin, D., Verbeuren, T., Michel, J.-B., & Jacob, M.-P. (2001). Smooth muscle cell modulation and cytokine overproduction in varicose veins. Anin situ study. *The Journal of Pathology*, *193*(3), 398–407. https://doi.org/10.1002/path.819

Baeyens, N., Bandyopadhyay, C., Coon, B. G., Yun, S., & Schwartz, M. A. (2016). Endothelial fluid shear stress sensing in vascular health and disease. *Journal of Clinical Investigation*, *126*(3), 821–828. https://doi.org/10.1172/JCI83083

Barallobre-Barreiro, J., Baig, F., Fava, M., Yin, X., & Mayr, M. (2017). Glycoproteomics of the extracellular matrix: a method for intact glycopeptide analysis using mass spectrometry. *JoVE (Journal of Visualized Experiments)*, (122), e55674.

Barallobre-Barreiro, J., Oklu, R., Lynch, M., Fava, M., Baig, F., Yin, X., ... & Mayr, M. (2016). Extracellular matrix remodelling in response to venous hypertension: proteomics of human varicose veins. *Cardiovascular research*, *110*(3), 419-430.

Barton, J. C., Edwards, C. Q., & Acton, R. T. (2015). HFE gene: Structure, function, mutations, and associated iron abnormalities. *Gene*, *574*(2), 179-192.

Barwari, T., Joshi, A., & Mayr, M. (2016). MicroRNAs in Cardiovascular Disease. *Journal of the American College of Cardiology*, *68*(23), 2577–2584. https://doi.org/10.1016/j.jacc.2016.09.945

Beebe-Dimmer, J. L., Pfeifer, J. R., Engle, J. S., & Schottenfeld, D. (2005). The epidemiology of chronic venous insufficiency and varicose veins. *Annals of epidemiology*, *15*(3), 175-184.

Beermann, J., Piccoli, M. T., Viereck, J., & Thum, T. (2016). Non-coding RNAs in Development and Disease: Background, Mechanisms, and Therapeutic Approaches. *Physiological reviews*, *96*(4), 1297–1325. https://doi.org/10.1152/physrev.00041.2015

Bergan, J. (2007). Molecular mechanisms in chronic venous insufficiency. *Annals of Vascular Surgery*, *21*(3), 260–266. https://doi.org/10.1016/j.avsg.2007.03.011

Bergan, J. J., Schmid-Schönbein, G. W., Smith, P. D., Nicolaides, A. N., Boisseau, M. R., & Eklof, B. (2006). Chronic venous disease. *The New England journal of medicine*, *355*(5), 488–498. https://doi.org/10.1056/NEJMra055289

Bergmann, A. (2002). Survival signaling goes BAD. *Developmental cell*, *3*(5), 607-608.

Berna-Erro, A., Jardin, I., Salido, G. M., & Rosado, J. A. (2017). Role of STIM2 in cell function and physiopathology. *The Journal of physiology*, *595*(10), 3111-3128.

Biranvand, A. S., Khosravi, M., Esfandiari, G., Poursaleh, A., Hosseini-Fard, S. R., Amirfarhangi, A., & Najafi, M. (2018). Associations between miR-661, miR-1202, lncRNA-HOTAIR, lncRNA-GAS5 and MMP9 in differentiated M2-macrophages of patients with varicose veins. *International angiology : a journal of the International Union of Angiology*, *37*(6), 451–456. https://doi.org/10.23736/S0392-9590.18.04022-1

Bjørklund, G., Svanberg, E., Dadar, M., Card, D. J., Chirumbolo, S., Harrington, D. J., & Aaseth, J. (2020). The role of matrix Gla protein (MGP) in vascular calcification. *Current Medicinal Chemistry*, *27*(10), 1647-1660.

Boisseau, M. R. (2007). Leukocyte involvement in the signs and symptoms of chronic venous disease. Perspectives for therapy. *Clinical hemorheology and microcirculation*, *37*(3), 277-290.

Boisseau, M. R. (2007). Recent finding in the pathogenesis of venous wall degradation. *Phlebolymphology*, *14*(2), 59-68.

Borghese, O., Pisani, A., & Di Centa, I. (2021). Endovenous radiofrequency for chronic superficial venous insufficiency: Clinical outcomes and impact in quality of life. *Journal de medecine vasculaire*, *46*(1), 3–8. https://doi.org/10.1016/j.jdmv.2020.11.003

Bosc, L. V. G., Wilkerson, M. K., Bradley, K. N., Eckman, D. M., Hill-Eubanks, D. C., & Nelson, M. T. (2004). Intraluminal pressure is a stimulus for NFATc3 nuclear accumulation: role of calcium, endothelium-derived nitric oxide, and cGMP-dependent protein kinase. *Journal of Biological Chemistry*, *279*(11), 10702-10709.

Boström, K., Zebboudj, A. F., Yao, Y., Lin, T. S., & Torres, A. (2004). Matrix GLA protein stimulates VEGF expression through increased transforming growth factor-β1 activity in endothelial cells. *Journal of Biological Chemistry*, *279*(51), 52904-52913.

Boukhalfa, A., Nascimbeni, A. C., Ramel, D., Dupont, N., Hirsch, E., Gayral, S., Laffargue, M., Codogno, P., & Morel, E. (2020). PI3KC2α-dependent and VPS34-independent generation of PI3P controls primary cilium-mediated autophagy in response to shear stress. *Nature communications*, *11*(1), 294. https://doi.org/10.1038/s41467-019-14086-1

Bradbury, A., Evans, C., Allan, P., Lee, A., Ruckley, C. V., & Fowkes, F. G. (1999). What are the symptoms of varicose veins? Edinburgh vein study cross sectional population survey. *BMJ (Clinical research ed.)*, *318*(7180), 353–356. https://doi.org/10.1136/bmj.318.7180.353

Brand, F. N., Dannenberg, A. L., Abbott, R. D., & Kannel, W. B. (1988). The epidemiology of varicose veins: the Framingham Study. *American journal of preventive medicine*, *4*(2), 96-101.

Bratton, S. B., & Salvesen, G. S. (2010). Regulation of the Apaf-1–caspase-9 apoptosome. *Journal of cell science*, *123*(19), 3209-3214.

Brier, L. W., Ge, L., Stjepanovic, G., Thelen, A. M., Hurley, J. H., & Schekman, R. (2019). Regulation of LC3 lipidation by the autophagy-specific class III phosphatidylinositol-3 kinase complex. *Molecular biology of the cell*, *30*(9), 1098–1107. https://doi.org/10.1091/mbc.E18-11-0743

Bruczko-Goralewska, M., Romanowicz, L., Bączyk, J., Wolańska, M., Sobolewski, K., & Kowalewski, R. (2019). Peptide growth factors and their receptors in the vein wall. *Journal of Investigative Medicine*, *67*(8), 1149-1154.

Buján, J., Jimenez-Cossio, J. A., Jurado, F., Gimeno, M. J., Pascual, G., Garcia-

Honduvilla, N., ... & Bellon, J. M. (2000). Evaluation of the smooth muscle cell component and apoptosis in the varicose vein wall. *Histology and Histopathology*, *15*(3), 745-752.

Buján, J., Pascual, G., & Bellón, J. M. (2008). Interaction between ageing, inflammation process, and the occurence of varicose veins. *Phlebolymphology*, *15*(4), 123-130.

Bush, R., Comerota, A., Meissner, M., Raffetto, J. D., Hahn, S. R., & Freeman, K. (2017). Recommendations for the medical management of chronic venous disease: the role of micronized purified flavanoid fraction (MPFF) recommendations from the Working Group in Chronic Venous Disease (CVD) 2016. *Phlebology*, *32*(1_suppl), 3-19.

Cadwell, K., Liu, J. Y., Brown, S. L., Miyoshi, H., Loh, J., Lennerz, J. K., Kishi, C., Kc, W., Carrero, J. A., Hunt, S., Stone, C. D., Brunt, E. M., Xavier, R. J., Sleckman, B. P., Li, E., Mizushima, N., Stappenbeck, T. S., & Virgin, H. W., 4th (2008). A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature*, *456*(7219), 259–263. https://doi.org/10.1038/nature07416

Caggiati, A., Rosi, C., Casini, A., Cirenza, M., Petrozza, V., Acconcia, M. C., & Zamboni, P. (2010). Skin iron deposition characterises lipodermatosclerosis and leg ulcer. *European Journal of Vascular and Endovascular Surgery*, *40*(6), 777-782.

Campbell, W. B., Niblett, P. G., Peters, A. S., MacIntyre, J. B., Sherriff, S., Palfreyman, S., Michaels, J. A., & REACTIV (Randomised and Economic Analysis of Conservative Treatment or Interventions for Varicose veins) Study Participants (2005). The clinical effectiveness of hand held Doppler examination for diagnosis of reflux in patients with varicose veins. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*, *30*(6), 664–669. https://doi.org/10.1016/j.ejvs.2005.05.037

Cario-Toumaniantz, C., Boularan, C., Schurgers, L. J., Heymann, M. F., Le Cunff, M., Léger, J., ... & Pacaud, P. (2007). Identification of differentially expressed genes in human varicose veins: involvement of matrix gla protein in extracellular matrix remodeling. *Journal of vascular research*, *44*(6), 444-459.

Cario-Toumaniantz, C., Evellin, S., Maury, S., Baron, O., Pacaud, P., & Loirand, G. (2002). Role of Rho kinase signalling in healthy and varicose human saphenous veins. *British journal of pharmacology*, *137*(2), 205.

Carman, T. L., & Al-Omari, A. (2019). Evaluation and management of chronic venous disease using the foundation of CEAP. *Current Cardiology Reports*, *21*, 1-8.

Carrasco, O. F., Ranero, A., Hong, E., & Vidrio, H. (2009). Endothelial function impairment in chronic venous insufficiency: effect of some cardiovascular protectant agents. *Angiology*, *60*(6), 763-771.

Castro-Ferreira, R., Cardoso, R., Leite-Moreira, A., & Mansilha, A. (2018). The role of endothelial dysfunction and inflammation in chronic venous disease. *Annals of vascular surgery*, *46*, 380-393.

Cavezzi, A. (2020). Medicine and phlebolymphology: Time to change? *Journal of Clinical Medicine*, *9*(12), 4091. https://doi.org/10.3390/jcm9124091

Cavezzi, A., Frullini, A., Ricci, S., & Tessari, L. (2002). Treatment of varicose veins by foam sclerotherapy: two clinical series. *Phlebology*, *17*(1), 13-18.

Chao, D. T., & Korsmeyer, S. J. (1998). BCL-2 family: regulators of cell death. *Annual review of immunology*, *16*(1), 395-419.

Charpentier, M. S., Christine, K. S., Amin, N. M., Dorr, K. M., Kushner, E. J., Bautch, V. L., ... & Conlon, F. L. (2013). CASZ1 promotes vascular assembly and morphogenesis through the direct regulation of an EGFL7/RhoA-mediated pathway. *Developmental cell*, *25*(2), 132-143.

Chen, S. S., & Prasad, S. K. (2009). Long saphenous vein and its anatomical variations. *Australasian Journal of Ultrasound in Medicine*, *12*(1), 28–31. https://doi.org/10.1002/j.2205-0140.2009.tb00004.x

Chen, Y., Peng, W., Raffetto, J. D., & Khalil, R. A. (2017). Matrix metalloproteinases in remodeling of lower extremity veins and chronic venous disease. *Progress in molecular biology and translational science*, *147*, 267-299.

Cheng, T. C., Hong, C., Akey, I. V., Yuan, S., & Akey, C. W. (2016). A near atomic structure of the active human apoptosome. *Elife*, *5*, e17755.

Chu, H. B., Yan, F., Zhao, J. H., Xu, Y. B., Wang, T., & Guo, W. J. (2013). Assessment of the infiltration of inflammatory cells in the walls of thrombotic varicose veins. *Angiology*, *64*(1), 69-72.

Coleridge Smith, P. D. (2001). Update on chronic-venous-insufficiency-induced inflammatory processes. *Angiology*, *52*(1_suppl), S35–S42. https://doi.org/10.1177/0003319701052001S05

Condrat, C. E., Thompson, D. C., Barbu, M. G., Bugnar, O. L., Boboc, A., Cretoiu, D., Suciu, N., Cretoiu, S. M., & Voinea, S. C. (2020). miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells*, *9*(2), 276. https://doi.org/10.3390/cells9020276

Cornu-Thenard, A., & Boivin, P. (2014). Chronic venous disease during pregnancy. *Phlebolymphology*, *21*(3), 138-145.

Cristóvão Porto, L., da Silveira, P. R. M., de Carvalho, J. J., & Brandao Panico, M. D. (1995). Connective tissue accumulation in the muscle layer in normal and varicose saphenous veins. *Angiology*, *46*(3), 243-249.

Cui, C., Liu, G., Huang, Y., Lu, X., Lu, M., Huang, X., Li, W., & Jiang, M. (2012). MicroRNA profiling in great saphenous vein tissues of patients with chronic venous insufficiency. *The Tohoku journal of experimental medicine*, *228*(4), 341–350. https://doi.org/10.1620/tjem.228.341

Danziger, N. (2007). Pathophysiology of pain in venous disease. *Journal des Maladies Vasculaires*, *32*(1), 1.

Davies, A. H. (2019). The seriousness of chronic venous disease: a review of real-world evidence. *Advances in therapy*, *36*(Suppl 1), 5-12.

Davies, P. F. (2009). Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nature clinical practice Cardiovascular medicine*, *6*(1), 16-26.

Derecka, M., Herman, J. S., Cauchy, P., Ramamoorthy, S., Lupar, E., Grün, D., & Grosschedl, R. (2020). EBF1-deficient bone marrow stroma elicits persistent changes in HSC potential. *Nature immunology*, *21*(3), 261-273.

Dorland, Y. L., & Huveneers, S. (2017). Cell–cell junctional mechanotransduction in endothelial remodeling. *Cellular and Molecular Life Sciences*, *74*, 279-292.

Douguet, D., Patel, A., Xu, A., Vanhoutte, P. M., & Honoré, E. (2019). Piezo ion channels in cardiovascular mechanobiology. *Trends in pharmacological sciences*, *40*(12), 956-970.

Dunn, J., Simmons, R., Thabet, S., & Jo, H. (2015). The role of epigenetics in the endothelial cell shear stress response and atherosclerosis. *The international journal of biochemistry & cell biology*, *67*, 167-176.

Eberhardt, R. T., & Raffetto, J. D. (2014). Chronic venous insufficiency. *Circulation*, *130*(4), 333–346. https://doi.org/10.1161/CIRCULATIONAHA.113.006898

Edwards, J. R., Yarychkivska, O., Boulard, M., & Bestor, T. H. (2017). DNA methylation and DNA methyltransferases. *Epigenetics & chromatin*, *10*(1), 1-10.

Eklof, B., Perrin, M., Delis, K. T., Rutherford, R. B., & Gloviczki, P. (2009). Updated terminology of chronic venous disorders: the VEIN-TERM transatlantic interdisciplinary consensus document. *Journal of vascular surgery*, *49*(2), 498-501.

Ellinghaus, E., Ellinghaus, D., Krusche, P., Greiner, A., Schreiber, C., Nikolaus, S., ... & Franke, A. (2017). Genome-wide association analysis for chronic venous disease identifies EFEMP1 and KCNH8 as susceptibility loci. *Scientific Reports*, *7*(1), 45652.

Elmore, S. (2007). Apoptosis: a review of programmed cell death. *Toxicologic pathology*, *35*(4), 495-516.

Elrod, H. A., & Sun, S. Y. (2008). Modulation of death receptors by cancer therapeutic agents. *Cancer biology & therapy*, *7*(2), 163-173.

Enari, M., Sakahira, H., Yokoyama, H., Okawa, K., Iwamatsu, A., & Nagata, S. (1998). A caspase-activated DNase that degrades DNA during apoptosis, and its inhibitor ICAD. *Nature*, *391*(6662), 43-50.

Engin, M., & Göncü, M.T. (2020). The role of plateletcrit and neutrophil lymphocyte ratio in showing the clinical severity of the disease in patients with chronic venous insufficiency. *Annals of Medical Research*.

Epstein, D., Bootun, R., Diop, M., Ortega-Ortega, M., Lane, T. R. A., & Davies, A. H. (2022). Cost-effectiveness analysis of current varicose veins treatments. *Journal*

of vascular surgery. Venous and lymphatic disorders, *10*(2), 504–513.e7. https://doi.org/10.1016/j.jvsv.2021.05.014

Farah, M. H., Nayfeh, T., Urtecho, M., Hasan, B., Amin, M., Sen, I., ... & Murad, M. H. (2022). A systematic review supporting the Society for Vascular Surgery, the American Venous Forum, and the American Vein and Lymphatic Society guidelines on the management of varicose veins. *Journal of vascular surgery: venous and lymphatic disorders*, *10*(5), 1155-1171.

Faringthon Reyes, L. O., & Sosa Veras, O. A. (2019). Chronic venous insufficiency and structural changes in the walls of the veins. *Synergy Journal of Medicine*, *4*(2), 3-20. https://doi.org/10.31434/rms.v4i2.172

Feldo, M., Woźniak, M., Wójciak-Kosior, M., Sowa, I., Kot-Waśik, A., Aszyk, J., ... & Bogucka-Kocka, A. (2018). Influence of diosmin treatment on the level of oxidative stress markers in patients with chronic venous insufficiency. *Oxidative medicine and cellular longevity*, *2018*.

Ferris, A. E., & Harding, K. G. (2019). An overview of the relationship between anaemia, iron, and venous leg ulcers. *International Wound Journal*, *16*(6), 1323-1329.

Ferris, A. E., & Harding, K. G. (2020). Does localized iron loss in venous disease lead to systemic iron deficiency? A descriptive pilot study. *Wound Repair and Regeneration*, *28*(1), 33-38.

Filis, K., Kavantzas, N., Isopoulos, T., Antonakis, P., Sigalas, P., Vavouranakis, E., & Sigala, F. (2011). Increased vein wall apoptosis in varicose vein disease is related to venous hypertension. *European Journal of Vascular and Endovascular Surgery*, *41*(4), 533–539. https://doi.org/10.1016/j.ejvs.2010.11.033

Fukaya, E., Flores, A. M., Lindholm, D., Gustafsson, S., Zanetti, D., Ingelsson, E., & Leeper, N. J. (2018). Clinical and genetic determinants of varicose veins: prospective, community-based study of≈ 500 000 individuals. *Circulation*, *138*(25), 2869-2880.

Gaenzer, H., Marschang, P., Sturm, W., Neumayr, G. Ü., Vogel, W., Patsch, J., & Weiss, G. Ü. (2002). Association between increased iron stores and impaired endothelial

function in patients with hereditary hemochromatosis. *Journal of the American College of Cardiology*, *40*(12), 2189-2194.

Gagliani, N., & Huber, S. (2017). Basic aspects of T helper cell differentiation. *T-cell differentiation: methods and protocols*, 19-30.

Galluzzi, L., & Green, D. R. (2019). Autophagy-Independent Functions of the Autophagy Machinery. *Cell*, *177*(7), 1682–1699. https://doi.org/10.1016/j.cell.2019.05.026

Galluzzi, L., Kepp, O., Trojel-Hansen, C., & Kroemer, G. (2012). Mitochondrial control of cellular life, stress, and death. *Circulation research*, *111*(9), 1198-1207.

Galluzzi, L., Vitale, I., Abrams, J. M., Alnemri, E. S., Baehrecke, E. H., Blagosklonny, M. V., ... & Kroemer, G. (2012). Molecular definitions of cell death subroutines: recommendations of the Nomenclature Committee on Cell Death 2012. *Cell Death & Differentiation*, *19*(1), 107-120.

Gloviczki, P., Comerota, A. J., Dalsing, M. C., Eklof, B. G., Gillespie, D. L., Gloviczki, M. L., Lohr, J. M., McLafferty, R. B., Meissner, M. H., Murad, M. H., Padberg, F. T., Pappas, P. J., Passman, M. A., Raffetto, J. D., Vasquez, M. A., Wakefield, T. W., Society for Vascular Surgery, & American Venous Forum (2011). The care of patients with varicose veins and associated chronic venous diseases: clinical practice guidelines of the Society for Vascular Surgery and the American Venous Forum. *Journal of vascular surgery*, *53*(5 Suppl), 2S–48S. https://doi.org/10.1016/j.jvs.2011.01.079

Gohel, M. S., & Davies, A. H. (2009). Pharmacological agents in the treatment of venous disease: an update of the available evidence. *Current vascular pharmacology*, *7*(3), 303–308. https://doi.org/10.2174/157016109788340758

González Cañas, E., Florit López, S., Vilagut, R. V., Guevara-Noriega, K. A., Santos Espí, M., Rios, J., Soto, S. N., & Giménez Gaibar, A. (2021). A randomized controlled noninferiority trial comparing radiofrequency with stripping and conservative hemodynamic cure for venous insufficiency technique for insufficiency of the great saphenous vein. *Journal of vascular surgery. Venous and lymphatic disorders*, *9*(1), 101–112. https://doi.org/10.1016/j.jvsv.2020.04.019

GORDON, M., & PAYNE, R. D. (1953). A controlled technique for vein stripping. *California medicine*, *79*(6), 425–427.

Gortat, A., Sancho, M., Mondragón, L., Messeguer, À., Pérez-Payá, E., & Orzáez, M. (2015). Apaf1 inhibition promotes cell recovery from apoptosis. *Protein & cell*, *6*(11), 833-843.

Graef, I. A., Chen, F., Chen, L., Kuo, A., & Crabtree, G. R. (2001). Signals transduced by Ca2+/calcineurin and NFATc3/c4 pattern the developing vasculature. *Cell*, *105*(7), 863-875.

Grosse, S. D., Nelson, R. E., Nyarko, K. A., Richardson, L. C., & Raskob, G. E. (2016). The economic burden of incident venous thromboembolism in the United States: A review of estimated attributable healthcare costs. *Thrombosis Research*, *137*, 3–10. https://doi.org/10.1016/j.thromres.2015.11.033

Grudzińska, E., & Czuba, Z. P. (2014). Immunological aspects of chronic venous disease pathogenesis. *Central European Journal of Immunology*, *39*(4), 525-531.

Grudzińska, E., Lekstan, A., Szliszka, E., & Czuba, Z. P. (2018). Cytokines produced by lymphocytes in the incompetent great saphenous vein. *Mediators of Inflammation*, *2018*, 1–8. https://doi.org/10.1155/2018/7161346

Guss, L. G., Javvaji, S., Case, J., Bs, B. B., Schaefer, K. N., Bs, R. G., ... & Housman, L. B. (2018). Differences in Inflammatory Cytokine Levels between Patients with Varying Severity of Chronic Venous Insufficiency. *J. Vasc. Med. Surg*, *6*, 1-6.

Hanley, K. P., Oakley, F., Sugden, S., Wilson, D. I., Mann, D. A., & Hanley, N. A. (2008). Ectopic SOX9 mediates extracellular matrix deposition characteristic of organ fibrosis. *Journal of Biological Chemistry*, *283*(20), 14063-14071.

Harada, H., Becknell, B., Wilm, M., Mann, M., Huang, L. J. S., Taylor, S. S., ... & Korsmeyer, S. J. (1999). Phosphorylation and inactivation of BAD by mitochondria-anchored protein kinase A. *Molecular cell*, *3*(4), 413-422.

Hardwick, J. M., & Soane, L. (2013). Multiple functions of BCL-2 family proteins. *Cold Spring Harbor perspectives in biology*, *5*(2), a008722.

Harraz, O. F., & Jensen, L. J. (2020). Aging, calcium channel signaling and vascular tone. *Mechanisms of ageing and development*, *191*, 111336.

Haupt, S., Berger, M., Goldberg, Z., & Haupt, Y. (2003). Apoptosis-the p53 network. *Journal of cell science*, *116*(20), 4077-4085.

Haviarová, Z., Weismann, P., Pavlíková, D., Durdík, Š., Kováč, P., Štvrtinová, V., & Mráz, P. (2002). Mast cell infiltration in the wall of varicose veins. *acta histochemica*, *104*(4), 357-360.

Hengartner, M. O. (2000). The biochemistry of apoptosis. *Nature*, *407*(6805), 770-776.

Horecka, A., Hordyjewska, A., Biernacka, J., Dąbrowski, W., Zubilewicz, T., Malec, A., ... & Kurzepa, J. (2021). Intense remodeling of extracellular matrix within the varicose vein: the role of gelatinases and vascular endothelial growth factor. *Irish Journal of Medical Science (1971-)*, *190*, 255-259.

Howlader, M. H., & Smith, P. D. C. (2003). Symptoms of chronic venous disease and association with systemic inflammatory markers. *Journal of vascular surgery*, *38*(5), 950-954.

Huveneers, S., Oldenburg, J., Spanjaard, E., van der Krogt, G., Grigoriev, I., Akhmanova, A., ... & de Rooij, J. (2012). Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. *Journal of Cell Biology*, *196*(5), 641-652.

Hyder, O. N., & Soukas, P. A. (2017). Chronic Venous Insufficiency: Novel Management Strategies for an Under-diagnosed Disease Process. *Rhode Island medical journal (2013)*, *100*(5), 37–39.

Imanaka-Yoshida, K., Yoshida, T., & Miyagawa-Tomita, S. (2014). Tenascin-C in development and disease of blood vessels. *The Anatomical Record*, *297*(9), 1747-1757.

Jacobs, B. N., Andraska, E. A., Obi, A. T., & Wakefield, T. W. (2017). *Pathophysiology of varicose veins. Journal of Vascular Surgery: Venous and Lymphatic Disorders, 5(3), 460–467.* doi:10.1016/j.jvsv.2016.12.014

Jaminon, A. M., Dai, L., Qureshi, A. R., Evenepoel, P., Ripsweden, J., Söderberg, M., ... & Stenvinkel, P. (2020). Matrix Gla protein is an independent predictor of both intimal and medial vascular calcification in chronic kidney disease. *Scientific reports*, *10*(1), 6586.

Jaworucka-Kaczorowska, A., Oszkinis, G., Huber, J., Wiertel-Krawczuk, A., Gabor, E., & Kaczorowski, P. (2015). Saphenous vein stripping surgical technique and frequency of saphenous nerve injury. *Phlebology*, *30*(3), 210–216. https://doi.org/10.1177/0268355514539316

Jiang, H., Lun, Y., Wu, X., Xia, Q., Zhang, X., Xin, S., & Zhang, J. (2014). Association between the hypomethylation of osteopontin and integrin β3 promoters and vascular smooth muscle cell phenotype switching in great saphenous varicose veins. *International Journal of Molecular Sciences*, *15*(10), 18747-18761.

Jin, Y., Xu, G., Huang, J., Zhou, D., Huang, X., & Shen, L. (2013). Analysis of the association between an insertion/deletion polymorphism within the 3′ untranslated region of COL1A2 and chronic venous insufficiency. *Annals of vascular surgery*, *27*(7), 959-963.

Jones, G. T., Marsman, J., Pardo, L. M., Nijsten, T., De Maeseneer, M., Phillips, V., ... & van Rij, A. M. (2019). A variant of the castor zinc finger 1 (CASZ1) gene is differentially associated with the clinical classification of chronic venous disease. *Scientific reports*, *9*(1), 14011.

Jones, W. S., Vemulapalli, S., Parikh, K. S., Coeytaux, R. R., Crowley, M. J., Raitz, G., ... & Sanders-Schmidler, G. D. (2018). Treatment Strategies for Patients with Lower Extremity Chronic Venous Disease (LECVD).

Kabnick, L. S., & Ombrellino, M. (2005). Ambulatory phlebectomy. *Seminars in interventional radiology*, *22*(3), 218–224. https://doi.org/10.1055/s-2005-921955

Kachlík, D., Lametschwandtner, A., Rejmontová, J., Stingl, J., & Vanek, I. (2002). Vasa vasorum of the human great saphenous vein. *Surgical and Radiologic Anatomy*, *24*(6), 376.

Kachlík, Stingl, Sosna, Straka, Lametschwandtner, Minnich, & Fára. (2008). Morphological features of vasa vasorum in pathologically changed human great saphenous vein and its tributaries. *Vasa*, *37*(2), 127-136.

Kakkos, S. K., Zolota, V. G., Peristeropoulou, P., & Apostolopoulou, A. (2003). Increased mast cell infiltration in familial varicose veins: pathogenetic implications?. *International angiology*, *22*(1), 43.

Kanta, J., & Zavadakova, A. (2020). Role of fibronectin in chronic venous diseases: A review. *Vascular Medicine*, *25*(6), 588-597.

Kazenwadel, J., Betterman, K. L., Chong, C. E., Stokes, P. H., Lee, Y. K., Secker, G. A., ... & Harvey, N. L. (2015). GATA2 is required for lymphatic vessel valve development and maintenance. *The Journal of clinical investigation*, *125*(8), 2979-2994.

Ke, N., Godzik, A., & Reed, J. C. (2001). Bcl-B, a novel Bcl-2 family member that differentially binds and regulates Bax and Bak. *Journal of biological chemistry*, *276*(16), 12481-12484.

Kim, B. C., Kim, H. T., Park, S. H., Cha, J. S., Yufit, T., Kim, S. J., & Falanga, V. (2003). Fibroblasts from chronic wounds show altered TGF-β-signaling and decreased TGF-β Type II Receptor expression. *Journal of cellular physiology*, *195*(3), 331-336.

Kim, J., Kundu, M., Viollet, B., & Guan, K. L. (2011). AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. *Nature cell biology*, *13*(2), 132–141. https://doi.org/10.1038/ncb2152

Kim, Y., Png, C. M., Sumpio, B. J., DeCarlo, C. S., & Dua, A. (2021, March). Defining the human and health care costs of chronic venous insufficiency. In *Seminars in Vascular Surgery* (Vol. 34, No. 1, pp. 59-64). WB Saunders.

Komarów, W., Hawro, P., Lekston, A., Urbanek, T., & Zagrodzki, P. (2015). Endothelial dysfunction in patients with chronic venous disease: an evaluation based on the flow-mediated dilatation test. *International Angiology: a journal of the International Union of Angiology*, *34*(1), 36-42.

Kondo, T., Nakano, Y., Adachi, S., & Murohara, T. (2019). Effects of tobacco smoking on cardiovascular disease. *Circulation Journal*, *83*(10), 1980-1985.

Kotb, M. M., Shakiban, H. K., & Sawaby, A. F. (2013). Foam treatment for varicose veins; efficacy and safety. *Alexandria journal of medicine*, *49*(3), 249-253.

Kowalewski, R., Malkowski, A., Sobolewski, K., & Gacko, M. (2010). Evaluation of transforming growth factor-β signaling pathway in the wall of normal and varicose veins. *Pathobiology*, *77*(1), 1-6.

Krasinski, Z., Krasińska, A., Markiewicz, S., & Zieliński, M. (2021). Patients with chronic venous insufficiency in the times of COVID-19 and the risk of thrombus formation - suggestions on conservative treatment of such patients based on the principles of pathophysiology. *Polski przeglad chirurgiczny*, *93*(2), 43–52. https://doi.org/10.5604/01.3001.0014.8500

Ku, K. H., Subramaniam, N., & Marsden, P. A. (2019). Epigenetic determinants of flow-mediated vascular endothelial gene expression. *Hypertension*, *74*(3), 467-476.

Kumar, B. V., Connors, T. J., & Farber, D. L. (2018). Human T cell development, localization, and function throughout life. *Immunity*, *48*(2), 202-213.

Labropoulos, N. (2019). How does chronic venous disease progress from the first symptoms to the advanced stages? A review. *Advances in Therapy*, *36*(S1), 13–19. https://doi.org/10.1007/s12325-019-0885-3

Langer, R. D., Ho, E., Denenberg, J. O., Fronek, A., Allison, M., & Criqui, M. H. (2005). Relationships between symptoms and venous disease: the San Diego population study. *Archives of internal medicine*, *165*(12), 1420–1424. https://doi.org/10.1001/archinte.165.12.1420

Lattimer, C. R., Kalodiki, E., Geroulakos, G., Hoppensteadt, D., & Fareed, J. (2016). Are inflammatory biomarkers increased in varicose vein blood?. *Clinical and Applied Thrombosis/Hemostasis*, *22*(7), 656-664.

Leci-Tahiri, L., Krušlin, B., Vučić, M., Sonicki, Z., & Lovričević, I. (2016). Assessment of apoptosis in the native vein used for hemodialysis access. *Croatian Medical Journal*, *57*(6), 540-547.

Lee, A. J., Robertson, L. A., Boghossian, S. M., Allan, P. L., Ruckley, C. V., Fowkes, F. G. R., & Evans, C. J. (2015). Progression of varicose veins and chronic venous insufficiency in the general population in the Edinburgh Vein Study. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, *3*(1), 18-26.

Lee, D.-K., Ahn, K.-S., Kang, C. H., & Cho, S. B. (2017). Ultrasonography of the lower extremity veins: Anatomy and basic approach. *Ultrasonography*, *36*(2), 120–130. https://doi.org/10.14366/usg.17001

Leu, H. J. (1980). Morphological aspects of changes in the venous wall. *Phlebologie*, *33*(2), 223-231.

Li, J., Hou, B., Tumova, S., Muraki, K., Bruns, A., Ludlow, M. J., ... & Beech, D. J. (2014). Piezo1 integration of vascular architecture with physiological force. *Nature*, *515*(7526), 279-282.

Li, M. X., & Dewson, G. (2015). Mitochondria and apoptosis: emerging concepts. *F1000prime reports*, *7*.

Li, X., Jiang, X. Y., Ge, J., Wang, J., Chen, G. J., Xu, L., Xie, D. Y., Yuan, T. Y., Zhang, D. S., Zhang, H., & Chen, Y. H. (2014). Aberrantly expressed lncRNAs in primary varicose great saphenous veins. *PloS one*, *9*(1), e86156. https://doi.org/10.1371/journal.pone.0086156

Ligi, D., Croce, L., & Mannello, F. (2018). Chronic venous disorders: The dangerous, the good, and the diverse. *International Journal of Molecular Sciences*, *19*(9), 2544. https://doi.org/10.3390/ijms19092544

Lim, C. S., & Davies, A. H. (2009). Pathogenesis of primary varicose veins. *Journal of British Surgery*, *96*(11), 1231-1242.

Lim, C. S., Gohel, M. S., Shepherd, A. C., Paleolog, E., & Davies, A. H. (2011). Venous hypoxia: a poorly studied etiological factor of varicose veins. *Journal of vascular research*, *48*(3), 185-194.

Lim, C. S., Kiriakidis, S., Paleolog, E. M., & Davies, A. H. (2012). Increased activation of the hypoxia-inducible factor pathway in varicose veins. *Journal of vascular surgery*, *55*(5), 1427-1439.

Lim, C. S., Kiriakidis, S., Sandison, A., Paleolog, E. M., & Davies, A. H. (2013). Hypoxia-inducible factor pathway and diseases of the vascular wall. *Journal of vascular surgery*, *58*(1), 219-230.

Lintermans, L. L., Stegeman, C. A., Heeringa, P., & Abdulahad, W. H. (2014). T cells in vascular inflammatory diseases. *Frontiers in immunology*, *5*, 504.

Lizcano, J. M., Morrice, N., & Cohen, P. (2000). Regulation of BAD by cAMP-dependent protein kinase is mediated via phosphorylation of a novel site, Ser155. *Biochemical Journal*, *349*(2), 547-557.

Lockhart, M. E., Sheldon, H. I., & Robbin, M. L. (2005). Augmentation in lower extremity sonography for the detection of deep venous thrombosis. *AJR.*

American journal of roentgenology, *184*(2), 419–422. https://doi.org/10.2214/ajr.184.2.01840419

Lohr, J. M., & Bush, R. L. (2013). Venous disease in women: epidemiology, manifestations, and treatment. *Journal of vascular surgery*, *57*(4), 37S-45S.

Lomonosova, E., & Chinnadurai, G. (2008). BH3-only proteins in apoptosis and beyond: an overview. *Oncogene*, *27*(1), S2-S19.

Lurie, F., Passman, M., Meisner, M., Dalsing, M., Masuda, E., Welch, H., Bush, R. L., Blebea, J., Carpentier, P. H., De Maeseneer, M., Gasparis, A., Labropoulos, N., Marston, W. A., Rafetto, J., Santiago, F., Shortell, C., Uhl, J. F., Urbanek, T., Van Rij, A., … Wakefield, T. (2020). The 2020 update of the CEAP classification system and reporting standards. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, *8*(3), 342–352. https://doi.org/10.1016/j.jvsv.2019.12.075

Lyons, O., Saha, P., Seet, C., Kuchta, A., Arnold, A., Grover, S., ... & Smith, A. (2017). Human venous valve disease caused by mutations in FOXC2 and GJC2. *Journal of Experimental Medicine*, *214*(8), 2437-2452.

Lystad, A. H., Carlsson, S. R., & Simonsen, A. (2019). Toward the function of mammalian ATG12-ATG5-ATG16L1 complex in autophagy and related processes. *Autophagy*, *15*(8), 1485–1486. https://doi.org/10.1080/15548627.2019.1618100

MacColl, E., & Khalil, R. A. (2015). Matrix metalloproteinases as regulators of vein structure and function: implications in chronic venous disease. *Journal of Pharmacology and Experimental Therapeutics*, *355*(3), 410-428.

Mace, P. D., & Riedl, S. J. (2010). Molecular cell death platforms and assemblies. *Current opinion in cell biology*, *22*(6), 828-836.

Martinou, J. C., & Youle, R. J. (2011). Mitochondria in apoptosis: Bcl-2 family members and mitochondrial dynamics. *Developmental cell*, *21*(1), 92-101.

Matsui, T., Jiang, P., Nakano, S., Sakamaki, Y., Yamamoto, H., & Mizushima, N. (2018). Autophagosomal YKT6 is required for fusion with lysosomes independently of syntaxin 17. *The Journal of cell biology*, *217*(8), 2633–2645. https://doi.org/10.1083/jcb.201712058

Mazzei, S., Sindoni, A., Fama, F., Buizon, N. J., & Shafei, M. A. (2020). Dehydrated human amnion/chorion membrane treatment of venous leg ulcers. *Indian journal of dermatology, venereology and leprology*, *86*(2), 212–214. https://doi.org/10.4103/ijdvl.IJDVL_175_19

McIlwain, D. R., Berger, T., & Mak, T. W. (2013). Caspase functions in cell death and disease. *Cold Spring Harbor perspectives in biology*, *5*(4), a008656.

Medical Advisory Secretariat (2011). Endovascular radiofrequency ablation for varicose veins: an evidence-based analysis. *Ontario health technology assessment series*, *11*(1), 1–93.

MedlinePlus. (n.d.). Varicose vein stripping. National Institutes of Health U.S. National Library of Medicine: MedlinePlus website. https://medlineplus.gov/ency/article/002952.htm. Accessed October 18, 2021.

Meissner, M. H., Moneta, G., Burnand, K., Gloviczki, P., Lohr, J. M., Lurie, F., Mattos, M. A., McLafferty, R. B., Mozes, G., Rutherford, R. B., Padberg, F., & Sumner, D. S. (2007). The hemodynamics and diagnosis of venous disease. *Journal of Vascular Surgery*, *46*(6), S4–S24. https://doi.org/10.1016/j.jvs.2007.09.043

Michiels, C., Arnould, T., & Remacle, J. (2000). Endothelial cell responses to hypoxia: initiation of a cascade of cellular interactions. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1497*(1), 1-10.

Mishra, M. K., Soni, R. K., Mohil, R. S., & Sinha, A. (2016). Comparative Study of Outcome of Duplex Ultrasound-Guided, Catheter-Directed Foam Sclerotherapy and Radio-frequency Ablation in the Management of Great Saphenous Varicose Veins. *The Indian journal of surgery*, *78*(5), 375–381. https://doi.org/10.1007/s12262-016-1485-y

Mizushima, N., & Komatsu, M. (2011). Autophagy: renovation of cells and tissues. *Cell*, *147*(4), 728–741. https://doi.org/10.1016/j.cell.2011.10.026

Moosavi, A., & Ardekani, A. M. (2016). Role of epigenetics in biology and human diseases. *Iranian biomedical journal*, *20*(5), 246.

Mosmiller, L. T., Steele, K. N., Shrader, C. D., & Petrone, A. B. (2017). Evaluation of inflammatory cell biomarkers in chronic venous insufficiency. *Phlebology*, *32*(9), 634-640.

Moura, R. M., Gonçalves, G. S., Navarro, T. P., Britto, R. R., & Dias, R. C. (2010). Relationship between quality of life and the CEAP clinical classification in chronic venous disease. *Brazilian Journal of Physical Therapy*, *14*, 99-105.

Mundy, L., Merlin, T. L., Fitridge, R. A., & Hiller, J. E. (2005). Systematic review of endovenous laser treatment for varicose veins. *The British journal of surgery*, *92*(10), 1189–1194. https://doi.org/10.1002/bjs.5142

Musil, D., Kaletova, M., & Herman, J. (2011). Age, body mass index and severity of primary chronic venous disease. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub*, *155*(4), 367-71.

Nakamura, N., Shi, X., Darabi, R., & Li, Y. (2021). Hypoxia in cell reprogramming and the epigenetic regulations. *Frontiers in Cell and Developmental Biology*, *9*, 609984.

Napolitano, G., Esposito, A., Choi, H., Matarese, M., Benedetti, V., Di Malta, C., Monfregola, J., Medina, D. L., Lippincott-Schwartz, J., & Ballabio, A. (2018). mTOR-dependent phosphorylation controls TFEB nuclear export. *Nature communications*, *9*(1), 3312. https://doi.org/10.1038/s41467-018-05862-6

Nelson, E. A., & Bell-Syer, S. E. (2014). Compression for preventing recurrence of venous ulcers. *The Cochrane database of systematic reviews*, *2014*(9), CD002303. https://doi.org/10.1002/14651858.CD002303.pub3

Nesbitt, C., Bedenis, R., Bhattacharya, V., & Stansby, G. (2014). Endovenous ablation (radiofrequency and laser) and foam sclerotherapy versus open surgery for great saphenous vein varices. *The Cochrane database of systematic reviews*, (7), CD005624. https://doi.org/10.1002/14651858.CD005624.pub3

Nicolaides, A. N. (2005). Chronic venous disease and the leukocyte-endothelium interaction: from symptoms to ulceration. *Angiology*, *56*(6_suppl), S11-S19.

Nicolaides, A. N., & Labropoulos, N. (2019). Burden and suffering in chronic venous disease. *Advances in therapy*, *36*, 1-4.

Nicolaides, A., Clark, H., Labropoulos, N., Geroulakos, G., Lugli, M., & Maleti, O. (2014). Quantitation of reflux and outflow obstruction in patients with CVD and correlation with clinical severity. *International angiology : a journal of the International Union of Angiology*, *33*(3), 275–281.

Nicolaides, A., Kakkos, S., Baekgaard, N., Comerota, A., de MAESENEER, M., Eklof, B., ... & Perrin, M. (2018). Management of chronic venous disorders of the lower limbs. Guidelines According to Scientific Evidence. Part I. *International angiology: a journal of the International Union of Angiology*, *37*(3), 181-254.

Nonomura, K., Lukacs, V., Sweet, D. T., Goddard, L. M., Kanie, A., Whitwam, T., ... & Patapoutian, A. (2018). Mechanically activated ion channel PIEZO1 is required for lymphatic valve formation. *Proceedings of the National Academy of Sciences*, *115*(50), 12817-12822.

O'Brien, M. A., & Kirby, R. (2008). Apoptosis: A review of pro-apoptotic and anti-apoptotic pathways and dysregulation in disease. *Journal of veterinary emergency and critical care*, *18*(6), 572-585.

Oh-hora, M. (2009). The calcium/NFAT pathway: role in development and function of regulatory T cells. *Microbes and infection*, *11*(5), 612-619.

Ojdana, D., Safiejko, K., Lipska, A., Sacha, P., Wieczorek, P., Radziwon, P., ... & Tryniszewska, E. (2009). The inflammatory reaction during chronic venous disease of lower limbs. *Folia Histochemica et Cytobiologica*, *47*(2), 185-189.

Ojdana, D., Safiejko, K., Milewski, R., Sacha, P., Wieczorek, P., Lipska, A., ... & Tryniszewska, E. (2009). Evaluation of the memory CD4+ and CD8+ T cells homeostasis during chronic venous disease of lower limbs. *Folia Histochemica et Cytobiologica*, *47*(3), 471-477.

Okamoto, O., & Fujiwara, S. (2006). Dermatopontin, a novel player in the biology of the extracellular matrix. *Connective tissue research*, *47*(4), 177-189.

O'Meara, S., Cullum, N., Nelson, E. A., & Dumville, J. C. (2012). Compression for venous leg ulcers. *The Cochrane database of systematic reviews*, *11*(11), CD000265. https://doi.org/10.1002/14651858.CD000265.pub3

Ono, T., Bergan, J. J., Schmid-Schönbein, G. W., & Takase, S. (1998). Monocyte infiltration into venous valves. *Journal of Vascular Surgery*, *27*(1), 158–166. https://doi.org/10.1016/S0741-5214(98)70303-9

Ordovás, J. M., & Smith, C. E. (2010). Epigenetics and cardiovascular disease. *Nature reviews. Cardiology*, *7*(9), 510–519. https://doi.org/10.1038/nrcardio.2010.104

Ortega, M. A., Asúnsolo, Á., Leal, J., Romero, B., Alvarez-Rocha, M. J., Sainz, F., ... & García-Honduvilla, N. (2018). Implication of the PI3K/Akt/mTOR pathway in the process of incompetent valves in patients with chronic venous insufficiency and the relationship with aging. *Oxidative Medicine and Cellular Longevity*, *2018*.

Ortega, M. A., Asúnsolo, Á., Pekarek, L., Alvarez-Mon, M. A., Delforge, A., Sáez, M. A., ... & García-Honduvilla, N. (2021). Histopathological study of JNK in venous wall of patients with chronic venous insufficiency related to osteogenesis process. *International Journal of Medical Sciences*, *18*(9), 1921.

Ortega, M. A., Asúnsolo, Á., Romero, B., Álvarez-Rocha, M. J., Sainz, F., Leal, J., ... & García-Honduvilla, N. (2019). Unravelling the role of MAPKs (ERK1/2) in venous reflux in patients with chronic venous disorder. *Cells Tissues Organs*, *206*(4-5), 272-282.

Ortega, M. A., Fraile-Martínez, O., Asúnsolo, Á., Martínez-Vivero, C., Pekarek, L., Coca, S., ... & Sainz, F. (2020). Chronic venous disease patients showed altered expression of IGF-1/PAPP-A/STC-2 axis in the vein wall. *BioMed Research International*, *2020*.

Ortega, M. A., Fraile-Martínez, O., Pekarek, L., Alvarez-Mon, M. A., Asúnsolo, Á., Sanchez-Trujillo, L., ... & Sainz, F. (2021). Defective expression of the peroxisome regulators PPARα receptors and lysogenesis with increased cellular senescence in the venous wall of chronic venous disorder. *Histol. Histopathol*, *36*, 547-558.

Ortega, M. A., Romero, B., Asúnsolo, Á., Sainz, F., Martinez-Vivero, C., Álvarez-Mon, M., ... & García-Honduvilla, N. (2018). Behavior of smooth muscle cells under hypoxic conditions: possible implications on the varicose vein endothelium. *BioMed Research International*, *2018*.

Ortega, M. A., Romero, B., Asúnsolo, Á., Sola, M., Álavrez-Rocha, M. J., Sainz, F., Álavrez-Mon, M., Buján, J., & García-Honduvilla, N. (2019). Patients with incompetent valves in chronic venous insufficiency show increased systematic lipid peroxidation and cellular oxidative stress markers. *Oxidative Medicine and Cellular Longevity*, *2019*, 1–9. https://doi.org/10.1155/2019/5164576

Ortega, M. A., Sánchez-Trujillo, L., Bravo, C., Fraile-Martinez, O., García-Montero, C., Saez, M. A., ... & García-Honduvilla, N. (2021). Newborns of mothers with venous disease during pregnancy show increased levels of lipid peroxidation and markers of oxidative stress and hypoxia in the umbilical cord. *Antioxidants*, *10*(6), 980.

Osawa, T., & Noda, N. N. (2019). Atg2: A novel phospholipid transfer protein that mediates de novo autophagosome biogenesis. *Protein science : a publication of the Protein Society*, *28*(6), 1005–1012. https://doi.org/10.1002/pro.3623

Pahwa, R., Goyal, A., Bansal, P., & Jialal, I. S. StatPearls Publishing; Treasure Island, FL, USA: 2020. *Chronic Inflammation.[Google Scholar]*.

Pal, S., & Tyler, J. K. (2016). Epigenetics and aging. *Science advances*, *2*(7), e1600584. https://doi.org/10.1126/sciadv.1600584

Palmieri, M., Impey, S., Kang, H., di Ronza, A., Pelz, C., Sardiello, M., & Ballabio, A. (2011). Characterization of the CLEAR network reveals an integrated control of cellular clearance pathways. *Human molecular genetics*, *20*(19), 3852–3866. https://doi.org/10.1093/hmg/ddr306

Pannier, F., & Rabe, E. (2012). The relevance of the natural history of varicose veins and refunded care. *Phlebology*, *27*(1_suppl), 23-26.

Parrish, A. B., Freel, C. D., & Kornbluth, S. (2013). Cellular mechanisms controlling caspase activation and function. *Cold Spring Harbor perspectives in biology*, *5*(6), a008672.

Pascual, G., Mendieta, C., García-Honduvilla, N., Corrales, C., Bellón, J. M., & Buján, J. (2007). TGF-β1 upregulation in the aging varicose vein. *Journal of vascular research*, *44*(3), 192-201.

Pastar, I., Stojadinovic, O., Krzyzanowska, A., Barrientos, S., Stuelten, C., Zimmerman, K., ... & Tomic-Canic, M. (2010). Attenuation of the transforming growth factor β-signaling pathway in chronic venous ulcers. *Molecular medicine*, *16*, 92-101.

Patel, J., Shah, P., & Gandhi, F. (2021). A study of chronic venous insufficiency in relation with body mass index and diameter of saphenofemoral junction and great saphenous vein. *Indian Journal of Vascular and Endovascular Surgery*, *8*(1), 58-62.

Piazza, G. (2014). Varicose veins. *Circulation*, *130*(7), 582-587.

Poredos, P., Spirkoska, A., Rucigaj, T., Fareed, J., & Jezovnik, M. K. (2015). Do blood constituents in varicose veins differ from the systemic blood constituents?. *European Journal of Vascular and Endovascular Surgery*, *50*(2), 250-256.

Powell, C. C., Rohrer, M. J., Barnard, M. R., Peyton, B. D., Furman, M. I., & Michelson, A. D. (1999). Chronic venous insufficiency is associated with increased platelet and monocyte activation and aggregation. *Journal of vascular surgery*, *30*(5), 844-853.

Rabe, E., Berboth, G., & Pannier, F. (2016). Epidemiology of chronic venous diseases. *Wiener Medizinische Wochenschrift*, *166*, 260-263.

Rabe, E., Guex, J. J., Puskas, A., Scuderi, A., Fernandez Quesada, F., & VCP Coordinators (2012). Epidemiology of chronic venous disorders in geographically diverse populations: results from the Vein Consult Program. *International angiology : a journal of the International Union of Angiology*, *31*(2), 105–115.

Raetz, J., Wilson, M., & Collins, K. (2019). Varicose veins: diagnosis and treatment. *American family physician*, *99*(11), 682-688.

Raffetto, J. D. (2016). Pathophysiology of wound healing and alterations in venous leg ulcers-review. *Phlebology: The Journal of Venous Disease*, *31*(1_suppl), 56–62. https://doi.org/10.1177/0268355516632998

Raffetto, J. D. (2018). Pathophysiology of chronic venous disease and venous ulcers. *Surgical Clinics of North America*, *98*(2), 337–347. https://doi.org/10.1016/j.suc.2017.11.002

Raffetto, J. D., & Mannello, F. (2014). Pathophysiology of chronic venous disease. International angiology : a journal of the International Union of Angiology, 33(3), 212–221.

Raffetto, J. D., Ross, R. L., & Khalil, R. A. (2007). Matrix metalloproteinase 2–induced venous dilation via hyperpolarization and activation of K+ channels: Relevance to varicose vein formation. *Journal of vascular surgery*, *45*(2), 373-380.

Raju, S., Knepper, J., May, C., Knight, A., Pace, N., & Jayaraj, A. (2019). Ambulatory venous pressure, air plethysmography, and the role of calf venous pump in chronic venous disease. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, *7*(3), 428–440. https://doi.org/10.1016/j.jvsv.2018.08.009

Raju, S., Knight, A., Lamanilao, L., Pace, N., & Jones, T. (2019). Peripheral venous hypertension in chronic venous disease. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, *7*(5), 706–714. https://doi.org/10.1016/j.jvsv.2019.03.006

Reček, Č. (2006). Conception of the venous hemodynamics in the lower extremity. *Angiology*, *57*(5), 556–563. https://doi.org/10.1177/0003319706293117

Recek, C. (2013). Calf pump activity influencing venous hemodynamics in the lower extremity. *International Journal of Angiology*, *22*(01), 023-030.

Recek, C., & Pojer, H. (2000). Ambulatory pressure gradient in the veins of the lower extremity. *VASA. Zeitschrift fur Gefasskrankheiten*, *29*(3), 187–190. https://doi.org/10.1024/0301-1526.29.3.187

Recek. (2010). Venous pressure gradients in the lower extremity and the hemodynamic consequences. *Vasa*, *39*(4), 292–297. https://doi.org/10.1024/0301-1526/a000052

Reily, C., Stewart, T. J., Renfrow, M. B., & Novak, J. (2019). Glycosylation in health and disease. *Nature Reviews Nephrology*, *15*(6), 346-366.

Roczniak-Ferguson, A., Petit, C. S., Froehlich, F., Qian, S., Ky, J., Angarola, B., Walther, T. C., & Ferguson, S. M. (2012). The transcription factor TFEB links mTORC1 signaling to transcriptional control of lysosome homeostasis. *Science signaling*, *5*(228), ra42. https://doi.org/10.1126/scisignal.2002790

Saberianpour, S., Modaghegh, M. H. S., Rahimi, H., & Kamyar, M. M. (2021). Role of mechanosignaling on pathology of varicose vein. *Biophysical Reviews*, *13*, 139-145.

Sabine, A., Bovay, E., Demir, C. S., Kimura, W., Jaquet, M., Agalarov, Y., ... & Petrova, T. V. (2015). FOXC2 and fluid shear stress stabilize postnatal lymphatic vasculature. *The Journal of clinical investigation*, *125*(10), 3861-3877.

Sahar, S., & Sassone-Corsi, P. (2013). The epigenetic language of circadian clocks. *Handbook of experimental pharmacology*, (217), 29–44. https://doi.org/10.1007/978-3-642-25950-0_2

Saigusa, R., Winkels, H., & Ley, K. (2020). T cell subsets and functions in atherosclerosis. *Nature Reviews Cardiology*, *17*(7), 387-401.

Salim, S., Machin, M., Patterson, B. O., Onida, S., & Davies, A. H. (2021). Global epidemiology of chronic venous disease: a systematic review with pooled prevalence analysis. *Annals of Surgery*, *274*(6), 971-976.

Sallustio, F., Gesualdo, L., & Gallone, A. (2019). New findings showing how DNA methylation influences diseases. *World Journal of Biological Chemistry*, *10*(1), 1.

Sansilvestri-Morel, P., Rupin, A., Badier-Commander, C., Fabiani, J. N., & Verbeuren, T. J. (2003). Chronic venous insufficiency: dysregulation of collagen synthesis. *Angiology*, *54*(1_suppl), S13-S18.

Santler, B., & Goerge, T. (2017). Chronic venous insufficiency - a review of pathophysiology, diagnosis, and treatment. *Journal der Deutschen Dermatologischen Gesellschaft = Journal of the German Society of Dermatology : JDDG*, *15*(5), 538–556. https://doi.org/10.1111/ddg.13242

Sapelkin, S. V., Timina, I. E., & Dudareva, A. S. (2017). Khronicheskie zabolevaniia ven: funktsiia klapanov i leĭkotsitarno-éndotelial'noe vzaimodeĭstvie, vozmozhnosti farmakoterapii [Chronic venous diseases: valvular function and leukocyte-endothelial interaction, possibilities of pharmacotherapy]. *Angiologiia i sosudistaia khirurgiia = Angiology and vascular surgery*, *23*(3), 89–96.

Saxton, R. A., & Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell*, *168*(6), 960–976. https://doi.org/10.1016/j.cell.2017.02.004

Sayer, G. L., & Smith, P. D. C. (2004). Immunocytochemical characterisation of the inflammatory cell infiltrate of varicose veins. *European journal of vascular and endovascular surgery*, *28*(5), 479-483.

Schmitz, S. U., Grote, P., & Herrmann, B. G. (2016). Mechanisms of long noncoding RNA function in development and disease. *Cellular and molecular life sciences : CMLS*, *73*(13), 2491–2509. https://doi.org/10.1007/s00018-016-2174-5

Schuller-Petrovic, S., Stessel, H., & Brunner, F. (2002). Ca2+ mobilization in saphenous vein smooth muscle cells derived from patients with primary varicosity. *European journal of clinical investigation*, *32*(9), 649-656.

Senra Barros, B., Kakkos, S. K., De Maeseneer, M., & Nicolaides, A. N. (2019). Chronic venous disease: From symptoms to microcirculation. *International Angiology*, *38*(3). https://doi.org/10.23736/S0392-9590.19.04116-6

Serra, R., Ssempijja, L., Provenzano, M., & Andreucci, M. (2020). Genetic biomarkers in chronic venous disease. *Biomarkers in Medicine*, *14*(2), 75-80.

Serralheiro, P., Novais, A., Cairrão, E., Maia, C., Costa Almeida, C. M., & Verde, I. (2017). Variability of MMP/TIMP and TGF-β1 receptors throughout the clinical progression of chronic venous disease. *International journal of molecular sciences*, *19*(1), 6.

Serralheiro, P., Soares, A., Costa Almeida, C. M., & Verde, I. (2017). TGF-β1 in vascular wall pathology: unraveling chronic venous insufficiency pathophysiology. *International journal of molecular sciences*, *18*(12), 2534.

Settembre, C., Di Malta, C., Polito, V. A., Garcia Arencibia, M., Vetrini, F., Erdin, S., Erdin, S. U., Huynh, T., Medina, D., Colella, P., Sardiello, M., Rubinsztein, D. C., & Ballabio, A. (2011). TFEB links autophagy to lysosomal biogenesis. *Science (New York, N.Y.)*, *332*(6036), 1429–1433. https://doi.org/10.1126/science.1204592

Settembre, C., Zoncu, R., Medina, D. L., Vetrini, F., Erdin, S., Erdin, S., Huynh, T., Ferron, M., Karsenty, G., Vellard, M. C., Facchinetti, V., Sabatini, D. M., & Ballabio, A. (2012). A lysosome-to-nucleus signalling mechanism senses and regulates the lysosome via mTOR and TFEB. *The EMBO journal*, *31*(5), 1095–1108. https://doi.org/10.1038/emboj.2012.32

Shadrina, A. S., Sharapov, S. Z., Shashkova, T. I., & Tsepilov, Y. A. (2019). Varicose veins of lower extremities: Insights from the first large-scale genetic study. *PLoS genetics*, *15*(4), e1008110.

Shalini, S., Dorstyn, L., Dawar, S., & Kumar, S. (2015). Old, new and emerging functions of caspases. *Cell Death & Differentiation*, *22*(4), 526-539.

Shanmugam, M. K., & Sethi, G. (2013). Role of epigenetics in inflammation-associated diseases. *Sub-cellular biochemistry*, *61*, 627–657. https://doi.org/10.1007/978-94-007-4525-4_27

Sharma, P., Mishra, A., & Ranjan, R. (2016). Comparative study of ultrasound guided foam sclerotherapy and surgical management for the treatment of varicose veins. *Journal of Scientific Research and Reports*, *9*(1), 1–8. https://doi.org/10.9734/JSRR/2016/17932

Sharma, S., Vashist, M., & Vashist, M. G. (2017). Family history as major predisposing factor in varicose veins disorder. *European Journal of Biomedical*, *4*(12), 392-396.

Shi, Y. (2004). Caspase activation: revisiting the induced proximity model. *Cell*, *117*(7), 855-858.

Shibuya, H., Okamoto, O., & Fujiwara, S. (2006). The bioactivity of transforming growth factor-β1 can be regulated via binding to dermal collagens in mink lung epithelial cells. *Journal of dermatological science*, *41*(3), 187-195.

Shingler, S., Robertson, L., Boghossian, S., & Stewart, M. (2013). Compression stockings for the initial treatment of varicose veins in patients without venous ulceration. *The Cochrane database of systematic reviews*, (12), CD008819. https://doi.org/10.1002/14651858.CD008819.pub3

Sichlau, M. J., & Ryu, R. K. (2004). Cutaneous thermal injury after endovenous laser ablation of the great saphenous vein. *Journal of vascular and interventional radiology : JVIR*, *15*(8), 865–867. https://doi.org/10.1097/01.RVI.0000136968.64870.07

Siddiqui, W. A., Ahad, A., & Ahsan, H. (2015). The mystery of BCL2 family: Bcl-2 proteins and apoptosis: an update. *Archives of toxicology*, *89*, 289-317.

Simka, M. (2004). Calf muscle pump dysfunction in the patients with severe chronic venous insufficiency. *Phlebolymphology*, *47*, 299-303.

Sindrilaru, A., Peters, T., Wieschalka, S., Baican, C., Baican, A., Peter, H., ... & Scharffetter-Kochanek, K. (2011). An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. *The Journal of clinical investigation*, *121*(3), 985-997.

Sladky, V., Schuler, F., Fava, L. L., & Villunger, A. (2017). The resurrection of the PIDDosome–emerging roles in the DNA-damage response and centrosome surveillance. *Journal of cell science*, *130*(22), 3779-3787.

Smetanina, M. A., Kel, A. E., Sevost'Ianova, K. S., Maiborodin, I. V., Shevela, A. I., Zolotukhin, I. A., ... & Filipenko, M. L. (2018). DNA methylation and gene expression profiling reveal MFAP5 as a regulatory driver of extracellular matrix remodeling in varicose vein disease. *Epigenomics*, *10*(08), 1103-1119.

Smetanina, M. A., Shevela, A. I., Gavrilov, K. A., & Filipenko, M. L. (2021). The genetic constituent of varicose vein pathogenesis as a key for future treatment option development. *Vessel Plus*, *5*(19), 2574-1209.

Snyder, D., Sullivan, N., Margolis, D., & Schoelles, K. (2020). *Skin Substitutes for Treating Chronic Wounds*. Agency for Healthcare Research and Quality (US).

Somasundaram, S. K., Weerasekera, A., Worku, D., Balasubramanian, R. K., Lister, D., Valenti, D., Rashid, H., & Singh Gambhir, R. P. (2019). Office Based Endovenous Radiofrequency Ablation of Truncal Veins: A Case for Moving Varicose Vein Treatment out of Operating Theatres. *European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery*, *58*(3), 410–414. https://doi.org/10.1016/j.ejvs.2019.05.020

Somers, P., & Knaapen, M. (2006). The histopathology of varicose vein disease. *Angiology*, *57*(5), 546-555.

Spáčil, J. (2015). Does body height affect the severity of chronic venous disease in lower extremities?. *Vnitrni Lekarstvi*, *61*(3), 202-206.

Sprague, A. H., & Khalil, R. A. (2009). Inflammatory cytokines in vascular dysfunction and vascular disease. *Biochemical Pharmacology*, *78*(6), 539–552. https://doi.org/10.1016/j.bcp.2009.04.029

Spreafico, G., Piccioli, A., Bernardi, E., Giraldi, E., Pavei, P., Borgoni, R., Ferrini, M., & Baccaglini, U. (2013). Six-year follow-up of endovenous laser ablation for great saphenous vein incompetence. *Journal of vascular surgery. Venous and lymphatic disorders*, *1*(1), 20–25. https://doi.org/10.1016/j.jvsv.2012.05.004

Stvrtinova, V., Jahnova, E., Weissova, S., Horvathova, M., & Ferencik, M. (2001). Inflammatory mechanism involving neutrophils in chronic venous insufficiency of lower limbs. *Bratislavske lekarske listy*, *102*(5), 235-239.

Sudoł-Szopińska, I., Bogdan, A., Szopiński, T., Panorska, A. K., & Kołodziejczak, M. (2011). Prevalence of chronic venous disorders among employees working in prolonged sitting and standing postures. *International journal of occupational safety and ergonomics*, *17*(2), 165-173.

Sukhovatykh, B. S., & Sukhovatykh, M. B. (2015). Perforating veins insufficiency in patients with varicose disease. *Khirurgiia*, (5), 14-18.

Tait, S. W., & Green, D. R. (2010). Mitochondria and cell death: outer membrane permeabilization and beyond. *Nature reviews Molecular cell biology*, *11*(9), 621-632.

Takahara, T., Amemiya, Y., Sugiyama, R., Maki, M., & Shibata, H. (2020). Amino acid-dependent control of mTORC1 signaling: a variety of regulatory modes. *Journal of biomedical science*, *27*(1), 87. https://doi.org/10.1186/s12929-020-00679-2

Takase, S., Schmid-Schönbein, G., & Bergan, J. J. (1999). Leukocyte activation in patients with venous insufficiency. *Journal of vascular surgery*, *30*(1), 148-156.

Takáts, S., Nagy, P., Varga, Á., Pircs, K., Kárpáti, M., Varga, K., Kovács, A. L., Hegedűs, K., & Juhász, G. (2013). Autophagosomal Syntaxin17-dependent lysosomal degradation maintains neuronal function in Drosophila. *The Journal of cell biology*, *201*(4), 531–539. https://doi.org/10.1083/jcb.201211160

Tamura, K., & Nakahara, H. (2014). MR Venography for the Assessment of Deep Vein Thrombosis in Lower Extremities with Varicose Veins. *Annals of vascular diseases*, *7*(4), 399–403. https://doi.org/10.3400/avd.oa.14-00068

Tansey, E. A., Montgomery, L. E., Quinn, J. G., Roe, S. M., & Johnson, C. D. (2019). Understanding basic vein physiology and venous blood pressure through simple physical assessments. *Advances in physiology education*.

Tarbell, J. M., & Cancel, L. M. (2016). The glycocalyx and its significance in human medicine. *Journal of internal medicine*, *280*(1), 97-113.

Teruya, T. H., & Ballard, J. L. (2004). New approaches for the treatment of varicose veins. *The Surgical clinics of North America*, *84*(5), 1397–ix. https://doi.org/10.1016/j.suc.2004.04.008

Tinel, A., & Tschopp, J. (2004). The PIDDosome, a protein complex implicated in activation of caspase-2 in response to genotoxic stress. *Science*, *304*(5672), 843-846.

Tisato, V., Zauli, G., Voltan, R., Gianesini, S., di Iasio, M. G., Volpi, I., ... & Secchiero, P. (2012). Endothelial cells obtained from patients affected by chronic venous disease exhibit a pro-inflammatory phenotype. *PloS one*, *7*(6), e39543.

Tucker, W. D., Arora, Y., & Mahajan, K. (2023). Anatomy, Blood Vessels. In *StatPearls*. StatPearls Publishing.

Uhl, J. F., & Gillot, C. (2015). Anatomy of the veno-muscular pumps of the lower limb. *Phlebology*, *30*(3), 180-193.

Ulloa J. H. (2019). Micronized Purified Flavonoid Fraction (MPFF) for Patients Suffering from Chronic Venous Disease: A Review of New Evidence. *Advances in therapy*, *36*(Suppl 1), 20–25. https://doi.org/10.1007/s12325-019-0884-4

Varicose vein stripping: Medlineplus medical encyclopedia. (n.d.). Retrieved November 17, 2023, from https://medlineplus.gov/ency/article/002952.htm

Vein. (n.d.). Servier Medical Art. Retrieved January 29, 2024, from https://smart.servier.com/smart_image/vein-cross-sectional/

Venous disease. (n.d.). Servier Medical Art. Retrieved January 29, 2024, from https://smart.servier.com/smart_image/smart-venous-disease-view/

Venous return. (n.d.). Servier Medical Art. Retrieved January 29, 2024, from https://smart.servier.com/smart_image/venous-return/

Vilagos, B., Hoffmann, M., Souabni, A., Sun, Q., Werner, B., Medvedovic, J., ... & Busslinger, M. (2012). Essential role of EBF1 in the generation and function of distinct mature B cell types. *Journal of Experimental Medicine*, *209*(4), 775-792.

VIu, B., Golovanova, O. V., Sergeeva, N. A., & Kuznetsov, A. N. (2011). Participation of leucocytes in pathogenesis of primary forms of lower limb chronic venous

disease. *Angiologiia i Sosudistaia Khirurgiia= Angiology and Vascular Surgery*, *17*(3), 71-75.

Vlajinac, H. D., Marinkovic, J. M., Maksimovic, M. Z., Matic, P. A., & Radak, D. J. (2013). Body mass index and primary chronic venous disease–a cross-sectional study. *European Journal of Vascular and Endovascular Surgery*, *45*(3), 293-298.

Vlajinac, H. D., Radak, Đ. J., Marinković, J. M., & Maksimović, M. Ž. (2012). Risk factors for chronic venous disease. *Phlebology*, *27*(8), 416-422.

Vuylsteke, M. E., & Mordon, S. R. (2012). Endovenous laser ablation: a review of mechanisms of action. *Annals of vascular surgery*, *26*(3), 424-433.

Vuylsteke, M. E., Colman, R., Thomis, S., Guillaume, G., Degrande, E., & Staelens, I. (2016). The influence of age and gender on venous symptomatology. An epidemiological survey in Belgium and Luxembourg. *Phlebology*, *31*(5), 325-333.

Vuylsteke, M. E., Colman, R., Thomis, S., Guillaume, G., Van Quickenborne, D., & Staelens, I. (2018). An epidemiological survey of venous disease among general practitioner attendees in different geographical regions on the globe: the final results of the Vein Consult Program. *Angiology*, *69*(9), 779-785.

Vuylsteke, M. E., Thomis, S., Guillaume, G., Modliszewski, M. L., Weides, N., & Staelens, I. (2015). Epidemiological study on chronic venous disease in Belgium and Luxembourg: prevalence, risk factors, and symptomatology. *European Journal of Vascular and Endovascular Surgery*, *49*(4), 432-439.

Wali, M. A., & Eid, R. A. (2001). Smooth muscle changes in varicose veins: an ultrastructural study. *Journal of Smooth Muscle Research*, *37*(5, 6), 123-135.

Wang, H. G., Pathan, N., Ethell, I. M., Krajewski, S., Yamaguchi, Y., Shibasaki, F., ... & Reed, J. C. (1999). Ca2+-induced apoptosis through calcineurin dephosphorylation of BAD. *Science*, *284*(5412), 339-343.

Wang, Y., Engels, I. H., Knee, D. A., Nasoff, M., Deveraux, Q. L., & Quon, K. C. (2004). Synthetic lethal targeting of MYC by activation of the DR5 death receptor pathway. *Cancer cell*, *5*(5), 501-512.

Whiston, R. J., Hallett, M. B., Davies, E. V., Harding, K. G., & Lane, I. F. (1994). Inappropriate neutrophil activation in venous disease. *Journal of British Surgery*, *81*(5), 695-698.

Wilmanns, C., Cooper, A., Wockner, L., Katsandris, S., Glaser, N., Meyer, A., ... & Zechner, U. (2015). Morphology and progression in primary varicose vein disorder due to 677C> T and 1298A> C variants of MTHFR. *EBioMedicine*, *2*(2), 158-164.

Xiao, Y., Huang, Z., Yin, H., Lin, Y., & Wang, S. (2009). In vitro differences between smooth muscle cells derived from varicose veins and normal veins. *Journal of vascular surgery*, *50*(5), 1149-1154.

Xu, H. M., Zhao, Y., Zhang, X. M., Zhu, T., & Fu, W. G. (2011). Polymorphisms in MMP-9 and TIMP-2 in Chinese patients with varicose veins. *Journal of Surgical Research*, *168*(1), e143-e148.

Xu, J., Li, K., & Zhou, W. (2019). Relationship between genetic polymorphism of MTHFR C677T and lower extremities deep venous thrombosis. *Hematology*, *24*(1), 108-111.

Xu, Y., Bei, Y., Li, Y., & Chu, H. (2017). Phenotypic and functional transformation in smooth muscle cells derived from varicose veins. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, *5*(5), 723-733.

Yan, Y., John, S., Ghalehnovi, M., Kabbani, L., Kennedy, N. A., & Mehrmohammadi, M. (2019). Photoacoustic Imaging for Image-guided Endovenous Laser Ablation Procedures. *Scientific reports*, *9*(1), 2933. https://doi.org/10.1038/s41598-018-37588-2

Yang, H., Jiang, X., Li, B., Yang, H. J., Miller, M., Yang, A., Dhar, A., & Pavletich, N. P. (2017). Mechanisms of mTORC1 activation by RHEB and inhibition by PRAS40. *Nature*, *552*(7685), 368–373. https://doi.org/10.1038/nature25023

Youn, Y. J., & Lee, J. (2019). Chronic venous insufficiency and varicose veins of the lower extremities. *The Korean journal of internal medicine*, *34*(2), 269–283. https://doi.org/10.3904/kjim.2018.230

Yuan, S., & Akey, C. W. (2013). Apoptosome structure, assembly, and procaspase activation. *Structure*, *21*(4), 501-515.

Yuan, S., Yu, X., Asara, J. M., Heuser, J. E., Ludtke, S. J., & Akey, C. W. (2011). The holo-apoptosome: activation of procaspase-9 and interactions with caspase-3. *Structure*, *19*(8), 1084-1096.

Zalewski, D. P., Ruszel, K. P., Stępniewski, A., Gałkowski, D., Bogucki, J., Komsta, Ł., Kołodziej, P., Chmiel, P., Zubilewicz, T., Feldo, M., Kocki, J., & Bogucka-Kocka, A. (2020). Dysregulations of MicroRNA and Gene Expression in Chronic Venous Disease. *Journal of clinical medicine*, *9*(5), 1251. https://doi.org/10.3390/jcm9051251

Zamboni, P. (2006). The big idea: iron-dependent inflammation in venous disease and proposed parallels in multiple sclerosis. *Journal of the royal society of medicine*, *99*(11), 589-593.

Zamboni, P., Izzo, M., Tognazzo, S., Carandina, S., De Palma, M., Catozzi, L., ... & Gemmati, D. (2006). The overlapping of local iron overload and HFE mutation in venous leg ulcer pathogenesis. *Free Radical Biology and Medicine*, *40*(10), 1869-1873.

Zamboni, P., Lanzara, S., Mascoli, F., Caggiati, A., & Liboni, A. (2008). Inflammation in venous disease. *Int Angiol*, *27*(5), 361-9.

Zöller, B., Ji, J., Sundquist, J., & Sundquist, K. (2014). Venous Thromboembolism and Varicose Veins Share Familial Susceptibility: A Nationwide Family Study in S weden. *Journal of the American Heart Association*, *3*(4), e000850.

Zolotukhin, I. A., Seliverstov, E. I., Shevtsov, Y. N., Avakiants, I. P., Nikishkov, A. S., Tatarintsev, A. M., & Kirienko, A. I. (2017). Prevalence and risk factors for chronic venous disease in the general Russian population. *European Journal of Vascular and Endovascular Surgery*, *54*(6), 752-758.

APPENDICES

Appendix A

NEAR EAST UNIVERSITY
SCIENTIFIC RESEARCH ETHICS COMMITTEE

RESEARCH PROJECT EVALUATION REPORT

Meeting date :25.01.2024
Meeting Number :2024/120
Project number :1783

The project entitled **"The expression profile of intrinsic apoptotic pathway genes in venous insufficiency."** (Project no: NEU/2024/120-1783), which will be conducted by Assoc. Prof. Dr. Mahmut Ergören has been reviewed and approved by the Near East University Scientific Research Ethical Committee.

Prof. Dr. Şanda Çalı
Near East University
Head of Scientific Research Ethics Committee

Committee Member	Role	Meeting Attendance *Attended(✓) Not attended(X)*	Decision *Approved(✓) Rejected(X)*
1. Prof. Dr. Şanda Çalı	Head	✓	✓
2. Assoc. Prof. Dr. Gulifeiya Abuduxike	Rapporteur	✓	✓
3. Prof. Dr. Tamer Yılmaz	Member	✓	✓
4. Prof. Dr. Şahan Saygı	Member	✓	✓
5. Prof. Dr. İlker Etikan	Member	✓	✓
6. Assoc. Prof. Dr. Mehtap Tınazlı	Member	✓	✓
7. Assoc. Prof. Dr. Dilek Sarpkaya Güder	Member	✓	✓
8. Prof. Dr. Burçin Şanlıdağ	Member	✓	✓

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