OPTIMISATION OF MINIMALLY INVASIVE THERAPY FOR PRIMARY VARICOSE VEINS

Barry Jonathan McAree

Submitted in accordance with the requirements for the degree of

Doctor of Medicine

The University of Leeds

Faculty of Medicine and Health Graduate School

April 2015

The candidate confirms that the work submitted is his/her own, except where work which has formed part of jointly-authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Chapter III and IV contain work which has formed the basis of jointly authored publications:

Comparative stability of sodium tetradecyl sulphate (STD) and polidocanol foam: impact on vein damage in an in-vitro model.

McAree B, Ikponmwosa A, Brockbank K, Abbott C, Homer-Vanniasinkam S, Gough MJ. Eur J Vasc Endovasc Surg. 2012 Jun; 43(6):721-5. Epub 2012 PMID: 22507925

The related experimentation was performed by me, Ms A. Ikponmwosa and Ms K. Brockbank with pathological analysis performed by Dr C. Abbott and I. Data analysis was performed by me and the paper written by me with input from Prof. S. Homer-Vanniasinkham and Prof. M.J. Gough.

ACKNOWLEDGEMENTS

I would like to thank Professor M.J. Gough for his guidance throughout my period of clinical research, his readiness to mobilise research funds and for his help in the preparation of this thesis. I would like to thank Dr. Cedric Abbott for his invaluable help and training in the analysis of venous histology. Without his input and generosity with his time this research would not have been possible.

I would like to extend my gratitude to Mike Shires and Martin Waterhouse, Leeds Institute of Molecular Medicine, for their help, advice and diligence in assisting with the pathological processing of tissue samples and subsequent provision of slides in both physical and computerised versions.

I would like to thank Prof. S Homer-Vanniasinkam for her advice and direction toward the staff at Northwick Park Hospital, London. There I would particularly like to thank Dr. Sandra Shurey for her practical assistance and knowledge and also Dr. Paul Sibbons for his help and direction.

My thanks also to the numerous patients who consented to be involved in this study and I would like to acknowledge the animals involved.

Last but not least I would like to thank my wife Orla for her support, patience and understanding and my parents for their encouragement.

ABSTRACT

Optimisation of minimally invasive therapy

for primary varicose veins

B.J. McAree

Submitted for degree of M.D. April 2015

Introduction: Primary varicose veins are common with a multitude of non-optimal treatments. Foam sclerotherapy has seen renewed interest but lacks efficacy versus more expensive modalities. The hypothesis of this thesis is that increasing the half-life of foams will improve efficacy as will mechanical adjuncts.

Methods: The most efficacious proprietary sclerosants are examined in terms of their foam half-life and histopathological effects in-vitro. The best proprietary foam has its half-life increased and histopathological effects of the three most promising resultant foams similarly assessed. Arterial cutting balloons are assessed as an adjunct for foam sclerotherapy in the same in-vitro human GSV model. The best foams are tested against each other and with cutting balloon adjuncts in an animal vein model with results established after three months.

Results: half-life of 3% polidocanol foam is longer than 3% STD. 3% STD damages the vein wall more than polidocanol. Longer lasting STD foams do not enhance its activity against vein wall *in-vitro*. Cutting balloons increase depth of penetration of foam into vein wall by affording it a deeper starting point *in-vitro*. Cutting balloons damage the structure of the vein wall leaving them varicose *in-vivo*. This is likely due to available cutting balloons being too large for tested pig veins. Longer lasting 0.15% xanthum and 3% STD foam outperformed proprietary 3% STD in causing vein occlusion in a pig model.

Conclusions: The active ingredient in sclerosant foams determine its efficacy *in-vitro* more so than the longevity of the foam however longer lasting 3% STD foam shows improved efficacy *in-vivo* in pigs as opposed to in an *in-vitro* human GSV model. Cutting balloons though promising *in-vitro* as adjuncts to foam sclerotherapy are likely best used as a guide to a more optimal mechanical adjunct.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

1 INTRODUCTION

Varicose veins are elongated, dilated and tortuous superficial veins.[1 2] Although varicose veins are common, not all patients are symptomatic and only a minority present for treatment.[3] Despite this the management of varicose veins represents a significant burden on the National Health Service (NHS) in the UK. Over 110,000 varicose vein procedures were performed in the NHS in England in 1999-2000 with around 40,000 performed in 2009-2010. Notwithstanding this evident reduction in the number of procedures, treatment of varicose veins remains a significant burden on healthcare resources. The cost of treating varicose veins and associated complications has been estimated at 1–3% of the total annual health care budget in European countries.[4] The treatment of complications of varicose veins has been estimated to cost about 2 per cent of the total NHS spending in the United Kingdom (UK).[5]

Primary varicose veins are those that occur due to superficial venous reflux alone. Primary varicose veins far outnumber secondary varicose veins, caused by deep venous incompetence. The aetiology and pathogenesis of primary varicose veins remains unclear.[1 6] The majority (60-70%) of patients with varicose veins have an incompetent sapheno-femoral junction (SFJ) and great saphenous vein (GSV) reflux.[7-10] It is known however that successful treatment depends on the abolition of reflux. The majority of patients in the UK are treated with surgery.[11] However, Hospital Episodes Statistics (HES) data from the NHS Information Centre for Health and Social Care display a reduction in varicose vein surgery in England from 1999-2000 when 48,587 surgical procedures were performed to 36,811 procedures in 2008-2009, a decrease of 24.2%. The

department of health state that this may be due to a greater proportion of varicose vein treatments performed in an outpatient setting, therefore being non-surgical.[11] Indeed the past decade has seen an explosion in the employment of new minimally invasive techniques to treat varicose veins which can be performed in the outpatient setting. Minimally invasive techniques include radiofrequency ablation (RFA), endovenous laser ablation (EVLA) and ultrasound guided foam sclerotherapy (UGFS). HES data show an increase in the employment of sclerotherapy from 2,231 procedures in 1999-2000 to 6,823 in 2009-2010. Transluminal operations (RFA and EVLA) have only been recorded separately in HES data since 2006-2007 and since this time the number of transluminal procedures has continually increased from 2,271 procedures to 10,612 in 2009-2010.[11]

In order to improve varicose vein therapy, better treatments should have improved efficacy and be more cost effective.

This thesis examines the potential for improving UGFS to meet these goals. In its current form despite disappointing recurrence rates of up to 20% at 3 years, this increasingly utilised treatment option compares favourably with other minimally invasive treatments especially in terms of procedure costs.[12 13 14] Little is known about its effect on human vein tissue.[15 16] This thesis examines the effects of the most commonly used foam sclerotherapy agents, modifications to these and potential adjuvant treatments in a human *in-vitro* vein model, with the aim of improving its efficacy.

The introduction presents an overview of the epidemiology, predisposing factors, aetiology, and pathogenesis of varicose veins. The relevant anatomy, symptoms and risks of varicose veins are discussed. A synopsis of the evidence base for the current treatment of varicose veins is reviewed, including

mode of action of treatments, their efficacy and where possible, their cost. The aims of this thesis are subsequently outlined.

1.1 EPIDEMIOLOGY OF VARICOSE VEINS

Varicose veins are often described as a “Western” disease as they seem to occur more frequently in industrialised than developing countries.[17-19] A number of large studies of adult populations of developed countries have shown that 30-50% have truncal varicose veins.[19] Epidemiological studies from disparate regions of the world report huge variation in the prevalence of varicose veins with variable prevalence with ethnicity within the same regions. Mekky *et al*observed a more than five-fold greater prevalence of varicose veins in English compared to Egyptian women.[20] In a community study in Jerusalem the prevalence of varicose veins was lower in subjects of North African descent compared with those of European, American or Asian descent. [21]

1.2 PREDISPOSING FACTORS TO VARICOSE VEINS

Several pre-disposing factors are thought to contribute to varicose veins. Age and family history are risk factors. Sex and pregnancy are often cited as the pre-disposing factors however there is variability between reports of different studies.

1.2.1 AGE

Prevalence of varicose veins increases with age.[3 17 22-25] The Edinburgh Vein Study reported a prevalence of 11.5% in those aged 18-24 years and 55.7% in those aged 55-64 years (p=0.001).[25] The Framingham Study reported a prevalence of 1% in men and 10% in women under 30 years and 57% and 77% in men and women respectively over the age of 70 years.[24]

1.2.2 FAMILY HISTORY

In a study of Japanese women, 42% of subjects with varicose veins reported a positive family history, compared with 14% in those without varicose veins.[26] A case-control study in the US reported that patients with varicose veins were 21.5 times more likely to report a positive family history.[27] Carpentier *et al*reported that family history of varicose veins in a first degree relative is the most important pre-disposing factor in both sexes.[18] Though representing a reasonable guide, the methodology of these studies can be questioned on the basis of self-reporting and that those with varicose veins are more attuned to related family history.

1.2.3 OTHER POTENTIAL RISK FACTORS

1.2.3.1 SEX

The vast majority of studies suggest that varicose veins are more common in women.[28] [29] [30] They are often quoted as affecting 10-15% of adult men and 25-33% of adult women in western countries.[31] On the basis of estimates of the San Diego epidemiological study, more than 11 million men and 22 million women in the United States had varicose veins in 2003.[32] However, these figures are largely based on studies involving self-reporting where women tend to be over-represented as women are more likely to present with varicose veins and to request treatment.[17] [33] The Edinburgh Vein Study which included 1,566 randomly selected subjects, found a reversal of this gender difference with 32% of women and 40% of men having truncal varicosities.[25] Clinical series always contain an excess of women (usually about 3:1) but age for age, varicose veins probably affect men and women equally.[34]

1.2.3.2 PREGNANCY

Pregnancy is associated with a number of anatomical and physiological changes which likely contribute to the development of varicose veins. [19 35-40] The risk of developing varicose veins increases with parity with Laurikka *et al*reporting a prevalence of 32, 38, 43, 48 and 59 percent in women with no, one, two, three and four pregnancies respectively.[29]

1.2.3.3 ELEVATED BODY-MASS-INDEX (BMI)

Most studies have shown that overweight and obese women are more likely to develop varicose veins.[20 22 24 27 40-45] However, there is no consistent indication that this relationship holds true for men.[40 46-48] These studies largely derive their data from self reporting. The gender difference reported, coupled with the fact that average body weight is higher in parous compared with nulliparous women cannot exclude the possibility that the observed associations between varicose veins development and obesity may be explained by a confounding effect of parity. [49] Elevated BMI appears to be an aggravating factor rather than a primary cause of varicose veins.[17]

1.2.3.4 OCCUPATION

The ergonomics and physical activity of an occupation may represent a contributing factor in the epidemiology of varicose veins. Most studies [20 21 29 50-53], but not all [54], indicate that working in a position resulting in protracted orthostasis may increase the prevalence and severity of disease.

1.2.3.5 OTHER POTENTIAL RISK FACTORS

Studies have examined associations between smoking, female hormone therapies, hypertension, diabetes mellitus, lack of dietary fibre, constipation, traumatic injury to the extremities, and coagulopathies.[17 24 27 28 30 38-40 48 50 55-58] No direct link has been proven.

1.3 AETIOLOGY AND PATHOGENESIS OF PRIMARY VARICOSE VEINS

The aetiology and pathogenesis of varicose veins remains unclear.[1 6 34 59] A normal venous system depends on the integrity of valves, vein wall and the haemodynamics of venous blood flow. These components are interdependent and disruption of one affects the integrity of the others. Blood flow in varicose veins is disrupted, resulting in blood stasis and reflux. The cause and sequence of events leading to such inefficient blood flow remain unclear, although the main hypotheses are: primary valvular incompetence and primary changes in the varicose vein wall causing weakness with venous dilatation. These categories are considered by many as primary and secondary valve failure with the latter being caused by a developmental weakness of the vein wall resulting in secondary widening of valve commissures and resultant valvular incompetence. There is increasing evidence that alteration of the vein wall is the primary abnormality in varicose veins.[58 60]

1.3.1 PRIMARY VALVULAR DYSFUNCTION / PRIMARY VALVE FAILURE

Absence and deformities, including tearing, thinning, thickening and scarring of the saphenous vein valves are found more frequently in varicose than non-varicose veins retrieved from surgery and during angioscopy.[61-63] Varicose veins also demonstrate hypotrophy of the valves and widening of the valvular

annulus compared with non-varicose veins.[61 64-66] The valves of varicose veins contain less collagen and lose the normal viscoelastic features typical of non-varices.[64] This long-standing hypothesis that a primary valve “lesion” is the initial pathological change occurring in varicose veins has been challenged more recently by several common ultrasonographic and histological findings in varicose veins. Varicosities are often observed below competent valves and not uncommonly found to precede valvular incompetence. Venous dilatation is also frequently seen distal to a valve rather than proximal, which one would expect if valvular dysfunction is the initial event.[58 67]

1.3.2 PRIMARY CHANGES IN VEIN WALL / SECONDARY VALVE FAILURE

The gravitational load on the vein wall and lack of an active mechanism of blood flow return means that elastic vein tissue is subjected to intense biomechanical stress, increasing its susceptibility to functional failure. Recent studies of varicose vein pathogenesis have focused on the structural and biochemical changes in the vein wall.[58 60 68] The underlying hypothesis is that the formation of varicose veins is secondary to defects in cellular and extracellular matrix (ECM) components, causing weakness and altered venous tone.[58 60 69] The triggers for these changes remain unclear, although several factors are likely to be involved, including hypoxia, mechanical stretch and low shear stress.[38 70] Numerous changes in cellular and extracellular matrix components of the vein wall have been identified, including intimal hyperplasia, smooth muscle cell dysfunction, changes to the collagen and elastin content and imbalances of matrix metalloproteinases and their tissue inhibitors. [58 60 71] These changes occur randomly rather than continuously or following a particular pattern. They are thought to cause an overall weakening and relaxation of vein wall leading to

venous dilatation, valve incompetence and reflux. Areas of irregular intimal hyperplasia with associated collagen deposits, smooth muscle cell (SMC) infiltration and plaques underneath the endothelial lining are common in varicose veins. Changes in the media including SMC proliferation and ECM degradation are seen more often in varicose than in non-varicose veins. Stereological studies by Travers *et al*compared the estimated collagen, elastin and smooth muscle content of varicose and control great saphenous veins. Varicose vein cross-sections had significantly larger wall areas ($p < 0.01$) and higher amounts of collagen and smooth muscle compared to controls ($p < 0.01$). There was a higher content of smooth muscle and elastin in varicose veins proximally compared to distally ($p < 0.05$). There was no significant difference in mean wall thickness or in elastin content between the 2 groups. These findings suggest that varicose veins are a dynamic response to venous hypertension and that in general they are not thin walled structures as previously thought.[72] Similarly the adventitia of varicose veins demonstrates areas of increased SMC, fibroblasts and connective tissue with regions of atrophy and devoid of vasa vasorum.[38 70] Vein wall hypoxia has been suggested as a contributing factor for varicose vein formation [58 60 73] Inadequate oxygen supply from luminal blood or vasa vasorum or both could potentially cause vein wall hypoxia. [74 75] Knaapen *et al*examined whether the changes that occur in varicose veins are associated with smooth muscle cell (SMC) hypertrophy, cellular proliferation or apoptosis. They concluded that cell death or proliferation of SMC do not, or only rarely, occur in varicose veins. However, remodelling of varicose veins can mainly be attributed to increased volumes of the SMC of the circular layer and this increase correlates with oestrogen receptor (ER) expression.[76]

The findings from duplex ultrasonography on the pattern and progression of venous dilatation and reflux strongly support vein wall changes as the primary event, although isolated primary valvular dysfunction may sometimes contribute.

1.4 VENOUS ANATOMY OF THE LOWER LIMB

The venous drainage of the lower limb is divided into the superficial and deep systems, the drainage areas of which are separated by the deep fascia. Thus the superficial veins, the great and small saphenous veins and tributaries of the perforating veins drain the skin and subcutaneous fat (the so-called superficial fascia) and the deep veins are responsible for venous return from muscle and other structures deep to the deep fascia. The volume of blood passing through the deep system far exceeds that through the superficial system, the function of the latter being mainly temperature regulation. The superficial veins communicate with the deep veins at the saphenopopliteal (SPJ) and saphenofemoral (SFJ) junctions and by way of the perforating veins through openings in the deep fascia.

1.4.1 THE SUPERFICIAL VEINS

The great saphenous (GSV) vein arises from the medial end of the dorsal arch of the foot, then passes in front of the medial malleolus and along the medial surface of the leg, enclosed in its own fascial sheath, the great saphenous canal. The GSV lies posteriorly at knee level, passing more anteriorly as it ascends the thigh and through the foramen ovale and the femoral triangle to join the common femoral vein, forming the SFJ. A variable number of tributaries join the GSV in this region, namely the anterior accessory GSV, posterior

accessory GSV, superficial circumflex iliac, superficial epigastric and external pudendal veins.

The small saphenous vein arises at the lateral end of the dorsal venous arch of the foot and passes behind the lateral malleolus entering the small saphenous canal (fascial sheath) to join the popliteal vein in the popliteal fossa forming the SPJ. This termination is variable and non-existent in up to a quarter of legs.[77] The Giacomini vein, is a proximal extension of the small saphenous vein into the thigh. It is present in 50-80% of the population and in >50% of those where it exists it terminates at the GSV in the thigh.[78-80]

Great or small saphenous vein duplication can occur, with a rate of 20 -50% being found in early studies.[81 82 83] Of late, these bifid trunks have been further delineated into “true” (within the saphenous canal) and “false” (within the superficial fascia) duplications and consequently a substantial lower rate of true GSV duplication has been found at 1% and 9%.[84 85 10] Labropoulos *et al*found a true duplication rate of both GSV and SSV of around 2% for patients with and without varicose veins.[10] Any superficial veins outside the saphenous canals are termed accessory or a tributary veins.[86] The majority of varicose veins are located in the saphenous vein tributaries or accessory veins. Zamboni *et al*detected a saphenous tributary as the primary varicosity in 98% of limbs.[87] Labropoulos *et al*in a prospective study of 739 patients with CVD and CEAP classification 2 to 6, found 94% (402/427) of those with CEAP class 2-3 and 84% (202/254) CEAP class 4-6 had varicosities arising from the accessory veins and tributaries.[10] Varicosities of the saphenous trunks are infrequent however there is a wide range of data on the actual occurrence. Caggiati *et al*reports a prevalence of saphenous trunk varicosities of 62% in a group with a mean age of 60 years and 39% in a group with a mean age of 30 years.[88]

However, the majority of other studies of primary varicosities have revealed a complete absence of varicosities within the saphenous trunks or at most segmental dilatations.[67] [89] [90] In their more contemporary study with bigger patient numbers Labropoulos *et al*found a prevalence of varicosities in either or both the GSV and SSV of <7%.[10]

1.4.2 THE PERFORATING VEINS

The perforating veins are those other than the great and small saphenous which penetrate the deep fascia, passing from superficial to deep. Between 50 and 100 in-direct perforating veins enter the muscles before joining the deep veins.[91]

1.4.3 THE DEEP VEINS

The deep veins of the lower leg are the paired venae comitantes of the anterior and posterior tibial and peroneal arteries, the gastrocnemius veins and the soleus venous arcades. These all join to form the popliteal vein, which receives the small saphenous vein. The popliteal vein becomes the femoral vein in the lower thigh. This large vein only contains two or three valves and has few tributaries apart from the Hunterian perforator, some muscle veins and the profunda femoris vein, which joins it to form the common femoral vein. The common femoral vein receives the termination of the GSV and often a deep pudendal branch.[91]

1.4.4 VALVES

Valves are present in both venous systems to ensure that blood flows in a single direction, from the superficial to the deep system, toward the heart and against gravity.[1] Valves are present at the junctions between the superficial and deep venous systems and within the perforator veins to prevent backflow from the deep to superficial systems.[91]

1.4.5 VEIN WALL STRUCTURE

The normal vein wall is composed of intimal, medial and adventitial layers. The content of each layer differs to other blood vessels reflecting the specific functions of veins as low pressure capacitance vessels. The intima is formed by a layer of endothelial cells which rest on a basal lamina. Beneath this is the thin subendothelial layer, which may not always be present, containing connective tissue and infrequent smooth muscle cells. Deep to this is the media comprising a network of type III collagen, elastin fibres and bundles of smooth muscle cells. The final and outermost layer of adventitia consists of longitudinally arranged type I collagen fibres. The intermittently placed valves within veins are composed of two semi-lunar endothelial lined folds of connective tissue.[92]

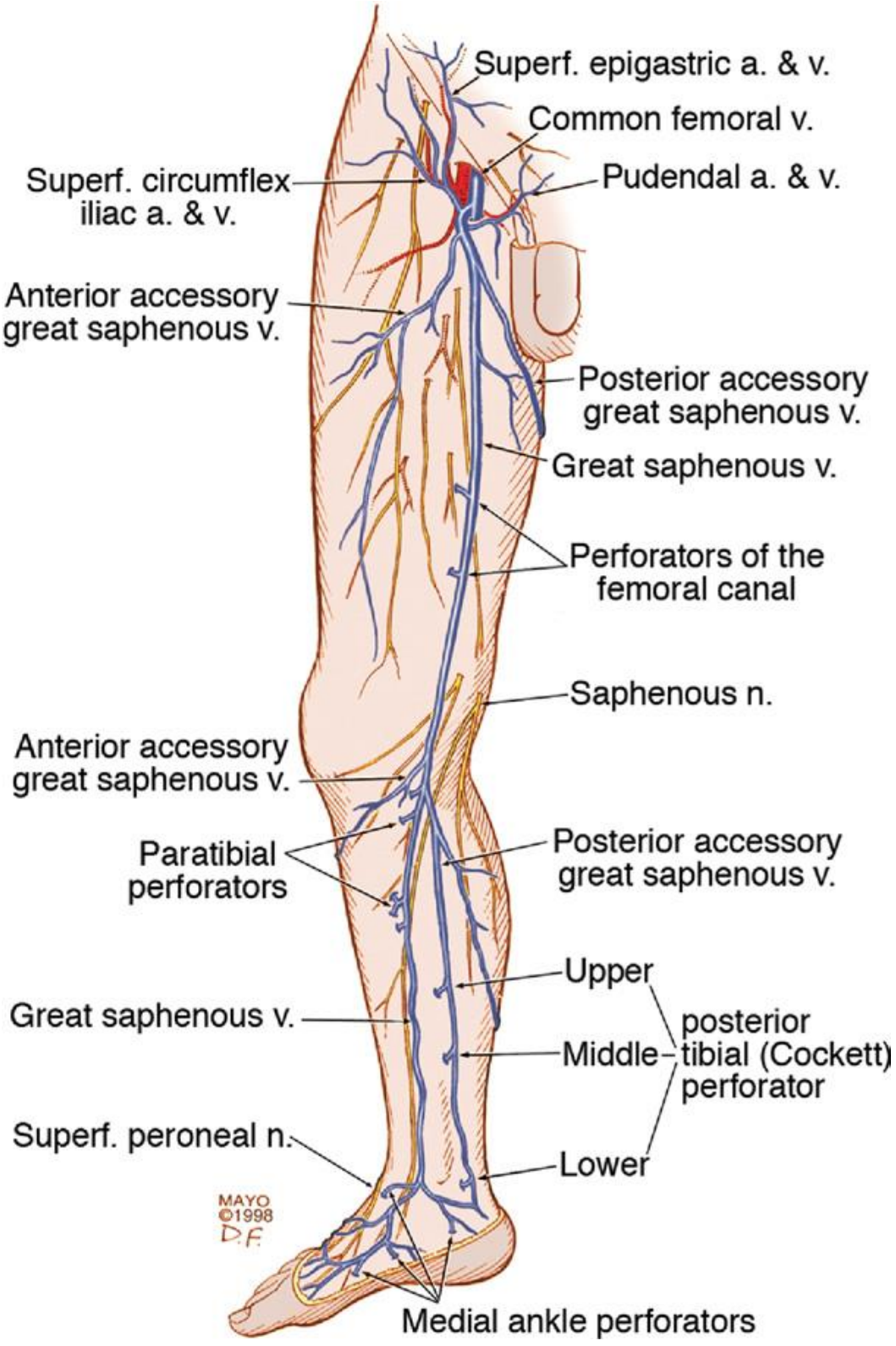


Figure 1.1: Anatomy of the Great Saphenous Vein, its accessory veins and tributaries, displaying the medial superficial and perforating veins of the lower limb. (Mayo Foundation for Medical Education and Research)

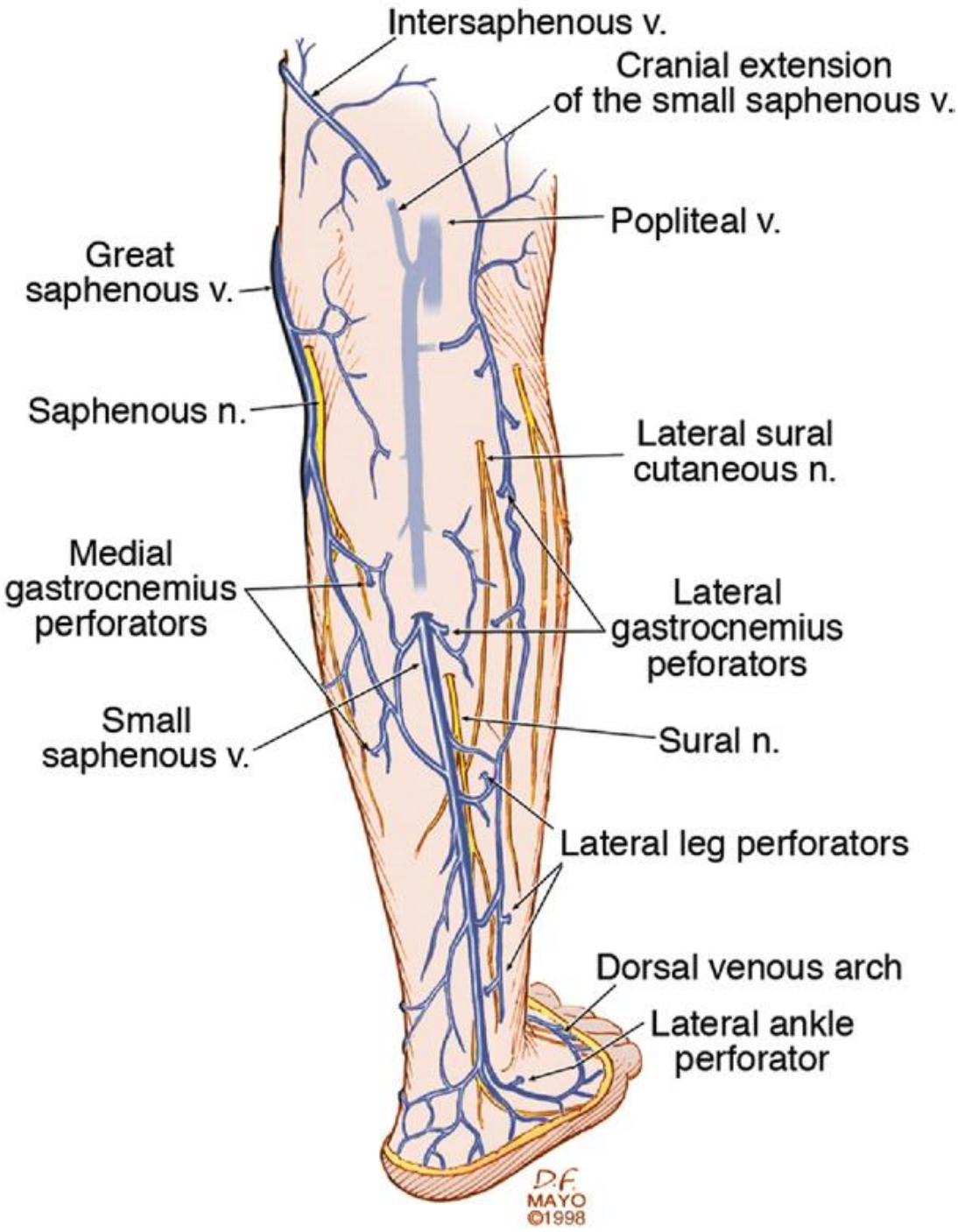


Figure 1.2: Anatomy of the Small Saphenous Vein, its accessory veins and tributaries, displaying the posterior superficial and perforating veins of the leg. (Mayo Foundation for Medical Education and Research)

1.5 SYMPTOMS OF VARICOSE VEINS

Varicose veins may cause symptoms of heaviness, swelling, aching, restless legs, cramps and itching in the legs.[3 93] However correlation between varicose veins and symptoms is controversial. The Edinburgh Vein Study reported pruritus (itch) as the only symptom significantly associated with the severity of truncal varices in men. In women, heaviness/tension, aching and itch all correlated with varicose vein occurrence. Not all patients with the condition are symptomatic and only a minority present for treatment. The Edinburgh Vein Study demonstrated that in the general population there is poor correlation between the presence of varicose veins detected on clinical examination and lower limb symptoms.[3] The presence of reflux on duplex scanning has a weak association with symptoms. Asymptomatic superficial venous reflux on duplex ultrasound is present in 7-39% of the population.[3 8 94 95] Even in the presence of trunk varices (varicose veins originating from an incompetent saphenous system), lower limb symptoms are likely to have a non-venous cause.[3 93] Symptomatic venous insufficiency (requiring treatment) affects 1-15% of adult men and 20-25% of adult women.[31]

1.6 RISKS OF VARICOSE VEINS

Complications of varicose veins include bleeding, superficial vein thrombosis (SVT) and ulceration.[96 97-99 100] Varicose vein bleeding and superficial vein thrombosis can have life threatening consequences however they are both thankfully very rare.[97 101] The POST (epidemiological) study and the CALISTO (randomised) study state that varicose veins are the main risk factor for SVT being involved in 86.5% and 88.6% of cases respectively.[102 103] van Langevelde

*et al*found a 3.9-fold increased risk of pulmonary embolism in clinically diagnosed superficial vein thrombosis.[101] However, ulceration is by far the most common serious complication of varicose veins and that which causes the greatest morbidity and has the greatest cost implications to healthcare agencies[5 97 101 104] Varicose veins are manifestations of chronic venous disease (CVD).[105 106] CVD includes various medical conditions of long duration, all involving morphological and functional abnormalities of the venous system. Varicose veins can progress to a more advanced form of chronic venous dysfunction such as chronic venous insufficiency (CVI).[107 108] In CVI, increased ambulatory venous hypertension initiates a series of changes in the subcutaneous tissue and the skin with activation of endothelial cells, extravasation of macromolecules and red blood cells, diapedesis of leukocytes, tissue oedema and chronic inflammatory changes most frequently noted at and above the ankles.[109] Limb swelling, varicose eczema, haemosiderin deposition, lipodermatosclerosis and venous ulceration can develop. Progression of primary varicose veins to severe CVI and venous ulceration is not rare as indicated by the North American subfascial endoscopic perforator surgery (SEPS) registry where more patients with advanced CVI had primary venous disease than post-thrombotic syndrome (PTS) (70% vs. 30%)[100] Varicose veins and associated complications such as ulcers may lead to chronic pain, disability, decreased quality of life (QOL), loss of working-days and early retirement.[104]

1.7 CLASSIFICATION OF CHRONIC VENOUS DISEASE

Chronic venous disease (CVD) is classified according to the CEAP classification. This scoring system initially created by the American venous forum in 1994 and modified in 2004, is used worldwide to descriptively classify CVD.[110] The fundamentals of the CEAP classification include a description of clinical (C) class based upon objective signs, the etiological (E), the anatomical (A) distribution of reflux and obstruction in the superficial, deep and perforating veins, and the underlying pathophysiology (P), whether due to reflux or obstruction. Seven clinical categories are recognised as shown in table 1.1 with the remaining breakdown of etiology, anatomical and pathophysiology shown in subsequent tables 1.2 to 1.6. Advanced CEAP pathophysiological classification is the same as basic with the addition that any of 18 named venous segments can be utilised as locators for venous pathology.

	CEAP Classification of CVD : Clinical
C0	No visible or palpable signs of venous disease
C1	Telangiectasies or reticular vein; veins less than 3 mm
C2	Varicose veins; veins greater than 3 mm
C3	Oedema
C4a	Pigmentation or eczema
C4b	Lipodermatosclerosis or atrophie blanche
C5	Healed venous ulcer
C6	Active venous ulcer
S	Symptoms including ache, pain, tightness, skin irritation, heaviness, muscle cramps, as well as other complaints attributable to venous dysfunction.
A	Asymptomatic

Table 1.1: CEAP Classification of CVD: Clinical

	CEAP Classification of CVD: Etiology
Ec	Congenital
Ep	Primary
Es	Secondary
En	No venous cause identified

Table 1.2: CEAP Classification of CVD: Etiology

	CEAP Classification of CVD: Anatomical
As	Superficial veins
Ap	Perforator veins
Ad	Deep veins
An	No location identified

Table 1.3: CEAP Classification of CVD: Anatomical

Basic CEAP	**CEAP Classification of CVD: Pathophysiology**
Pr	Reflux
Po	Obstruction
Pro	Reflux and obstruction
Pn	No pathophysiology identified

Table 1.4: Basic CEAP Classification of CVD: Pathophysiology

Advanced CEAP	**CEAP Classification of CVD: Pathophysiology**
Superficial veins	1. Telangiectasies/reticular veins
	2. GSV above knee
	3. GSV below knee
	4. GSV
	5. Non-saphenous veins
Deep veins	6. IVC
	7. Common iliac vein.
	8. Internal iliac vein
	9. External iliac vein
	10. Pelvic: gonadal, broad ligament veins, other
	11. Common femoral vein
	12. Deep femoral vein
	13. Femoral vein
	14. Popliteal vein
	15. Crural: anterior tibial, posterior tibial, peroneal veins (all paired)
	16. Muscular: gastrocnemial, soleal veins, other
	17. Thigh
	18. Calf

Table 1.5: Advanced CEAP Classification of CVD: Pathophysiology

	CEAP Classification of CVD: Level of investigation
L1	Clinical or doppler
L2	Non-invasive (duplex plethysmography)
L3	Invasive or complex (venography, CT, or MRI)

Table 1.6: CEAP Classification of CVD: Level of investigation

1.8 TREATMENT OPTIONS

Optimum treatment of varicose veins should aim to abolish venous reflux and relieve symptoms.[111]

1.8.1 NON - INVASIVE TREATMENT

1.8.1.1 COMPRESSION HOSIERY

Graded compression hosiery or graduated elastic compression stockings (GECS) apply controlled pressure to the leg. This supports the superficial venous system by exerting an external pressure that is greatest at the ankle (minimum of 14 mmHg) and decreases up the leg.[112] The hypothesis is that by reducing venous capacity and increasing venous velocity in the deeper veins, venous stasis and reflux are reduced helping to reduce the severity of varicose veins and the associated symptoms.[113] GECS are often used as a first-line treatment for varicose veins in primary care. However despite there being a lot of trial data regarding the use of compression hosiery much of this is inconclusive except to say that where symptoms were assessed, many studies appeared to show a subjective improvement in participants wearing stockings.[114 115] The lack of published methodology in all of these studies makes these conclusions subject to bias.[114] There is no good evidence that GECS are beneficial in terms of slowing progression or preventing recurrence of varicose veins.[115] Benefits for symptoms appear restricted to the period during which the stocking is worn.[116] Palfreyman *et al's* systematic review concluded that wearing compression improved symptom management, but even this could be confounded by the exclusion of a high number of non-compliant patients within the trials. Compliance with compression is variable and difficult to assess. It has been reported that only 37-47% of patients continue to wear stockings one

year after DVT or for the long-term prevention of venous ulceration.[117] [118] A retrospective patient reported study of compliance with compressions hosiery by Raju *et al*found that of new patients attending a CVD tertiary referral centre, compliance was relatively better at 50% in those giving a prior history of DVT (n=675) compared to 35% in those without such a prior history (n=2,437) ($p<0.001$). They found no difference between the compliance of men and women (39% vs. 38% respectively) and that compliance increased with longevity of symptoms: <1year, 25%; 1-5years, 34%, 6-10years, 40%; >10years, 44% ($p<0.003$).[119] They reported that symptoms of CVD persisted in about a third (37%) of patients despite apparent compliance with prescribed stockings. Ragu *et al*concluded that non-compliance with compression hosiery in patients with CVD is very high regardless of age, sex, aetiology of CVD, duration of symptoms or disease severity. They state the reasons for non-compliance as being under two interdependent major categories: wear-comfort factors and intangible sense of restriction imposed by the stockings.[119]

Recently the commonly held opinion that elastic compression stockings should have a graduated decrease in pressure application from the ankle upward has been questioned. Rather than graduated elastic compression stockings (GECS), inversely graduated or “progressive” elastic compression stockings (PECS) have been assessed. Beneficial results have been reported in sports applications as well as in patients with venous disease.[120] [121] In a double-blind multicentre randomised control trial in patients with mild chronic venous insufficiency (CEAP C0-C2), it was demonstrated that PECS are equally effective as conventional stockings concerning an improvement of subjective symptoms. Additionally, they reported that PECS were easier to put on, were more comfortable to wear and had a better compliance compared to GECS.[121]

Mosti *et al*report using strain-gauge plethysmography, that the mean increase in calf ejection fraction produced by PECS (vs. without stocking) was +75% compared to +32% with GECS (vs. without stocking) with a significant correlation between calf ejection fraction and the stocking pressure measured at calf level during standing and walking. Thereby they concluded that stockings exerting a higher pressure on the calf than on the ankle show greater efficacy in increasing the venous ejection fraction from the leg. They hypothesise that this should translate to benefits for patients with varicose veins.

A randomised trial comparing surgery and conservative treatment for varicose veins showed that surgical treatment produced better results than conservative measures in terms of health-related quality of life, symptomatic relief, anatomical extent and patient satisfaction.[122] The limitations of conservative management, relying largely on the use of compression stockings, are further confirmed by the large proportion of patients (52%) in this study who had crossed over to the surgical arm by three years.

Compression stockings have not been included in cost-effectiveness analyses of varicose vein treatments. An approximate cost to the NHS of two single stockings lasting three to six months is £36.95. [123]

1.8.2 MINIMALLY INVASIVE TREATMENTS FOR VARICOSE VEINS

Minimally invasive techniques include radiofrequency ablation (RFA), endovenous laser therapy (EVLT) and ultrasound-guided foam sclerotherapy (UGFS). These procedures are increasingly employed in the treatment of varicose veins in the NHS.

The development of minimally invasive procedures has primarily been led by short-term trials that demonstrate equivalence of outcome with conventional surgery, with less post-operative discomfort, fewer operative side-effects and a speedier return to normal activity.[124-126] In addition these new techniques can be employed under local anaesthetic, often in an out-patient setting, thereby freeing operating theatre time and potentially improving cost-effectiveness.[127]

An analysis of the cost-effectiveness of minimally invasive and traditional surgical treatments of varicose veins suggests that either RFA or EVLA performed in an outpatient or office based setting with staged UGFS for residual varicosities, were likely to be as cost-effective as traditional GSV surgery.[13] In this analysis UGFS alone had the least expensive initial cost, but this advantage was partly offset by high expected rates of re-intervention at 3 months for residual reflux or varicosities with a corresponding loss in HRQoL (Health related quality of life score [EQ-5D™ index (EuroQol Group, Rotterdam, The Netherlands)]) for the patient, and the additional cost to the health service. However this analysis is based upon non-favourable results which have subsequently been highlighted to be disingenuous regarding UGFS.[14 128 129]

All treatment modalities fall within the NHS threshold of £20,000 per quality-adjusted life years (QALY) however there clearly exists a huge range of cost-implications in deciding upon treatment modality.[13]

Current innovations and developments in minimally invasive varicose treatments include the use of endovenous steam, cyanoacrylate adhesive (Sapheon®[super-glue]) and mechano-chemical (Clarivein®) devices.[130-132]

1.8.2.1 ULTRASOUND GUIDED FOAM SCLEROTHERPAY (UGFS)

Sclerotherapy has been used to treat varicose veins from as early as 1835 according to the records of Massachusetts General Hospital.[133] However it was not until 1963 that the technique of sclerotherapy was described and popularised by Fegan whose name has become synonymous with the procedure.[134] Although liquid sclerotherapy has been used for treating reticular veins or telangiectasia (≤3 mm diameter) for almost a century, foam (1939) and mixing the sclerosant with air (1944) are more recent innovations.[135] [136] [137] However foam has only become more commonly used since the work of Cabrera in the early 1990s. Ultrasound guided foam sclerotherapy (UGFS) has now become common-place in the management of varicose veins.[138] Foam displaces blood from the vein and increases the surface area and duration of contact between the sclerosant and the vein wall.[104] [139] In 2000 Tessari proposed the method of creating foam that remains the most widely used to date.[140]

The goal of sclerotherapy is to obliterate the lumen of the incompetent varicose vein. This requires both intravascular thrombosis and significant damage to the vascular endothelium at the very least.[133] It is now clear that foam is much more effective than traditional liquid sclerosants in achieving sclerosis of the saphenous trunk. [141,142] [143] Compared with liquid, foam has increased contact with the venous endothelium and remains in the vein lumen for a longer period of time. This increases the efficacy of the procedure by enhancing contact between active agent and vein wall to maximise vein wall damage. The use of foam also allows the sclerosant to be visualised with duplex imaging so that the

injection can be placed more accurately. UGFS technique has further enabled the use of sclerotherapy in the treatment of GSV incompetence. The procedure can be carried out using little or no local anaesthetic, takes less than 30 minutes and is a true out-patient procedure. Side effects may occur including superficial thrombophlebitis (10%) and skin staining (17%).[144] Visual disturbance, migranous attacks, allergic reactions and deep vein thrombosis occur infrequently.[12,145] In addition there have been reports of transient ischaemic attack and stroke invariably following the entry of foam into the arterial circulation via a patent foramen ovale.[146-148] In the UK NICE (National Institute for Health and Clinical Excellence) has examined the evidence from a large number of patient series and concluded that foam is safe for routine use, stating that audit of late results is recommended.[127 149]

The most commonly used sclerosants worldwide are polidocanol and sodium tetradecyl sulphate (STD), the latter being the most common in the UK with the former not licenced in the UK.[150 151] Polidocanol and STD are detergent agents containing a hydrophilic and a hydrophobic pole. They act by altering the surface tension around endothelial cells. The hydrophobic pole binds to the cell surface, whereas the hydrophilic portion attracts water into the cell, resulting in a rapid and intense cell hydration.[152] Endothelial damage is enhanced by greater concentrations of sclerosant in smaller veins and delivery as foam prolongs the time of contact and amplifies the effect of the agent.[104 139] It is accepted that STD is a more potent sclerosant than polidocanol in the order of 3:1.[153-155]

Regarding the practical use of UGFS, once complete obliteration of the saphenous trunk has been obtained (which may take more than one treatment),

it is likely that the risk of recanalisation of GSV and SSV trunks is up to 10-20% within the first year, hence the need for early and on-going follow-up especially in the first year.[14 129]

Using 2007-2008 data, estimated NHS cost of the initial UGFS procedure is around £202 with an estimated total cost of £429 over 5 years due to expected re-interventions.[13 156] Gohel *et al*state that the ICER (Incremental cost-effectiveness ratio) for UGFS being £1400 per QALY (Quality-adjusted life year) is the least of all varicose vein therapies, however other treatments may offer greater expected benefits for the additional cost and as such may be considered better value for money.[13] Gohel *et al*state that if GSV occlusion rates from UGFS could be improved (from those used in their analysis; Wright *et al)*[14] it would become a cost-effective treatment and a huge cost saver to health services. According to Gohel *et al,* if the probability of occlusion of the GSV 3 months after UGFS was the same as that for surgery, then UGFS would be cost-effective with an ICER per QALY of £1,000 versus conservative care. Had Gohel *et al*used other data available on UGFS outcomes[128 157] or been able to use data from Darvall *et al*published subsequently, showing 97% occlusion rates after a single UGFS treatment with 93.7% without recanalisation at 12 months, they would according to their own hypothesis have found in favour of UGFS being cost-effective.[129]

Beyond this Gohel *et al*did not assess the potential societal costs of treatments. The different treatments may lead to greatly differing times away from work, especially when comparing the most and least invasive treatments. Absence from work in the range of 1–4 weeks following surgical treatment has been

reported in several studies, whereas minimally invasive treatments usually lead to absence from work of a few days, maximally.[158] Failure to consider such costs does not fully reflect the advantage of less invasive treatments. In a recent non-controlled follow-up study, 43·2 per cent of patients had returned to work within 24 hours after UGFS, but none after surgery ($p < 0{\cdot}001$). Respective proportions after 1 week were 77·4 and 23 per cent ($p < 0{\cdot}001$).[158]

A recent Cochrane review of the efficacy of minimally invasive treatment and traditional surgery for varicose veins concluded that existing evidence on UGFS is insufficient upon which to base an informed decision on its benefits.[159] More robust randomised controlled trials, for example the CLASS study in the UK (Comparison of Laser, Surgery and foam Sclerotherapy), are currently recruiting and will hopefully make treatment choice better informed.[160]

Modifications of sclerotherapy, some listed below, have been attempted with varying levels of success.

1.8.2.1.1 CURRENT UGFS MODIFICATIONS

1.8.2.1.1.1 CATHETER DIRECTED FOAM SCLEROTHERAPY

Catheter directed foam sclerotherapy (CDFS) was initially described in 1997.[161] CDFS involves the use of a long catheter to deliver a foam sclerosing agent into a target vessel (saphenous trunks or venous malformations) under ultrasound guidance.[161] Williamsson *et al*report 70% occlusion of treated segments at one year, in their series of 100 patients, with 15% completely recanalised. They performed CDFS using 10mLs of Tessari formed 3% polidocanol foam (2mL liquid: 8mL air), delivered along the GSV while the catheter was withdrawn. At two weeks and at one year after treatment the patients were evaluated. They

report that 84% of the patients were satisfied with the treatment at one year and there were no serious side-effects.[162] Asciutto *et al* report that of the 188 patients who completed the one year follow up examination post CDFS, 67 % had a complete and 14 % a near complete obliteration of the treated vessel. They report that 92% of patients were satisfied with the results of treatment. Tan *et al*report on their series of 66 legs treated in 62 patients with CDFS with Tessari foam using 3% STD : air (1:3), where they occluded the DUS identified SFJ by digital pressure. 62 of the 66 treated veins were completely thrombosed at day one, an immediate success rate of 94%. 34 legs were scanned at 12 months, of which 19 (56%) of legs had an occluded GSV with partial recanalisation noted in 8 limbs (24%). 7 (20%) of legs had a patent GSV.

1.8.2.1.1.2 SFJ OCCLUSION

Balloon catheters have previously been considered for use as adjuncts to UGFS by using them as SFJ occlusion devices. They were used in an attempt to prevent the escape of sclerosant into the deep veins theoretically protecting the deep veins and enhancing the efficiacy of treatment by increasing the time that foam is in contact with the treated vein wall.[163] [164] Reich-Schupke *et al*report a pilot study of their use of a triple-lumen, double-balloon catheter again born from the hypothesis that in order to minimise the risk of a foam transfer beyond the SFJ into the deep venous system the following two techniques are of use: when using an angiography-catheter the SFJ is compressed by the duplex probe during and some minutes after the foam injection[165] [166] and using a single balloon catheter the balloon at the proximal tip of the catheter is placed at the SFJ sealing the junction.[163] [164] However Reich-Schupke *et al*hypothesised that in both the methods foam escape into the distal part of the vein treated is possible. This they felt would reduce the foam volume required to treat the

desired area provoking incomplete filling of the vessel resulting in the contact time of the foam with vessel wall being shortened inhibiting a successful occlusion of the GSV. Reich-Schupke *et al*therefore initiated a pilot study to survey the safety and efficacy of a triple-lumen double-balloon catheter (Stopflow-catheter Systems, Clinical Plastic Products SA, La Chaux-de-Fonds, Swiss), aiming to diminish the transfer of the foam to the distal part of the vein and hopefully improve the efficacy of the sclerotherapy of the varicose GSV.[166] They used Tessari foam (2 mL 3% polidocanol: 8 mL air) applied via the third lumen of the catheter exiting via nine small holes in the catheter between the proximal and distal balloons. After three minutes the foam was aspirated then first the distal, then the proximal balloon deflated and the catheter removed. 1 day, 1 week and 6 weeks after the treatment, 95% (19/20) of the GSV had a complete occlusion of the treated vein segment. Only eight of the patients treated attended after six months of which 75% (6/8) had a successful occlusion and after 12 months only 50% (4/8) had an occlusion of the treated GSV. Ten patients (50%) complained about a feeling of pressure at the site of the two filled balloons during the treatment. In 10% of the patients (2/20), the retraction of the catheter in the vasospastic vessel was painful. Limitations of this treatment include: the length of the vessel that can be treated is limited by the fixed distance between the two balloons; the time for the treatment with the double-balloon catheter is higher than that compared with the use of an angiography catheter; the double-balloon catheter has possible specific additional side-effects compared with a simple angiography catheter (e.g. rupture of the balloons). Subsequently it appears that attempts to occlude the SFJ during UGFS may in fact increase the leakage of foam into the deep veins by opening the SFJ which in the majority of those with truncal varices is not

actually incompetent. At the very least balloon occlusion, manual compression of the SFJ and even ligation of the SFJ prior to UGFS have been found not to prevent leakage of foam into the deep veins, hence these techniques have been largely abandoned.[167] [168]

1.8.2.1.1.3 DENUDATION

1.8.2.1.1.3.1 MECHANOCHEMICAL

Endovenous mechanochemical ablation is a new technique with minimal data thus far. Its mechanism of action is stated to be a combination of mechanical damage of the endothelium and scarring of the vein due to liquid sclerosant. One qualitative histological *in-vitro* study of five 8-10cm lengths of GSV removed at SJF ligation and subsequently treated with ClariVein® only, revealed a qualitative result of >50% but <100% endothelium remaining intact in test samples with 100% endothelium remaining in control segments p=0.004 (as per publication).[169] Mechanochemical ablation does appear to have the advantage that it does not involve thermal damage and thus does not require tumescent anaesthesia. Two pilot studies have been published, one using 1.5% STD and the other 1.5% polidocanol, both using the only commercially available device, ClariVein® endovenous mechanochemical ablation.[170] [171] The single-use, disposable ClariVein® infusion catheter contains a rotating dispersion wire that extends through its lumen. At the end of the wire is an angled tip that protrudes 2 cm. A small ball attached to the tip enhances ultrasonographic visibility and is stated to mechanically damage the endothelial layer as it rotates at 3500 revolutions per minute and disperses sclerosant into the bloodstream and onto the vessel wall. The catheter is connected to a 9 volt battery motorised handle unit that controls wire rotation. A 5 mL syringe mounted on the handle delivers sclerosant. The catheter is inserted through a 4-6-Fr micropuncture set inserted

into the truncal vein under ultrasound guidance via an 18 gauge intravenous access needle. One study reports that 6 weeks following polidocanol in combination with Clarivein®, starting 2 cm distal from the SFJ, with a mean treatment length of 40cm, a mean sclerosant volume of 6.8mLs sclerosant, the GSV was completely obliterated in 26 (87%) of 30 veins. Two patients had partial recanalisation of the proximal GSV, 15 and 18 cm respectively from the SFJ whilst another had partial recanalisation of the distal GSV. One total recanalisation of the GSV was successfully treated in a redo procedure 7 weeks after initial treatment. The STD study reports a longer follow-up of six months with a mean treatment length of 37.5cm. All patients received 12mLs of liquid 1.5% STD. DUS findings were similar at one and six months with only one recanalisation in 30 GSVs, a primary closure rate of 97.6%.[132 170 171] It would appear likely that this treatment modality offers similar recanalisation rates to more recently reported UGFS follow-up. [129]

1.8.2.1.1.3.2 BALLOON DENUDATION

Fogarty balloons have previously been assessed at Leeds Vascular Institute for their potential as adjunctive treatment in UGFS. This work was based on the hypothesis that “trawling” an inflated Fogarty balloon through the GSV prior to UGFS may potentiate its effects by denuding the endothelium, thus enhancing the penetration of foam into the subendothelial layer. Unfortunately, though balloon denudation did increase the overall endothelial cell loss, it did not increase the depth of injury caused by the foam.[16]

This thesis examines the potential for cutting balloons to act as adjuncts to UGFS. The cutting balloon was first designed by Barath *et al*.[172] Cutting balloons are designed for arterial atherosclerotic stenoses that are unlikely to

respond to balloon angioplasty. Cutting balloons feature three or four atherotomes (microsurgical blades), which are 3-5 times sharper than conventional surgical blades.[173] The atherotomes, which are fixed longitudinally to the outer surface of a non-complaint balloon, expand radially and deliver longitudinal incisions in the vessel, designed to relieve the radial stress of an atherosclerotic stenosis. The atherotomes are mounted along the length of the cutting balloon. The unique design of the cutting balloon is engineered to protect the vessel from the edges of the atherotomes when it is deflated. This minimises the risk of trauma to the artery as the balloon is passed to and from a target lesion. We hypothesise that cutting balloon atherotomes (used immediately prior to UGFS) will cut the GVS media in up to four places (depending on the balloon design) allowing influx of foam sclerosant deeper into the vein media, thereby increasing the efficacy of UGFS.

1.8.2.2 THERMAL ABLATION TECHNIQUES

RFA and EVLA are thermal ablation techniques which use heat to damage the vein causing non-thrombotic venous occlusion by inducing endothelial damage, collagen denaturation and focal coagulative necrosis.[174,175] These techniques usually access the GSV at the level of the knee thus avoiding a groin incision and the potential morbidity associated with this component of a surgical procedure. Controlled trials show that the GSV is obliterated in over 90% of patients.[174,175] Although the early GSV occlusion rates with thermal ablation techniques are encouraging, long term recurrence rates have not been established.[176,177] Thermal ablation is usually possible only in long, straight veins, meaning that typically it is only the truncal vein that is treated thermally. An alternative method is often used to obliterate residual varices, although many patients obtain symptomatic improvement without this additional step.

Thermal ablation is usually carried out under tumescent local anaesthesia, in which the local anaesthetic agent injected not only provides pain relief but also via volume effect, moves the vein away from the nerve and skin to reduce the chance of thermal damage to these structures. Thermal ablation techniques require additional skills namely duplex ultrasonography and duplex-guided venous cannulation. There are several different laser and RFA devices and specific training and experience are required with each for their use.[127]

1.8.2.2.1 ENDOVENOUS LASER ABLATION (EVLA)

A relatively novel technique, EVLA was invented by Dr. Robert Min at Cornell University, New York.[178] It involved placement under ultrasound guidance of an intravenous catheter into the GSV at knee level, with a sterile bare-tipped laser fibre inserted via the catheter up to the SFJ. Delivery of continuous wave laser energy commences 1-2cm distal to the SFJ. The laser fibre tip is withdrawn at a rate of 3mm/second distributing approximately 70J/cm along the length of the GSV. Corcos *et al*undertook a study assessing the effects of application of an 808nm diode laser. Specimens were stained with haematoxylin and eosin and with Weigert for elastic fibres. The depth of tissue penetration in terms of thermal damage was evaluated in 29 vein specimens. Full thickness penetration was found in the intimal layer, partial to full thickness in the media in the majority of specimens and minimal effects in the adventitia. The thermal alterations in tissue were described as necrosis, vacuolation, delamination, coagulation and tissue loss. The detection of vaporised, coagulated blood further supports the theory of heat transfer to the vein wall via the production of steam and subsequent thrombus development as postulated by Proebstle *et al*.[179,180] Contemporary EVLA commonly involves the use of higher wavelength lasers e.g. 1470nm diode laser directing thermal energy at the water within the cells of the veins wall. This results in decreased perforations and bruising seen

with earlier lasers. Treatment now also involves ablating from the lowest point of reflux which provides maximum improvement in symptom severity scores (Aberdeen varicose vein symptom score) and maximum spontaneous resolution of varicosities with only 17% have residual varicosities after GSV EVLA alone.[181] van den Bos *et al* report in their 2009 meta-analysis that EVLA was significantly more effective compared with surgery (AOR, 1.13; 95% CI, 0.40-1.87), foam sclerotherapy (AOR, 1.02; 95% CI, 0.28-1.75), and radiofrequency ablation (AOR, 0.71; 95% CI, 0.15-1.27).[125] After 3 years, the estimated pooled success rates (with 95% confidence intervals [CI]) for stripping, foam sclerotherapy, radiofrequency ablation, and laser therapy were about 78% (70%-84%), 77% (69%-84%), 84% (75%-90%), and 94% (87%-98%), respectively.

When offered as an out-patient/office based procedure, the cost of initial EVLA is estimated at around £698 with total cost at 5 years, accounting for expected re-interventions being £1,021 with an ICER per QALY of around £5,800.[13]

1.8.2.2.2 RADIOFREQUENCY ABLATION (RFA)

RFA was introduced in Europe in 1998 and first described in clinical practice by Goldman in 2000.[182,183] RFA involves heating the GSV wall to 85°C with radiofrequency delivered via catheter electrodes with a temperature feedback loop. The catheter is placed percutaneously into the GSV at knee level and advanced to the SFJ under duplex ultrasound guidance. The catheter is then withdrawn treating sequential sections (c.7cm with VNUS ClosureFAST™) with a microprocessor radiofrequency generator ensuring delivery of the minimum power needed to maintain the target temperature at the treatment site. RFA is thought to achieve fibrotic occlusion by direct heating of the vein wall inducing collagen denaturation.[184 185] The most popular RFA system is the VNUS®

ClosureFAST™ (VNUS Medical technologies, San Jose, California, USA) segmental ablation catheter, which has superseded earlier continuous withdrawal catheters.[126] van den Bos *et al*reported in their meta-analysis that RFA was as effective as surgery and foam sclerotherapy, however less-so than EVLA.[125]

When offered as an out-patient/office based procedure, the cost of initial RFA is estimated at around £776 with total cost at 5 years, accounting for expected re-interventions being £1,110 with an ICER per QALY of around £17,350.[13]

1.8.2.3 CURRENT ENDOVENOUS INNOVATIONS

1.8.2.3.1 ENDOVENOUS STEAM

The exact mechanism of action of endovenous laser is not fully understood however several theories exist such as the “steam bubble theory,” the “direct contact theory,” the “heat pipe,” and “direct light energy absorption” hypotheses.[186] Obviously several factors play an important role in the mechanism of endovenous laser ablation. Direct energy absorption by the vein wall appears the most efficient mechanism.[186] With a view to extrapolating the potential ablative action of intravascular “steam bubbles”, steam itself has been used as an ablatant in a new method of thermal vein ablation. Its objective is to achieve a potentially safer and easier method of thermal ablation that has fewer side effects. Only one study has been reported thus far with histological reports from animal studies on sheep and a pilot study in humans. The Steam Vein Sclerosis (SVS) system (CERMA SA, Archamps France) was used. The SVS system consists of a steam generator and a hand-piece that injects micropulses of steam into a catheter that delivers the steam into the vein to be treated. They calculated that approximately 2258 J is released when 1 gram of steam condenses. In EVLA, it is considered consensus to apply about 50 to 60

Joules/cm. To occlude 30 cm of vein with steam ablation, theoretically, 1 to 1.5 mL of water is needed. In practice, 2 to 5mLof water is likely to be required, because not all steam condenses at the vein wall. The steam is produced by means of piston pressing a fixed amount of water (76µL=diameter piston x stroke) through a heated element located just before the catheter. By keeping the lumen diameters very small and the exit holes even smaller, pressure is maintained and loss of energy is limited. The volume of the steam depends on the pressure and temperature. As the energy is transferred to the vein, the steam cools and condenses to the same volume of water used to produce the steam. The steam starts to cool and condense when it leaves the catheter due to the drop in pressure and the exchange of energy with the surroundings. This process is dynamic. The theoretic amount of energy of one pulse of steam is 174 J. The measured amount released at the tip of the catheter is 60 J per pulse. Results in sheep veins treated with steam ablation displayed fibrosis and destruction of the vein was confirmed by histology, and ongoing fibrotic contraction was confirmed by shrinking of vein diameter on US imaging. The similar histological changes found after heating using the RFA technique are stated by the authors to show that their study design was valid and in line with other minimally invasive thermal ablation modalities. The human pilot study comprised 19 patients treating 20 veins. The mean treated length of the veins was 25 cm, with an average of 50 pulses of steam were administered per treated vein. All treated veins were occluded on US examination at 1 week. At 3 months, 1 of the 20 treated varicose veins showed a small segment of several centimetres with minimal blood flow. At 6 months, flow was observed on US examination in 7 of 20 treated saphenous veins, but this was segmental (<10 cm). Subsequent work by this team has led them to recommend an increase

dose of steam to induce veins i.e. 2 instead of 1 steam pulse/cm.[187] Though this potential treatment modality is very much in its infancy, it appears to show promise.

1.8.2.3.2 CYANOACRYLATE

Cyanoacrylate (CA), commonly referred to as ‘‘superglue,’’ has gained momentum in recent years for intravascular use to treat conditions such as arteriovenous malformations (AVMs),

varicoceles among other pathologies.[188] [189-191] Although its use is off patent for peripheral applications, the properties of CA make it potentially useful for a number of vascular occlusion procedures. When used intravascularly, CA triggers a robust inflammatory reaction in the vessel wall. Anionic substances such as plasma, blood, or saline stimulate polymerisation of the CA adhesive upon contact, leading to occlusion. The resultant polymerisation damages the vascular intima and induces an acute immunological response.[192] After polymerisation is complete, gradual resorption of the occlusive polymer takes place. Within approximately one month, the response progresses to granuloma formation with giant cells and eventually fibrosis.[193] [194] Preliminary studies have been carried out in animal models and a small number of human subjects though the later has not yet been reported. These studies have employed the Sapheon® CA adhesive delivery catheter (Sapheon, Santa Rosa, California). The dispenser gun delivers a fixed volume (0.16mL) of glue per trigger pull, which is repeated every 3cm. Animal studies using a porcine model report endpoints of histological findings at 30 and 60 days as being, venous closure, segmental wall thickening and fibrosis. They state that intravascular injection of CA is feasible for closure of superficial veins in animal models and clarify that these findings warrant further animal studies of this proprietary CA to assess efficacy, safety and its effects on perivenous structures. They have already

conducted human trials on eight subjects with incompetent GSVs and signs or symptoms of chronic venous disease. They report that the mean treatment time was 17 minutes, the mean treatment length was 31 cm, and the mean CA injected volume was 1.58 mL. Results declared are that all veins were closed at 1 month and there were apparently no serious adverse events. The authors conclude that CA adhesive is a feasible alternative treatment for incompetent saphenous veins and state that a more robust study began in March 2011.[195] Human studies have begun in other units.[196]

1.8.3 SURGICAL TREATMENT

1.8.3.1 SFJ LIGATION (CROSSECTOMY), GSV STRIPPING, PHLEBECTOMIES

The most common method of treatment of varicose veins in the UK remains surgical ligation of the saphenofemoral junction (SFJ) with subsequent stripping of the great saphenous vein (GSV).[11] Conventional varicose vein operations are high volume and low risk and are typically carried out under general anaesthesia. These operations have changed little over decades. Although surgery is highly effective in the short term, the 5-year recurrence rates are approximately 30% for GSVs and 50% for SSVs, which may be due to neovascularisation.[197] Campbell *et al*report a 10 year recurrence rate post-surgery of 70% and Winterborn *et al*report recurrence at 11 years in the order of 60%.[198] [199] The Randomised and Economic Assessment of Conservative and Therapeutic interventions for Varicose Veins (REACTIV) trial suggests that the cost of varicose vein surgery in the UK is within National Institute of Health and Clinical Excellence (NICE) guidelines for quality-adjusted life years.[122] [127] When offered as a day case procedure, the cost of initial surgery is estimated at around £980 with total cost at 5 years, accounting for expected re-interventions

being £1,242 with an ICER per QALY of around £19,012.[13] Costs are greatly increased by inpatient status and according to the review of cost-effectiveness of varicose vein treatments by Gohel *et al*negate the probability that the treatment is cost-effective at the NHS threshold of £20,000 per QALY.

1.8.3.2 MODIFICATIONS TO STANDARD SURGICAL TREATMENT

Over more than 20 years a number of attempts at conservative surgery for saphenous truncal incompetence have been reported.[200] [201] [202] A technique which has fallen from favour is flush sapheno-femoral ligation combined with multiple phlebectomies without stripping of the GSV. Some short term reports suggested that this resulted in similar outcomes to stripping operations.[202] The evidence in favour of stripping is suggestive but not overwhelming. Stripping of the GSV is postulated to reduce recurrence by preventing neovascularisation in the groin joining up with the residual trunk of the GSV in the upper thigh and producing significant GSV reflux in the lower limb. Neovascularisation has been shown to be the cause of recurrence in up to two-thirds of patients[198] but this same trial showed no difference in rates of recurrence between legs that had undergone stripping and those that had undergone simple SFJ ligation. Despite this finding of equal rates of recurrent varicosities, those patients in the stripping group had fewer re-do operations for recurrent varicose veins, a finding that is not explained.[198] [203]

SFJ ligation without GSV stripping is often confused with the Haemodynamic Correction procedure (CHIVA, Cure Conservatrice et He´modynamique de l'Insuffisance Veineuse en Ambulatoire, Ambulatory Conservative Haemodynamic Management of Varicose Veins).

1.8.3.2.1 CURE CONSERVATRICE ET HEMODYNAMIQUE DE L´INSUFFICIENCE VEINEUSE EN AMBULATOIRE (CHIVA) / CONSERVATIVE HAEMODYNAMIC CURE OF VENOUS INSUFFICIENCY.

In 1988, Franceschi described conservative haemodynamic cure of venous insufficiency, known by the French acronym “CHIVA”.[204] CHIVA was designed to allow treatment of varicose veins without sacrificing the superficial vein network, and consists of minimally invasive surgical procedures under local anaesthesia that are based on findings of careful haemodynamic analysis of the venous network of the lower limb using duplex imaging. CHIVA is based on the fact that, even though varicose disease is associated with weakness of the vein wall, clinical manifestations occur only under certain orthostatic haemodynamic conditions.[204-206] If these haemodynamic abnormalities are corrected by breaking the pressure column and suppressing veno-venous shunting, manifestations disappear while preserving runoff from superficial tissue via the superficial vein network. Therefore, the goal of CHIVA for superficial venous insufficiency is to relieve the hydrostatic pressure column by stopping veno-venous shunts of the saphenous vein or disruption of drainage of superficial tissue.[207] The aim of CHIVA is not only to preserve the GSV for use as a future vascular graft, but also to maintain its drainage eliminating reflux points with change of compartments.[204 206 208 209] CHIVA aims to treat the varicose vein disease by creating a draining saphenous system. A single randomised trial comparing the outcome of CHIVA with saphenous stripping operations has been reported. In those treated with surgery their treatment involved: flush sapheno-femoral ligation, GSV stripping from groin to knee, multiple phlebectomies of the tributaries and subfascial ligation of thigh perforating veins under general or spinal anaesthesia. Haemodynamic correction (CHIVA) in keeping with the philosophy of CHIVA treatment was performed using what is

termed a minimally invasive technique Haemodynamic Correction type 1 (CHIVA 1).[202] This consists of flush SFJ ligation, disconnection from the GSV of the varicose tributaries and their avulsion through cosmetic incisions. Reverse drainage of both the GSV and competent tributaries toward the re-entry perforating vein is produced. Incompetent tributaries were disconnected from the GSV and partially removed, the residual distal segments of these tributaries drain separately though their own re-entry perforating vein. All CHIVA operations were performed as day surgery cases and under local anaesthesia. The rate of ultrasound confirmed recurrence was significantly higher in the stripping group, 35% compared to 18% in the CHIVA group, respectively ($p = 0.038$, Fisher's exact test). The relative risk of recurrence in the stripping group is doubled at 10 years compared to CHIVA (OR 2.2; 95% CI 1-5, $p = 0.04$, Fisher's exact test). The main difference between the two groups after 10 years is the 22% of newly formed varicose veins found in the stripping group, without any detectable reflux point. The authors believe that this type of recurrence is attributable to the lack of a draining saphenous system encouraging neovascularisation. The maintenance of drainage seems to be a decisive factor in avoiding neovascularisation after varicose vein surgery.[210] This observation is confirmed when CHIVA treatment is not correctly performed leading to post-operative GSV thrombosis and occlusion. A non-draining GSV, despite conservative surgery, increases the number of recurrences in comparison to draining GSV systems.[209] CHIVA presents difficulties as the operating surgeon must also be competent at duplex ultrasonography and capable of analysing patterns of varicose veins by duplex ultrasonography undertaken before the operation. In countries where surgeons usually perform their own duplex ultrasound examinations such as in Italy and Spain, the CHIVA technique is

more commonly used. In Spain, a national survey demonstrates this technique is used in about 50 % of cases.[207] In contrast, in France the same kind of evaluation assessed CHIVA performed in less than 5 % of cases.[211]

1.8.3.2.2 AMBULATORY SELECTIVE VARICES ABLATION UNDER LOCAL ANESTHESIA (ASVAL*)*

As discussed previously numerous biochemical, anatomical and pathological studies suggest a parietal or secondary valve failure, rather than primary valvular failure hypothesis as the origin of varicose veins.[58 60 212-214] According to this hypothesis, valvular insufficiency is caused by the dilatation of the vein. Anatomical and clinical studies indicate that in the presence of varices, not only is the SFJ competent in >50% of cases,[215 216] but moreover the GSV is often partially or wholly competent.[9 217] All of these observations collectively challenge the sole culpability of the GSV in the initial development of saphenous vein disease. The correlation between the extent of the saphenous reflux, age, and clinical stage is described in the literature[9 79 88] and supports the theory of the development of superficial venous insufficiency starting from the suprafascial / superficial venous network in an ascending or multifocal fashion.[9 67 88 217-220] Pittaluga *et al*hypothesised that it appeared logical therefore to treat the superficial varicosities alone in an attempt to reverse the changes in leading to further and worsening varicose veins.[220] They report a 4 year follow-up on a cohort of patients treated by ASVAL. ASVAL was done with phlebectomies (reducing the varicose reservoir ≅ number of zones to be treated [NZT; of 32 potential zones per leg]) through staged incisions under tumescent local anaesthesia. They treated one cohort with ASVAL and another with high (SFJ) ligation and GSV stripping (High ligation and strip; HLS). Retrospective comparison of the two cohorts confirmed that they had as planned been selective in choosing patients for ASVAL. They reserved this more limited

surgical treatment for the least evolved stage of the varicose disease hoping to obtain clinical and hemodynamic reversibility. The comparison of the cohorts undergoing ASVAL and HLS revealed significant differences: In the ASVAL group the mean age was younger, the proportion of women was greater, the mean body mass index (BMI) was lower, the CEAP C4 to C6 class was less frequent (33% asymptomatic and 91% had no skin changes), the mean varicose reservoir was smaller (the mean number of zones to be treated [NZT] was lower) and the incidence of asymptomatic lower limbs was higher. The hemodynamic status of the lower limbs undergoing ASVAL also revealed significant differences: the SFJ was competent more often, its diameter was smaller, and the saphenous reflux was less often complete from the SFJ to the malleolus. They report a recurrence of 11.5% at 4 years with 78% having symptom improvement. They state that this is favourable when compared to recurrence rates observed after HLS [221] [222] (15% to 30%) or after RFA [223] [224] (22% to 23%) after 3 or 4 years of follow-up. They highlight that it in their ASVAL cohort, recurrences were accompanied by an absence of saphenous reflux in 17 of 24 cases, and that the reflux was unchanged compared with the preoperative situation in only three limbs, stating that this outcome is comparable with the midterm results of RFA in which approximately half of the recurrences appeared despite permanent obliteration of the GSV.[223] [224] They surmise that this finding further suggests an evolution of the varicose recurrence from the suprafascial venous network, unrelated to any persistent or recurrent GSV reflux, but further reflecting the secondary valvular/primitive ascending/ multifocal evolution of varicose veins. They conclude that the performance of isolated phlebectomy with the conservation of a refluxing GSV may be effective over the mid-term against the symptoms of superficial venous

insufficiency, for the disappearance of the varices, and for saphenous haemodynamics, with attainment of non-significant postoperative reflux in two-thirds of ASVAL treated limbs. The magnitude of the volume of the varicose reservoir appears to be the determining factor for the clinical and hemodynamic efficacy of this more limited surgical approach. They highlight that further long-term prospective studies will be necessary to delineate the indications for this therapeutic approach. Pittaluga *et al*calculated the cost of the procedures from insurance company reimbursement. The mean cost for each procedure was €758.88 ± 10.20 for ASVAL vs. €1143.96 ± 12.42 for HLS ($p < 0.05$), yielding a mean saving of €387.08 ± 14.35 per procedure for the ASVAL group. This treatment modality shows promise but is likely only useful in the treatment of minor to moderate varicose veins.

1.9 SUMMARY OF TREATMENTS AT OUTSET OF THIS THESIS

In 2008 Luebke *et al*performed a systematic review and meta-analysis of endovenous radiofrequency obliteration, endovenous laser therapy, and foam sclerotherapy for primary varicose veins. Twenty-nine EVLA studies, 32 RFA studies and 22 foam sclerotherapy trials were included. They reported RFA was associated with the worst short and long-term safety and efficacy results compared to EVLA and foam sclerotherapy regarding complete occlusion at the end of follow-up, phlebitis, deep vein thrombosis, and paraesthesia. EVLA had the best results concerning the long-term effectiveness parameters for occlusion at the end of follow-up and recanalisation, recurrence or development of new veins, compared to RFA and foam sclerotherapy. They reported that foam sclerotherapy of varicose veins is associated with a higher recurrence rate in patients with SFJ incompetence compared to the rates after EVLA or RFA treatment.[225]

A 2009 meta-analysis by van den Bos *et al*comparing minimally invasive varicose vein treatments with surgery reported on 64 eligible studies including 12,320 limbs, with a mean follow-up of 32.2 months compiles the majority of data available to guide treatment of varicose veins in the UK during the time period of this research. They report estimated pooled success rates (with 95% confidence intervals [CI]) for stripping, UGFS, RFA and EVLT were 78% (70%-84%), 77% (69%-84%), 84% (75%-90%) and 94% (87%-98%) respectively. After adjusting for follow-up, UGFS and RFA were as effective as surgical stripping (adjusted odds ratio [AOR], 0.12 [95% CI, -0.61 to 0.85] and 0.43 [95% CI, -0.19 to 1.04] respectively). EVLT was significantly more effective compared

with stripping (AOR, 1.13; 95% CI, 0.40-1.87), UGFS (AOR, 1,02; 955 CI, 0,28-1.75) and RFA (AOR, 0.71; 955 CI, 0.15-1.27).[125] van den Bos *et al*acknowledge that large, long-term comparative randomised controlled trials (RCTs) that include patient-reported outcomes, cost-effectiveness analyses and safety assessment are need to achieve level 1 medical evidence for the treatment of varicose veins.

1.10 SUMMARY OF MORE RECENT EVIDENCE

The CLASS trial was designed to compare the clinical effectiveness and cost-effectiveness of foam sclerotherapy, endovenous laser ablation (EVLA) and surgery for the treatment of patients with varicose veins.[226] The study was designed as a parallel-group randomised controlled trial (RCT) without blinding. Eleven UK specialist vascular centres were included. In total seven hundred and ninety-eight patients with primary varicose veins were included (foam, n = 292; surgery, n = 294; EVLA, n = 212). Patients were randomised between all three treatment options (eight centres) or between foam and surgery (three centres). Primary outcome measures being disease-specific [Aberdeen Varicose Vein Questionnaire (AVVQ)] and generic [European Quality of Life-5 Dimensions (EQ-5D), Short Form questionnaire-36 items (SF-36) physical and mental component scores] quality of life (QoL) were assessed at 6 months. Cost-effectiveness was examined as cost per quality-adjusted life-year (QALY) gained. Secondary outcome measures were quality of life at 6 weeks; residual varicose veins; Venous Clinical Severity Score (VCSS); complication rates; return to normal activity; truncal vein ablation rates; and costs. Considerations of both the 6-month clinical outcomes and the estimated 5-year cost-effectiveness suggest that EVLA should be considered as the treatment of choice for suitable patients.

Current NICE guidelines (2013)[227] on the treatment of varicose veins are that for people with confirmed varicose veins and truncal reflux:

- Offer endothermal ablation (radiofrequency ablation or endovenous laser treatment of the long saphenous vein).
- If endothermal ablation is unsuitable, offer ultrasound-guided foam sclerotherapy.
- If ultrasound-guided foam sclerotherapy is unsuitable, offer surgery.
- If incompetent varicose tributaries are to be treated, consider treating them at the same time.

1.11 COMPARISON OF HISTOLOGICAL CHANGES INDUCED BY MINIMALLY INVASIVE TREATMENTS

Duplex ultrasound follow-up after truncal vein foam sclerotherapy demonstrates recanalisation and recurrent reflux in 20-32% of limbs at 1-3 years, compared to a rate of 1-16% following RFA and EVLA.[228 229 230 14 125] It is likely that the more favourable early and mid-term occlusion rates offered by these techniques are the result of a greater damage inflicted upon the vessel wall. What remains unclear is whether the vessel damage produced by UGFS is likely to be sufficient to produce similar results to other minimally invasive varicose vein treatments. The above recanalisation rates suggest that the principle mode of action of UGFS is to promote thrombotic occlusion rather than permanent vein wall injury.

EVLA and RFA inflict tissue injury by thermal-dependent changes in collagen in the vein wall with loss of periodicity, dissolution and fusion of fibres, coagulation of collagen bundles and shrinking of smooth muscle, resulting in vessel contraction.[179 184 185] It therefore appears that denaturing collagen is necessary for sustainable vessel closure.[231] Whilst this has been demonstrated with EVLA

and RFA, associated with high ablation rates, little is known about the ability of either STD or polidocanol to achieve this.[232] [233]

Effective sclerosis (fibrosis) of a vein requires endothelial destruction and exposure of subendothelial collagen fibres to the sclerosant. This initiates the intrinsic pathway of blood coagulation by activating factor XII. Nevertheless the aim of sclerotherapy is not to merely achieve vein thrombosis, which may be amenable to recanalisation, but to achieve transformation into a fibrous cord.[104] [234] The latter is more likely to occur where the damage inflicted upon the vein wall involves both the endothelium and the subendothelial, medial layer.

Although sodium tetradecyl sulphate (STD) and polidocanol are the two most widely used sclerosant foams for treating varicose veins[150] [151] minimal data is available displaying the damage that they inflict upon the vein wall. Endothelial damage is enhanced by greater concentrations of sclerosant in smaller veins and delivery as foam prolongs the time of contact and amplifies the effect of the agent.[104] Longevity of the foam could therefore be a factor in determining the efficacy of these treatments. Differences between the longevity of the respective proprietary foams have been found by others using different strengths of these sclerosants.[235] STD foam is more stable (longer-lasting) than polidocanol at lower comparable concentrations and this finding is reversed with increasing concentrations of each. This may be related to the larger molecular size of polidocanol thus enhancing its surfactant and foaming properties at higher concentrations. Most literature (including product information) recommends a concentration of 3% for both sclerosants in the treatment of large superficial varicose or incompetent truncal veins.[139] [236] [237] Their relative stabilities at the 3% concentrations have not been compared though should this hold true it ought to be beneficial to the efficacy of polidocanol because of a longer contact

time between the sclerosant and the endothelial cells.[104] On the other hand in the treatment of varicose veins and telangiectasia with sclerotherapy there is some evidence that STD may be more effective than polidocanol.[153-155] Nevertheless the EASI study (using liquids not foams) showed that polidocanol 0.5% and 1% were as effective as STD in the treatment of patients with reticular and spider veins, and that more patients were satisfied with the effect of polidocanol because of more adverse events with STD.[234] Similarly, Goldman treated patients with varicose and telangiectatic veins (none with truncal vein incompetence) with either polidocanol liquid (0.5%, 1%) or STD liquid (0.25%, 0.5%) and observed that polidocanol and STD were equally effective although tissue necrosis and swelling was less common in the polidocanol group.[154] Rao et al, in the only study using foam also found that the two sclerosants had similar efficacy, tolerability and patient satisfaction.[155]

STD is invariably described as more potent than polidocanol in the order of 3:1, however most literature (including product information) recommends a concentration of 3% for both sclerosants in the treatment of large superficial varicose or incompetent truncal veins.[139 236 237] This was confirmed as common practice in a survey of members of the Vascular Society of Great Britain and Ireland in 2007.[238] Therefore this research compares STD with polidocanol each at a concentration of 3% as this is what is used and recommended in the majority of clinical practice.

Although there is much work describing the in-vitro effects of sclerosants on coagulation and cultured cell lines using normal arterial or superficial venous tissue there is minimal data on their effect on the superficial veins of patients with varicose veins.[239 16 240 241] Further there is no literature comparing the effects of STD and polidocanol on human veins.

Orsini *et al*described changes in the vein wall of GSV treated with 3% STD (in-vivo), reporting complete loss of endothelium after 2 minutes with subendothelial oedema developing after 15 minutes exposure to STD.[15] Ikponmwosa *et al*found almost complete endothelial cell loss after exposure to 1% and 3% STD after two minutes but with minimal subendothelial damage and no collagen disruption.[16]

At the very least a sclerosant which causes maximal endothelial destruction is likely to have a longer lasting therapeutic effect. Nevertheless even when complete endothelial loss is achieved re-endothelialisation may still occur via circulating endothelial progenitor cells.[242] [243] Early treatment failure following sclerotherapy is associated with recanalisation of the treated vein. For long-term success it is probable that denaturation of the cells in this layer (which results in contraction and fibrosis of the treated vein) is required (as per EVLT and RFA). It is possible that if the foam was in contact with the vein for longer that greater vessel injury may occur. To that end research preceding this, performed at Leeds Vascular Institute resulted in the development of a technique that increases the half-life of foam from around 160 seconds to greater than 15 minutes. Balloon mechanical denudation was also formerly assessed as a potential adjunct to foam sclerotherapy to enhance damage to both endothelium and media. [16]

1.12 RESEARCH QUESTIONS

Below are the main research questions to be examined in this thesis with a discussion as to how these questions have arisen and how the research aims to answer them.

The aims of this research t*o improve the efficacy of UGFS are*:

- assess the half-lives of the two most commonly used sclerotherapy foams worldwide
- assess *in-vitro* (using sections of GSV excised from patients undergoing varicose vein surgery) the histological effects of proprietary (STD and Polidocanol) foam sclerosants on vein wall integrity, to ascertain the optimal proprietary sclerosant
- assess *in-vitro* the histological effects of constituent parts of the proprietary sclerosant foams on vein wall integrity, to ascertain the most active ingredient
- assess *in-vitro* the histological effects of ‘longer-lasting’ foam sclerosants on vein wall integrity, to ascertain that producing maximal effect on the vein wall
- assess *in-vitro* the histological effects of cutting balloons upon vein wall integrity, to assess their possibility as mono-therapy for varicose veins or as adjuvant to enhance foam sclerotherapy.
- Identify the most promising treatment(s) from the above and apply this in an animal model

1.12.1 WHAT IS THE HALF-LIFE OF FOAMS CURRENTLY USED FOR UGFS AND DOES THIS AFFECT EFFICIACY?

This research focuses on the use of foams for treating truncal varicosities of the great or small saphenous systems. As stated previously STD is widely regarded as more potent than polidocanol, in the order of 1:3 respectively.[152-155] Despite the acknowledged difference in potency, the majority of world literature (including product literature) recommends a concentration of 3% for both sclerosants in the treatment of truncal varicosities.[139 236 237 244-246] In a survey of members of the Vascular Society of Great Britain and Ireland in 2007, the majority of those who responded used 3% (STD or polidocanol) for the treatment of truncal varicosities.[238] 3% is therefore the concentration of each sclerosant that were used for studies comparing their relative half-lives.

Sodium tetradecyl sulphate (STD; Molecular Formula $C_{14}H_{30}O_4S$) is listed on PubChem (National Centre for Biotechnology Information; USA) as a sclerosing solution.[247] PubChem describes STD as an anionic surface-active agent used for its wetting properties in industry and used in medicine as an irritant and sclerosing agent for haemorrhoids and varicose veins as well as gastric varices, oesophageal varices and in peptic ulcer haemorrhage.[247] It is made by a number of manufacturers most commonly marketed in the UK as Fibro-vein™ (STD Pharmaceutical products Ltd, Hereford, UK).

Polidocanol (Molecular Formula $C_{14}H_{30}O_2$) is listed on PubChem as a detergent and sclerosing solution, the former described as purifying or cleansing agents, usually salts of long-chain aliphatic bases or acids, that exert cleansing (oil-dissolving) and antimicrobial effects through a surface action that depends on possessing both hydrophilic and hydrophobic properties. Polidocanol was first synthesised in 1936 and marketed as a topical and local anaesthetic.[150] This urethane local anaesthetic differs from classic ester and amide anaesthetics

agents as it lacks an aromatic ring. The ability of polidocanol to sclerose blood vessels without significant damage to surrounding tissues led to its use as a sclerosant in the 1960s. In 1967 it was registered in the Federal Republic of Germany as aethoxysklerol (Kreussler & Co. GmbH) and is now the only sclerosant registered for use in Germany.[150] Polidocanol (Asclera injection, Bioform Medical Inc, San Mateo, Calif) was approved for use in the USA in 2010.[104] Though currently unlicensed in this country, polidocanol is certainly used by a number of practitioners in the UK.[238]

A comparison of the chemical structure of polidocanol and STD in Figures 1-1, 1-2 and 1-3 shows that the polymer backbone of polidocanol is longer than STD. This tends to provide greater stabilisation of polidocanol foam through steric and electric repulsion at the gas-liquid interface, reducing coalescence and disproportionation.

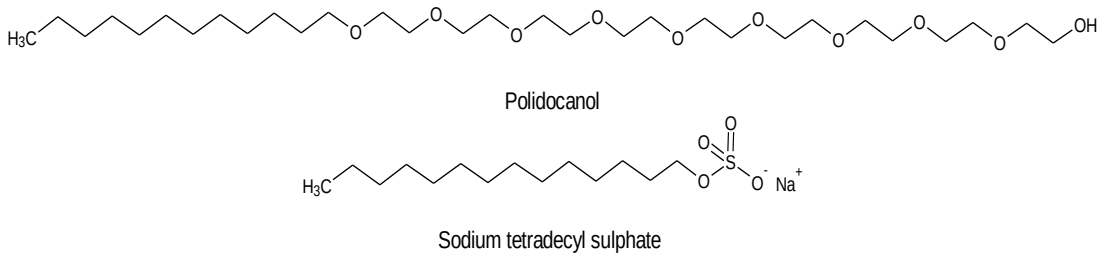


Figure 1.3: Chemical structures of polidocanol and sodium tetradecyl sulphate.

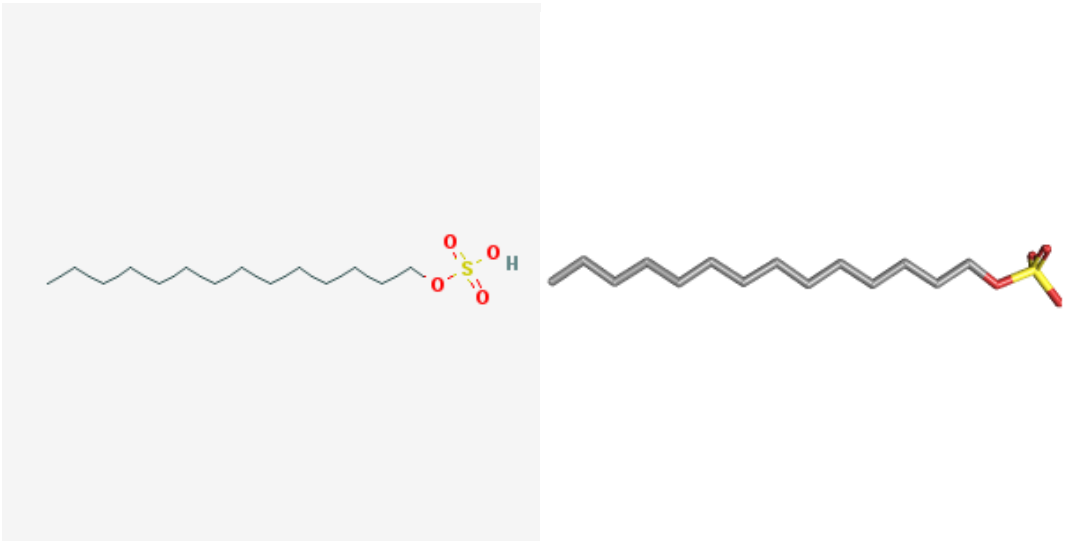


Figure 1.4: Pubchem images of STD in 2D and 3D

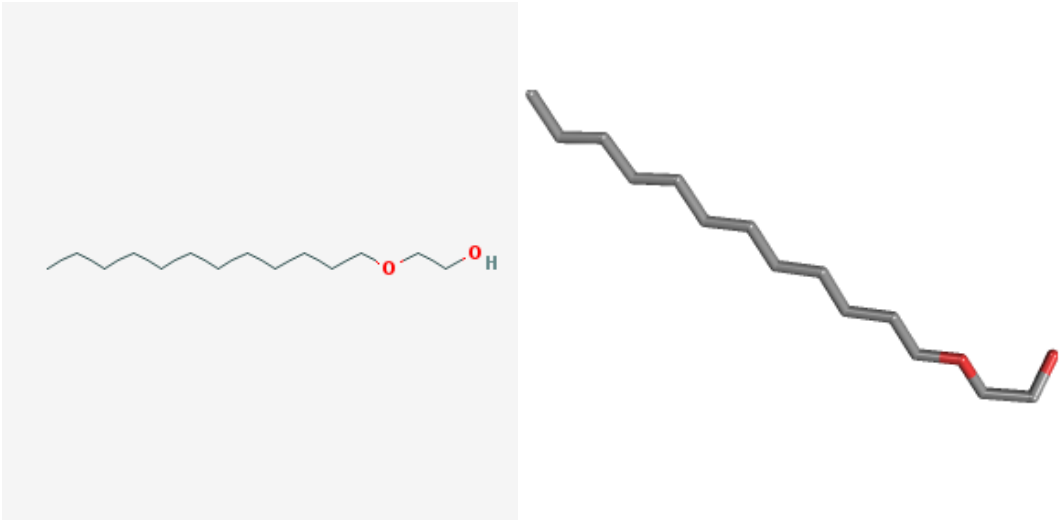


Figure 1.5: Pubchem images of polidocanol in 2D and 3D

UGFS as the name implies utilises sclerotherapy in the form of foam to enhance the efficacy of sclerotherapy by increasing the surface area of the treated vein contacted by the sclerosant. A question previously unanswered in the literature is how the half-lives of the two most commonly used sclerosants world-wide compare and similarly how they compare in *in-vitro* testing regarding damage to the vein wall. The first of these questions is answered in chapter three and the second in chapter four with the method of experimentation presented below.

Foam is commonly made from gas and liquid in a ratio of four parts of gas and one of liquid sclerosant. This is based on physical characteristics produced by different ratios of foam.

The most commonly used technique for preparation of sclerosant foams was developed by Tessari *et al*using a three-way-tap with ratios from 1:3 to 1:5, sclerosant to air (or using physiological gas e.g. CO_2 & O_2 mixture).[104 139 248] The most commonly used gas in clinical practice is room air.[249] At the 2003 European Consensus Meeting on Foam Sclerotherapy, sclerosing foam was defined as a dispersion of gas in a liquid sclerosing solution where the gas is physiologically tolerated at therapeutic doses.[250] The 2nd European Consensus Meeting on Foam Sclerotherapy 2006 stated that “The preferred ratio of liquid to gas for preparing sclerosant foam for all indications is 1:4 (1 part liquid : 4 parts gas).”[139] However, much of the evidence upon which they based this decision was research done using polidocanol and a modification of Tessari’s, Tourbillion technique.[139 251 252] They also state that for large truncal vessels a viscous foam should be utilised.[139] Previous research at Leeds Vascular Institute has tested ratios of 1:3 and 1:4 liquid (1% and 3%) STD:air and found for both concentrations of STD, 1:3 forms more stable foam than 1:4. 3% STD or polidocanol foams have a longer half-life than their respective 1% foams. This is expected due to the intrinsic surfactant properties of these detergents.[253] Hence for the purposes of the foam stability studies in this research, foam was prepared using Tessari’s technique (1:3 ratio of sclerosant : air) with 20 passes through a double syringe system (Figure 2.4) and immediately transferred to a preheated (37°C) 15 ml graduated polyester tube (Figure 2.5).

Previous research on foam stability and degradation was performed at Leeds Vascular Institute in conjunction with The Institute for Pharmaceutical Innovation at Bradford University. This confirmed that the rate of destabilisation of these foams is non-linear displaying periods of apparent stability before sudden decreases in the volume of foam as demonstrated by the experimental data in Figure 2.6 showing the degradation of three runs of STD 1% foam. McMaster describes this from the perspective of liquid reformation, which though stated to be predictable, does not progress at a constant rate.[254]

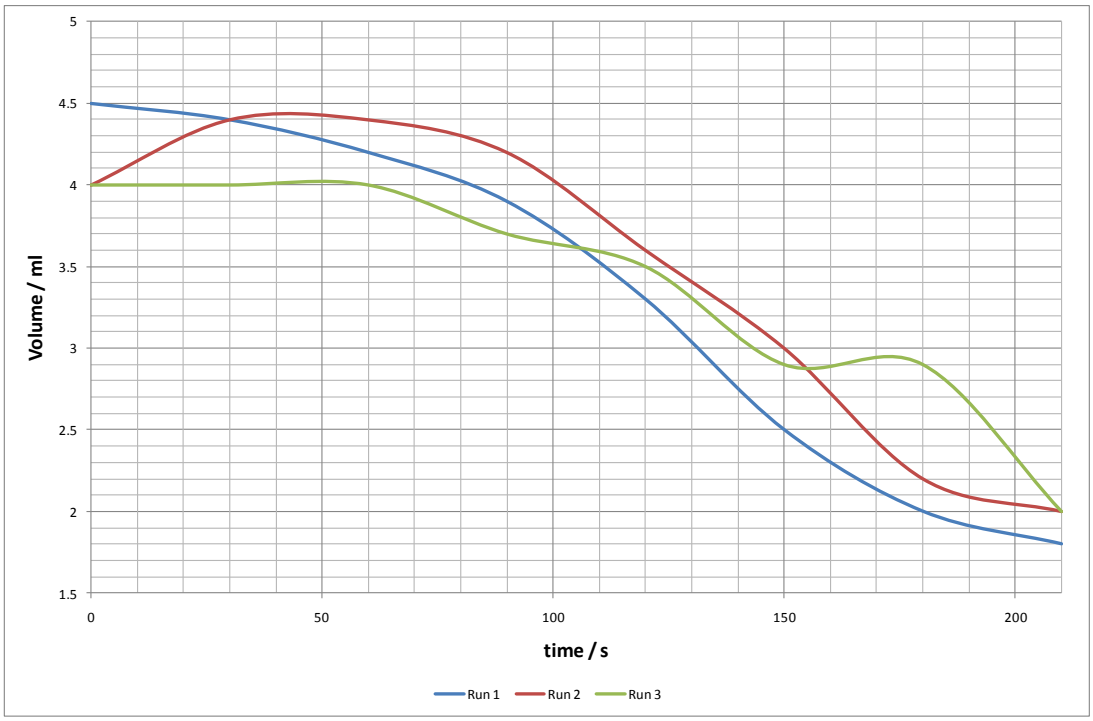


Figure 1.6: Volume vs. Time for fibro-vein™ (STD) 1% (1:3), results demonstrate the foam destabilisation is non-linear

Further complications arise because the initial volumes of foam often vary between experiments. The rate of change (in the foam volume) cannot therefore be used as a measure of foam stability. Instead the experimental data was converted to percentage (of foam remaining) as a function of time with the time taken for a 10% decrease in the overall volume (T_{90}) and for a 50% decrease in

the overall volume of the foam / liquid (T_{50}) used as a measure of the foam stability, with higher values meaning a more stable foam. A high foam fraction is however essential for maximum effectiveness of Fibro-Vein™ (STD), in order to maintain contact with the vein walls. This is not necessarily indicated by a high value for T_{50}. T_{90} was therefore also calculated as an additional indicator of foam stability.

We determined the T_{90} and T_{50} values for STD (Fibro-Vein™) and polidocanol (Sclerovein®) 3% solution for comparison. The raw experimental data does not allow comparison of the results. All experimental data was therefore converted to percentage (of foam) vs. time data. The time taken for the volume of foam to drop by 10% (T_{90}) and 50% (T_{50}) were then calculated from the converted experimental data. These are similar values sought in other research into foam degradation. The T_{50} should not be confused with the term “Foam half time” (FHT) which is the time (in seconds) it takes to form a liquid layer at the bottom of the foam column that is half the volume of the original liquid.[253]

A hypothetical plot of the percentage volume of the foam (**X**) against time (**T**) is shown in Figure 1.7. The value **z** is the cut off value for the percentage of the foam, i.e. 50% or 90% and T_z is the time taken for the volume of the foam to reach this percentage. It is this value which is used for evaluation of the stability of the foam where **z** equals 90% and 50% of foam volume at 30 seconds. (Volume at 0 seconds is not used as the foam takes time to settle).

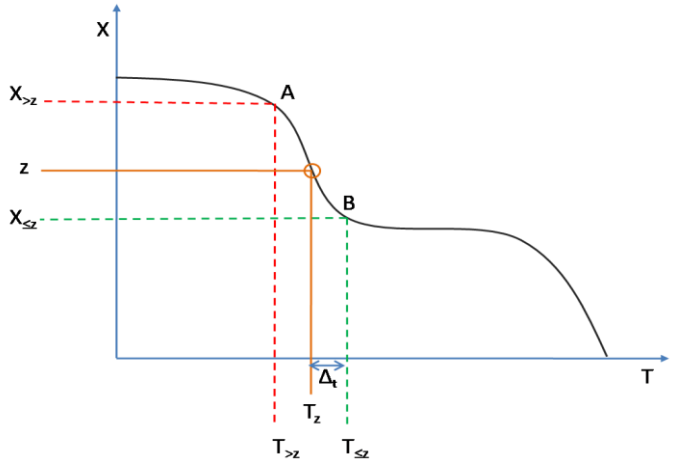


Figure 1.7: A hypothetical plot of the percentage volume of the foam (x) against time (t).

$X_{>z}$,$X_{\leq z}$ and **$T_{>z}$,$T_{\leq z}$** are known experimental points whose values are closest to **z** (and **T_z**) (**z** must fall between these values). $X_{>z}$ is the percentage of foam greater than Z and $T_{>z}$ is the corresponding time as shown in Figure 2.7, while **$X_{\leq z}$** is the percentage of foam lower or equal to Z and **$T_{\leq z}$** is the corresponding time i.e. the time taken for the volume of the foam to drop before Z. From these values the gradient of the graph between point A and point B can be calculated using equation 1.

EQUATION 1

$$\frac{\mathbf{X_{\leq z} - X_{>z}}}{\mathbf{T_{\leq z} - T_{>z}}} = \mathbf{\Delta x}$$

From the gradient of the graph the time taken (Δt) for the volume of the foam to drop from Z (90%) to $X_{\leq z}$ (86.36%) can be calculated using equation 2.

EQUATION 2

$$\frac{\mathbf{z - X_{\leq z}}}{\mathbf{\Delta x}} = \mathbf{\Delta t}$$

This value can then be used to calculate T_z using EQUATION 3. In terms of experimental data, an excel spread sheet template was used to perform the

necessary calculations, however $X_{>z}$, $X_{\leq z}$ and $T_{>z}$, $T_{\leq z}$ still had to be identified for each experimental run as this function is not available with excel.

EQUATION 3

$$\mathbf{T_z = T_{\leq z} + \Delta t}$$

Figure 2.8 shows a cross section of a results template, the experimental values for calculating $\mathbf{T_z}$ where **z** equals 90% are shown. As can be seen from the table these values are selected because **z** falls within the associated range for the percentage volume. The values for $\mathbf{T_{>z}}$ and $\mathbf{T_{\leq z}}$ are the corresponding values for time. An example of the calculations using the values from Figure 2.8 is given below:

$$\mathbf{\Delta x = \frac{X_{\leq z} - X_{>z}}{T_{\leq z} - T_{>z}}} = \frac{86.36 - 95.45}{120 - 90} = \mathbf{-0.30\%\ s^{-1}}$$

$$\mathbf{\Delta t = \frac{z - X_{\leq z}}{\Delta x}} = \frac{90 - 86.36}{-0.30} = \mathbf{-12.0\ s}$$

$$\mathbf{T_z = T_{\leq z} + \Delta t} = 120 + -12.0 = \mathbf{108.0s}$$

Time /s	Volume / ml			Percentage		
	1	2	3	1	2	3
0				-	-	-
30	4.5	4.4	4.3	100.00	100.00	100.00
60	4.5	4.4	4.3	100.00	100.00	100.00
90	4.3	4.2	4.2	95.56	95.45	97.67
120	3.9	3.8	3.8	86.67	86.36	88.37
150	3.2	3.2	3.2	71.11	72.73	74.42
180	2.6	2.6	2.6	57.78	59.09	60.47
210	2.2	2.2	2.1	48.89	50.00	48.84
240	1.9	1.9	1.9	42.22	43.18	44.19
270	1.7	1.7	1.6	37.78	38.64	37.21

$T_{>z}$ $T_{\leq z}$ $X_{>z}$ $X_{\leq z}$

Figure 1.8: Section of results spread sheet showing values used to calculate Tz where z is 90%.

As T_z is a calculated value it should be noted that this value is an approximation and that the experimental value may differ slightly.

This method was used to calculate T_{90} and T_{50} for both sclerosants via 10 runs used per foam. Median values (with inter-quartile ranges) were used for statistical analysis.

In summary, the rate of destabilisation of foam is non-linear with periods of apparent stability before sudden decreases in the volume of foam.[254] Further, the initial volume of the foam varied slightly between experiments. Thus, rate of change in foam volume cannot be used as a measure of foam stability. Instead the experimental data was converted to percentage of foam remaining against time with the time taken for a 10% (T_{90}) and 50% (T_{50}) decrease in volume used as a measure of foam stability.

Hence the methods details experiments undertaken to compare the half-lives of the two foams.

1.12.2 DOES INCREASING THE HALF-LIFE OF FOAM IMPROVE ITS EFFICACY?

We hypothesize that foam sclerosants with a longer foam half-life are more active against the vein wall and therefore potentially more efficacious. Longer-lasting foams were previously developed through Leeds Vascular Institute in

conjunction with the Institute for Pharmaceutical Innovation at Bradford University. These included STD 3%/pluronic 5%, STD 3%/carbomer 0.45%, and STD 3%/xanthum 0.15%. Polymers such as pluronic cause stabilisation of foams through electric and steric repulsion.[255] Xanthan gum and carbomer are viscosity enhancing agents which aid foam stabilisation by increasing the viscosity of the continuous phase which inhibits the key mechanisms of destabilisation – drainage, coalescence and disproportionation.[256] These products were the most promising of many longer-lasting sclerosant foams tested at Bradford University and the strengths used were those which provided the longest lasting foams.

These foams were used in experiments detailed in chapter two to test the hypothesis that longer-lasting foams would be more efficacious in the treatment of varicose veins.

1.12.3 DO CUTTING BALLOONS INCREASE THE EFFICACY OF UGFS?

Previous research at Leeds Vascular Institute demonstrated that despite Fogarty balloon denudation increasing the overall endothelial cell loss when used as an adjunct to foam sclerotherapy, it did not increase the depth of injury caused by the foam.[16] Those experiments used 5 Fr LeMaitre® embolectomy catheters with a balloon diameter 13.0 mm**.**

This thesis examines the potential for cutting balloons, those licensed for arterial work, to act as adjuncts to UGFS. Cutting balloons have three or four atherotomes (microsurgical blades) mounted along the length of a non-complaint balloon, which expand radially and deliver longitudinal incisions to the vessel, designed to relieve the radial stress of arterial atherosclerotic stenoses. The cutting balloon is designed to protect the vessel from the edges of the

atherotomes when it is deflated minimising the risk of trauma to the vessel as the balloon is passed to and from a target lesion. We hypothesise that cutting balloon atherotomes used immediately prior to foam sclerotherapy will cut the GVS media in up to four places (depending on the balloon design) allowing influx of foam sclerosant deeper into the vein media, thereby increasing the damage caused by the foam sclerosant and thereby enhance its efficacy.

1.12.4 HUMAN VARICOSE VEIN MODEL

It is widely believed that to effectively sclerose (fibrose) venous tissue, sclerosants must produce total endothelial destruction resulting in the exposure of subendothelial collagen fibres (an obligate step in thrombogenesis) to sclerosants, which initiates the intrinsic pathway of blood coagulation by activating factor XII.[257] The purpose of sclerotherapy is not merely to achieve thrombosis of the vessel, which per se may be amenable to recanalisation, but definitive transformation into a fibrous cord.[234] The latter is more likely to occur where damage inflicted upon the vein wall initially occurs not only to the endothelium but to the subendothelial medial layer of vein wall. This is the case with other minimally invasive treatments including endovenous laser ablation (EVLA) and radiofrequency ablation (RFA).[232 233 258-260] Foam sclerotherapy has early recanalisation rates of up to 32% as well as poor medium to long-term occlusion rates compared to these other minimally invasive treatments.[261-263] Therefore one might expect that the more localised superficial venous damage caused by detergent foam, the more likely it is have better early and durable occlusion rates. Obviously a dose response curve is also likely whereby this benefit is limited by production of side-effects. Much work has been published on the in-vitro effects of detergent sclerotherapy agents on coagulation, cultured cell lines or animal studies or using normal arterial or superficial venous

tissue.[239-241] [264-266] However there is minimal data on the effect on human varicose vein tissue.[15] [16] There is no literature comparing the effects of STD to polidocanol, the two most widely used sclerosants worldwide, in human varicose vein tissue in-vitro.

The majority of experimentation for testing treatments for varicose veins is performed on in-vitro cell lines or in-vivo in animal models.[131] [232] [233] [239] [240] In-vivo animal models are expensive and ideally reserved until preliminary research in other models directs the most useful animal studies, for monetary and ethical reasons. There is remarkably little published research using human vein in-vitro models for assessing varicose vein treatments.[185] Rotter *et al*described a model using vein left over from cardiac bypass surgery.[241] This has the disadvantage of having a different make up to GSV in patients with varicose veins as it is recognised that such veins have different medial collagen type and deposition. A model for varicose vein experimentation was developed in recent years at Leeds Vascular Institute which uses sections of proximal GSV dissected out pre-stripping at varicose vein surgery. This model has the advantage of using GSV tissue from patients with proven varicose veins on clinical and duplex examination with all having documented significant SFJ incompetence.[16]

1.10.5 ANIMAL VEIN MODEL

Upon obtaining results from the above experimentation the aim is to test any promising modifications of UFGS and related adjuncts in an animal model. The objective of this research section will be to ascertain the haemodynamic, ultrasonographic and histopathological changes of superficial veins treated with licensed foam sclerotherapy, “long-lasting” (unlicensed for human use) foam sclerotherapy and endovenous catheter balloon (fogarty and cutting) therapy (unlicensed for human use), versus control. The study endpoints were ideally to be:

1. Haemodynamic changes: patency, reflux and ultrasonographic changes
2. Histopathological analysis: degree and extent of tunica intima and media damage

This research focuses on the treatment of GSV truncal varicosities therefore directing treatment at the GSV. An animal model was sought that would mimic human GSV. It is well documented that varicose veins are a disease of homo-sapiens and do not naturally occur in other animals.[267] Anatomical and clinical studies in humans indicate that in the presence of varices, not only is the SFJ competent in >50% of cases,[215 216] but moreover the GSV is often partially or wholly competent.[9 217] The majority of studies of patients with primary varicosities have revealed a complete absence of varicosities within the saphenous trunks or at most segmental dilatations.[67 89 90] Labropoulos *et al*found a prevalence of varicosities in either or both the GSV and SSV of <7%.[10] Hence for the purposes of this research it was decided not to attempt to

create an incompetent animal model as has been previously used in other related research.[267]

A suitable animal model required a superficial truncal vein which entered the deep venous system in a similar fashion to the SFJ in humans. It requires a similar structure and dimension to human superficial truncal veins. It also requires to be affected by gravity i.e. vertical leg vein as opposed to veins that have been used in some animal models, e.g. superficial epigastric veins, which are horizontal to the ground and would not be subject to the same gravitational forces or those of compression etc caused by movements of the leg and calf and thigh muscle groups.[131] Animal models previously used for varicose vein treatment research include pigs, sheep and goats.[131 267 130 233 268] Overarching many of the requirements of the animal vein model necessary were the fact the animal needed to be amenable to having procedures performed whether they were under sedation or general anaesthetic and therefore that the animal would withstand these procedures. As well as requiring the veins and animal to be of a suitable size at initial treatment, it was also necessary that the animal be of an appropriate size after an adequate time to allow response to treatment. An interval of 12 weeks / 3 months was chosen as the time interval before treated vein segments were to be harvested.

The assessment of suitable models involved practical experimentation and is included in chapter two.

1.13 RESEARCH QUESTION; AIMS AND OBJECTIVES

Despite 150 years of unregulated human experimentation encompassing a range of more or less toxic agents, the “perfect sclerosant,” free from complications and causing permanent vein occlusion has not been found. All sclerosants represent a compromise between efficacy and toxicity.[150] UGFS has vastly improved the efficacy of this treatment and shows much promise and versatility in the treatment of varicose veins. It can be used to treat thread veins and superficial as well as truncal related varicose veins. It is cheap, quick, relatively easily performed and easily repeatable. It remains to be seen whether clinicians and health services as well as patients, deem the current requirement for some patients to require repeat treatments as being preferable to other treatments that at least currently appear to need fewer treatments to achieve “completion”. If it is possible to improve the longevity of results achieved with UGFS it would surely represent a much more favourable treatment option for patients, clinicians and health services alike.

This thesis examines the hypothesis that if the foam itself can be made to last longer or an additional synergistic treatment be performed in the treated vein segment this should allow increased time for delivery or penetration of the active ingredient into the vein wall thereby hopefully improving the efficacy of UGFS.

We hypothesise that it may be possible to improve its efficacy by:

1. increasing the half-life of the foam
2. using adjuncts to synergistically assist the activity of the foam

Modified longer lasting foams have been developed at Leeds Vascular Institute and this thesis tests each of these longer lasting foams in an in-vitro human model with the aim to test the best performing in an in-vivo animal model. Similarly balloon denudation has been assessed at Leeds Vascular Institute as an adjunct to UGFS, and this work shall be continued with cutting balloons and again tested in an in-vitro human model and in an in-vivo animal model.

Before performing these tasks it is necessary to review our current knowledge about sclerosants foams with respect to:

1. their longevity
2. what injury do they inflict upon the veins
3. which sclerosant appears to inflict the most damage

This study is designed to complete the aforementioned research at Leeds Vascular Institute providing information on the activity of proprietary foams on the vein wall, as well as that of Longer lasting sclerosant foams and that of balloons including cutting balloon adjuncts.

CHAPTER II

METHODS

2 METHODS

This chapter presents the methods used in all experiments conducted as part of this research.

2.1 IN-VITRO FOAM HALF-LIFE STUDIES

This research focuses on the use of foams for treating truncal varicosities of the great or small saphenous systems. Despite the acknowledged difference in potency, the majority of world literature (including product literature) recommends a concentration of 3% for both sclerosants in the treatment of truncal varicosities.[139 236 237 244-246] 3% is therefore the concentration of each sclerosant that were used for studies comparing their relative half-lives.

Previous research on foam stability and degradation was performed at Leeds Vascular Institute in conjunction with The Institute for Pharmaceutical Innovation at Bradford University. This confirmed that the rate of destabilisation of these foams is non-linear displaying periods of apparent stability before sudden decreases in the volume of foam. A problem with these experiments was that the initial volume of foam often varied between experiments. The rate of change (in the foam volume) cannot therefore be used as a measure of foam stability. Instead the experimental data was converted to percentage (of foam remaining) as a function of time with the time taken for a 10% (T_{90}) or 50% decrease in the overall volume of the foam / liquid (T_{50}) used as a measure of the foam stability, with higher values meaning a more stable foam. A high foam fraction is considered essential for maximum effectiveness of Fibro-Vein™ (STD), in order to maintain contact with the vein walls. This is not necessarily indicated by a

high value for T_{50}. T_{90} was therefore also calculated as an additional indicator of foam stability.

We determined the T_{90} and T_{50} values for STD (Fibro-Vein™) and Polidocanol (Sclerovein®) 3% solution for comparison. The experimental data was converted to the percentage (of foam) vs. time data, as indicated above. The time taken for the volume of foam to drop by 10% (T_{90}) and 50% (T_{50}) were then calculated from the converted experimental data. These are similar parameters to those measured in other research into foam degradation. The T_{50} should not be confused with the term “Foam half time” (FHT) which is the time (in seconds) it takes to form a liquid layer at the bottom of the foam column that is half the volume of the original liquid.[253]

Thus the initial foam volume was recorded and subsequent readings were taken every 30 seconds until the volume of the foam fell below 25% of its original volume. Experiments were conducted at 37^{o}C (Stuart Incubator S16D, Bibby Scientific Ltd, Staffordshire, UK), (Figure 2.1). 10 runs were performed for both 3% STD and 3% polidocanol foams.

2.1.1 FOAM HALF-LIFE EXPERIMENTAL METHOD

3% STD and 3% polidocanol foams were produced using the Tessari technique with a ratio of 1:3 (1 part liquid to 3 parts air) (Tessari 2000):

- 2mL of liquid 3% STD (Fibro-vein®, STD Pharmaceutical Products Ltd, England) or polidocanol (Sclerovein, Resinag Pharmaceutical and Health Care Products, Switzerland) drawn into the first of two 10mL syringes (BD Elastipac, Becton Dickinson Infusion Therapy, Helsingborg, Sweden) using an 18 gauge drawing up needle (Terumo Medical Products, USA).
- 6mLs of air drawn into the second syringe.
- Syringes connected at 90^{o} three-way tap (BD connecta™, Becton Dickinson Infusion Therapy, Helsingborg, Sweden)
- Contents mixed to produce foam by 20 passes of each syringe plunger. (Figure 2.2)

2mL of liquid was used to produce foam in 10mL syringes. This volume was selected in order to facilitate recording of foam volumes during the experiments. On completion the foam was transferred to a preheated 15mL graduated polyester tube. The initial foam volume was recorded and subsequent readings were taken every 30 seconds until the remaining volume of the foam fell below 25% of its original volume. (Figure 2.3) All experiments were conducted at 37 ^{o}C using a Stuart Incubator (Bibby Scientific Ltd, Staffordshire UK).

Figure 2.1: Stuart Incubator S16D (Bibby Scientific Ltd, Staffordshire, UK), At 37^{o}C

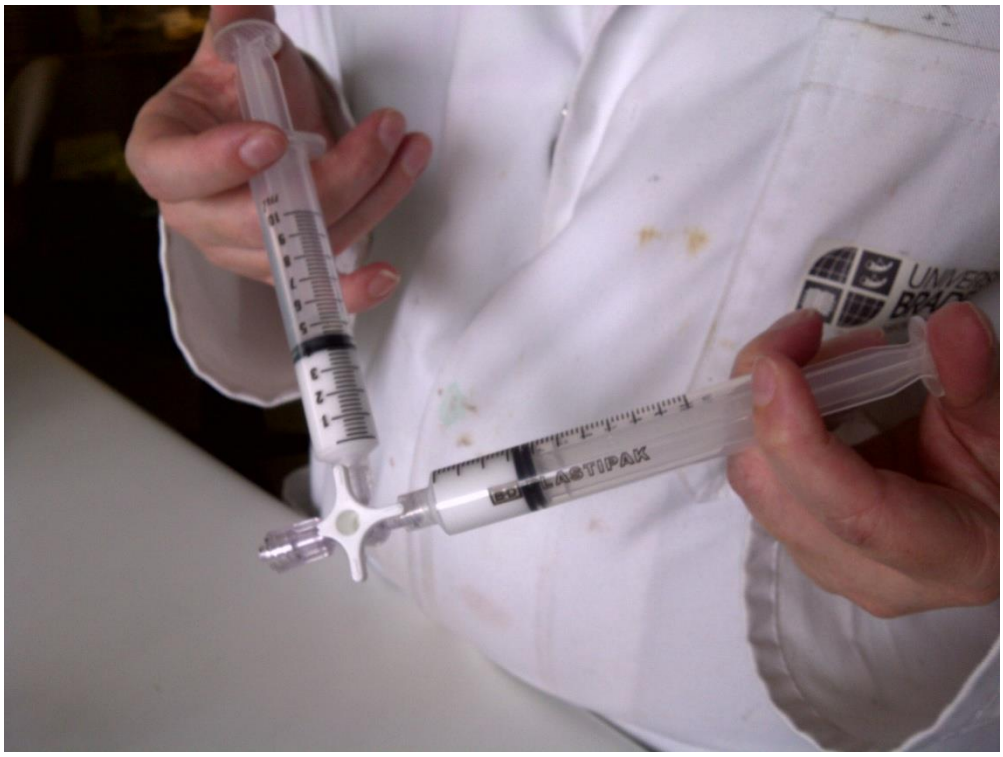


Figure 2.2: Foam preparation for stability studies; Tessari's technique 1:3 sclerosant: air, 20 passes via fully open three way tap without filter.

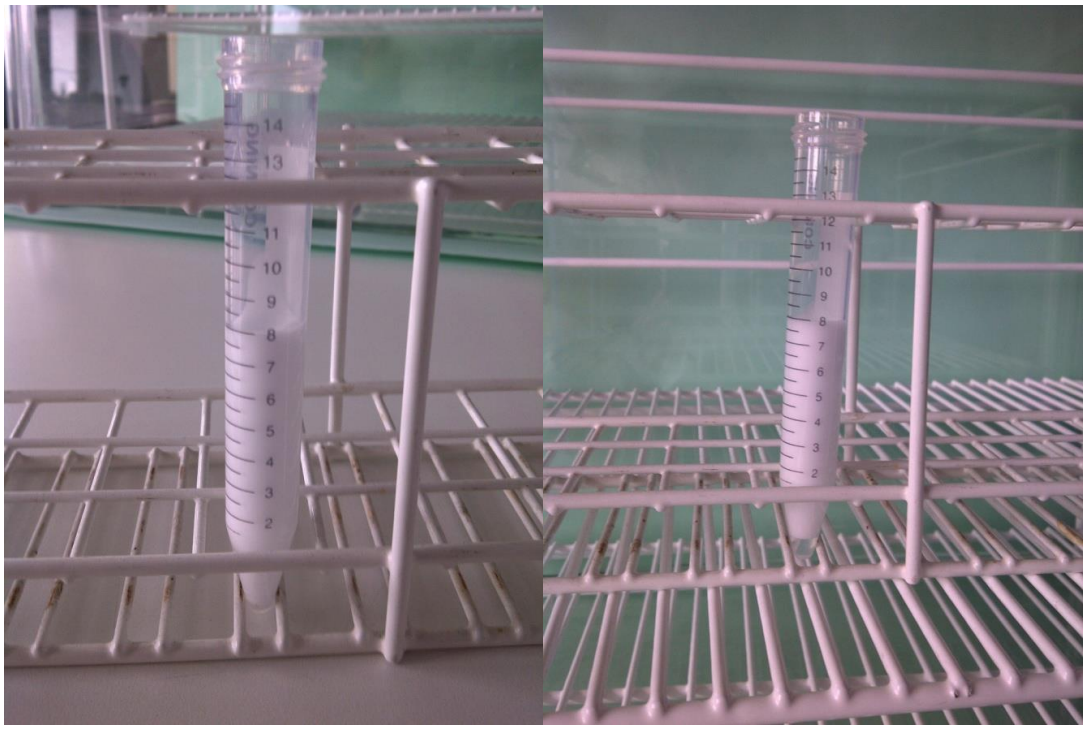


Figure 2.3:pre-heated graduated polyester tube (incubator at 37°c)

2.2 *IN-VITRO* HUMAN GSV EXPERIMENTS

Very little literature exists examining the effects of sclerotherapy *in-vitro*, foam or liquid, on human vein wall. Beyond this the basis of this thesis was to examine whether foam sclerosants with a longer foam half-life inflicted a greater injury on the vein wall and thus be potentially more efficacious. The experiments detailed below display the method used to obtain human GSV to allow *in-vitro* experimentation. Foam sclerosants including the two proprietary agents most commonly used world-wide were tested using our *in*-vitro experimental model.

Longer-lasting foams developed through Leeds Vascular Institute in conjunction with the Institute for Pharmaceutical Innovation at Bradford University were developed using the experimental model described. These included 3% STD and 5% pluronic, 3% STD and 0.45% carbomer and 3% STD and 0.15% xanthum, each of which was tested for 5 and 15 minutes as per section 2.2.2. Polymers such as pluronic cause stabilisation of foams through electric and steric repulsion.[255] Xanthan gum and carbomer are viscosity enhancing agents which aid foam stabilisation by increasing the viscosity of the continuous phase which inhibits the key mechanisms of destabilisation – drainage, coalescence and disproportionation.[256] These products were the most promising regarding their longevity, of many longer-lasting sclerosant foams tested at Bradford University and the strengths used were those which provided the longest lasting foams. It should be noted that addition of stabilising agents reduces the strength of the sclerosant. The proportions are detailed in Table 2.1. Hence the actual strength of STD in each of the products is 2.857% STD and 5% Pluronic, 2.988% STD and 0.4% Carbomer and 2.995% STD and 0.15% xanthum. Experiments were carried out for 5 and 15 minutes each as per section 2.2.2.

	% additive	Dosage of STD in 3% solution after additive
	0.1	2.997
Xanthum	**0.15**	**2.995**
	0.2	2.996
	0.3	2.991
Carbomer	**0.4**	**2.988**
	0.5	2.985
	1	2.970
	2	2.941
	3	2.912
	4	2.884
Pluronic	**5**	**2.857**
	6	2.830
	7	2.803
	8	2.777
	9	2.752
	10	2.727

Table 2.1: Relative strength of "3%" STD in longer-lasting foams produced at Bradford institute for pharmaceutical innovation

2.2.1 PATIENT RECRUITMENT

Patients with primary varicose veins with documented ultrasound evidence of SFJ incompetence and GSV reflux, listed for surgery (SFJ ligation and GSV stripping) were approached prior to surgery. All participants were provided with an information sheet and written informed consent was obtained to allow laboratory analysis on a segment of GSV removed at surgery and for a blood

sample to be taken for use during experiments (Appendix I). Ethical approval was granted by the local research and ethics committee.

2.2.2 HARVESTING OF HUMAN GSV FOR IN-VITRO EXPERIMENTS

In summary a groin skin crease incision was made medial to the femoral pulse, the superficial fascia incised and the GSV identified. After ligation and division of minor tributaries, the SFJ was identified and standard SFJ ligation was performed. *In-vitro* experiments required a 3-5cm length of proximal GSV to act both as test and control section. Upon disconnection of the SFJ this proximal section of the GSV was excised with minimal trauma and instrumentation during SFJ ligation prior to placement of the pin stripper in order to minimise the risk of mechanical injury (Figure 2.4). The use of diathermy was avoided. Once harvested, (Figure 2.5) the section was divided into two; one half test, the other half control segment.

A heparinised syringe with 22Gauge needle was used to extract blood from the distal GSV prior to stripping. This was used to control for pressure effects in controls and for tissue preservation (Figure 2.6).

Experiments were performed on the vein segment in a side-room off the operating theatre in order to minimise the time between excision and experimentation.

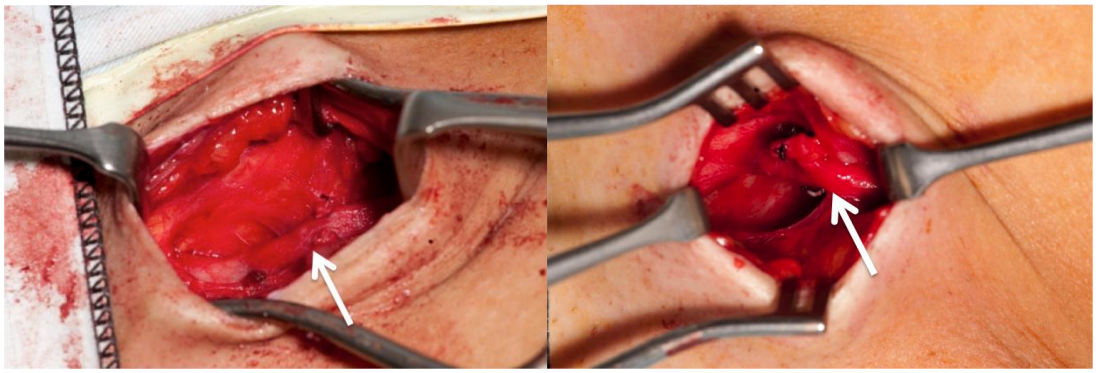


Figure 2.4: Dissection of the proximal gsv using minimal instrumentation to obtain a 3-5cm segment

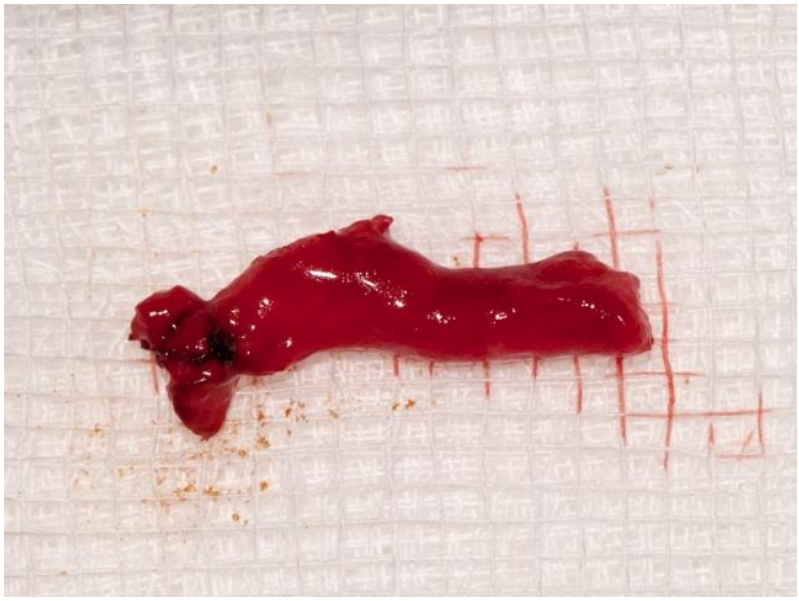


Figure 2.5: 3-5cm segment of proximal GSV to be divided into test and control segment

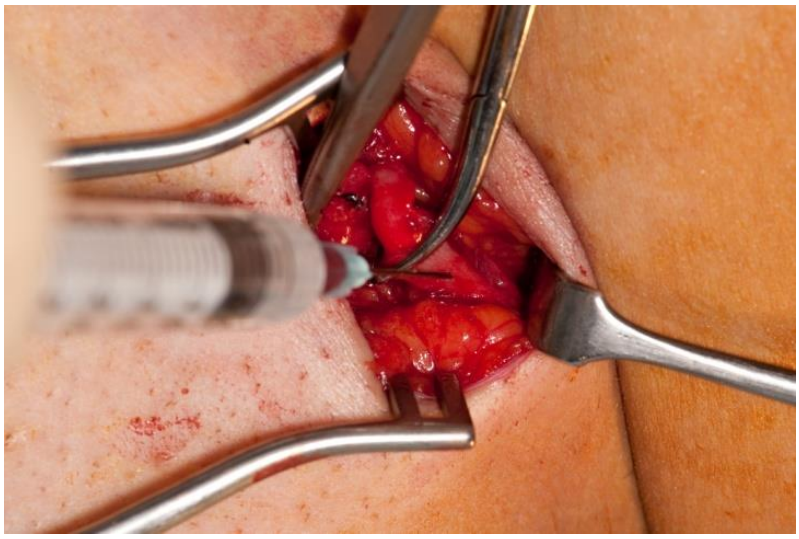


Figure 2.6: Heparinised syringe used to extract blood (for controls and tissue preservation) from distal GSV prior to stripping

2.2.3 PROTOCOL FOR IN-VITRO EXPERIMENT TECHNIQUE FOR COMPARISON OF TESTS CHEMICALS WITH CONTROLS

- Vascutek® clip or small artery clip placed at one end of the test specimen to occlude it.
- Foam produced using the Tessari technique (Figure 2.7).
- 0.5mL of liquid sclerosant drawn into the first of two 2mL syringes (BD Elastipac, Becton Dickinson Infusion Therapy, Helsingborg, Sweden) using an 18 gauge drawing up needle (Terumo Medical Products, USA).
- 1.5mLs of air drawn into the second syringe.
- Syringes connected at 90^{o} three-way tap (BD connecta™, Becton Dickinson Infusion Therapy, Helsingborg, Sweden)
- Contents mixed to produce foam by 20 passes of each syringe plunger.
- Foam placed in the lumen of the vein specimen via a foreshortened 20 Gauge IV cannulae (B Braun) with finger versus thumb pressure occluding the open end of the vein around the cannula (Figure 2.8).
- A second clip is placed to occlude the opposite end of the vein specimen (Figure 2.9). Foam (or other test substance depending on the experiment; all test liquids were put through the Tessari technique) left in-situ for 5 or 15 minutes depending on the experiment.
- Control sections were simultaneously filled with heparinised blood (Figure 2.6) to control for pressure and mechanical effects.
- Test and control sections were simultaneously placed in 2-5mLs of patient's heparinised blood and left in-situ for either 5 or 15 minutes depending on the experiment.

- After the allotted time the lumens were rinsed with 2 – 3 mLs heparinised blood and the centre segments, remote from the clips to prevent inclusion of traumatised vein, sectioned for histological fixation (Figure 2.10).

Five vein segments were collected per group for each of the products tested. This figure was derived from previous research at Leeds Vascular Institute which indicated that five vein samples would give an accurate assessment of a given product whilst allowing for the large number of products to be tested within an appropriate time frame obtaining the high number of patients required to enable completion of the complete research project in the allotted research period. Previous *in-vitro* experimentation with foam sclerotherapy undertaken at Leeds was based on experiments lasting 2 minutes. However, despite the fact that these time frames, especially 15 minutes, are never likely to be achieved with *in-vivo* use of this treatment, it was felt that 5 and more-so 15 minute experiments were necessary to discover if prolonged sclerosant foam, vein lumen contact, conferred any conceivable benefit to the efficacy of foam sclerotherapy.

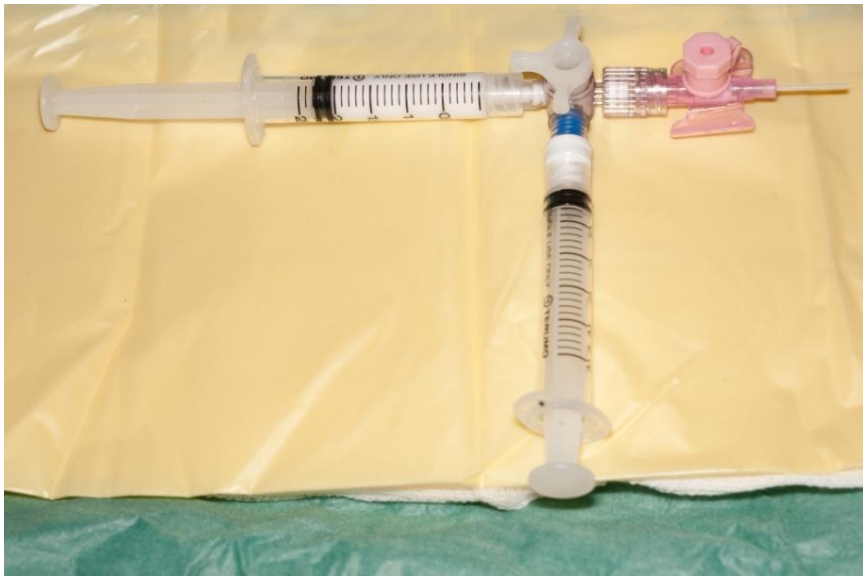


Figure 2.7: Tessari foam made using fully opened 3-way tap without filter. Shorthened 20G iv cannula (B Braun) for atraumatic insertion into test segment

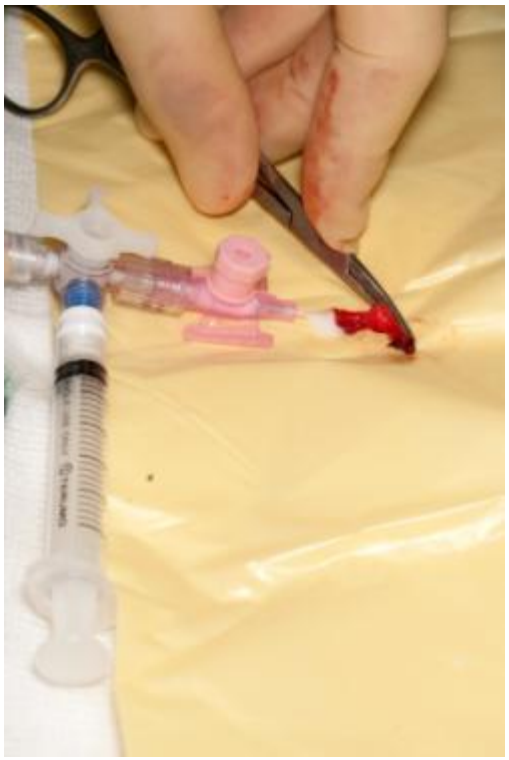


Figure 2.8: Foam made via the Tessari technique being injected into a test segment of vein with one end closed with a small artery clip

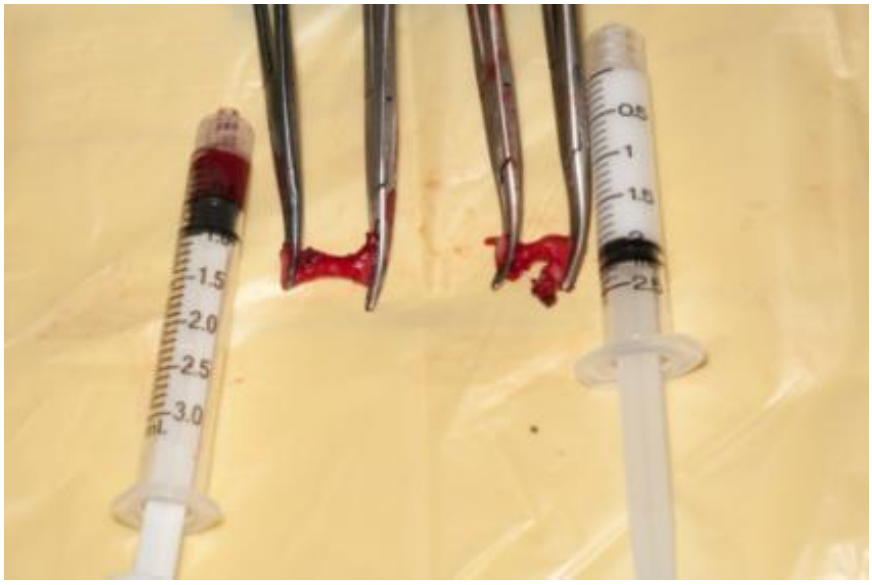


Figure 2.9: control vein segment (containing heparinised blood) and test vein segment containing operator "blinded" foam.

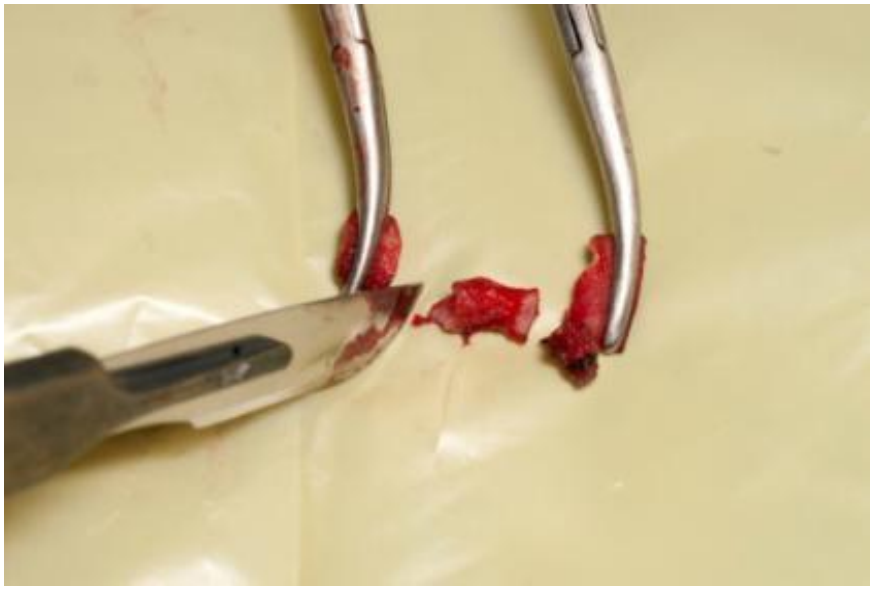


Figure 2.10: central vein segment (remote from the clips to prevent inclusion of traumatised vein) sectioned for histological fixation

Specimens were immediately fixed in 10% buffered formaldehyde (Figure 2.11). Each case was allocated a random number (from a computerised random number generator) to ensure blinding during histological analysis.

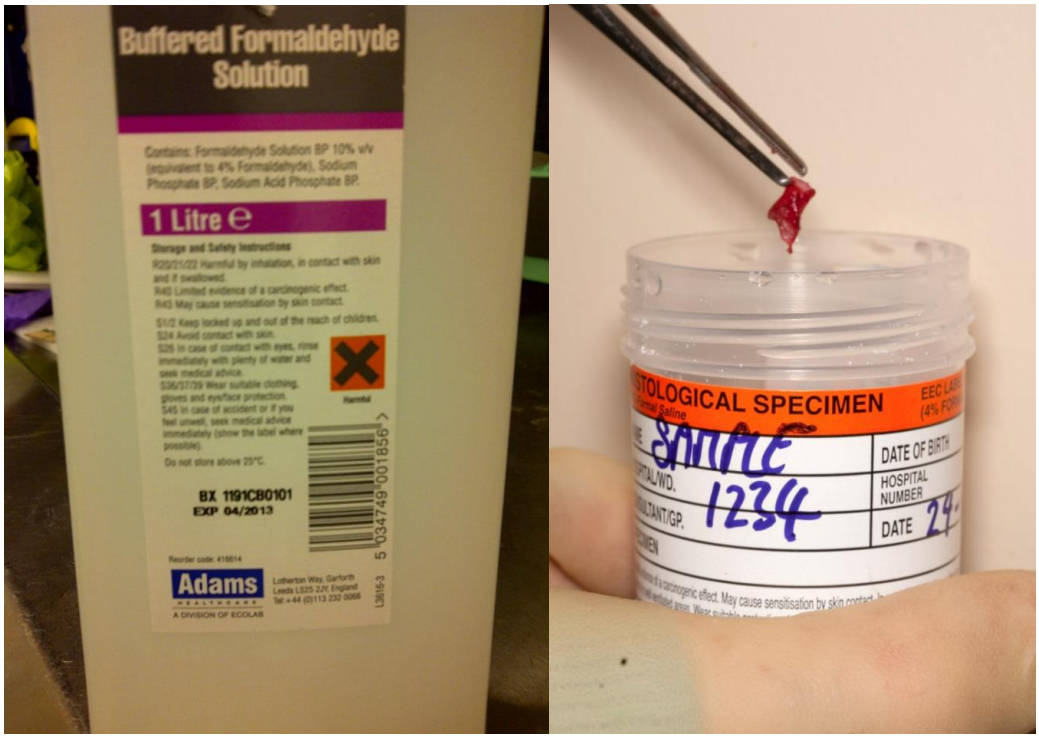


Figure 2.11: vein specimens (allotted a random number to maintain blinding) immediately fixed in 10% buffered formaldehyde.

2.2.4 ASSESSMENT OF EXPERIMENTAL TECHNIQUE: EXAMINATION OF THE HISTOLOGICAL EFFECTS OF HEPARINISED BLOOD CONTROL VERSUS NORMAL SALINE

In order to assess this experimental technique and the control model, the histological effects on the vein wall of heparinised blood controls were compared to those of normal saline. However experimentation was performed as per all subsequent experiments except that in this case 10 samples (each with test [heparinised blood] and control [normal saline]) were tested for the 15 minute time frame only, as per protocol in section 2.2.2.

2.2.5 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF PROPRIETARY FOAM (3% STD AND 3% POLIDOCANOL) SCLEROSANTS ON IN-VITRO GSV WALL INTEGRITY

3% STD and 3% polidocanol (Figure 2.12) foams were used on the test segments. Experiments were carried out for 5 and 15 minutes each as per protocol in section 2.2.2.

Figure 2.12: 3% STD as Fibro-vein™ (STD pharmaceutical products Ltd, England), 3% polidocanol as Sclerovein (Resinag Pharmaceutical And Health Care Products, Switzerland)

2.2.6 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF CONSTITUENT PARTS OF THE PROPRIETARY SCLEROSANT FOAMS ON IN-VITRO GSV WALL INTEGRITY, TO ASCERTAIN THE MOST ACTIVE INGREDIENT

The constituent parts of proprietary 3% STD foam were applied to the test segments. These were buffered 3% STD alone and 2% benzyl alcohol. Experiments were carried out for 5 and 15 minutes each as per protocol in section 2.2.2. Experimentation was not undertaken on the constituent parts of polidocanol as results of vein wall damage showed that polidocanol did not damage the vein media.

2.2.7 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF 'LONGER-LASTING' FOAM SCLEROSANTS ON IN-VITRO GSV WALL INTEGRITY, TO ASCERTAIN THAT PRODUCING MAXIMAL EFFECT ON THE VEIN WALL

The "longer-lasting" 3% STD foams included STD 3% and pluronic 5%, STD 3% and carbomer 0.4%, and STD 3% and xanthum 0.15%. Results revealed that STD 3% and xanthum 0.15% caused a greater degree of injury than the other two longer-lasting foams hence its constituent parts were assessed individually. These were phosphate buffer pH 7.6, normal saline 0.9% and buffered xanthum alone 5%. Experiments were carried out for 5 and 15 minutes each as per protocol in section 2.2.2.

2.2.8 ASSESSMENT OF CUTTING BALLOONS FOR USE AS MONO-THERAPY OR ADJUVANT TREATMENT WITH FOAM SCLEROTHERAPY FOR TREATMENT OF TRUNCAL VARICOSE VEINS

Due to cost implications and initially to examine our hypothesis, cutting balloons previously used for arterial angioplasty were tested on *in-vitro* GSV sections obtained as per section 2.2.2. Test and control segments were similarly separated initially. Balloon sizes over 4mm diameter were used as these have a minimum circumference of 12.56mm. Respective diameters of balloons used were: 4mm = 12.56mm circumference; 5mm = 15.7mm circumference; 6 mm = 18.85mm circumference and 7mm = 21.99 mm circumference. Cutting balloons over 4mm also have four atherotomes as opposed to cutting balloons smaller than 4mm diameter, which have only three. Atherotomes measure 15mm to 20mm in length by 0.25mm in height extending 0.127mm from the balloon when it is inflated.

Available balloons (Peripheral Cutting Balloons; Boston Scientific, USA) were 4.0mm/1.5cm (that is 4mm balloon diameter when inflated to recommended pressure)/1.5cm (length of blades running longitudinally on the surface of the balloon), 5.0mm/2.0cm (Figure 2.20), 6.0mm/2.0cm and 7.0mm/2.0cm.

Each experiment in this group involved 5 GSV segments (each with test and control). The test segments were wrapped inside a saline soaked surgical swab held snuggly with finger thumb opposition beside the vein segment/balloon in order to mimic resistance of the subcutaneous fat as would be the case *in-vitro*. Other models designed to provide external pressure were assessed but were unsuitable. Cutting balloons utilised for these experiments were already used

once (on arteries) hence they were used a maximum of twice more to prevent significant blunting of their blades. As vein segments are of differing dimension and so too are cutting balloons as recorded above, balloons were matched to vein segments according to vein diameter.

2.2.9 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF CUTTING BALLOONS ALONE ON IN-VITRO GSV WALL INTEGRITY

Experiments involved:

1. Cutting balloon (matched to size of vein) inflated in lumen of test vein segment for 10 seconds then deflated and removed.

   Inflation was performed using an Encore™26 Inflation Device (Boston Scientific, USA; Figure 2.19) to a pressure of 6.0 Atmospheres (ATM) / 608 kilo Pascals (kPa) (pressure recommended by the manufacturers for each of the balloons)
2. Cutting balloon (matched to size of vein) inflated to a pressure of 6.0 ATM / 608 kPa in lumen of test vein segment for 10 seconds. Balloon then withdrawn (pulled) from inside the test segment whilst inflated. deBakey (non-traumatic forceps) placed at one end of the specimen used to anchor the vein to allow withdrawal of the cutting balloon whilst inflated.

2.2.10 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF CUTTING BALLOONS IN COMBINATION WITH PROPRIETARY FOAM SCLEROTHERAPY ON IN-VITRO GSV WALL INTEGRITY

Experiment:

1. Cutting balloon inflated to a pressure of 6.0 ATM / 608 kPa in lumen of test vein segment for 10 seconds. Balloon then withdrawn (pulled) from inside the test segment whilst inflated. deBakey (non-traumatic forceps) placed at one end of the specimen used to anchor the vein to allow

withdrawal of the cutting balloon whilst inflated. Then 3% STD or 3% Polidocanol foam was placed (as per experiments in 2.2.2) into the test segment, remaining in the lumen for 5mins. Controls for this experiment had the same balloon treatment as test segments and were then filled with heparinised blood to control for pressure as per previous experiments.

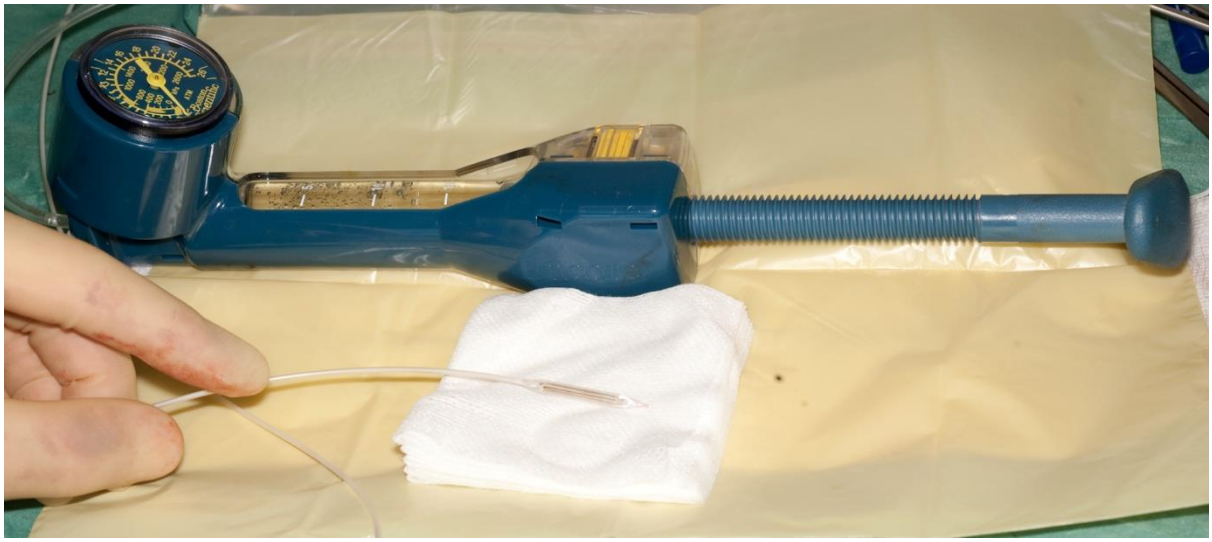


Figure 2.13: Encore™26 Inflation Device (Boston Scientific, USA)

Figure 2.14: 5.0mm/2.0cm Peripheral Cutting Balloon (Boston Scientific, USA)

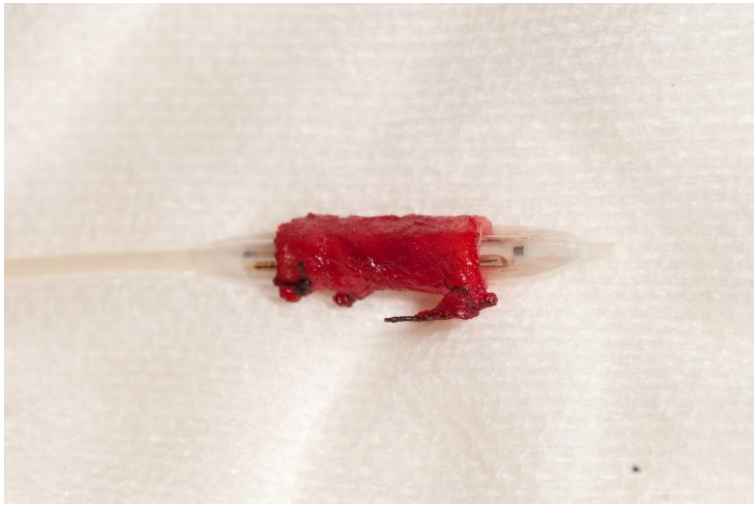


Figure 2.15: (Experiment 2.2.9.1) cutting balloon placed un-inflated into the vein test segment

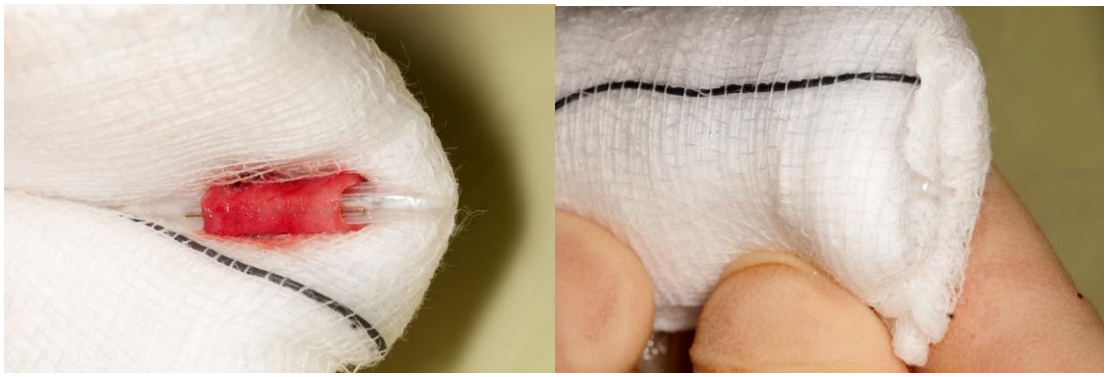


Figure 2.16: (Experiment 2.2.9.1) vein segment wrapped in normal saline soaked surgical swab to mimic resistance of the subcutaneous fat

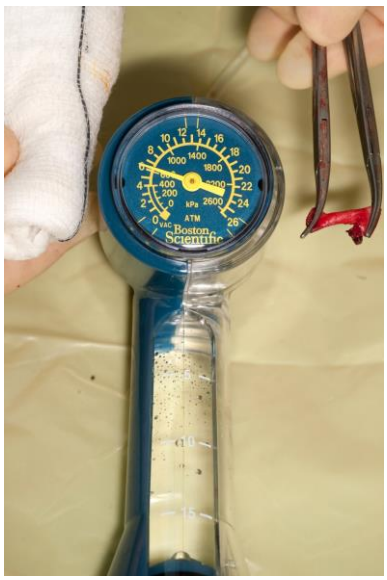


Figure 2.17: (Experiment 2.2.9) balloon inflated for 10 seconds to 6 atm with swab held snuggly at side of vein/balloon. Control segments filled with heparinised blood

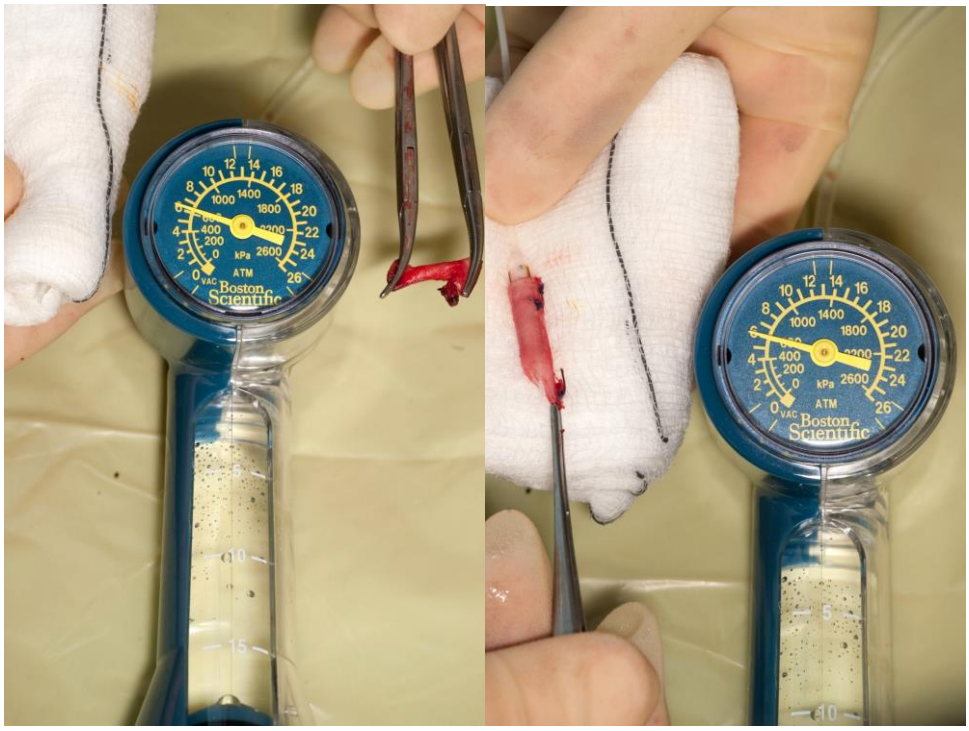


Figure 2.18: (experiment 2.2.9.2) as for experiment 1 but balloon inflated inside swab then vein segment anchored with deBakey forcep and inflated balloon withdrawn. Control segments filled with heparinised blood

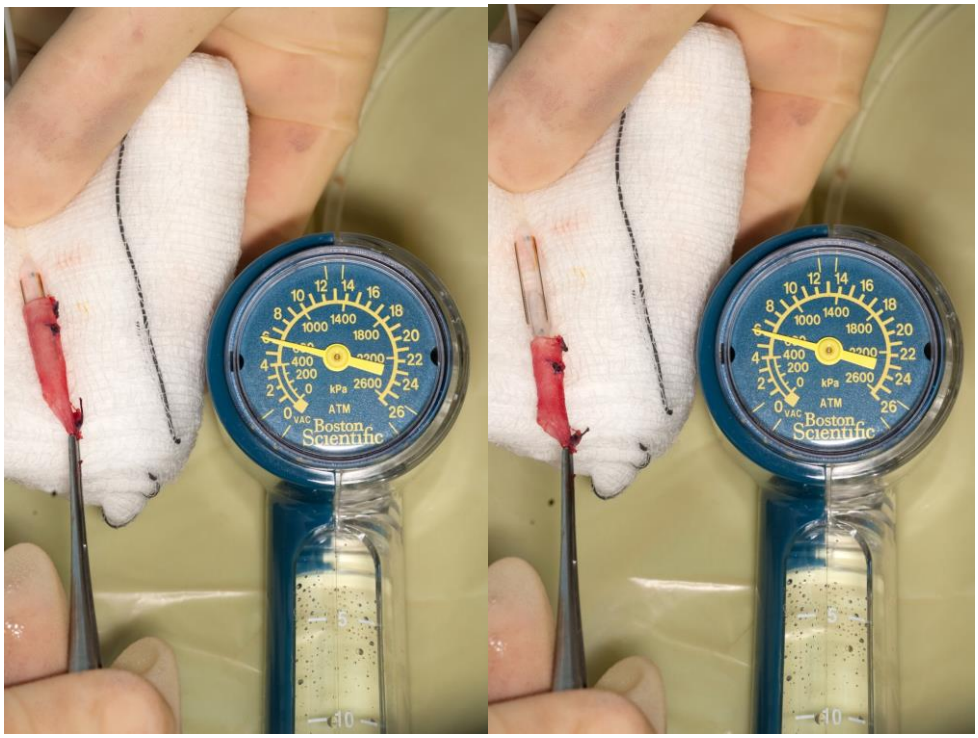


Figure 2.19: (experiment 2.2.9.2) vein segment anchored with deBakey forcep and inflated (6atm) cutting balloon withdrawn

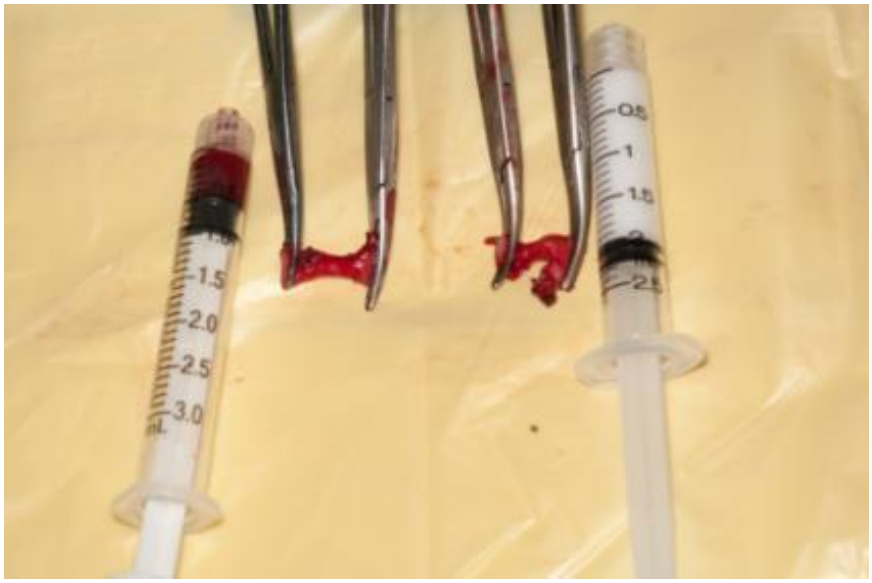


Figure 2.20: (experiment 2.2.10) as per experiment 2. Then test segment filled with either 3% STD or 3% polidocanol for 5 mins. After the same balloon treatment, controls were filled with heparinised blood

2.3 IN-VIVO ANIMAL STUDIES

2.3.1 ASSESSMENT OF A SUITABLE ANIMAL MODEL

Clinical team members at a Home Office accredited animal research facility were consulted regarding their knowledge on the matter of choice of animal model with the published literature also taken into account. A porcine or sheep model was suggested due to their availability, animal husbandry requirements, size of animal and their respective veins, limbs and anatomy. Appropriate thickness and accessibility through tissue overlying the vein segments was also taken into account. This was necessary for USS, experimental treatments and dissection. Preliminary visits to the facility by the lead researcher were performed in-order to assess the most favourable of the models. It became clear that neither animal model would allow pre-procedure USS, preparation of the area with shaving, or experimental treatments to be undertaken, only using sedation. Hence the requirement for general anaesthetic was agreed which impacted upon the accuracy of USS due to the animals being supine. This matter was overcome to an extent by occluding the superficial veins proximally with hand pressure and subsequently using USS in the distal vein for the purpose of vein identification and assessment of competence.

2.3.1.1 ASSESSMENT OF A OVINE MODEL

Sheep were initially assessed as a potential model. Beier *et al*used sheep (female merino land sheep, 24-26kg) fore-limb saphenous vein for research into tissue engineered vascularised grafts. In accordance with the very few anatomical descriptions depicting the medial aspect of the groin in sheep, they refer to this neurovascular bundle in the following as saphenous artery, vein(s), and nerve (Figure: 2.21).[269] They describe its exposure as requiring little muscular dissection as it was constantly found in the epifascial soft tissue layer beneath the superficial fascia. They report only sparse side branching of the saphenous artery and vein(s) with only marginal diminution of vessel diameter from proximal to distal noted. They found the saphenous vessels to constantly emerge proximally in the groin by passing through a muscular hiatus, formed by the tendinous origins of the sartorious muscle anteriorly and the gracilis muscle posteriorly (Figure 2.21). For their graft purposes they found dissectible length of the saphenous artery and vein was at least 16 cm, reaching from the groin to the knee joint with an average arterial and venous calibre of 2.5–3 mm and 2–2.5 mm, respectively. In seven out of eight sheep, there was just one concomitant vein, whereas one sheep had two saphenous veins. This calibre of vein though potentially useful for foam studies was deemed likely too small for Fogarty and cutting balloon experiments in this research. The sheep available for this research project were of a larger size than those used by Beier *et al*hence examinations of sheep as a potential animal model were undertaken.

Animals that were being terminated by lethal injection (Sodium pentobarbital 120mg/mL) for other studies were given analgesia and subsequently general anaesthetic via inhaled gas to enable assessment of their fore and hind-limb superficial venous systems using both USS and open surgical dissection. The appropriate anatomical sites (medial aspect of all four limbs and their junctions with the body) were shaved and USS performed. This investigation proved difficult due to the relatively low soft tissue mass within the legs of the sheep and due to the fact that the animals were supine. Fore-limb veins were impossible to delineate on USS in one animal with only arteries seen and the other animal died before any useful information could be obtained on USS from its fore-limbs. The largest superficial veins seen on USS (2-3mm) (Figure 2.22) originated from the hind limbs but they were deep within pre-abdominal tissue (Figure 2.23). These deep areas (Figure 2.23) as well as those which would be necessary anatomically for experimental purposes were explored surgically and this revealed very small vessels (≤2mm) running as paired veins on each side of their respective superficial artery on each hind leg (Figure 2.24) becoming 3mm diameter at maximum deep in pre-abdominal tissue. One hind-limb vein was cannulated with great difficulty with a 2.5Ch Fogarty balloon catheter but larger catheters required for the purpose of experimentation could not be inserted. In the fore limbs the superficial veins were c.2mm for a length of >10cm but the majority of this was traversing deep and next to bone (Figure 2.25). This procedure was repeated in two other sheep revealing similar anatomy in each (Figure 2.26-2.28).

Beyond these anatomical problems, staff at the animal research facility highlighted the fact that sheep are more likely to die under general anaesthetic

than the majority of other animals they use for research purposes, usually due to cardiac arrhythmias. This coupled with the anatomical problem meant that sheep were deemed an unsuitable model for this research purpose.

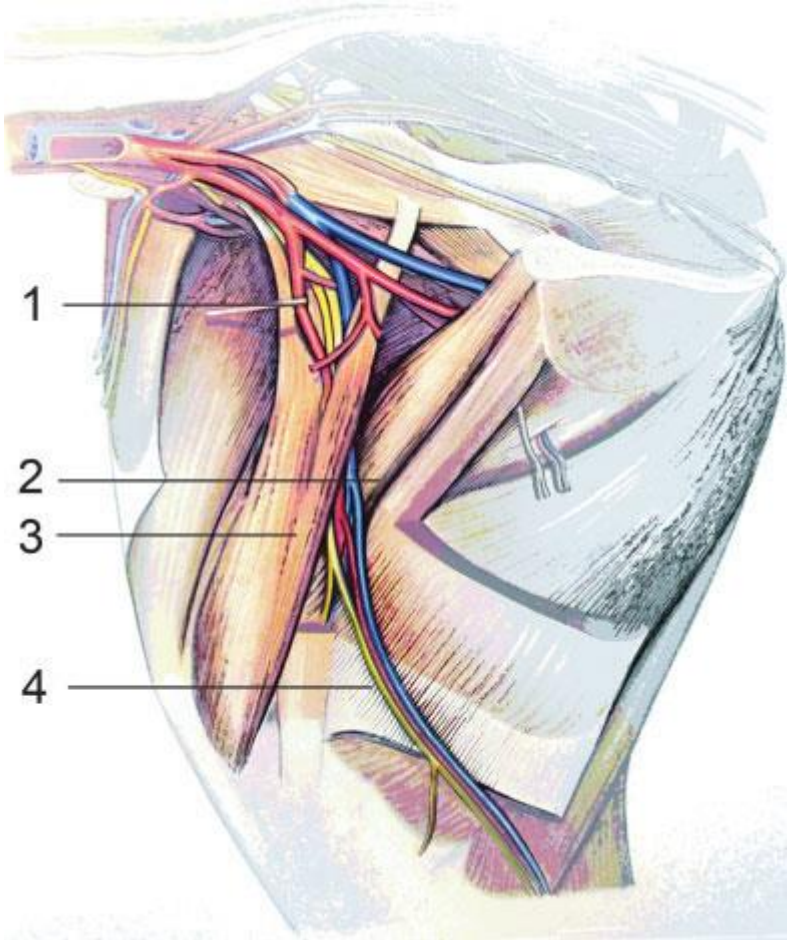


Figure 2.21: anatomy of the sheep's groin and medial thigh with sapheneous artery (1) passing between the gracilis muscle (2) and the sartorius muscle (3), accompanied by the saphenous nerve (4). From Beier *et al*[269]

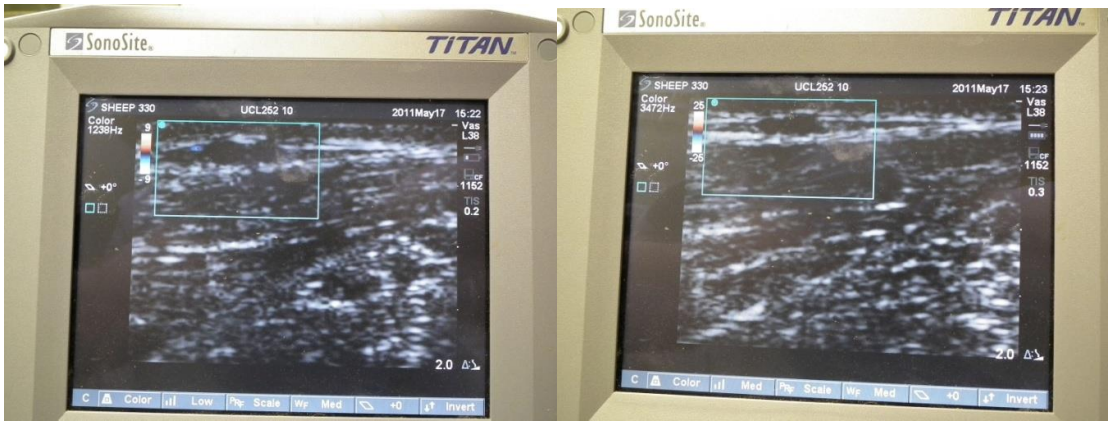


Figure 2.22: USS pictures from two posterior abdominal wall/upper hind limb junctions in a supine sheep under general anaethetic with proximal finger pressure to dilate the veins; artery with paired veins alongside

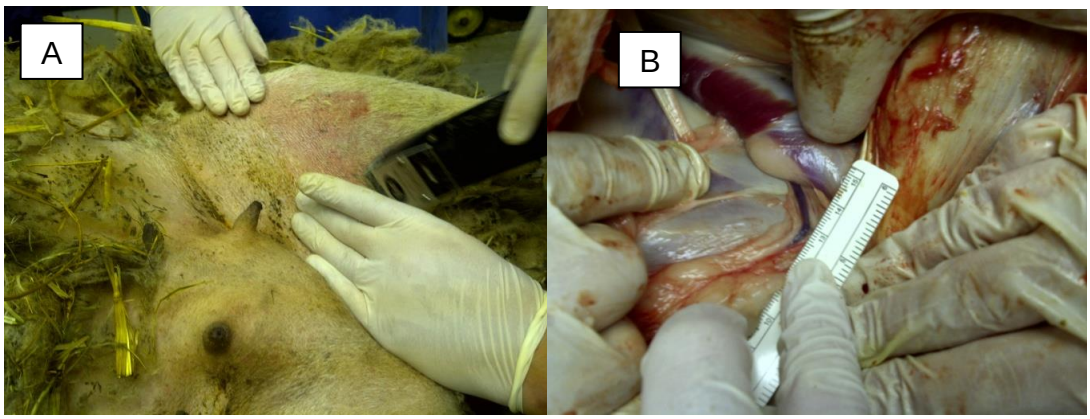


Figure 2.23: sheep 1) posterior abdominal wall/upper hind limb junctions in a supine sheep under general anaesthetic being prepared (A) for uss and surgery (B) revealing a deep running 3mm vein requiring significant dissection.

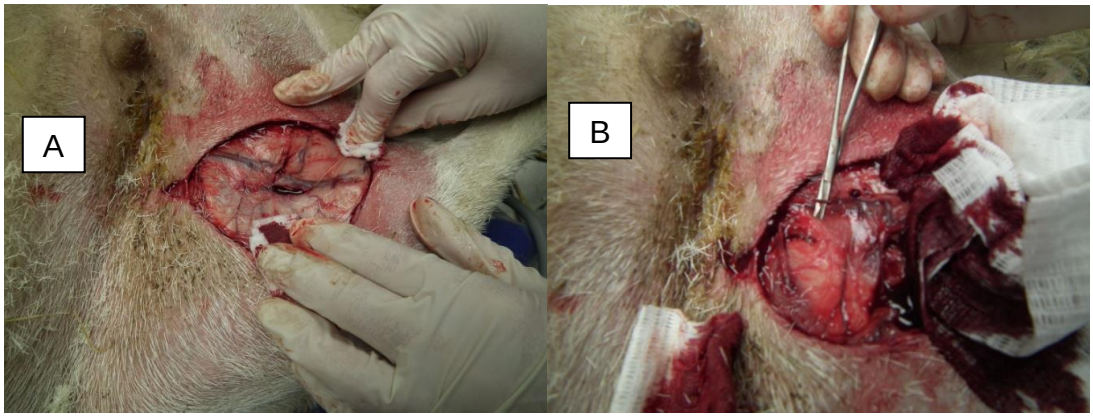


Figure 2.24: sheep 1) inner hind limb thigh level <1mm veins; running each side of artery (A) and vein dissected off artery (B)

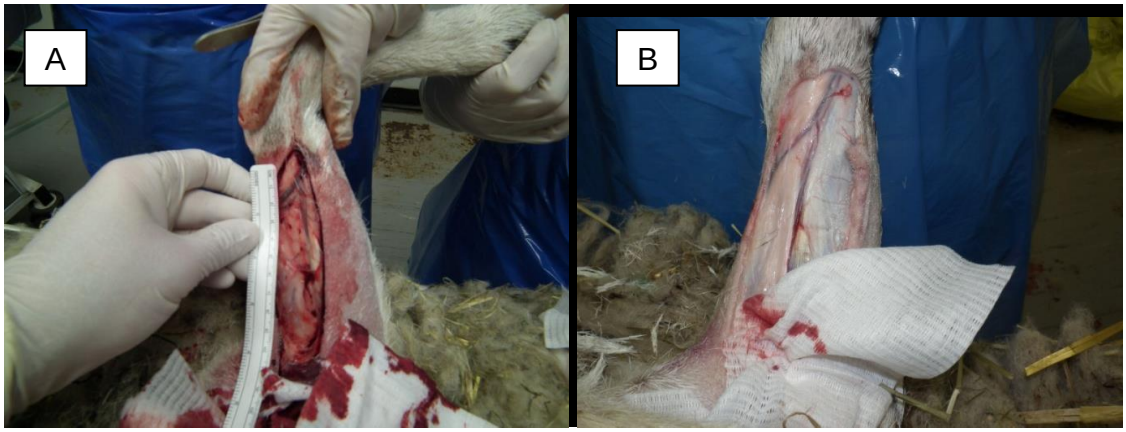


Figure 2.25 : sheep 1) fore limb superficial vein >10cm length available (A), and after deeper dissection with 1mm vein deep to tendon and close to bone (B)

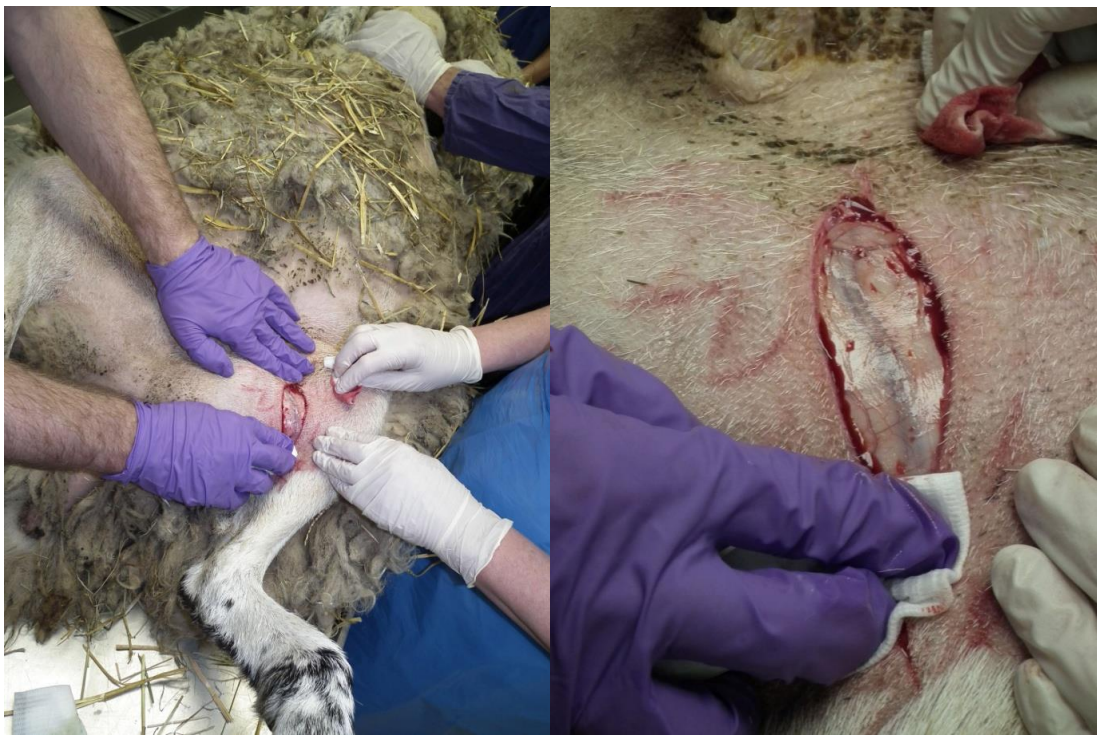


Figure 2.26: sheep 2) <1mm hind limb veins

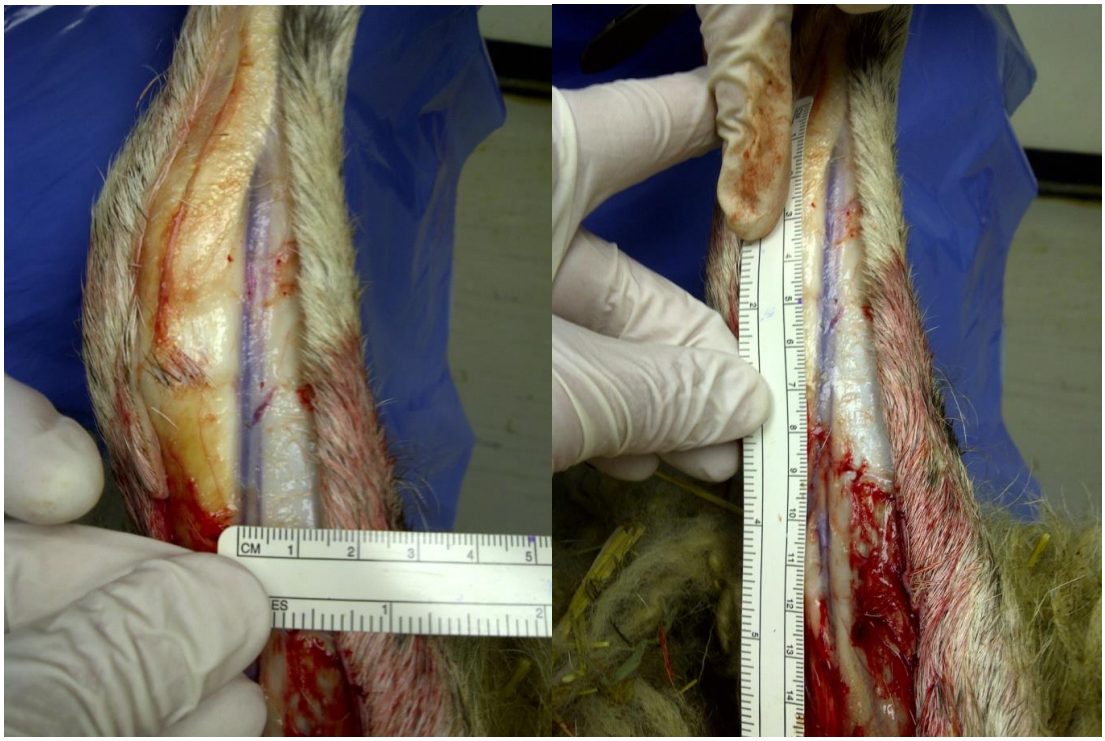


Figure 2.27: sheep 2) 1mm forelimb veins running with artery

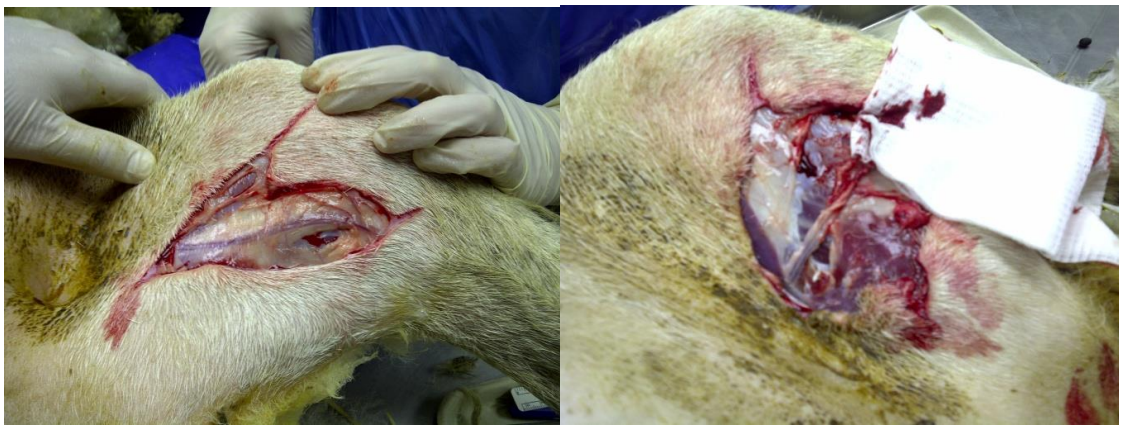


Figure 2.28: sheep 3) <1mm hind limb veins

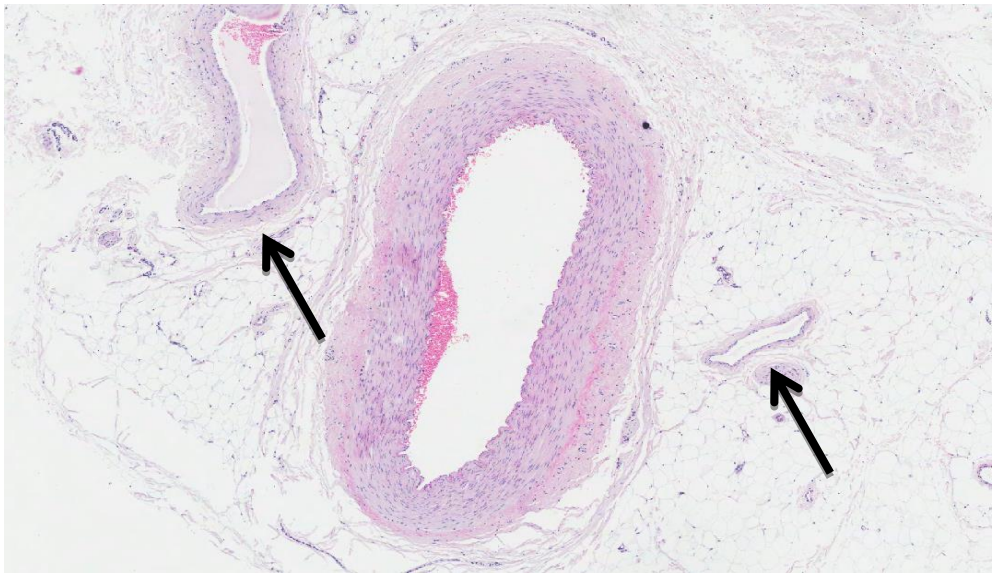


Figure 2.29: H&E stain of section from front leg of sheep 2 right front leg. Deemed unsuitable due to paired small veins (arrows)

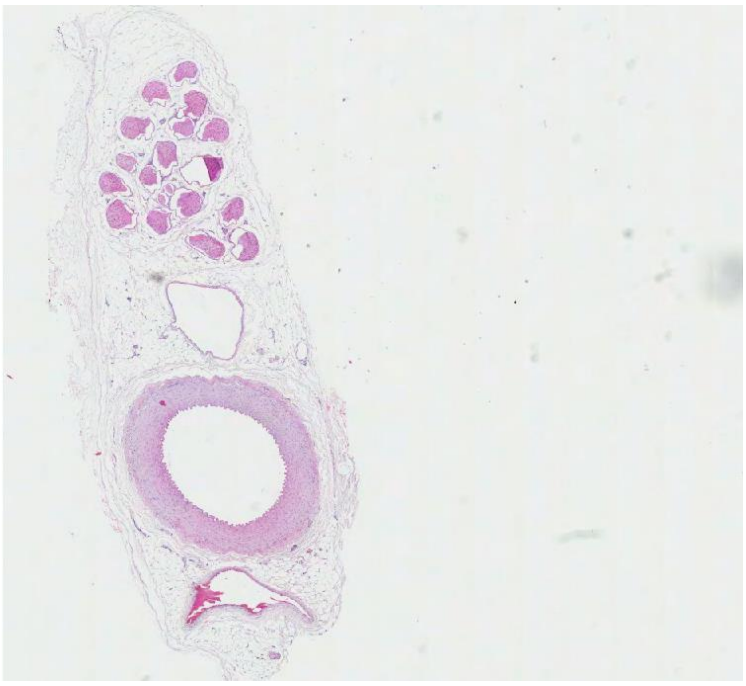


Figure 2.30: H&E stain of section from neurovascular bundle in sheep 2 left fore- leg

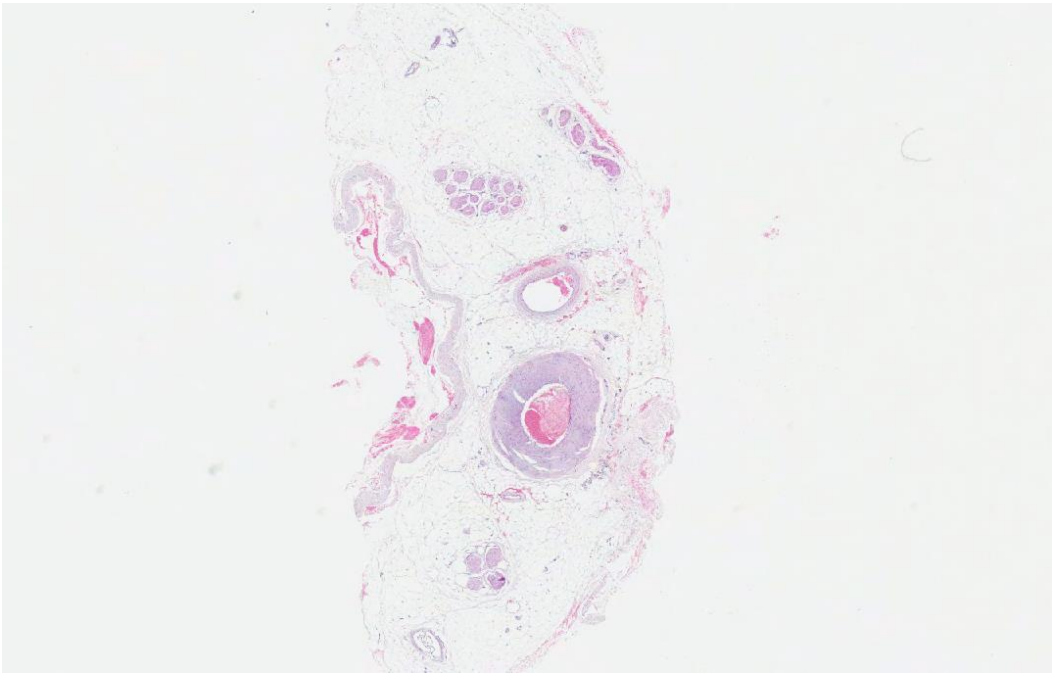


Figure 2.31: H&E stain of section from sheep 2 left hind limb with smaller vessels cf fore-limbs.

2.3.1.2 ASSESSMENT OF A PORCINE MODEL

Pigs were then assessed as a potential animal model. Advice given by the faculty at the Home Office accredited animal research facility was that porcine superficial veins are similar structurally and anatomically to superficial human veins though smaller. Animals of c.50kg were likely to be the minimum size required to obtain veins in the order of 4-5mm diameter in the knowledge that over an intervening 12 week period these animals could triple their body weight rendering any follow-up procedures logistically difficult. Fore-limb veins were stated likely to be of most use but hind-limbs could be of a useful size. The staff felt that it should be possible to treat a 10cm length of superficial vein in each of the four limbs without a significant risk of sclerosant entering the deep venous system in great volume. Beyond this they stated that porcine superficial veins are reasonably easily accessible for treatment and USS assessment and they converge with the deep veins in a similar fashion to human superficial lower limb veins. In addition they stated that pigs would have more suitable limbs than sheep for comparable bandaging post-procedure as would be performed following foam sclerotherapy in humans.

Jones *et al*reported in their normal porcine controls (weight c.100kg) that the superficial venous tributaries of the thigh of the hind-limbs communicated with the saphenous veins via a perforating junction approximately six to eight cm distal to the junction with the femoral vein (SFJ).[267] They state that the saphenous venous drainage comprises two venous channels, one usually larger than the other, lying on each side of the saphenous artery and communicating with each other via a series of bridging veins. These vessels are surrounded by a distinct fascial condensation forming the saphenous bundle (Figure 2.33). The saphenous veins remained within the saphenous fascia

before penetrating the fascia cribrosa and joining the femoral vein separately. Each length of saphenous vein consistently contained eight to 10 bicuspid valves preventing reflux from the deep system.[267] Again a preliminary study was carried out on a pig of the size that could be used for this research. This was performed on a pig that was being terminated for other research purposes. It was again assessed under general anaesthetic with analgesia, in the supine position as the experimental animals would be in this study. Shaving was unnecessary in the pig model. USS proved difficult but revealed more favourably lying and better sized fore-limb veins (c. 4-5mm) in the animal examined. Surgical exploration confirmed this and demonstrated that despite the fore-limb veins running deep to thick fibrous tissue, they were accessible and not as proximal to bone as in sheep (Figure 2.25 A&B). Surgical exploration revealed hind limb veins that were slightly larger than those in the sheep however they were still small at ≤2mm. The advantage with their hind limb veins versus sheep were that they ran a suitably long course superficially before entering deep tissue in the abdomen. Veins in the hind legs were again in pairs running with the limb's main superficial artery as per Jones *et al*[267] (Figure 2.33). A porcine animal model using superficial epigastric veins has been used by others in varicose vein treatment research however this was not considered in this case due to the altered gravitational and anatomical effects on this vein in pigs (Figure 2.34).[131]

Hence a porcine model using all four limb superficial veins was chosen as the animal model for this research. Due to the costs involved in an animal model the study population in the first instance was two animals (4 veins: one per limb) studied over 12 weeks to confirm whether the model is feasible. Should this

model prove feasible the initial plan was that further animals could be included in the study.

Animals of a suitable size and weight (50-60kg) were selected on the basis that they have 4mm diameter fore-limb superficial veins and c. 2-3mm diameter hind-limb superficial veins, the latter of which would likely be used for foam studies alone if the veins were unsuitable for balloon experiments. The fore-limb veins would likely be suitable for all balloon studies. Animals of this size would likely be c.150kg 12 weeks later and at that stage still manageable in the research facility. Larger animals would have presented logistical problems, likely being unmanageable due to their size and weight.

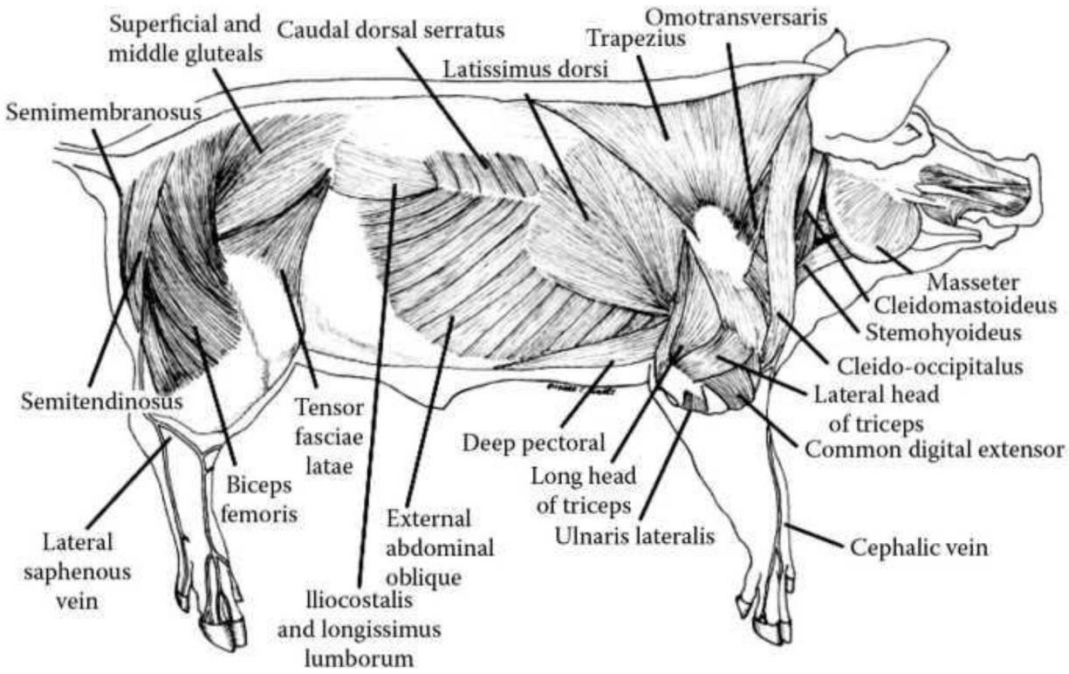


Figure 2.32: Superficial venous anatomy of pig limbs

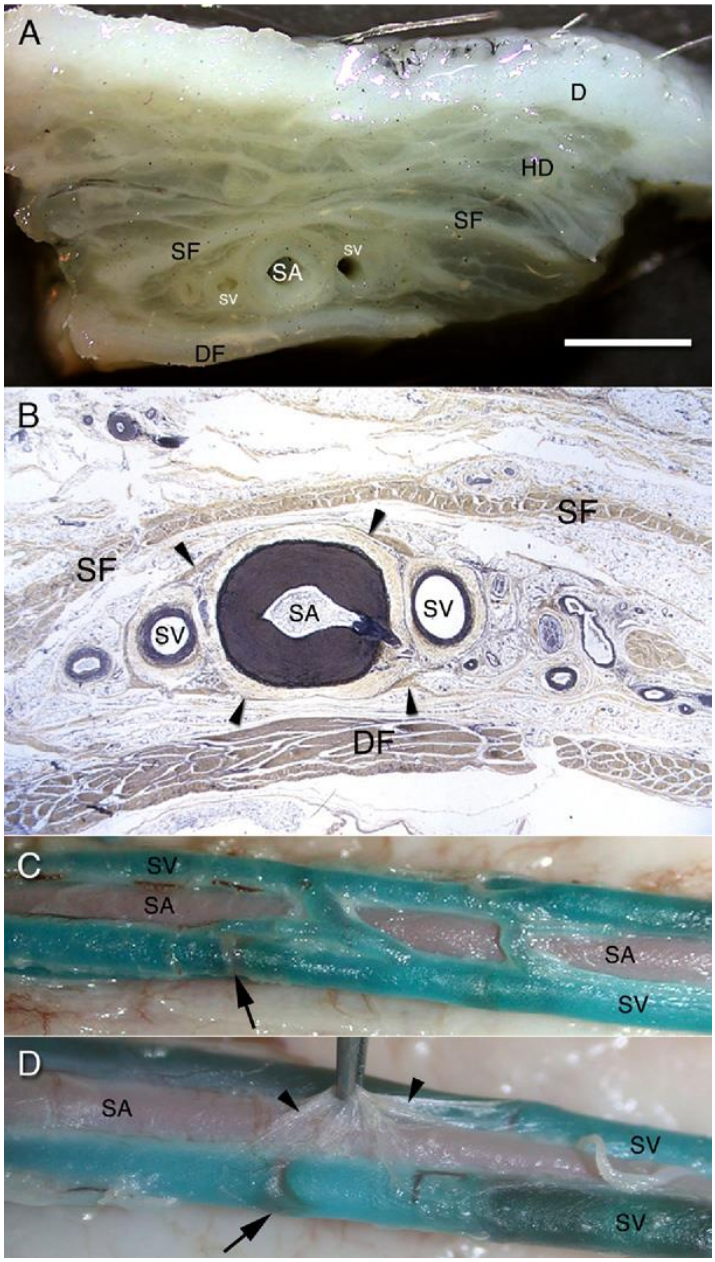


Figure 2.33: The porcine saphenous bundle from Jones *et al.*[267] *T*ransverse en bloc preparation of the saphenous bundle and overlying skin, mid thigh. Scale bar equals 3 mm. (b) histological section (Verhoeff's elastic stain) of the same block. (c-d) resin vascular cast of the saphenous venous network, demonstrating the relationship of both the superficial (sf) and deep fascia (df) to the saphenous fascia (arrow heads). Within the saphenous compartment, the saphenous artery (sa) is bordered by the saphenous veins (sv). Note the numerous valves (arrows) and communicating veins within the saphenous network.

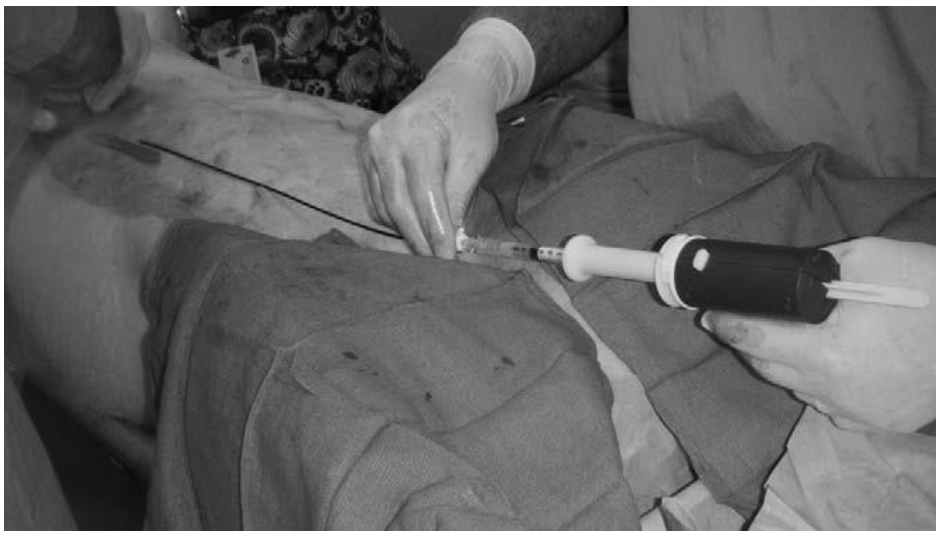


Figure 2.34: Superficial Epigastric Veins Used For Cyanoacrylate Research In A Porcine Model. From Almedia *Et Al.*[131]

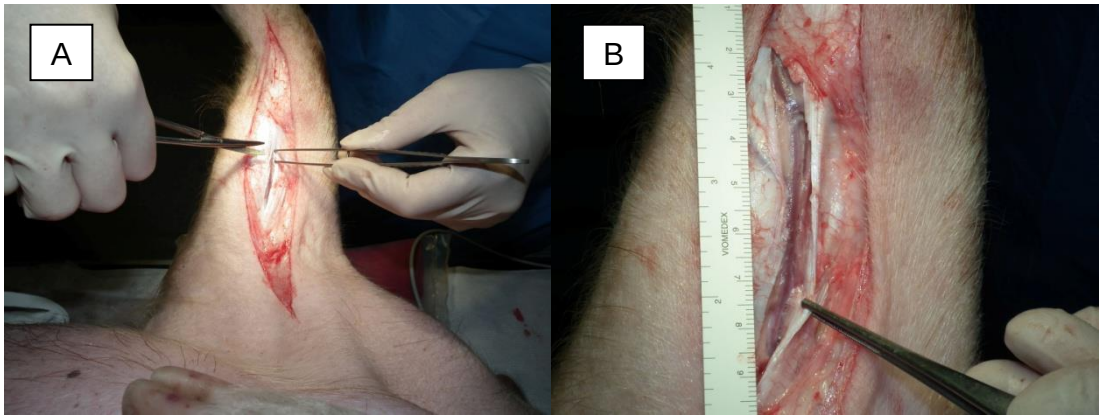


Figure 2.35: fore limb veins in a pig (a) running deep to a thick fascial layer (b)

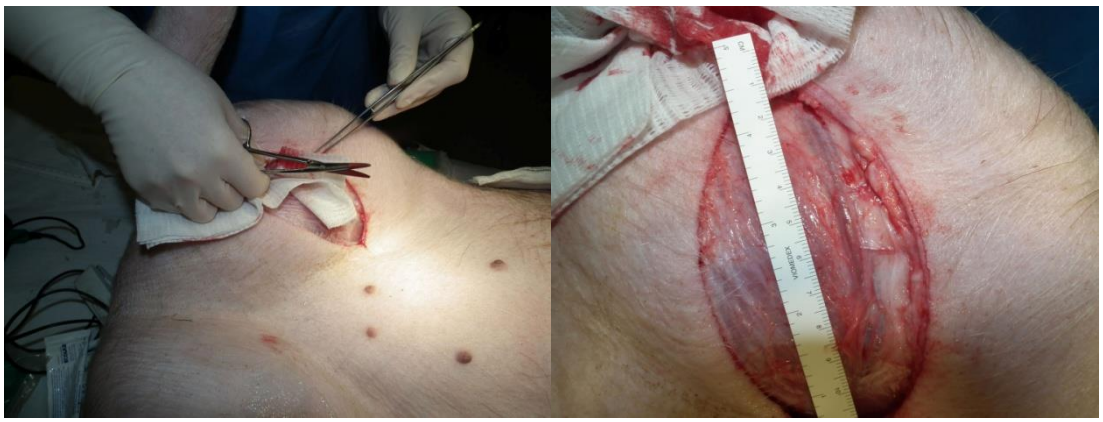


Figure 2.36: hind limb pig veins running paired with an artery and accessible for a length >10cm

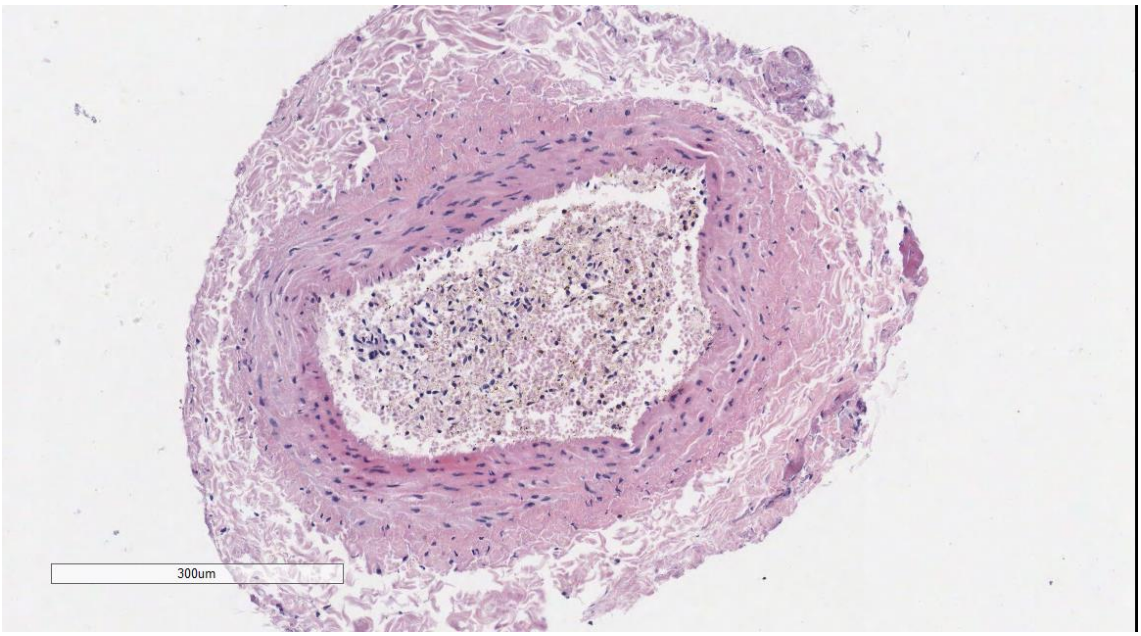


Figure 2.37: H&E stain of section from right fore- limb vein of pig 1

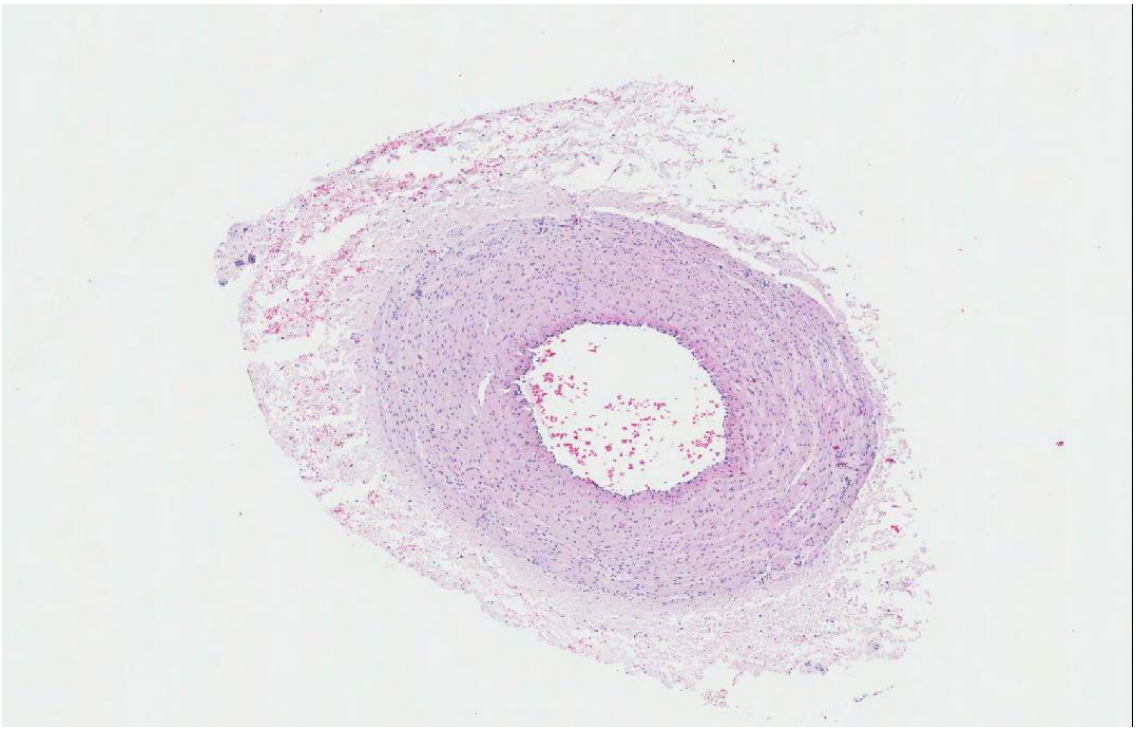


Figure 2.38: H&E stain of section from right hind limb of pig 1

2.3.2 ASSESSMENT OF THE THERAPEUTIC EFFECTS OF PROPRIETARY FOAM SCLEROTHERAPY, "LONG-LASTING" FOAM SCLEROTHERAPY AND ENDOVENOUS CATHETER BALLOONS (FOGARTY AND CUTTING) IN PORCINE SUPERFICIAL VEINS.

The protocol for this stage of the study was established by the research team in conjunction with the Home Office accredited animal research clinical team and given approval by Home Office officials **(Appendix II)**. This study was undertaken in a licensed Home Office facility, with all aspects of the following procedures undertaken by licensed staff.

The animals were given anaesthetic pre-medication and were then fully anaesthetised in a theatre environment. The animals were given relevant prophylactic antibiotics and long-acting analgesic medication. The veins to be utilised were initially scanned by USS to ensure patency. Controls for these experiments would be vein tissue excised at the initial experimental surgery and untreated vein tissue excised at the 12 week stage.

All parts of the procedures were carried out by a licensed animal practitioner under the guidance of the lead researcher.

The following procedure was that which was planned:

Fogarty balloon x5 passes (control); Hind-limb

Fogarty balloon x5 passes; STD foam; Hind-limb

Fogarty balloon x5 passes; cutting balloon x3 passes; Fore-limb

Fogarty balloon x5 passes; cutting balloon x3 passes; STD foam; Fore-limb

However, during the procedures it was evident that the neither Fogarty nor cutting balloon catheter could be inserted into the hind-limb veins of the two animals treated due to the small diameter hind-limb veins veins. Hence to maximise use of the animal 3% STD foam (the more potent proprietary foam) and 3%STD/0.15% xanthum foam were tested on the hind-limbs of both animals.

Post procedure the limbs were dressed with compression bandaging with swabs overlying the superficial vein territory, to apply increased pressure in these areas hence mimicking the post-procedure dressing of humans treated with foam sclerotherapy for great saphenous varicose veins.

The animals were monitored during their animal husbandry period on the farm before returning to the research facility after a 12-week period for surgical excision of the treated vein segments for histological analysis. The animals were terminated immediately post-operatively whilst still anaesthetised and analgised with other organs removed surgically pre-termination for use by other research groups to maximise use of the animal.

2.3.2.1 ANIMAL 1 (BM 1): OP PROCEDURE; WEIGHT: 55KG; DATE: 13/06/11

Pig anaesthetised

USS all 4 limbs (>10cm length of vein found in all limbs and all patent).

Tattoo 10cm length in all four limbs

Open cut down begun distal to distal mark on all limbs to allow access to full 10cm length.

1. **Right fore-leg (Branch taken as control sample):**

   Fogarty Ch3 x5 passes; Cutting balloon 4x1.5cm x1 pass (5cm only); STD 3%foam

   (Half of right forelimb treated as catheter would not go any further and this size balloon has no guide wire hence weakened catheter allowed only one pass. Hole in vein repaired with x3 6-0 sutures 1cm distal to catheter entry. More holes present 4cm distal to catheter entry. Entry point 1cm distal to tattoo.

   Vein side-branch taken for histology.

2. **Left fore-leg:**

   Fogarty Ch3 x5; Cutting balloon 4x1.5cm x1 pass (c.10cm)

   Catheter point sealed with 6/0 prolene

3. **Right hind-leg:**

   1 mL 0.15% Xanthum and 3% STD foam via lateral branch into more proximal half of visible vein (that just lateral to artery), distally no foam

4. **Left hind-leg:**

   1 mL STD 3% foam into more proximal vein (medial to artery) with distal half avulsed therefore removed for histology sample; vein tacking suture to hold out to length.

   Vein side-branch taken for histology.

Extra tattoo line placed to mark catheter insertion point.

All wounds closed with 3-0 silk interrupted horizontal mattress sutures.

Cicatrin® (Neomycin) antibiotic powder applied to all four wounds.

Adhesive compression bandages applied to fore-limbs.

Operation time: 3hr 30min

Post-Op:

14/6/11: Pig had removed bandages after 24hours.

15/6/11: Pig seems fine.

16/6 11: Slight swelling on right hind limb over wound (fluid?). Slight limp?

17/6/11: Swelling still present, (no inflammation present). Pig is using right hind limb a little stiffly – internal bruising?

20/6/11: Slight swelling still present in right hind limb but pig is no longer limping.

27/6/11: Swelling still present but much reduced.

12/9/11: All veins harvested at 3 months

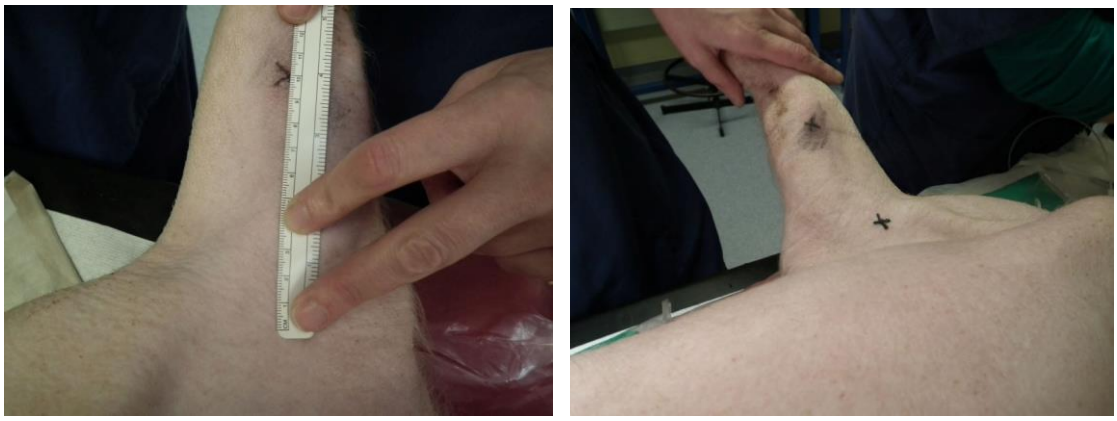


Figure 2.39: BM1; pre-op tattoos marking 10cm superficial vein in right fore-limb

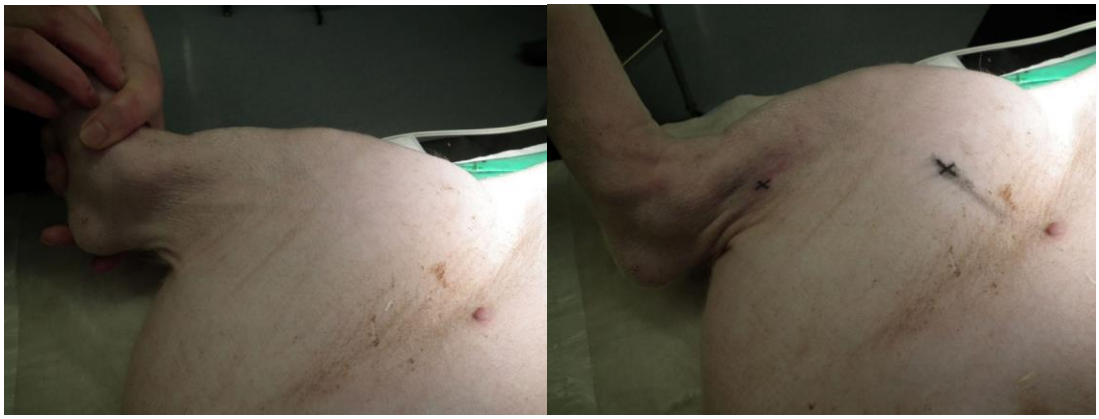


Figure 2.40: BM1; pre-op tattoos marking 10cm superficial vein in right hind-limb

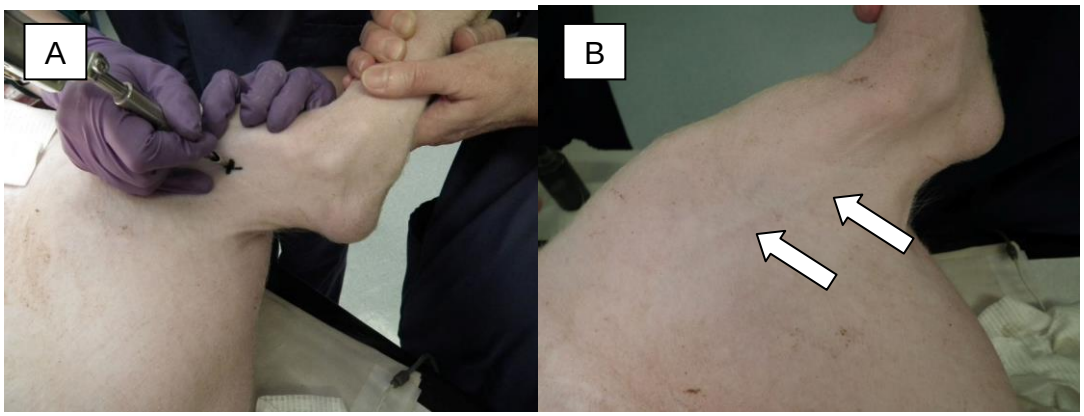


Figure 2.41: BM1: pre-op tattooing (a) marking 10cm superficial vein in left hind-limb; artery easily seen (b; arrows) with paired superficial veins

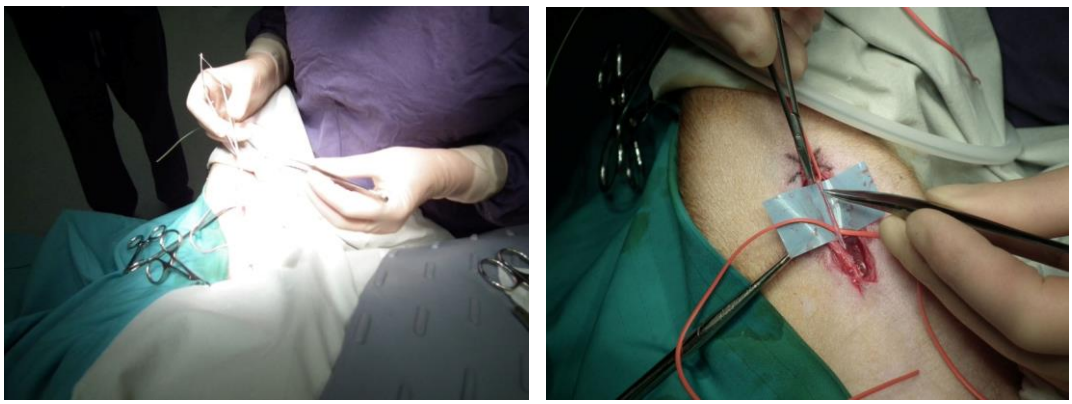


Figure 2.42: BM1; exposure of left fore-limb superficial vein

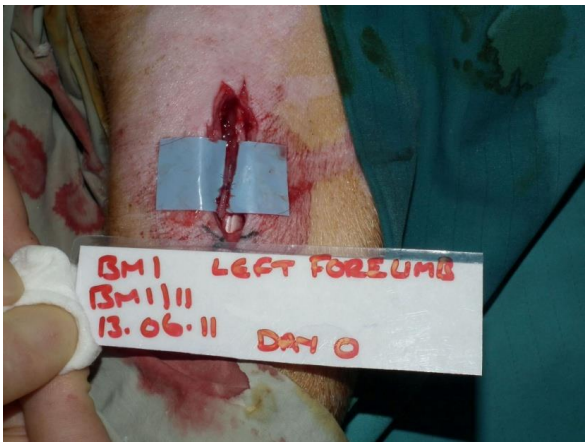


Figure 2.43: BM1; left fore-limb experimental rx: fogarty ch3 x5; cutting balloon 4mmx1.5cm (x1 pass [10cm])

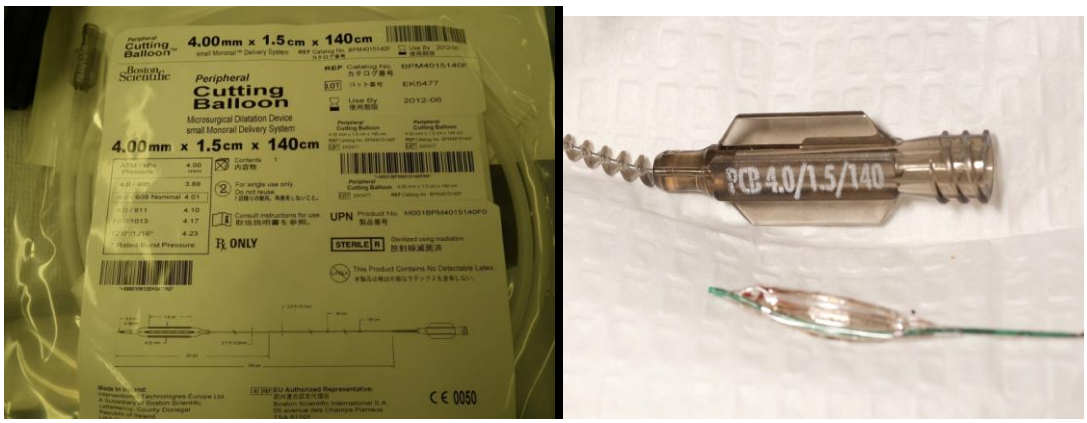


Figure 2.44: BM1; 4.0mm x 1.5cm peripheral cutting balloon (boston scientific, usa) used on forelimbs of bm1

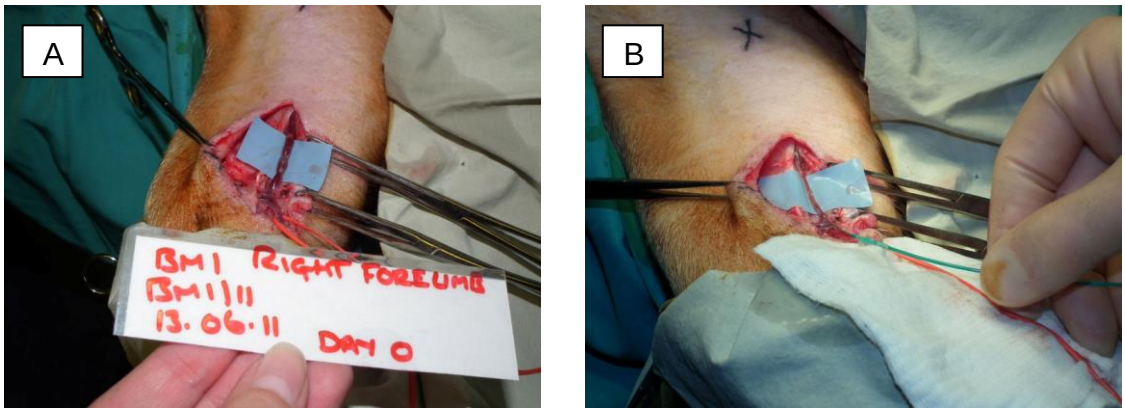


Figure 2.45: BM1; right fore-leg experimental rx: fogarty ch3 x5 passes; cutting balloon 4mmx1.5 cm (x1 pass (5 cm only)); std 3%foam 3ch fogarty balloon trawled through vein (b)

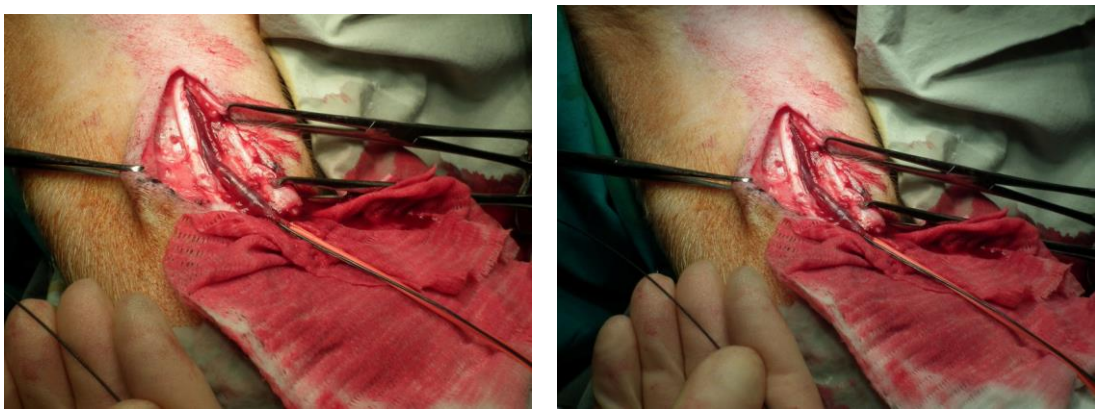


Figure 2.46: Cutting balloon being withdrawn through vein

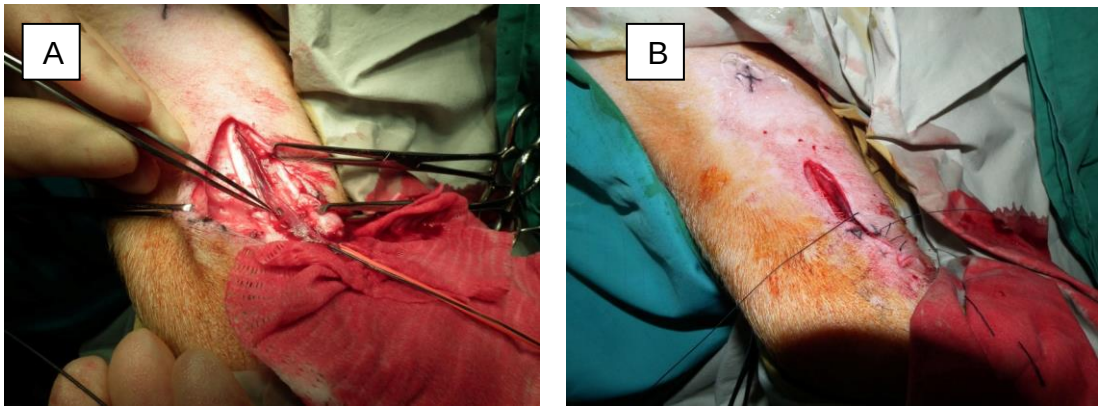


Figure 2.47: Cutting balloon deflated and removed from vein; small venotomy created (a). fascia and skin closed (b)

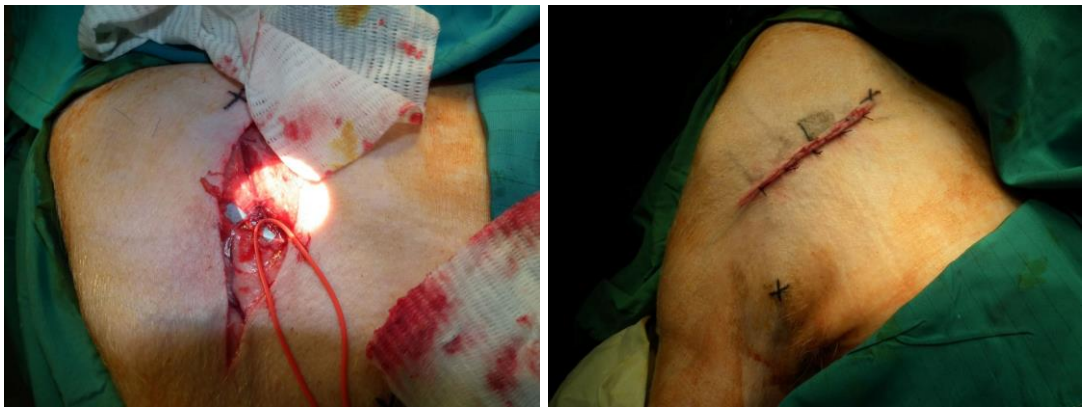


Figure 2.48: BM1; right hind-leg experimental rx: 1 ml 0.15%xanthum/3%STD foam into vein medial to artery

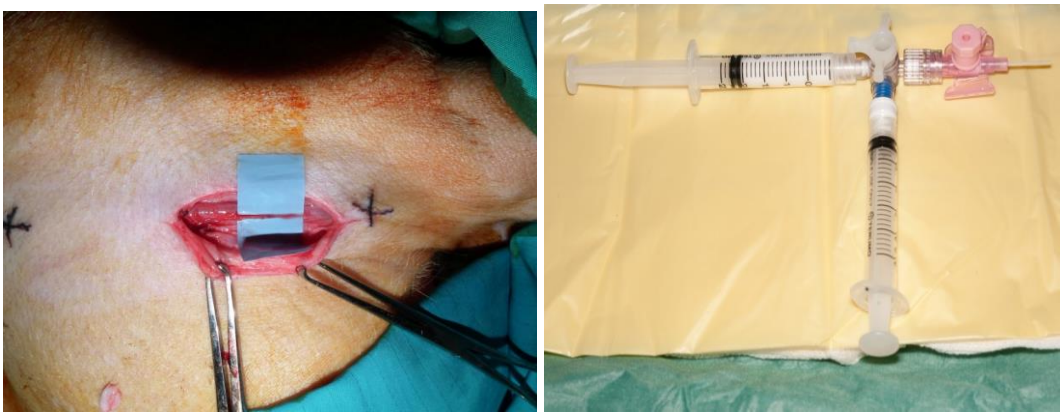


Figure 2.49 : BM1; left hind-leg experimental rx: 1 ml 3% STD foam into vein medial to artery

2.3.2.2 ANIMAL 2 (BM 2): OP PROCEDURE; WEIGHT: 50KG; DATE: 13/06/11

Pig anaesthetised

USS all 4 limbs (>10cm length of vein found in all limbs and all patent).

Tattoo 10cm length in all four limbs

Open cut down begun distal to distal mark on all limbs to allow access to full 10cm length.

1. **Right fore-leg** (Branch taken as sample):

   Fogarty Ch3 x4 passes and Ch4 x1; Cutting balloon 3mmx1.5cm x1 pass (10cm) x3passes (c.8cm); 1.5 mLs STD 3%foam

   Entry point 2.5cm distal to distal tattoo.

   Branch taken as sample

2. **Left fore-leg:**

   Fogarty Ch3 x5 passes; Cutting balloon 5mmx2cm x1 pass in 2cm steps with deflation between Rxed c.10cm, vein burst and repaired with prolene

   Difficulty getting guide wire into vein.

   Caused large holes in the proximal vein x3 repaired with 6/0 prolene continuous stitch.

3. **Right hind-leg:**

   1 mL Xanthum/3%STD foam via lateral branch into more proximal half of visible vein (that just lateral to artery), distally no foam

4. **Left hind-leg:**

   1 mL STD 3% foam via lateral branch crossing over artery into more proximal half of visible vein (that just medial to artery), distally no foam

Extra tattoo line placed to mark catheter insertion point.

All wounds closed with 3-0 silk interrupted horizontal mattress sutures.

Cicatrin® (Neomycin) antibiotic powder applied to all four wounds.

Adhesive compression bandages applied to fore-limbs.

Operation time: 2hr 35min

Post-Op

14/6/11: Pig had removed bandages after 24 hours.

15/6/11: Pig fine

16/6/11: Pig fine

17/6/11: Pig fine

21/6/11: Right forelimb, swelling under sutured area – seems to be fluid filled (~50ml)

Slight redness. Pig is not limping and the area does not seem to be painful.

Slight swelling also on left fore-limb.

22/6/11: Swelling on both limbs starting to recede.

27/6/11:Swelling on left fore-limb receded but there appears to be a single small stitch abscess – antibiotics given

12/9/11: All veins harvested at 3 months

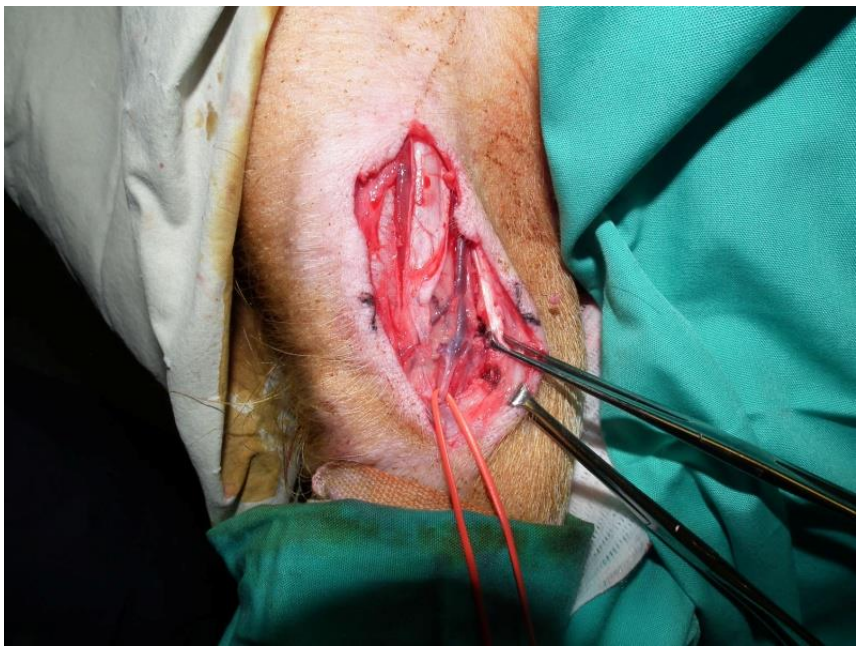
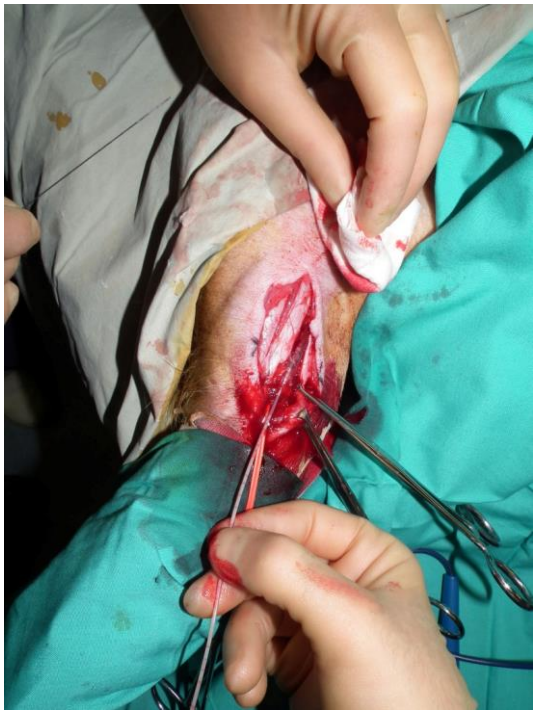


Figure 2.50: BM2; left fore-leg experimental rx:fogarty ch3 x5; cutting balloon 5mmx2cm (x1 pass in 2cm steps)



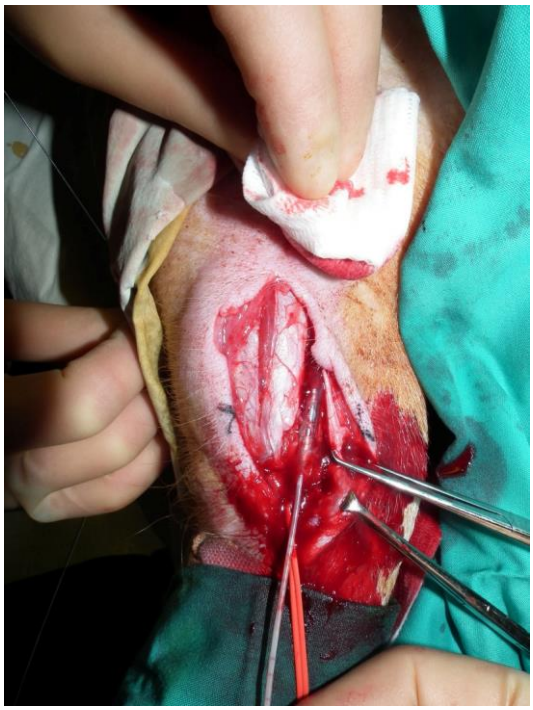


Figure 2.51: 5mmx2cm cutting balloon perforating distal vein on removal

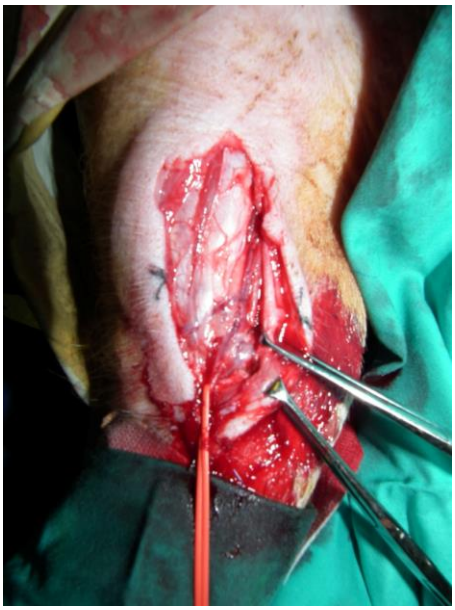


Figure 2.52: Vein perforation repaired with 6-0 prolene pre-closure

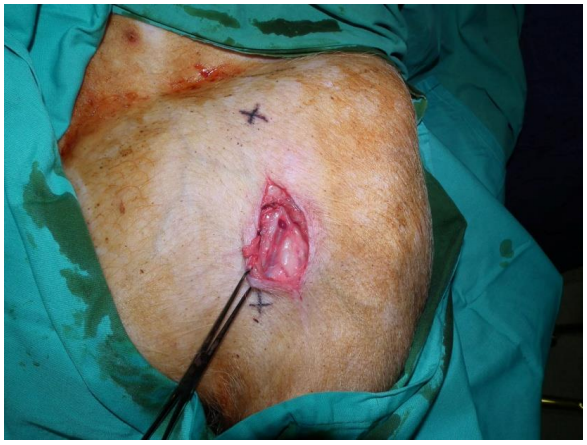


Figure 2.53: BM2; left hind-leg: 1 ml std 3% foam via branch into vein medial to artery

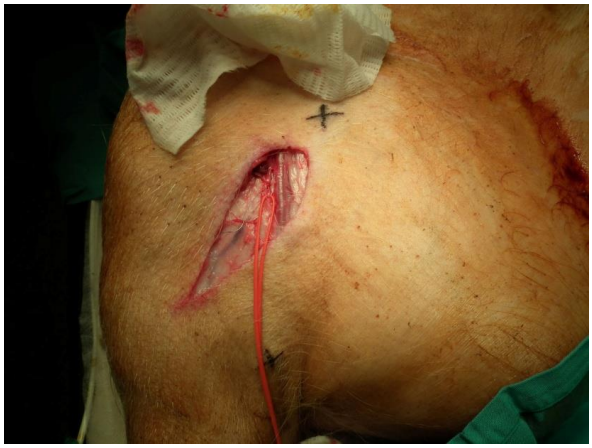


Figure 2.54: BM2; right hind-leg experimetal rx: 1 ml 0.15% xanthum/3%STD foam via branch into vein lateral to artery

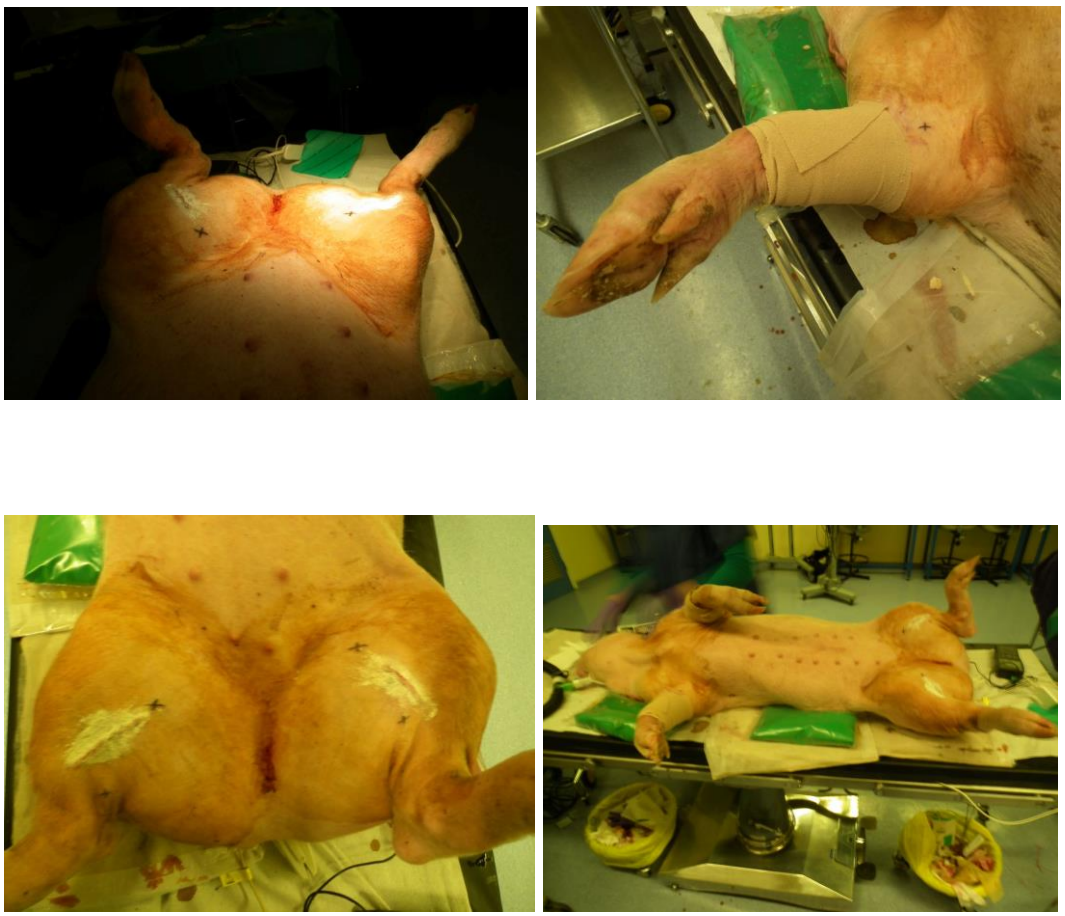


Figure 2.55: All wounds treated with cicatrin® powder and treated region of fore-limbs dressed with adhesive compression bandages

2.3.3 TERMINATION PROCEDURES

At the end of the 12 week period the animals were again anaesthetised as previously.

USS was performed on each of the limbs pre-termination to assess their patency and ultrasonographic appearance. In both animals all arteries were palpable and showed normal flow on USS. All treated veins in both animals were occluded but this procedure was difficult due to the size of the vessels and the animal being supine.

Each 10cm length of superficial vein was removed surgically, as atraumatically as possible. Dissection proved difficult due to scar tissue. Vascular tissue (arteries and vein) were removed en-bloc to prevent damage to the treated segments. These tissue segments were then prepared, marked and processed in formalin for histopathological processing and analysis. The tissue was tacked out to length on histology specimen card and with both mapping and photographs of specimens used to note orientation for future analysis.

Organs were removed from the animals for other research studies. The animals were then euthanised in accordance with Home Office regulations.

A record of the procedures for each animal is recorded below.

2.3.3.1 ANIMAL 1 (BM 1): TERMINATION PROCEDURE WEIGHT: 150KG; DATE: 12/9/2011

Pig anaesthetised (Figure 2.56).

USS all 4 limbs: No venous treated segments patent; arteries palpable with normal flow.

Open cut down between tattoo markings.

Difficult dissections especially forelegs due to scar tissue hence tissues removed en-bloc.

Vascular tissue removed en-bloc was prepared, marked and immediately fixed in 10% buffered formal saline.

Harvest Time: 4 hours.

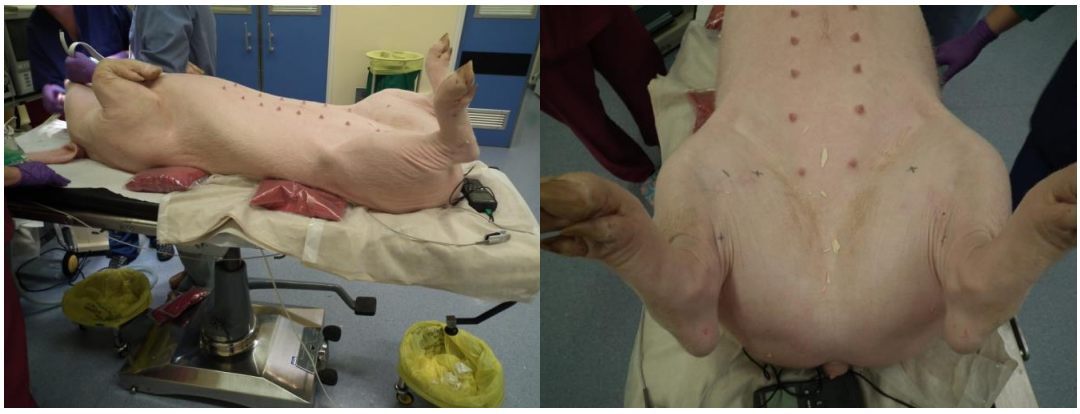


Figure 2.56: Animal 1 (BM1) being anaesthetised

Figure 2.57: BM1; right fore-leg

Figure 2.58: BM1; left fore-limb

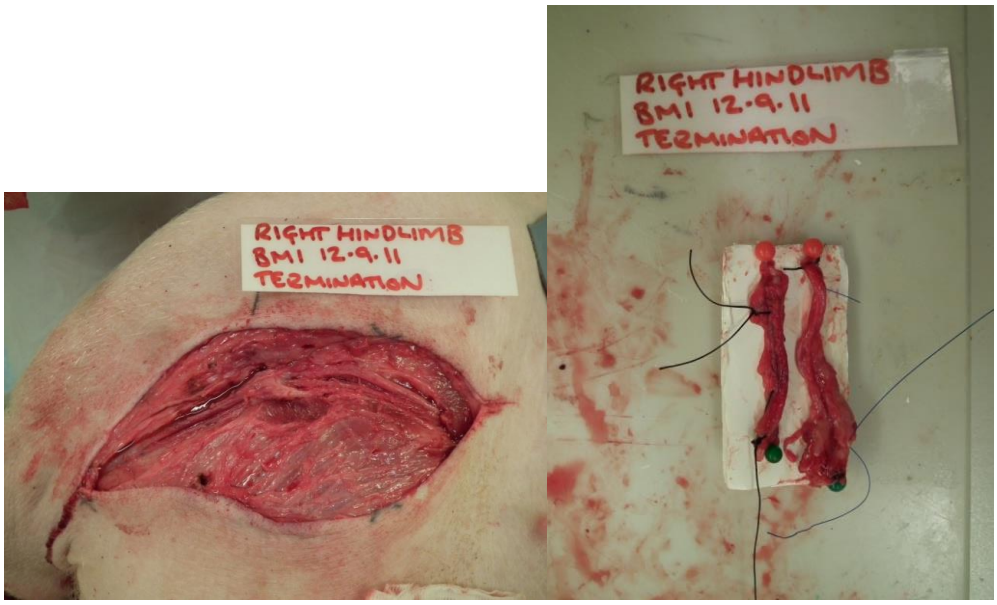


Figure 2.59: BM1; right hind-limb

Figure 2.60: BM1; left hind-limb

2.3.3.2 ANIMAL 2 (BM 2): TERMINATION PROCEDURE: WEIGHT: 150KG; DATE: 12/9/2011

Pig anaesthetised.

USS all 4 limbs: No venous treated segments patent; arteries palpable with normal flow.

Open cut down between tattoo markings.

Difficult dissections especially forelegs due to scar tissue hence tissues removed en-bloc.

Vascular tissue removed en-bloc was prepared, marked and immediately fixed in 10% buffered formal saline.

Harvest Time: 3 hours.

Figure 2.61: BM2; right fore-limb

Figure 2.62: BM2; left fore-limb

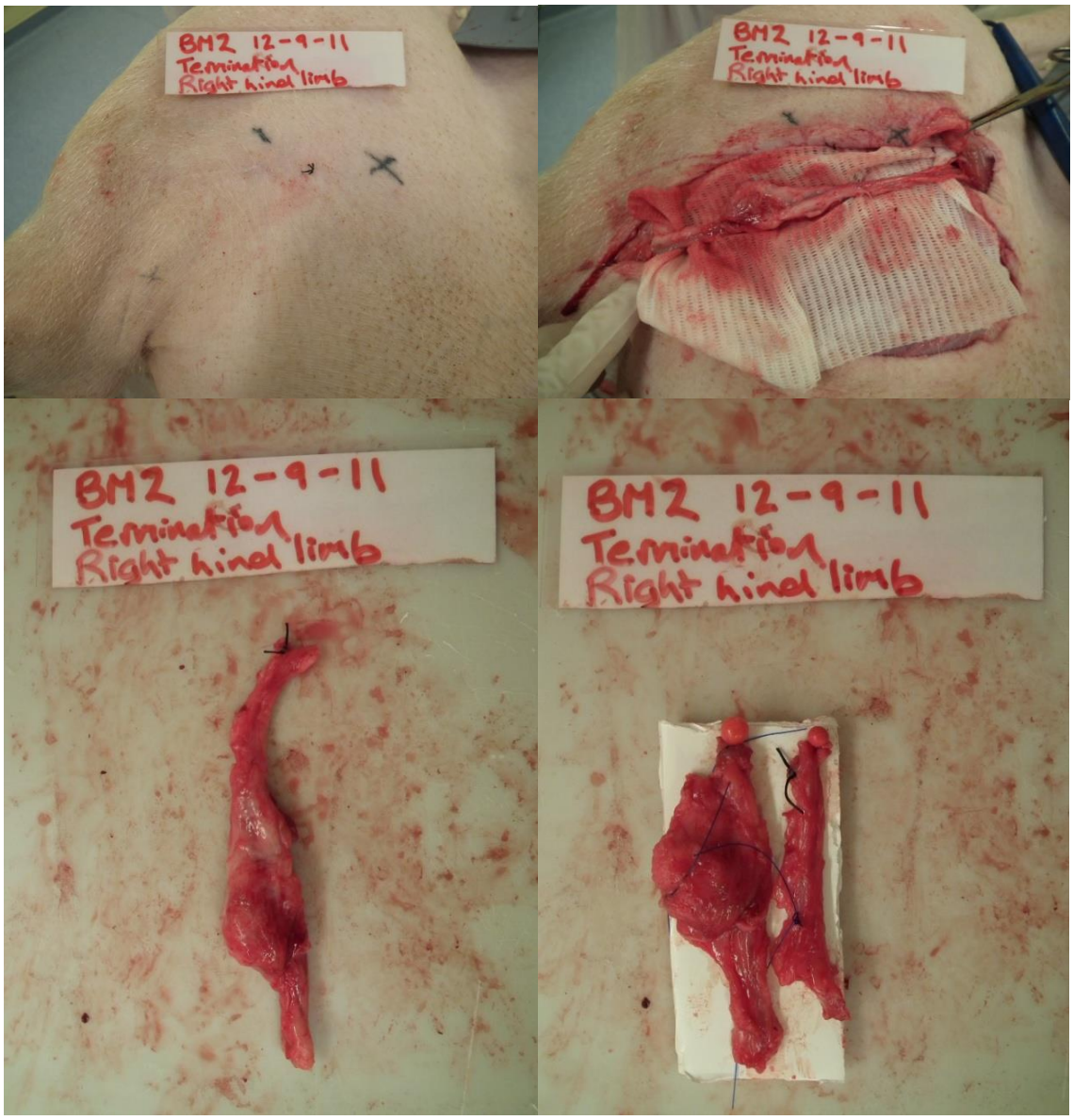


Figure 2.63: BM2; right hind-limb

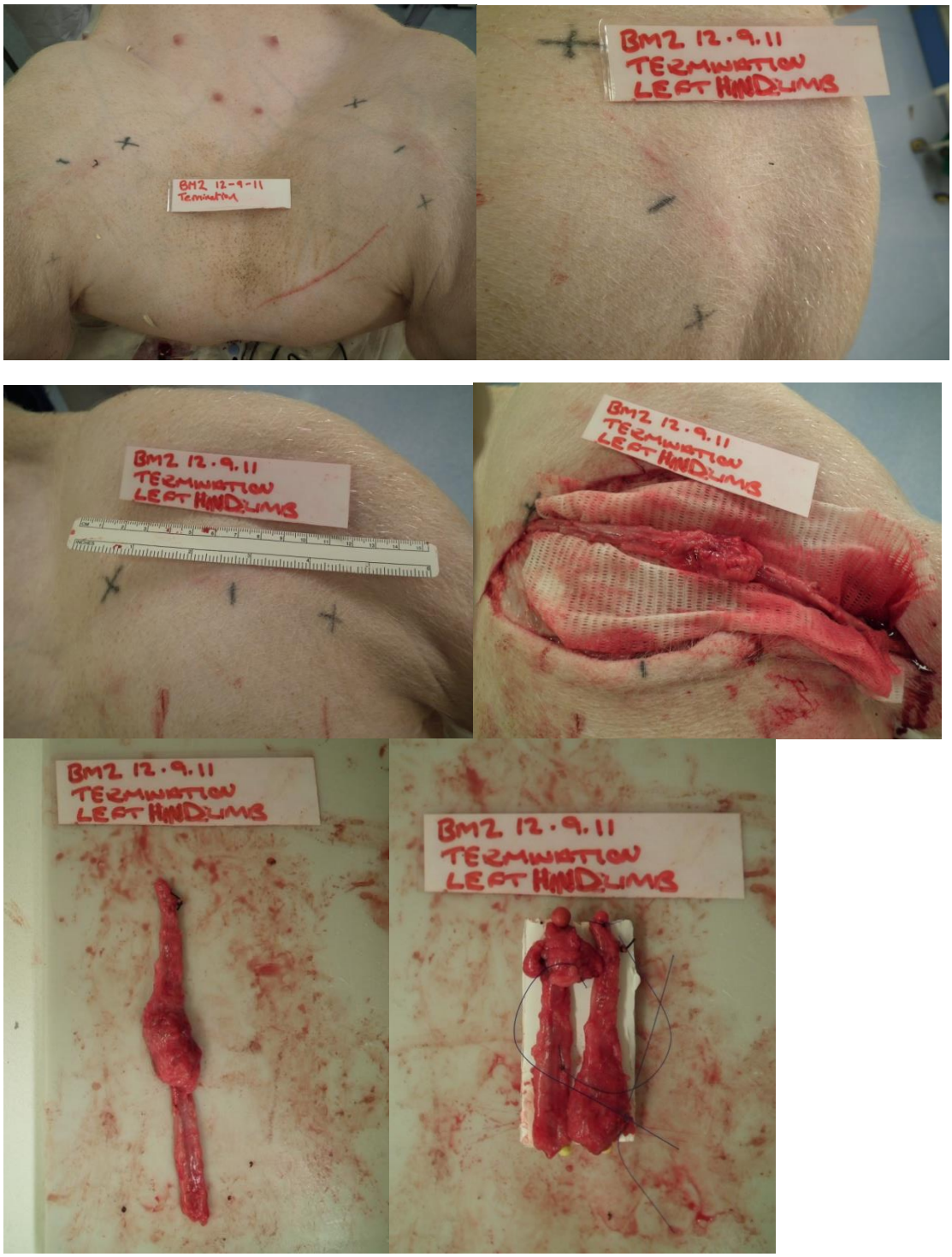


Figure 2.64: BM2; left hind-limb

2.4 HISTOLOGICAL PROCESSING

2.4.1 HUMAN TISSUE

Following immediate fixation in formalin (Figure 2.17: Human tissue) specimens were stored at room temperature for at minimum one week. All human tissue was processed as per section 2.4.3

2.4.2 ANIMAL TISSUE

Following immediate fixation in formalin, animal tissue was sectioned prior to a further fixation period. Tissue was cut with a scalpel into 5mm wide blocks by the lead researcher. Each was stored in individual numbered containers each traceable to that section from which it came. The sections were again stored in formalin for an additional week. Due to the abundance of tissue every other segment was then processed as per section 2.4.3.

2.4.3 PROCESSING OF HUMAN AND ANIMAL TISSUE SEGMENTS

Tissue was processed in the histopathology laboratory as follows and in Table 2.2: fixed tissue was transferred to a cassette labelled with a unique laboratory ID number, and processed into paraffin wax in a Leica ASP2000 tissue processing machine (Leica Microsystems, Milton Keynes, Bucks, UK) using the routine overnight programme below:

Reagent	Time	Temperature
70% ethanol	30 min	37°C
80% ethanol	30 min	37°C
90% ethanol	30 min	37°C
95% ethanol	30 min	37°C
100% ethanol	1:00h	37°C
100% ethanol	1:00h	37°C
100% ethanol	1:30h	37°C
Xylene	1:00h	37°C
Xylene	1:30h	37°C
Xylene	1:30h	37°C
Wax	1:00h	65°C
Wax	1:00h	65°C
Wax	1:00h	65°C

Table 2.2: Histopathology processing of vein/tissue segments (sequence runs from top to bottom)

Tissue wax embedded on a Leica EG1150C embedding station in Cellwax plus wax (Cellpath Ltd, Newtown, Powys, UK). Tissue was sectioned transversely on a Leica RM 2235 microtome (Figure 2.27) at 4µm and placed on a glass microslide (Solmedia Ltd, Shrewsbury, UK; Figure 2.28). After drying on a hot plate for 2 hours at 70°C, slides were de-waxed through 4 changes of xylene

(VWR International Ltd, Lutterworth, Leicestershire, UK) and 4 changes of absolute ethanol (Sigma, Poole, Dorset, UK) before then being rehydrated in running tap water before staining with Mayers Haematoxylin for 2 minutes and Eosin for 2 minutes. Haematoxylin and Eosin was used to demonstrate overall vein architecture and had been found in preceding research at Leeds University to be the most useful staining method to gain the necessary information on damage inflicted chemically and mechanically on vein samples. The previous research at Leeds University had also used van Geison and Picrosirus Red staining to demonstrate the elastin fibres and collagen respectively, however for the purpose of damage assessment these had previously been deemed superfluous to requirements. Transmission electron microscopy was deemed unnecessary by the pathologist who had taken part in all previous mechano-chemical vein damage experiments in Leeds. Sections were then dehydrated in 4 changes of absolute ethanol and three changes of xylene before coverslipping in DPX (Solmedia). Analysis was performed on glass slides using light microscopy examination. All processed H&E sections were digitally scanned using Aperio Scanscope/Imagescope (Version 10.2.2.2319), Aperio Technologies, Vista, California) to produce images for subsequent observer blinded analysis. These images allowed simultaneous inspection and measurement of areas of interest (FIGURES 2.69 and 2.70).

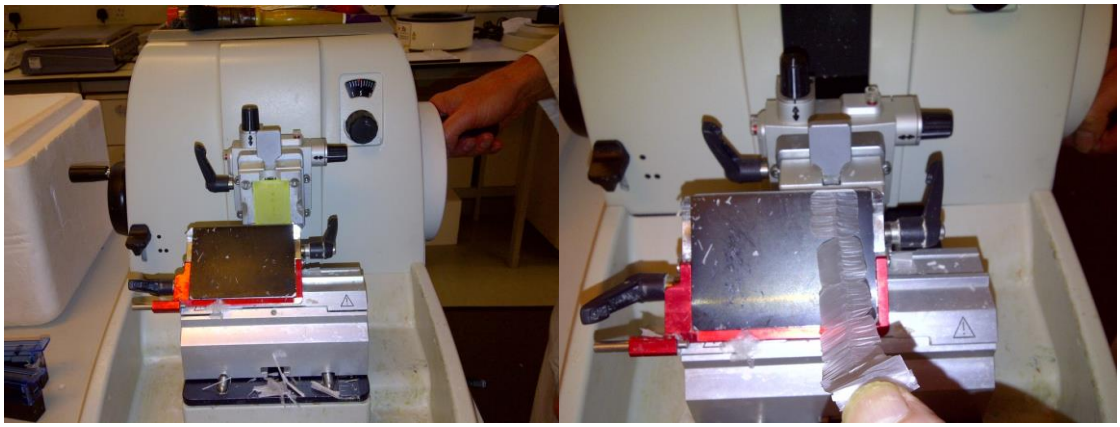


Figure 2.65: Tissue was sectioned transversely at 4µm using a Leica RM 2235 microtome

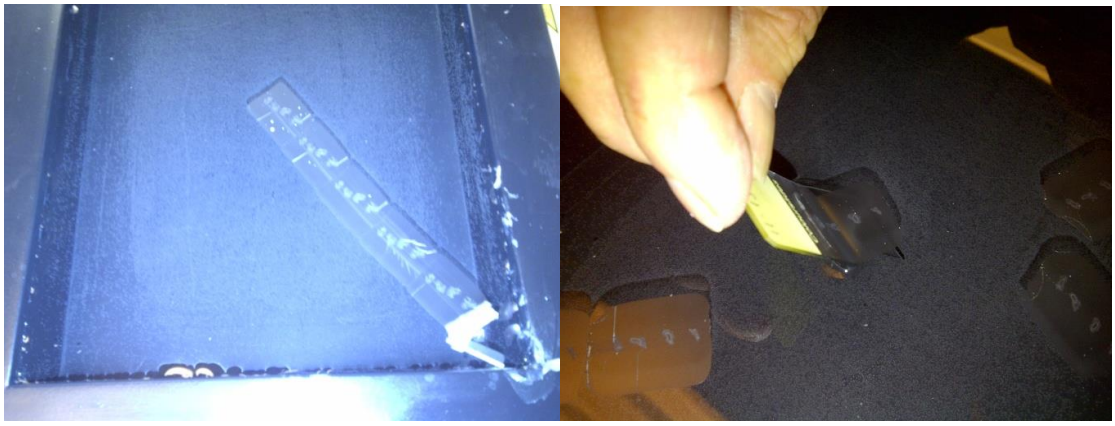


Figure 2.66: 4µM wax embedded sectioned tissue placed on glass microslides (Solmedia Ltd, Shrewsbury, UK)

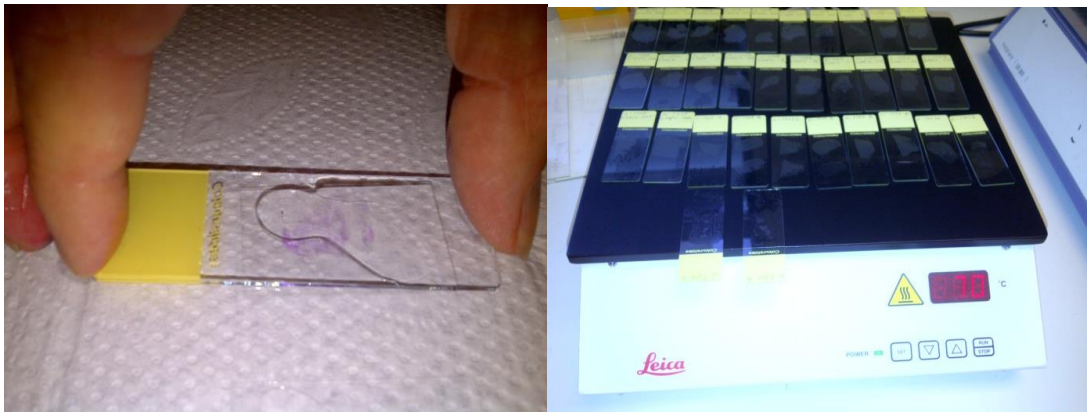


Figure 2.67: Sectioned tissue on a glass microslide dried on tissue prior to drying on a hot plate for 2 hours at 70°c slides

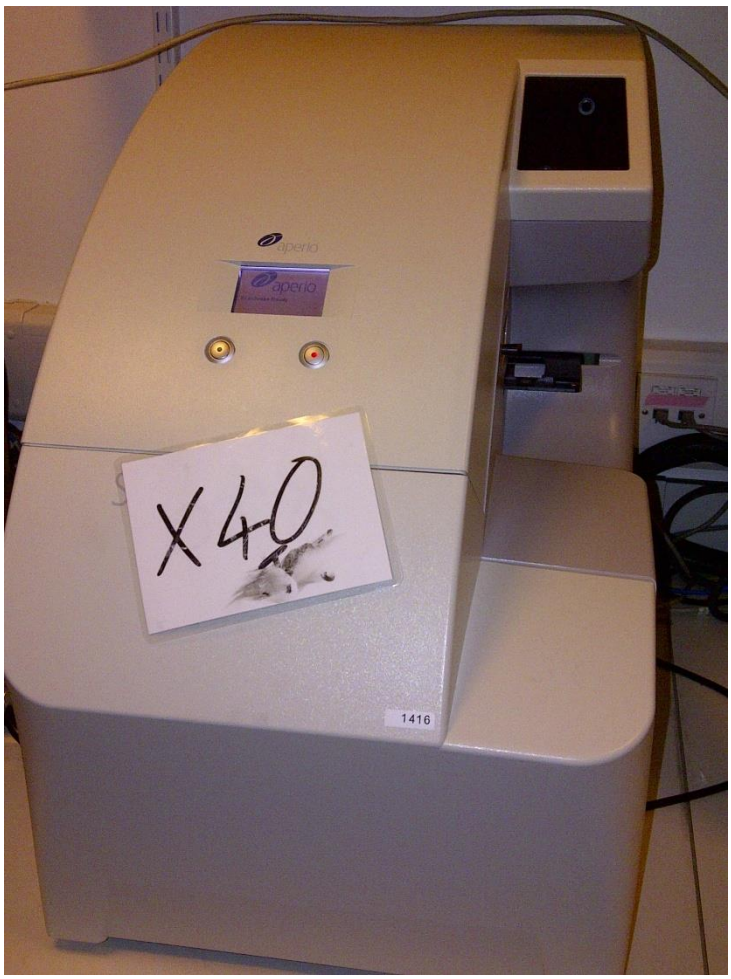


Figure 2.68 : Slides were digitised using an Aperio Scanscope and the images stored on a server for quantitative histological analysis using 20x magnification sections analysed using Aperio Imagescope v.10.2.2.2319 (Aperio technologies, Inc. Vista, CA, USA)

2.5 HISTOLOGICAL ANALYSIS

2.5.1 HUMAN TISSUE

Haematoxylin and Eosin (H&E) staining was performed for identification of the intimal, medial and adventitial layers. Interpretation was performed by a consultant cardiovascular pathologist blinded to which specimens were the test or control segments and to the nature of tests performed. All qualitative analysis was performed by the pathologist along with the lead researcher with blinding removed after all batches relevant to an experimental category had been assessed. Slides from previous research were used prior to commencement of these analyses to allow the consultant pathologist to train the lead researcher in the assessment of normal, chemically and mechanically damaged vein segments both for the purposes of qualitative and quantitative analysis. A minimum of two sections were included per slide hence per experiment and two were used to determine the results. 30% of quantitative analyses were performed in isolation both by the pathologist and the lead researcher. There was no evidence of significant inter- or intra-observer variation hence the lead researcher performed the remaining 70% of quantitative studies with randomly assigned checks or advice on difficult cases performed by the pathologist.

2.5.1.1 QUALITATIVE ANALYSIS

Qualitative analysis was performed on all specimens at 20x magnification using the original glass slides and a microscope. For each specimen two transverse sections underwent examination. The qualitative outcome measures recorded were:

1. proportion of endothelial cell loss (as a percentage of entire endothelial surface on the slide
2. depth of cell injury (measured in cell depths)
3. comment on any subendothelial nucleolar vacuolation or opacification of nuclei
4. valve interference with foam activity
5. other comments

2.5.1.2 QUANTITATIVE ANALYSIS

Quantitative data were collated from 20x scanned computerised histology slides analysed via Aperio ImageScope v.10.2.2.2319 (histological analysis computer programme). For each specimen two transverse sections underwent examination. The quantitative outcome measures were endothelial cell loss and measurement of the depth of vein wall injury.

2.5.1.2.1 MEASUREMENT OF ENDOTHELIAL CELL COVERAGE/LOSS

Endothelial cell loss (Figure 2.69) measurements were performed at 20 x magnification (to reduce bias) using the Aperio “Pen Tool” to measure the luminal surface and the collective length of endothelial cells remaining in micrometres (µm). Where veins were cross-sectioned the luminal surface was measured as the complete ring and where vein was displayed as an incomplete ring, cut ends of vein were excluded from analysis and the intervening

endothelium measured as the “endothelial width”. Endothelial cell loss as a proportion of luminal surface present was derived as a percentage figure.

The extent of endothelial cell coverage was determined by first measuring the total luminal circumference. The intima was then closely inspected and intact, densely stained endothelial cells that remained in contact with the basement were manually marked by the observer and the percentage luminal endothelial cell loss calculated.

The range of measurements between the consultant pathologist and the lead researcher was within 10%, demonstrating minimal spread. Comparison of paired measurements did not reveal any significant difference (inter-observer p=0.821, intra-observer p=0.615).

2.5.1.2.2 MEASUREMENT OF DEPTH OF VEIN WALL INJURY

Depth of injury (Figure 2.70) measurements were performed at 20 x magnification (to reduce bias) using the Aperio “Pen Tool” to measure depth of injury and depth of media in micrometres (µm), at the “12 points” of a clock-face of in cross-sectional histological images of “tubular” vein at the “12 points” of a clock-face of. Where vein was displayed as an incomplete ring, cut ends of vein were excluded from the analysis and 12 measurements were taken at evenly spaced intervals across the remaining non-mechanically damaged luminal vein surface. From these quantitative data, the median and inter-quartile ranges of depth of injury were calculated for each specimen tested.

The depth of vein wall injury was classified in two ways:

1) the absolute depth of injury, and

2) the depth of injury as a proportion of the thickness of the media expressed as a percentage.

The width of the tunica media was measured. This was defined as the point from the lumen to the outer layer of smooth muscle. Twelve measurements were taken per section, evenly spaced around the circumference of the intima. This approach was used as the intima and tunica media are the most constant structures, whilst the internal elastic lamina may be absent in places and the edge of the adventitia may be partially removed during vein harvesting. The percentage depth of injury was then established by measuring the depth that sub-endothelial vacuolation extended from the intima (vein lumen). These changes are characterised by swollen, pale smooth muscle cells, with an unravelling nucleolus, as opposed to the densely stained, small nuclei exhibited by normal cells. It is important to quantify the injury sustained by smooth muscle cells as this potentially represents collagen damage within that region. At each tunica media measurement, the depth of injury was established and a measurement taken (the absolute depth of injury) and the median tunica media width and median depth of injury was derived for each transverse section. The percentage depth of injury was then calculated. The median percentage depth of injury and the median absolute depth of injury for each experimental group were obtained.

Reproducibility of the measurement techniques was assessed by comparison of inter- and intra-observer measurements. The range of measurements between the consultant pathologist and the lead researcher was within 10%, thereby again demonstrating minimal spread. Comparison of paired measurements did not reveal any significant difference (inter-observer $p=0.799$, intra-observer $p=0.589$).

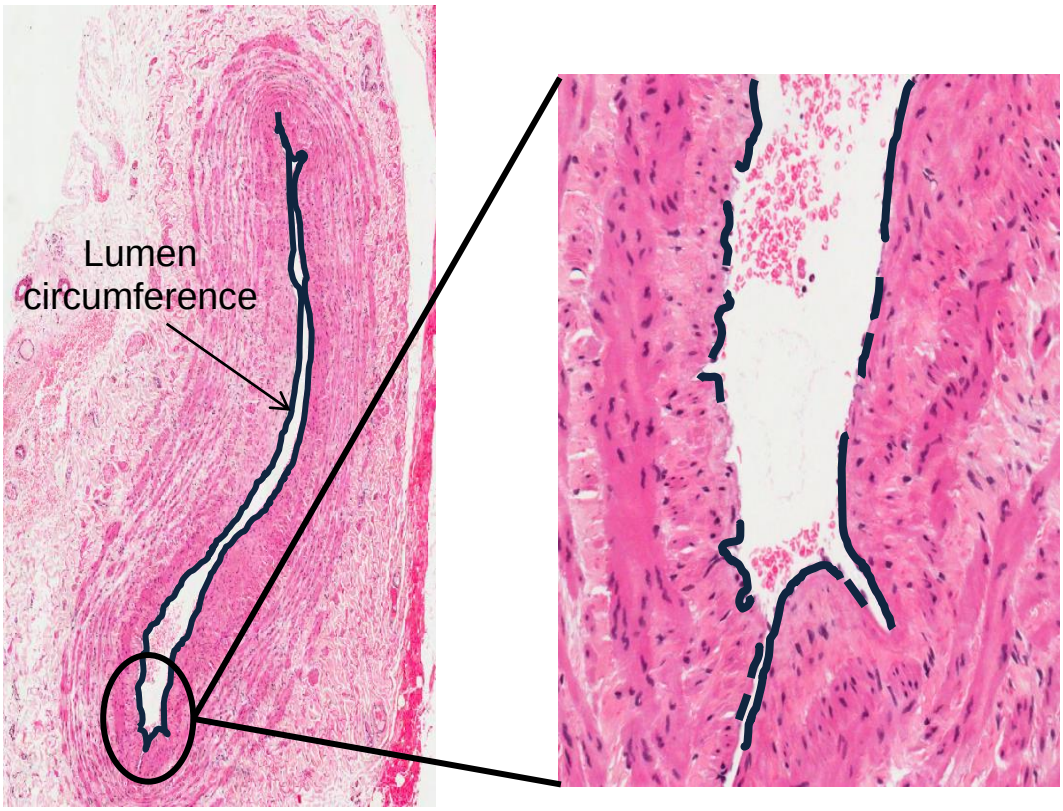


Figure 2.69: Method of measuring luminal circumference and endothelial cell loss using Aperio Imagescope v.10.2.2.2319

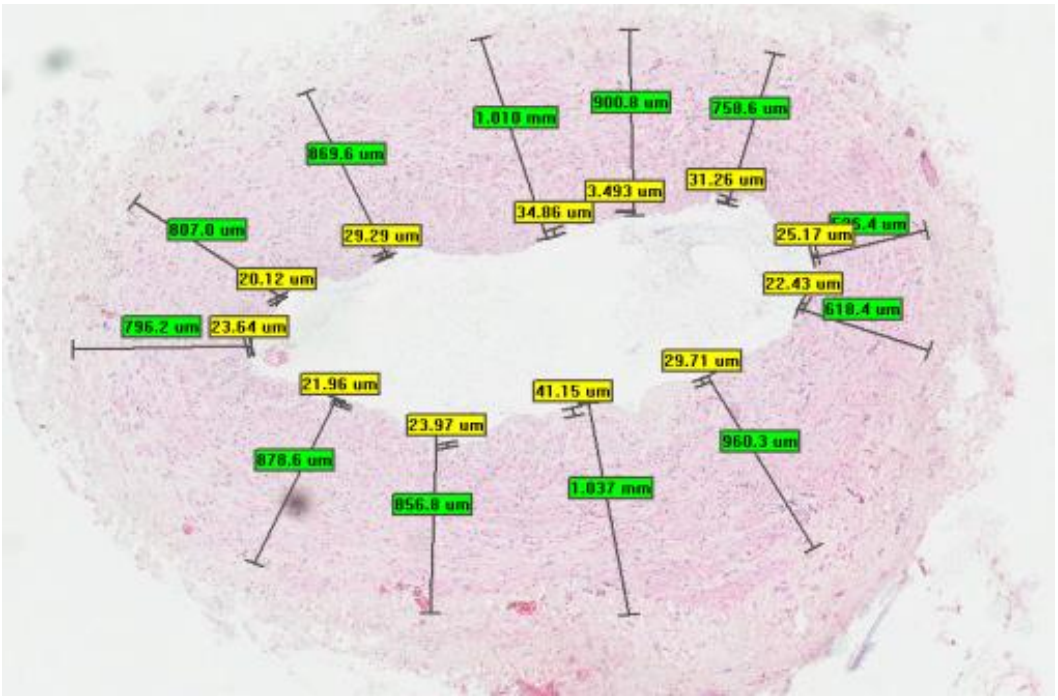


Figure 2.70: Method of measuring media depth and depth of injury at 12 points using Aperio Scanscope v.10.2.2.2319

2.5.2 ANIMAL TISSUE

Haematoxylin and Eosin (H&E) staining was again performed for identification of intimal, medial and adventitial layers. Preliminary sections were performed on porcine vein tissue during the establishment of the animal vein model, to ensure that findings were comparable to those in human tissue. Porcine vein showed similar histological findings to human with the same structures highlighted in a similar fashion by H&E stain. Interpretation of test sections was performed by a consultant cardiovascular pathologist blinded to the nature of tests performed however the pathologist was aware that the tissue was from animal studies. Similar to experimentation on human tissue each sample had an experiment number and was then assigned a laboratory number which was that used during histological analysis. These numbers were converted back to the initially assigned experimental number only when all analyses had been performed to keep both the pathologist and lead researcher blind to the nature of the test. All qualitative and quantitative analyses of animal tissue were performed by the pathologist along with the lead researcher.

Animal tissue was sectioned every 5mm, and every other section was analysed due to the amount of tissue. Initial qualitative results, examined blindly, were recorded by the lead researcher and consultant vascular pathologist and then in-order to more accurately assess samples the blinding was removed and the samples grouped together in the order in which they were both resected and hence sectioned. This was necessary to decipher each section’s relation to one another by allowing a representation of the full length of the specimen. In order

to assess the tissue and treatment and control sections the en-bloc tissue sections were orientated correctly in relation to each other to ensure that the correct vein was being assessed. This was necessary more so with paired hind-limb veins with one acting as test and the other control and where veins were occluded.

Data were gained from a minimum of six and maximum of eight sections per treatment or control with two, of a maximum four cuts, analysed to derive the both the qualitative score and quantitative results.

Analysis was performed on all specimens at 20x magnification using the original glass slides and a light microscope and from 20x scanned computerised histology slides analysed via Aperio Scanscope/Imagescope (Version 10.2.2.2319), Aperio Technologies, Vista, California) (histological analysis computer programme).

Stereology was considered as a potential method that could be utilised for this experimentation. However we felt stereology would add little at this stage but if this animal model and experimentation did yield promising results, any further animal experiments could be assessed using stereology. Similarly immunohistochemistry was considered but having been used in previous vein damage research in Leeds to no avail, was deemed an unnecessary methodology as well as expense.

Fore-limb veins in both animals were treated with a 3Ch Fogarty balloon catheter and a cutting balloon with the right fore-limbs additionally treated with 3% STD foam. The proximally treated segment of vein was compared histologically to the distally untreated segment acting as control. This control was selected because to remove vein at the initial experimental stage would have changed the anatomy and physiology of the limb and to use other veins whilst retrieving tissue for final analysis examples would have introduced significant variance.

Hind-limb veins were treated with either 3% STD foam or 0.15% xanthum and 3% STD foam. The hind-veins are paired hence the paired vein acted as control.

2.5.3 QUALITATIVE ANALYSIS

Details of what was present in the specimen and the nature of damage caused were recorded. Two attempts were made at developing qualitative based scoring systems. Initially samples were scored using the criteria in table 2.3 with a minimum score of 1 and maximum of 20 (TABLE 2.3). Subsequent to removing the blinding it was found that using pathology based findings in isolation gave little comparable information. Hence this was adjusted to an ordinal scale of damage from what we would have liked most to have happened to the treated veins, i.e. obliteration/occlusion, through to what would be deemed treatment failure i.e. no change or worse still varicose vein formation. Thereby a second qualitative scale was formed on this basis with the lowest score of 1 delineating the most effective treatment and a score of 7 the least effective i.e. the treatment itself resulting in causing varicosities of the treated vein (TABLE 2.4).

EFFECT	SCORE
Congestion of vessel with blood	1
Dilated veins with thickened wall	2
Inflammation (acute and local)	2
Inflammation (chronic)	3
Fibrous repair(mature)	2
Proliferative repair(cellular)	4
Organisation and recanalisation (per vessel)	3
Dilated and tortuous vein	3

Table 2.3: Animal vein damage qualitative pathology score 1

Effect	Points
Vein obliteration with no additional effects	1
Vein obliteration with additional effects	2
Recanalisation without additional effects	3
Recanalisation with additional effects	4
Vein patent without additional effects	5
Vein patent with additional effects	6
Vein varicosity	7

Table 2.4: Animal vein damage qualitative pathology score 2

Additional effects included, acute inflammation, chronic inflammation, fibrosis and proliferative repair

2.5.4 QUANTITATIVE ANALYSIS

Previous experiments using human GSV allowed measurement of the vein circumference and endothelial loss for comparison of test versus control segments. Similarly depth of media injury was measurable as an absolute figure and as a percentage of the media. These measures were both obvious due to the acute nature of the changes that had been induced by treatments inflicted upon the vein segments. It was clear on initial blinded qualitative analysis of animal tissue which had been subjected to treatment three months previous, that there was no such marked change in the media or endothelial loss per say that could be measured. Attempts were made to furnish multiple data points including vein wall thickness and vein diameter, however given the nature of the treatments these figures could not be obtained with any regularity and were therefore not useful for comparison purposes. The only useful measure that could be made was the patent luminal circumference or PLC. The patent luminal circumference could be measured whether the vein remained patent, occluded, recanalised, expanded or collapsed with an occluded vein having a PLC measure of 0.

Quantitative analysis was carried out by the lead researcher using Aperio Imagescope after confirmation of technique with the pathologist.

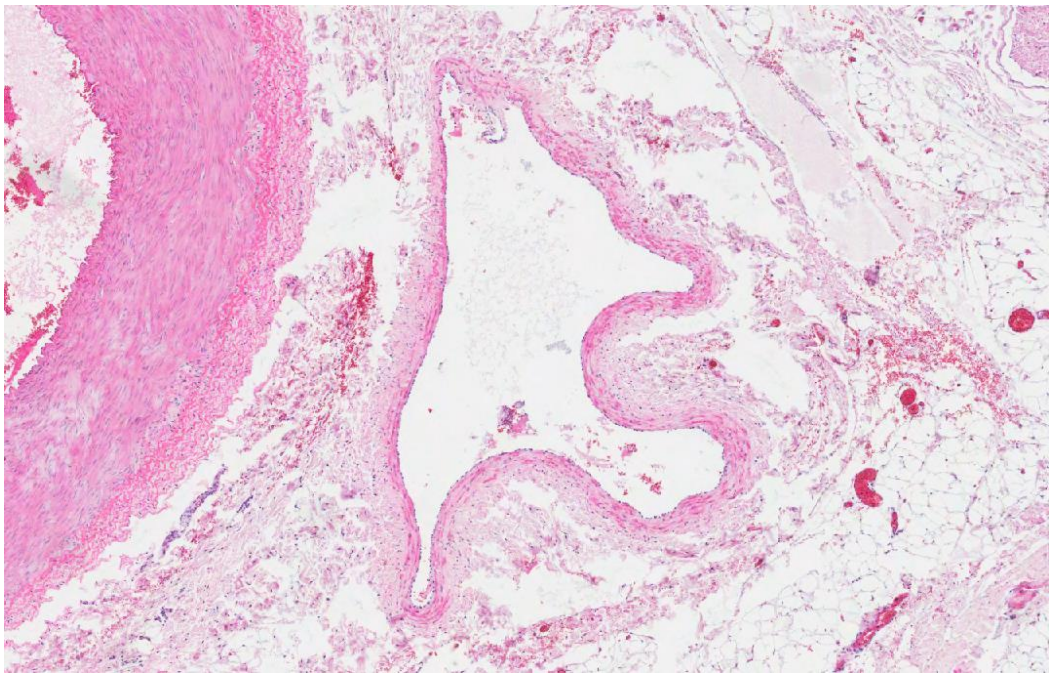


Figure 2.71: x3 Example of forelimb vein experiments with varicosity created by cutting balloon and 3%STD treatment

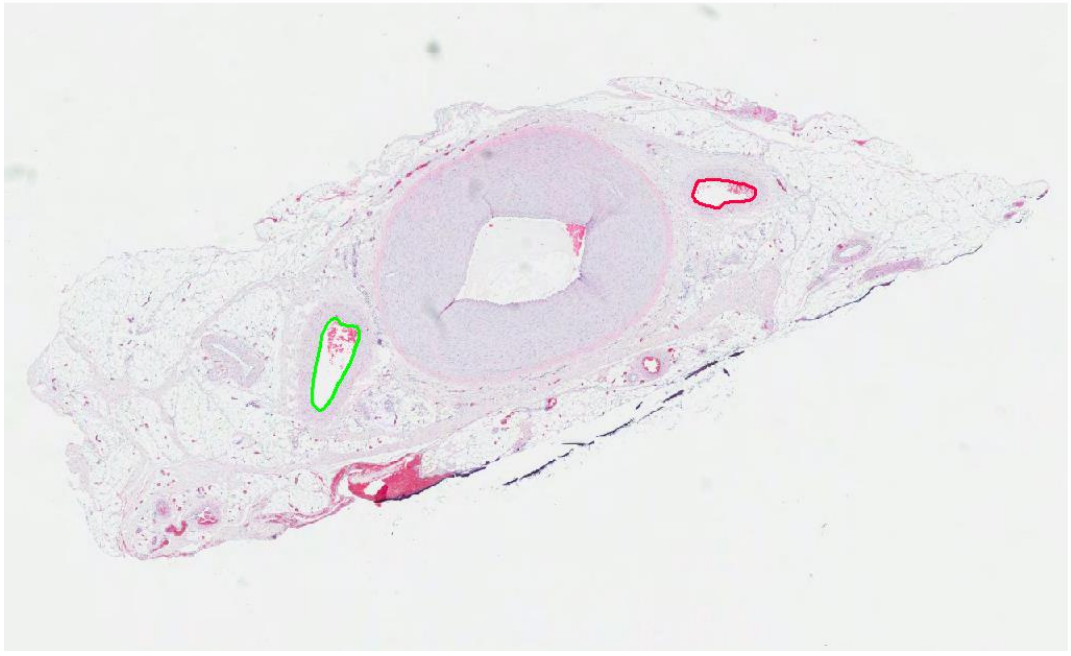


Figure 2.72: X1 Example of patent lumen circumference measurements of a distal segment in a hind-limb with patent test and control veins in a limb tested with 3% STD foam into more proximal aspect of the vein highlighted in green and the control limb vein lumen in red.

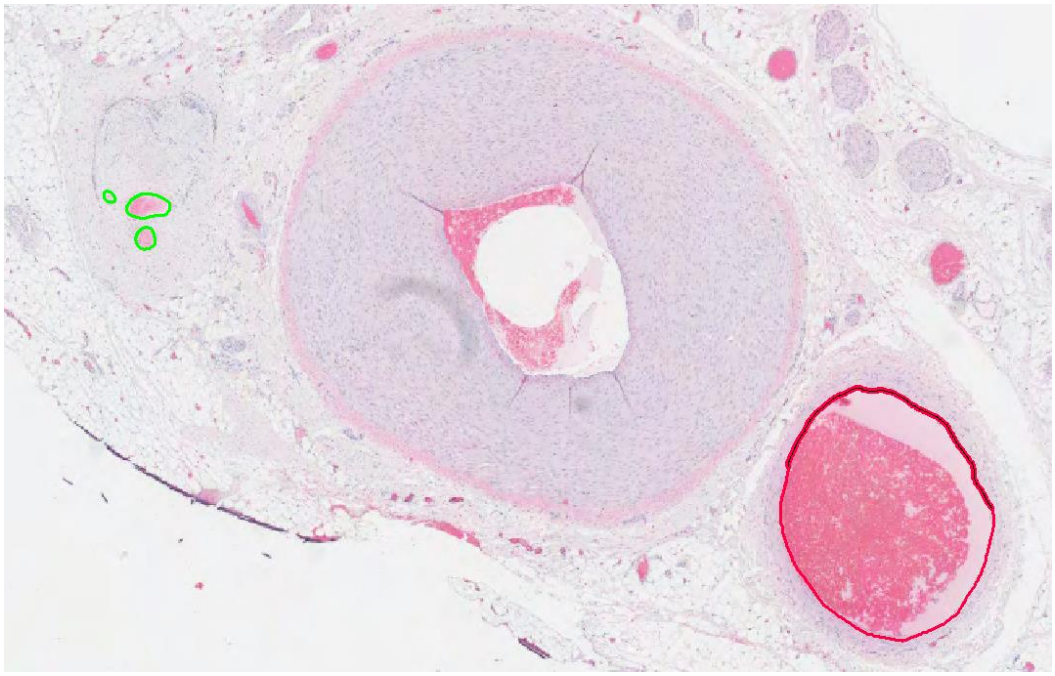


Figure 2.73: x2 Example of recanalisation of 3% STD treated test vein on left with neo-lumens marked in green. Control vein lumen is marked in red.

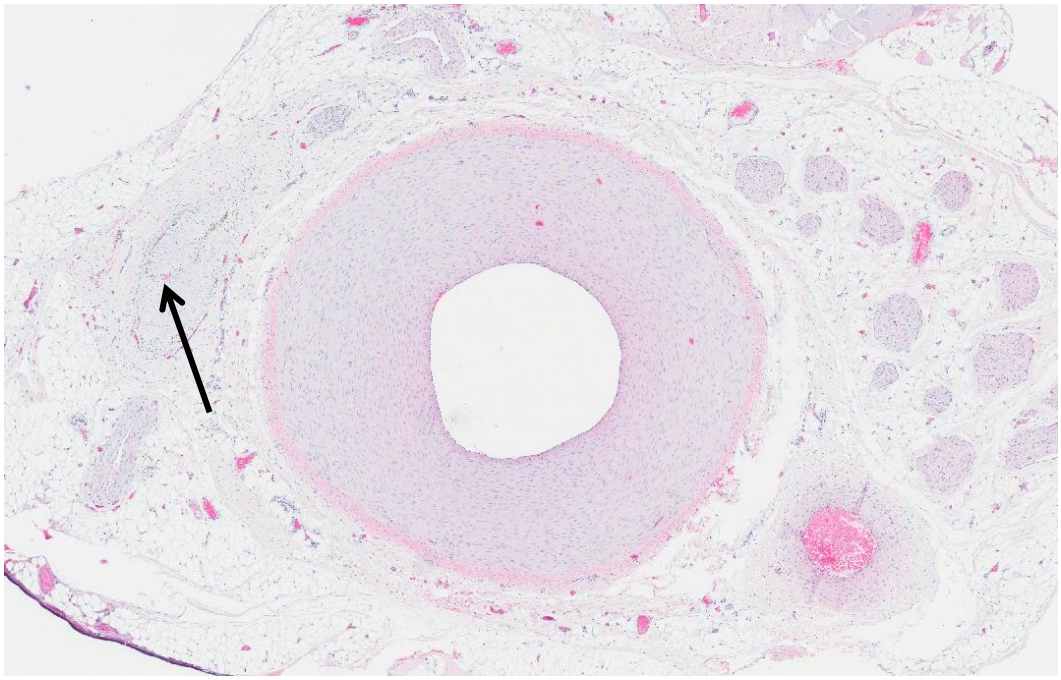


Figure 2.74: x2 Example of test vein obliteration using 3% STD (arrow) with patent control vein

2.6 STATISTICAL ANALYSIS

Analysis was performed using PASW (SPSS, Statistical Package for Social Sciences Inc, Chicago, Illinois, USA) version 18.0. A p value of <0.05 was considered significant.

2.6.1.1 PARAMETRIC DATA

Data was subjected to the Shapiro-Wilk test in order to confirm whether the set was normally distributed. If all criteria were met, inter-group comparisons between 2 groups were performed using the unpaired or paired t test, where appropriate. Data is presented using means and standard deviation.

2.6.1.2 NON-PARAMETRIC DATA

Inter-group comparisons were performed using the Mann-Whitney U, Kolmogorov-Smirnov or Kruskal Wallis test where appropriate. Data is presented using medians and inter-quartile ranges (IQR)

2.6.1.3 REPRODUCIBILITY

Agreement and variability between inter and intra-observer scores were assessed using the Bland-Altman plot and paired t test.

CHAPTER III

IN-VITRO FOAM STABILITY STUDIES

3 *IN-VITRO* FOAM STABILITY STUDIES

UGFS utilises sclerotherapy in the form of foam to enhance its efficacy by increasing the surface area of the treated vein contacted by the sclerosant. A previously unanswered question in the literature is how the half-lives of the two most commonly used sclerosants compare and how they compare in in-vitro testing regarding damage to the vein wall. This chapter presents the half-life of 3% STD foam versus that of 3% polidocanol. Chapter four examines the damage caused by the respective foams on in-vitro vein segments.

3.1 IN-VITRO FOAM STABILITY EXPERIMENTS

For the purposes of the foam stability studies in this research, foam was prepared using Tessari's technique (1:3 ratio of sclerosant : air) with 20 passes through a double syringe system and immediately transferred to a preheated (37°C) 15ml graduated polyester tube. As stated in the methods section, the rate of change in foam volume cannot be used as a measure of foam stability. Instead the experimental data was converted to percentage of foam remaining against time with the time taken for a 10% (T_{90}) and 50% (T_{50}) decrease in volume used as a measure of foam stability. Thus the initial foam volume was recorded and subsequent readings were taken every 30 seconds until the volume of the foam fell below 25% of its original volume. Experiments were conducted at 37^{o}C (Stuart Incubator S16D, Bibby Scientific Ltd, Staffordshire, UK). 10 runs were performed for both 3% STD and 3% polidocanol foams.

3.1.1 3% STD AND 3% POLIDOCANOL FOAM STABILITY RESULTS

T_{90} and T_{50} values are shown in the Table 3.1 and Figure 3.1. The median T_{90} and T_{50} for 3% polidocanol were significantly higher than those for 3% STD (p=0.008 and p=0.004 respectively). Thus 3% polidocanol shows greater stability than 3% STD foam.

3% STD Run No.	**T_{90} (sec)**	**T_{50} (sec)**		**3% Polidocanol Run No.**	**T_{90} (sec)**	**T_{50} (sec)**
1	130	255		1	175.01	412.5
2	100	204		2	137.26	295.01
3	115.01	228.76		3	116.25	277.5
4	77.65	185.99		4	98.5	244.98
5	104	220		5	110.24	221.25
6	94.33	206.25		6	244.59	465
7	90	200		7	190.46	450
8	106	232.5		8	123	247.5
9	114	240		9	123.59	255
10	75	200		10	106.5	210
Median time (seconds)	**102.0 (IQR 91.1 - 112)**	**213.1 (IQR 201 - 231.6)**		**Median time (seconds)**	**123.30 (IQR 111.7 - 165.6)**	**266.3 (IQR 245.6 - 383.1)**

Table 3.1: T_{90} and T_{50} values forSTD and polidocanol 3% foams

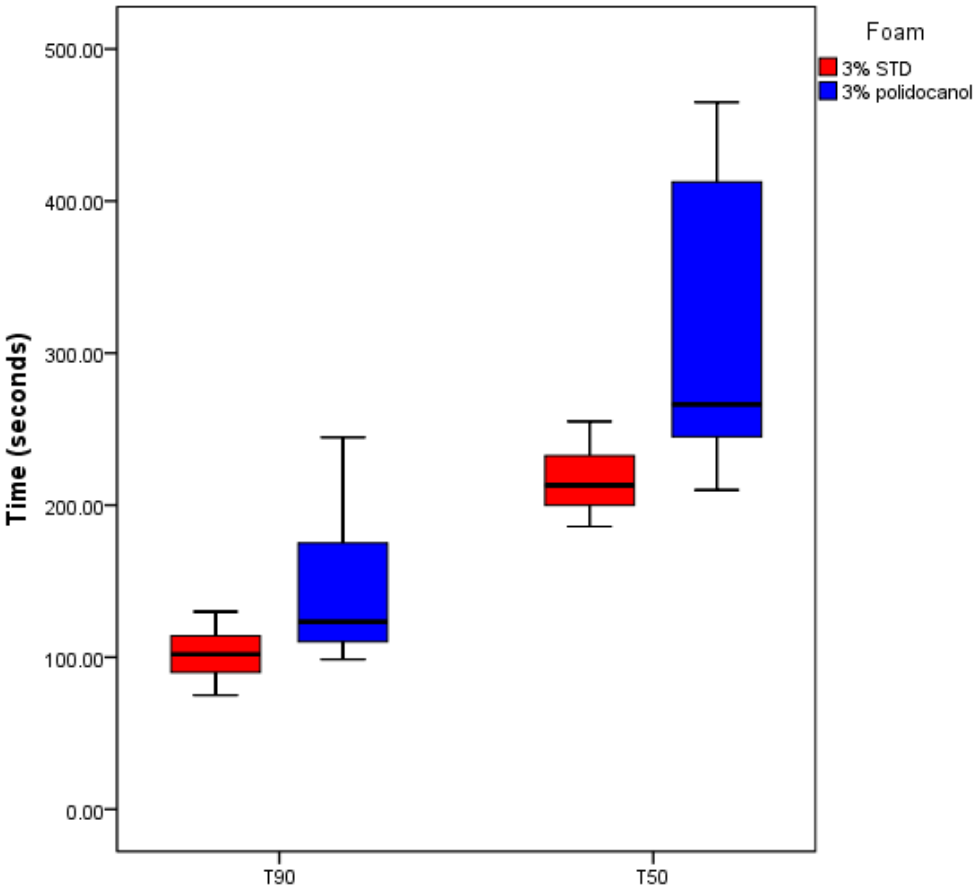


Figure 3.1: T_{90} and T_{50} (sec) for 3% STD and 3% polidocanol foams

3.2 DISCUSSION

Varying initial volumes of foam were observed during all experiments. This may have occurred for multiple reasons. These include that although a graduated syringe was used to draw up the liquid to a volume of 1mL for foaming, there may have been small volume variation above or below this. This also applies to the volume of air drawn up in the second syringe to produce the foam. A smaller syringe, with smaller increment markings could have been used to draw up the initial volumes and then this transferred to a 10mL syringe. It would however have proved difficult to transfer the liquid completely as viscous liquids adhere to the syringe wall.

The results show that 3% polidocanol foam lasts longer than 3% STD foam with a median difference of 21.3 s and 53.2 s for T_{90} and T_{50} respectively. Similar differences between the longevity of the respective foams have been found by others using different strengths of these sclerosants.[235] This may be related to the larger molecular size of polidocanol thus enhancing its surfactant and foaming properties. This ought to be beneficial given that foam sclerotherapy is considered more efficacious than liquid sclerotherapy because of a longer contact time between the sclerosant and the endothelial cells.[104] However as detailed in chapter four, experiments comparing the damage caused to *in-vitro* sections of human great saphenous vein by these proprietary sclerosants revealed that despite its longer foam half-life, polidocanol caused a lesser degree of damage to the vein wall than STD foam.

CHAPTER IV

IN-VITRO HUMAN GREAT SAPHENOUS VEIN EXPERIMENTS

4 *IN-VITRO* HUMAN GREAT SAPHENOUS VEIN EXPERIMENTS

4.1 ASSESSMENT OF EXPERIMENTAL TECHNIQUE: EXAMINATION OF THE HISTOLOGICAL EFFECTS OF HEPARINISED BLOOD CONTROL VERSUS NORMAL SALINE

In order to assess this experimental technique and the control model, the histological effects on vein wall of heparinised blood control were compared to those of normal saline. 10 samples (each with test [heparinised blood] and control [normal saline]) were tested for 15 minutes.

N.Saline 15 min	% Endothelial cell loss		Heparinised blood 15 min	% Endothelial cell loss
Median	8.04		Median	3.84
IQR1	2.92		IQR1	1.42
IQR3	10.83		IQR3	12.56

Table 4.1: Percentage endothelial cell loss at 15 minutes using normal saline and heparinised blood

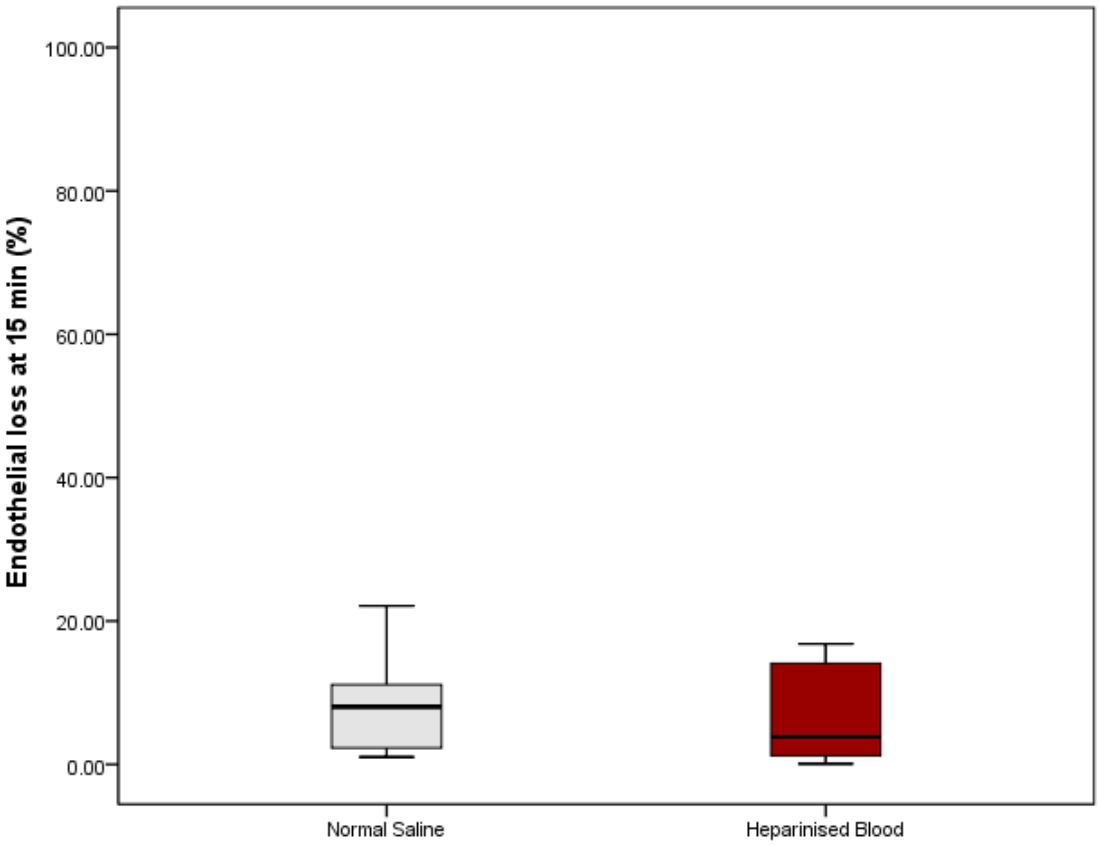


Figure 4.1: Percentage endothelial cell loss at 15 minutes using normal saline and heparinised blood

Comparison of medians across vein samples treated with normal saline for 15 minutes versus those treated for the same time span with heparinised blood revealed no difference in percentage endothelial loss p=0.45 (Mann Whitney U).

Injury to the media was not detected in samples treated for 15 minutes with either normal saline or heparinised blood with median, IQR1 and IQR3 for all 10 test segments using both agents, being 0µm and 0% depth of injury respectively.

Heparinised blood was selected as the control agent for all further experiments of this type as though there was no difference in its effect on endothelial cells versus normal saline. There was in fact a trend toward a lesser median

endothelial loss using heparinised blood (3.84%), versus normal saline (8.04%).

This was not a statistically significant difference.

4.2 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF PROPRIETARY FOAM (3% STD [FIBROVEIN®] AND 3% POLIDOCANOL [SCLEROVEIN®]) SCLEROSANTS ON *IN-VITRO* GSV WALL INTEGRITY

3% STD and 3% polidocanol foams were used on the test segments. Experiments were carried out for 5 and 15 minutes.

3% STD 5 min	% Endothelial Cell loss	Control 5 min	% Endothelial cell loss		3% Polidoc. 5 min	% Endothelial cell loss	Control 5 min	% Endothelial cell loss
Median	86.3	Median	7.26		Median	63.48	Median	7.18
IQR1	84.75	IQR1	3.44		IQR1	62.15	IQR1	3.27
IQR3	93.73	IQR3	7.97		IQR3	82.81	IQR3	14.38

Table 4.2: Percentage endothelial cell loss using 3% STD versus 3% polidocanol and their respective heparinised blood controls at 5 minutes

3% STD 15 min	% Endothelial Cell loss	Control 15 min	% Endothelial cell loss		3% Polidoc 15 min	% Endothelial cell loss	Control 15 min	% Endothelial cell loss
Median	97.64	Median	6.71		Median	85.85	Median	5.7
IQR1	97.25	IQR1	5.7		IQR1	83.83	IQR1	3.1
IQR3	97.83	IQR3	8.13		IQR3	92.48	IQR3	6.42

Table 4.3: Percentage endothelial cell loss using 3% STD versus 3% polidocanol and their respective heparinised blood controls at 15 minutes

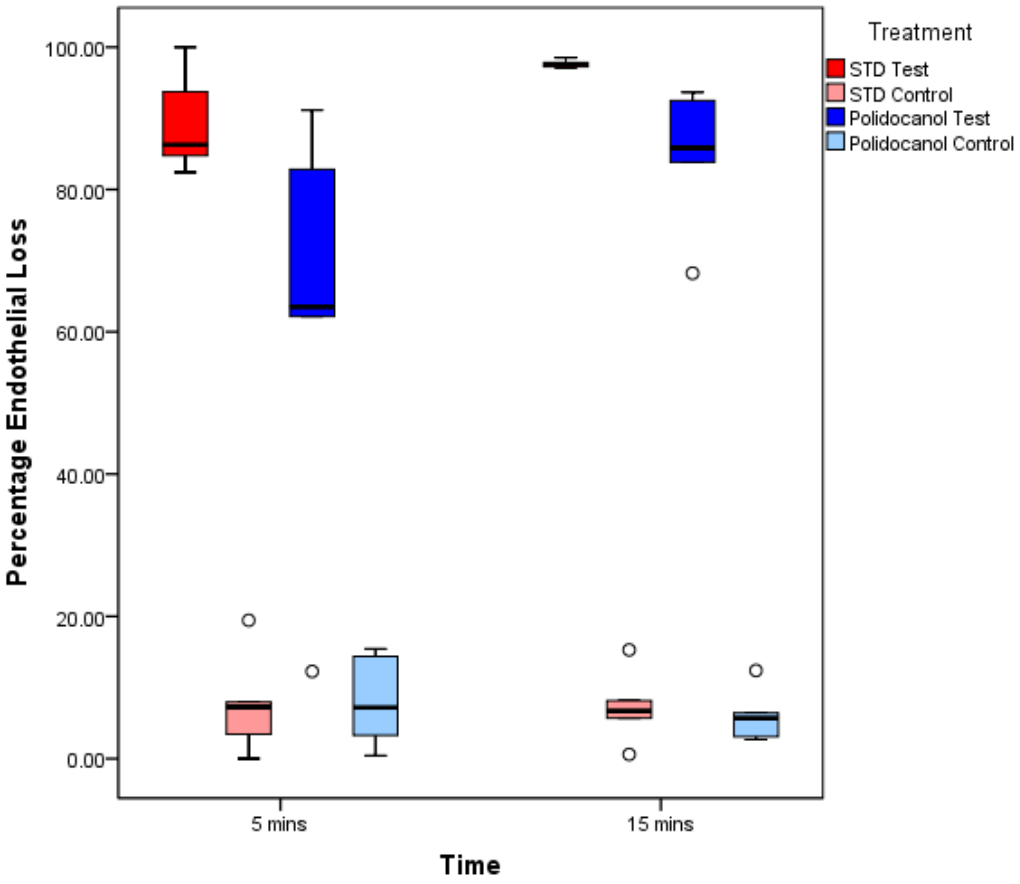


Figure 4.2: Percentage endothelial cell loss using 3% STD versus 3% polidocanol and their respective heparinised blood controls at 5 and 15 minutes

3% STD 5 min	Median depth of injury (µm)	Quantitative depth of injury vs media thickness %		3% Polidoc. 5 min	Median depth of injury (µm)	Quantitative depth of injury vs media thickness %
Median	37.35	3.45		Median	0	0
IQR1	35.31	3.12		IQR1	0	0
IQR3	45.76	3.59		IQR3	0	0

Table 4.4: Vein wall injury data for 3% STD and 3% polidocanol at 5 minutes

	STD 5 min	Polidocanol 5 min	p
Median endothelial cell loss (%)	86.3 (84.8-93.7)	63.48 (62.2-82.9)	= 0.095
Median depth of injury (µm)	37.4 (35.3-45.8)	0 (0-0)	< 0.01
Median depth of injury as % of media thickness	3.5 (3.1-3.6)	0 (0-0)	< 0.01

Table 4.5: Comparison of vein wall injury data for 3% STD and 3% polidocanol at 5 minutes

3% STD 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% Polidoc. 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	43.39	5.3		Median	0	0
IQR1	42.05	3.72		IQR1	0	0
IQR3	46.68	5.96		IQR3	0	0

Table 4.6: Vein wall injury data for 3% STD and 3% polidocanol at 15 minutes

	STD 15 min	Polidocanol 15 min	p
Median endothelial cell loss (%)	97.6 (97.3-97.9)	85.9 (83.8-92.5)	< 0.01
Median depth of injury (µm)	43.4 (42.1-46.7)	0 (0-0)	< 0.01
Median depth of injury as % of media thickness	5.3 (3.7-6.0)	0 (0-0)	< 0.01

Table 4.7: Comparison of vein wall injury data for 3% STD and 3% polidocanol at 15 minutes

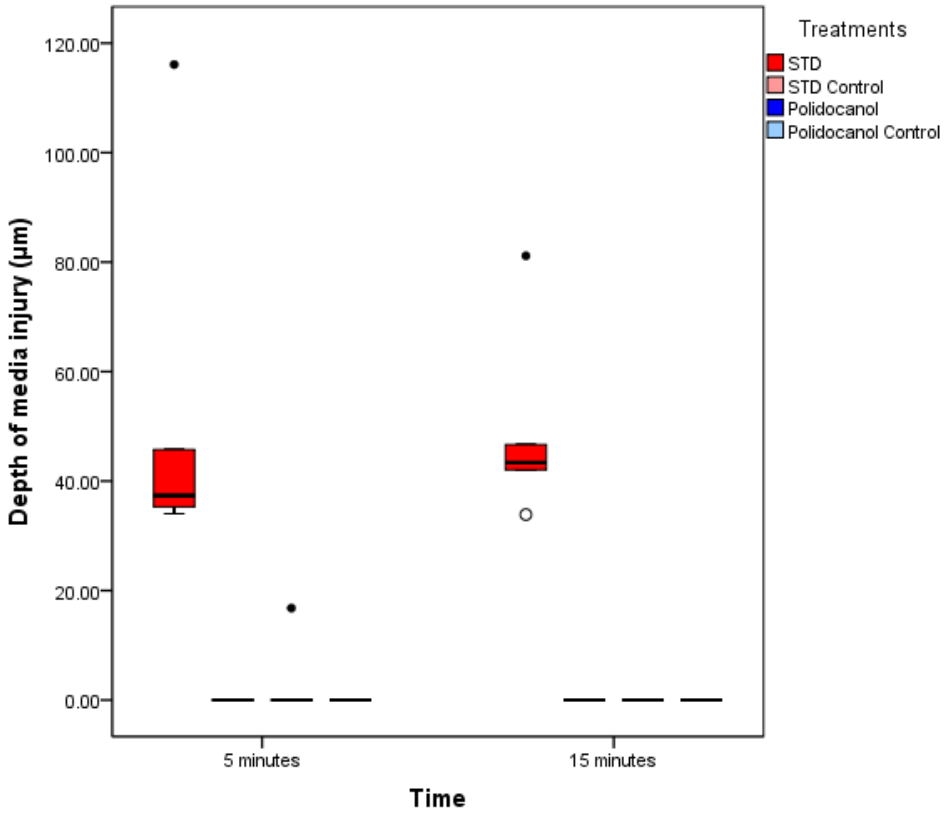


Figure 4.3: Depth of media injury (µm) using 3% STD versus 3% polidocanol and their respective heparinised blood controls at 5 and 15 minutes.

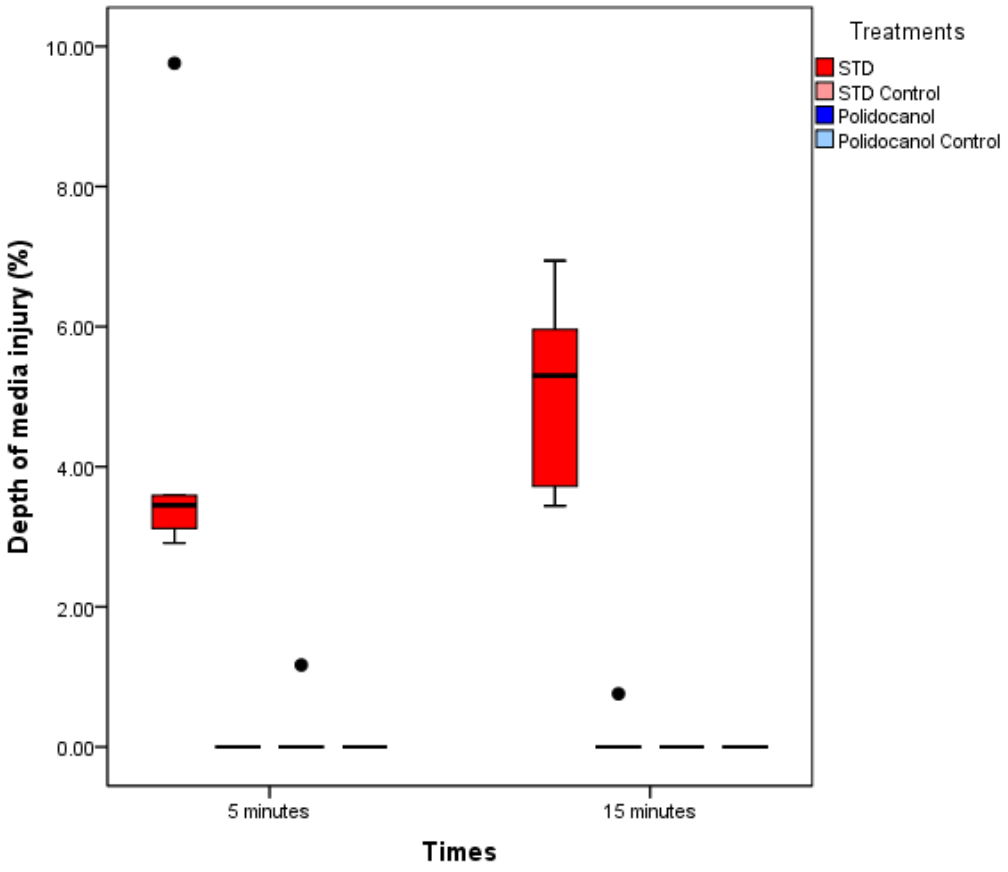


Figure 4.4: Percentage of media depth injured using 3% STD versus 3% polidocanol and their respective heparinised blood controls at 5 and 15 minutes.

Median endothelial cell loss was significantly greater with 3% STD (86.3% [84.8-93.7]) versus controls (7.26% [3.44, 7.97]) at 5 mins p=0.008 and at 15 mins: 3% STD (97.6% [97.3-97.9]) versus controls (6.71% [5.7, 8.13]) p=0.008. Similarly 3% polidocanol produced a significantly greater endothelial loss versus controls at 5 mins, 63.48% (62.15, 82.81) versus 7.18% (3.27, 14.38) p=0.032 and at 15 mins (polidocanol 85.85% (83.83, 92.48) versus controls 5.7% (3.1, 6.42) p=0.008 respectively. Comparison of median endothelial loss at 5 mins with 3% STD (86.3% [84.75, 93.73]) vs 3% polidocanol (63.48% [62.15, 82.81]) revealed no significant difference (p=0.095), but at 15 mins there was a statistically significant different endothelial loss p=0.008, with 3% STD having a median of 97.64% (97.25, 97.83) endothelial loss versus 85.85% (83.83, 92.48) using polidocanol. The enhanced endothelial loss seen with 3% STD between 5 mins (86.3% [84.75, 93.73]) compared with 15 mins (97.64% [97.25, 97.83]) is not statistically significant p=0.151. Similarly the enhanced endothelial loss seen with 3% polidocanol between 5 mins (63.48% [62.15, 82.81]) and 15 mins (85.85% [83.83, 92.48]) is not statistically significant p=0.095.

There was no difference found across the range of controls for both the 5 and 15 minute experiments inclusive (p=0.494: Independent samples median test). Hence additional time *in-vitro* with controls alone did not affect the endothelial lining.

The median depth of media injury and percentage depth of media injured was greater with 3% STD (median depth of injury at 5 (37.4µm [35.3-45.8]) and 15 min (43.4 µm [42.1-46.7]) compared to 3% polidocanol (0 µm [0,0] for 5 and 15 min experiments) p = 0.008 for both. Similarly percentage depth of media injury is greater with 3% STD at 5 (3.5% [3.1-3.6]) and 15 mins (5.3% [3.7-6.0])

compared to 3% polidocanol (0% [0,0] for 5 and 15 min experiments) p=0.008 for both.

Depth of media injury with 3% STD at 5 (37.4µm [35.3-45.8]) versus 3% STD at 15mins (43.4 µm [42.1-46.7]) showed no difference p=0.841. There was also no difference in the percentage depth of injury with 3% STD at 5 (3.5% [3.1-3.6]) and 15 mins (5.3% [3.7-6.0]) p=0.310.

No media injury occurred in controls for any of these experiments hence again the additional time 10 minutes between 15 min and 5 min experiments *in-vitro* had no effect on the media.

In summary the significant loss of endothelial lining in test segments compared to relatively unaffected controls is similar between 3% STD and 3% polidocanol except with longer experiment times where endothelial loss is enhanced with 3% STD compared to 3% polidocanol (but not compared to itself).

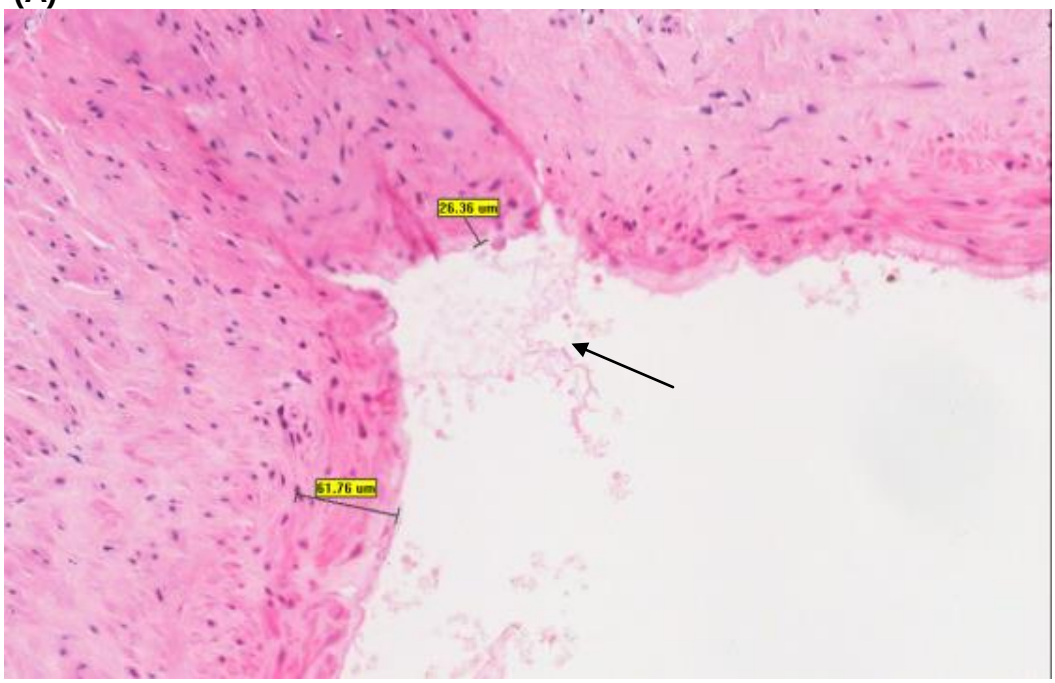
The depth of media injury in micrometres and as a percentage of the vein media is significantly higher for 3% STD as none occurred with 3% polidocanol.

If these differences also occur *in vivo then* 3% STD may be more effective in causing permanent vein damage than polidocanol during the treatment of varicose veins,

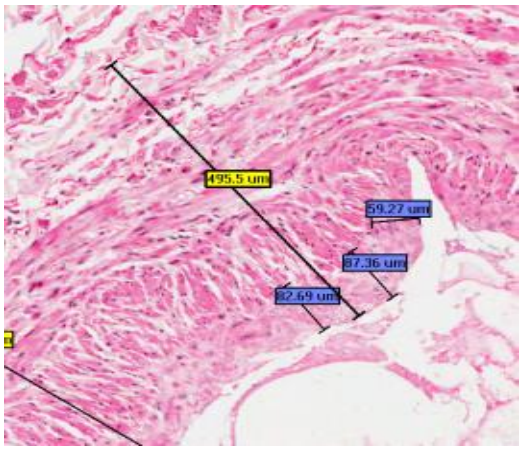
Below are examples of 20x and 40x magnified *in-vitro* GSV test samples stained with H&E. The first (Figure 4.5 A-C) are examples of GSV treated with STD for 15 minutes with an arrow pointing to “preserved” foam and the depths of injury clearly marked to the depth of the most superficial viable nucleus, with a 40x example of obvious sub-endothelial vacuolation. The second (Figure 4.6)

is an example of GSV treated with polidocanol for 15 minutes with large areas of intact endothelium (arrows) and no injury to the media. This slide is typical of those vein segments treated with polidocanol.

(A)



(B):



(C):

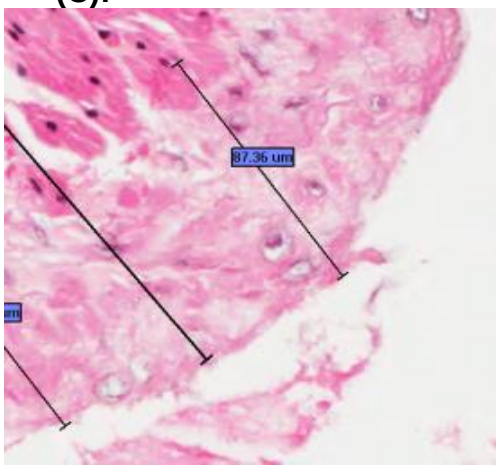


Figure 4.5: Example of *in-vitro* GSV treated for 15 minutes with 3 % STD foam

(a): arrow pointing to STD foam remaining in contact with damaged media. The two measurements of depth of injury are marked reflecting the depth of the most superficial viable nuclei (20x)
(b): close-up of individual "clock-face" measurements (20x)
(c): sub-endothelial vacuolation highlighting the depth of media injury (40x)

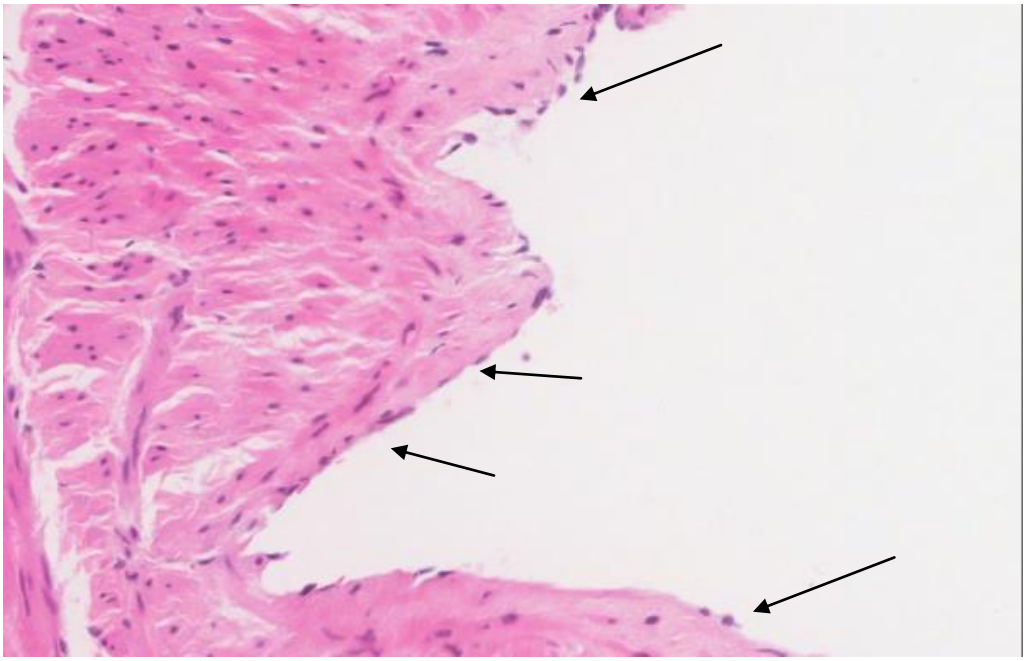


Figure 4.6: *In-vitro* GSV treated for 15 minutes with 3% polidocanol foam demonstrating intact endothelium with no discernible injury to the media (20x) (arrows showing intact endothelium)

4.3 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF CONSTITUENT PARTS OF PROPRIETARY SCLEROSANT FOAM (3% STD) ON *IN-VITRO* GSV WALL INTEGRITY, TO ASCERTAIN THE MOST ACTIVE INGREDIENT

The constituent parts of proprietary 3% STD foam were applied to the test segments. These are buffered 3% STD alone and 2% benzyl alcohol.

Buffered non-proprietary 3% STD 5 min	**% Endothelial cell loss**	**Control 5 min**	**% Endothelial cell loss**		**Phosphate Buffer pH 7.6 5 min**	**% Endothelial cell loss**	**Control 5 min**	**% Endothelial cell loss**
Median	85.28	Median	6.99		Median	1.93	Median	5.62
IQR1	83.15	IQR1	6.79		IQR1	1.02	IQR1	4.45
IQR3	87.66	IQR3	7.68		IQR3	8.98	IQR3	7.35
			p=0.008					p=0.421

Table 4.8: Percentage endothelial cell loss using buffered non-proprietary 3% STD versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 5 minutes

Buffered 2% Benzyl Alcohol 5 min	**% Endothelial Cell loss**	**Control 5 min**	**% Endothelial cell loss**		**Phosphate Buffer pH 7.6 5 min**	**% Endothelial cell loss**	**Control 5 min**	**% Endothelial cell loss**
Median	13.86	Median	4.94		Median	1.93	Median	5.62
IQR1	9.32	IQR1	2.93		IQR1	1.02	IQR1	4.45
IQR3	17.05	IQR3	5.67		IQR3	8.98	IQR3	7.35
			p=0.095					p=0.421

Table 4.9: Percentage endothelial cell loss using buffered 2% benzyl alcohol versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 5 minutes

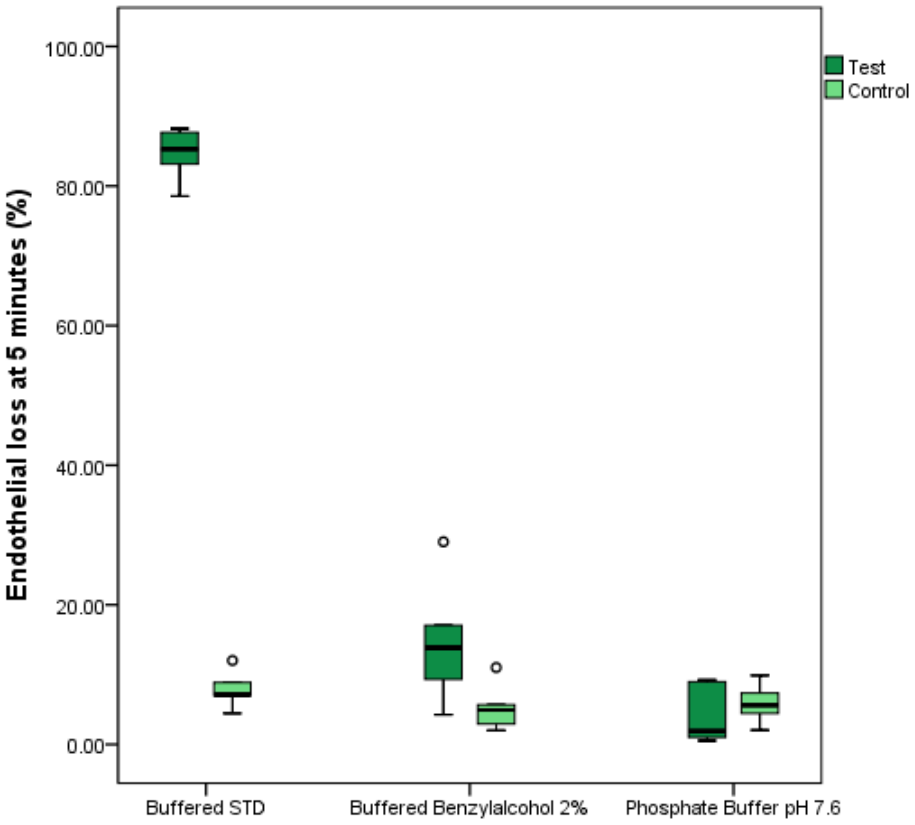


Figure 4.7: Percentage endothelial cell loss using buffered non-proprietary 3% STD versus buffered 2% benzyl alcohol versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 5 minutes

Buffered 2% Benzyl Alcohol 15 min	% Endothelial Cell loss	Control 15 min	% Endothelial cell loss		Phosphate Buffer pH 7.6 15 min	% Endothelial cell loss	Control 15 min	% Endothelial cell loss
Median	15.67	Median	9.78		Median	4.97	Median	8.91
IQR1	11.09	IQR1	8.88		IQR1	3.02	IQR1	7.98
IQR3	18.72	IQR3	12.55		IQR3	8.76	IQR3	12.23

Table 4.10: Percentage endothelial cell loss using buffered 2% benzyl alcohol versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 15 minutes

Buffered non-proprietary 3% STD 15 min	% Endothelial Cell loss	Control 15 min	% Endothelial cell loss		Phosphate Buffer pH 7.6 15 min	% Endothelial cell loss	Control 15 min	% Endothelial cell loss
Median	83.12	Median	9.45		Median	4.97	Median	8.91
IQR1	75.34	IQR1	6.72		IQR1	3.02	IQR1	7.98
IQR3	94.32	IQR3	10.31		IQR3	8.76	IQR3	12.23

Table 4.11: Percentage endothelial cell loss using buffered non-proprietary 3% STD versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 15 minutes

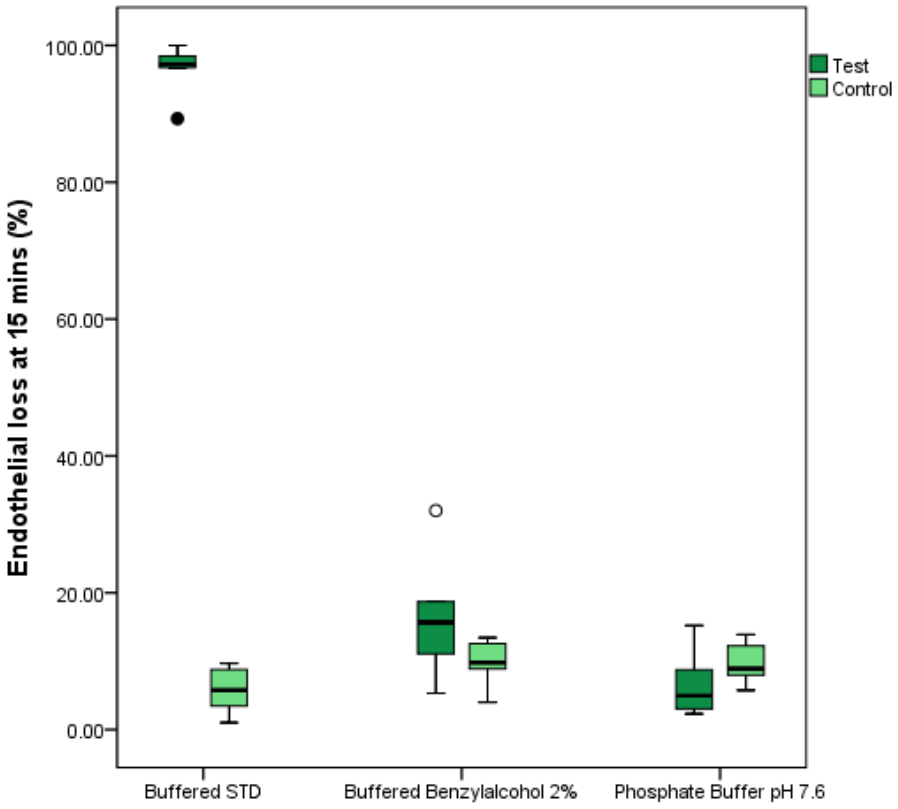


Figure 4.8: Percentage endothelial cell loss using buffered non-proprietary 3% STD versus buffered 2% benzyl alcohol versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 15 minutes

Buffered non-proprietary 3% STD 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %		Phosphate Buffer pH 7.6 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	0	0		Median	0	0
IQR1	0	0		IQR1	0	0
IQR3	0	0		IQR3	0	0

Table 4.12: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using buffered (non-proprietary) 3% STD versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 5 minutes.

Buffered Benzyl Alcohol 2% 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %		Phosphate Buffer pH 7.6 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	0	0		Median	0	0
IQR1	0	0		IQR1	0	0
IQR3	0	0		IQR3	0	0

Table 4.13: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using buffered benzylalcohol 2% versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 5 minutes.

Buffered (non-proprietary) 3% STD 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %		Phosphate Buffer pH 7.6 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	0	0		Median	0	0
IQR1	0	0		IQR1	0	0
IQR3	0	0		IQR3	0	0

Table 4.14: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using buffered (non-proprietary) 3% STD versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 15 minutes.

Buffered Benzyl Alcohol 2% 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %		Phosphate Buffer pH 7.6 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	0	0		Median	0	0
IQR1	0	0		IQR1	0	0
IQR3	0	0		IQR3	0	0

Table 4.15: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using buffered benzylalcohol 2% STD versus phosphate buffer pH 7.6 and their respective heparinised blood controls at 15 minutes.

5 minute experiments comparing the percentage endothelial loss using buffered non-proprietary 3% STD (85.28% [83.15, 87.66]) to its controls (6.99% [6.79, 7.68]) and to the phosphate buffer (1.93% [1.02,8.98]) displays a significant difference for both p=0.008. Comparing percentage endothelial loss using 3% STD (85.28% [83.15, 87.66]) to buffered 2% benzyl alcohol (13.86% [9.32, 17.05]) shows again a statistically significant difference p=0.008. Comparing endothelial percentage loss with buffered 2% benzyl alcohol (13.86% [9.32, 17.05]) to phosphate buffer (1.93% [1.02, 8.98]) also reveals a significant difference p=0.032. However this is less marked in comparison to the difference between buffered non-proprietary 3% STD and phosphate buffer.

Comparing endothelial loss across all controls for buffered 3% STD, buffered 2% benzyl alcohol and phosphate buffer experiments indicates that they are all comparatively low p=0.153 (Independent samples median test).

Therefore 5 minute experiments reveal that during this time frame buffered non-proprietary 3% STD causes much more endothelial loss (85.28% [83.15, 87.66]) than buffered 2% benzyl alcohol (13.86% [9.32, 17.05]) p=0.008 with the later causing no more endothelial loss than its controls (4.94% [2.93, 5.67]) p=0.095.

Similarly for 15 minute experiments, endothelial loss using buffered 3% STD (83.12% [75.34, 94.32]) was significantly more compared with buffered 2% benzyl alcohol (15.67% [11.09,18.72]) p=0.008 and in comparison to phosphate buffer (8.91% [7.98, 12.23]) p=0.008 Mann-Whitney U test.

Comparison of medians across groups of heparinised blood controls for buffered 3% STD, buffered 2% benzyl alcohol and phosphate buffer 15 minute experiments again showed no significant difference p=0.343

These results suggest that the most active ingredient in proprietary STD is, as expected STD.

Regarding depth of injury experiments, these results can be summarised by stating that neither of the constituent parts of sclerosant foams, buffered 3% STD and 2% benzyl alcohol, when used separately, caused any damage to the *in-vitro* vein media for either 5 or 15 minute experiments.

Comparing the endothelial loss using buffered 3% STD in 5 minute experiments (85.28% [83.15, 87.66]) versus 15 min experiments (83.12% [75.34, 94.32]), there was no statistical difference p=0.841. Therefore increasing the contact time of buffered 3% STD with the *in-vitro* endothelium did not inflict more damage. Similarly buffered 2% benzyl alcohol did not inflict more damage on the endothelium with the longer experiments: 5 min (13.86% [9.32, 17.05]) versus 15 min (15.67% [11.09, 8.72]) p=0.690. Therefore for either agent additional time in contact with the endothelium did not equate to more damage.

Endothelial loss with proprietary 3% STD at 5 mins is 86.3% (84.8-93.7) versus 85.28% (83.15, 87.66) with non-proprietary buffered 3% STD p=0.310 and in 15 min experiments is 97.6% (97.3-97.9) versus 83.12% (75.34, 94.32) p=0.548.

Therefore when used separately the constituent parts of STD foam damage the endothelium but not the media. Additional time in contact with the endothelium did not equate to any increase in damage inflicted to the endothelium. Hence either the combination of STD and benzyl alcohol in proprietary STD is synergistic, or our buffering mechanism was at least interfering with the activity of STD against the media.

4.4 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF 'LONGER-LASTING' FOAM SCLEROSANTS ON *IN-VITRO* GSV WALL INTEGRITY, TO ASCERTAIN THAT PRODUCING MAXIMAL EFFECT ON THE VEIN WALL

These are the results for longer lasting STD foams.

4.4.1 ENDOTHELIAL CELL LOSS WITH LONGER LASTING FOAMS

3% STD 5 min	% Endothelial Cell loss	Control 5 min	% Endothelial cell loss		3% STD+ 5% Pluronic 5 min	% Endothelial cell loss	Control 5 min	% Endothelial cell loss
Median	86.3	Median	7.26		Median	96.26	Median	12.98
IQR1	84.75	IQR1	3.44		IQR1	84.16	IQR1	7.18
IQR3	93.73	IQR3	7.97		IQR3	90.12	IQR3	10.08

Table 4.16: Percentage endothelial cell loss with 3% STD versus 3% STD + 5% pluronic and their respective heparinised blood controls at 5 minutes

3% STD 15 min	% Endothelial Cell loss	Control 15 min	% Endothelial cell loss		3% STD+ 5% Pluronic 15 min	% Endothelial cell loss	Control 15 min	% Endothelial cell loss
Median	97.64	Median	6.71		Median	86.32	Median	6.71
IQR1	97.25	IQR1	5.7		IQR1	85.52	IQR1	5.7
IQR3	97.83	IQR3	8.13		IQR3	85.92	IQR3	6.21

Table 4.17: Percentage endothelial cell loss with 3% STD foam versus 3% STD + 5% pluronic foam and their respective heparinised blood controls at 15 minutes

The "longer-lasting" 3% STD foams included STD 3% and pluronic 5%, STD 3% and carbomer 0.45%, and STD 3% and xanthum 0.15%. Results revealed that STD 3%/xanthum 0.15% caused a greater degree of injury than the other two longer-lasting foams hence its constituent parts were assessed individually.

These were phosphate buffer pH 7.6, normal saline 0.9% and buffered xanthum alone 5%.

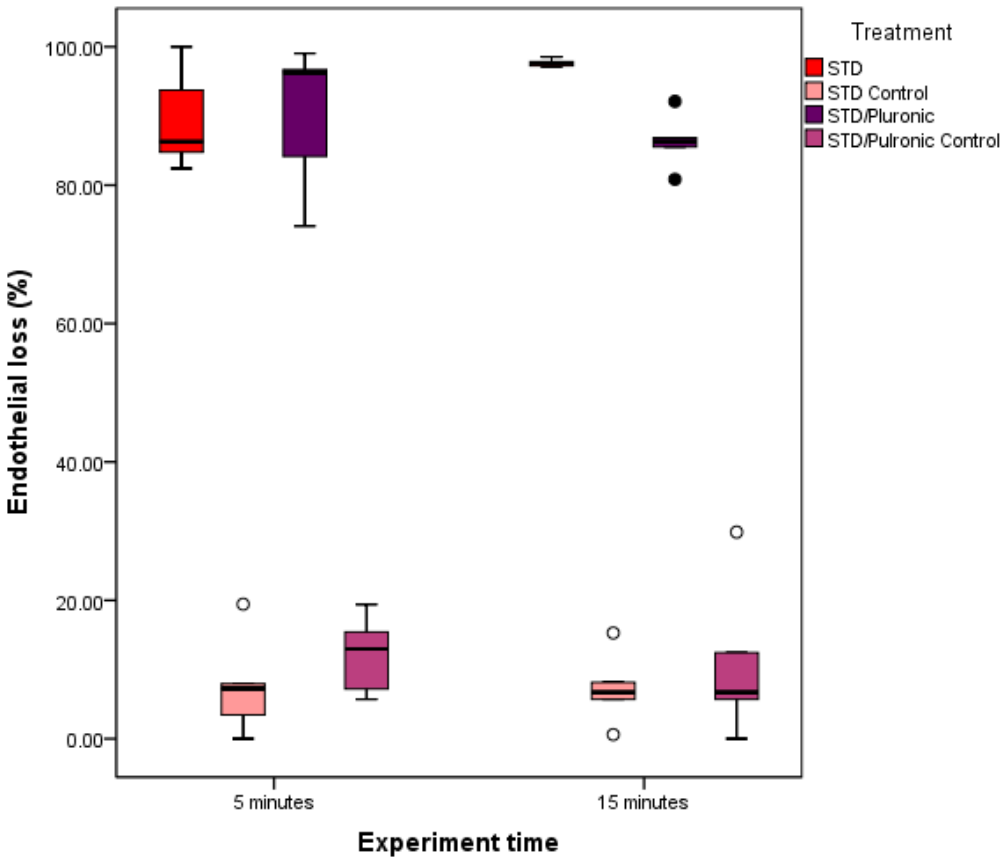


Figure 4.9: Percentage endothelial loss with 3% STD foam versus 3% STD + 5% pluronic foam and their respective heparinised blood controls at 5 and 15 minutes.

5 minutes experiments comparing endothelial loss with 3% STD versus STD/Pluronic revealed no difference ($p=1.0$). However in the 15 minute experiments STD/Pluronic causing less endothelial cell loss than 3% STD ($p<0.01$; Mann Whitney U). .

3% STD 5 min	% Endothelial Cell loss	Control 5 min	% Endothelial cell loss		3% STD+ 0.45% Carbomer 5 min	% Endothelial cell loss	Control 5 min	% Endothelial cell loss
Median	86.3	Median	7.26		Median	84.14	Median	8.56
IQR1	84.75	IQR1	3.44		IQR1	57.16	IQR1	5.78
IQR3	93.73	IQR3	7.97		IQR3	85.32	IQR3	10.21

Table 4.18: Percentage endothelial cell loss with 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 5 minutes

3% STD 15 min	% Endothelial Cell loss	Control 15 min	% Endothelial cell loss		3% STD+ 0.45% Carbomer 15 min	% Endothelial cell loss	Control 15 min	% Endothelial cell loss
Median	97.64	Median	6.71		Median	88.64	Median	4.62
IQR1	97.25	IQR1	5.7		IQR1	86.28	IQR1	3.1
IQR3	97.83	IQR3	8.13		IQR3	91.72	IQR3	12.39

Table 4.19: Percentage endothelial cell loss with 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 15 minutes

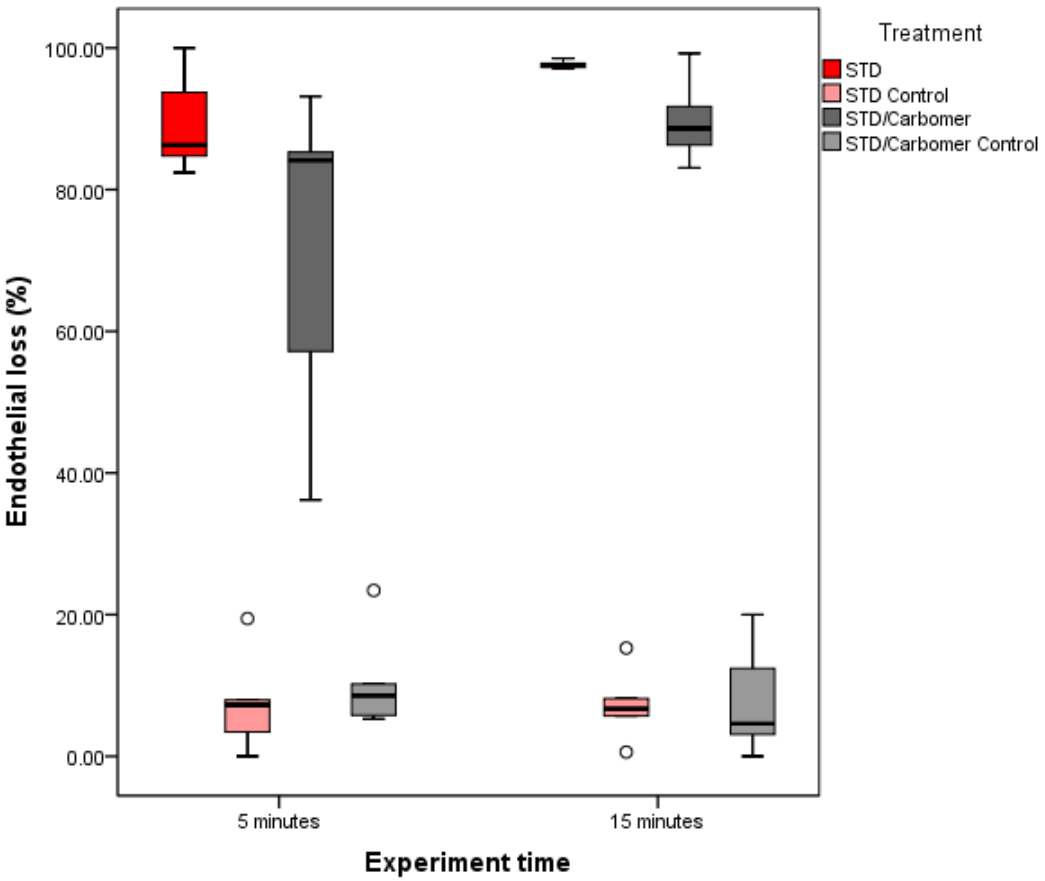


Figure 4.10: Percentage endothelial loss with 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 5 and 15 minutes

Neither the 5 nor 15 minute experiments comparing endothelial loss with 3% STD versus STD/Carbomer showed a difference between the foams (p=0.222 and p=0.151 respectively; Mann Whitney U).

3% STD 5 min	% Endothelial Cell loss	Control 5 min	% Endothelial cell loss		3% STD+ 0.15% Xanthum 5 min	% Endothelial cell loss	Control 5 min	% Endothelial cell loss
Median	86.3	Median	7.26		Median	98.01	Median	12.98
IQR1	84.75	IQR1	3.44		IQR1	87.8	IQR1	9.29
IQR3	93.73	IQR3	7.97		IQR3	98.22	IQR3	15.78

Table 4.20: Percentage endothelial cell loss with 3% STD versus 3% STD + 0.15% xanthum and their respective heparinised blood controls at 5 minutes

3% STD 15 min	% Endothelial Cell loss	Control 15 min	% Endothelial cell loss		3% STD+ 0.15% Xanthum 15 min	% Endothelial cell loss	Control 15 min	% Endothelial cell loss
Median	97.64	Median	6.71		Median	94.71	Median	8.96
IQR1	97.25	IQR1	5.7		IQR1	91.59	IQR1	6.42
IQR3	97.83	IQR3	8.13		IQR3	95.21	IQR3	16.35

Table 4.21: Percentage endothelial cell loss with 3% STD versus 3% STD + 0.15% xanthum and their respective heparinised blood controls at 15 minutes

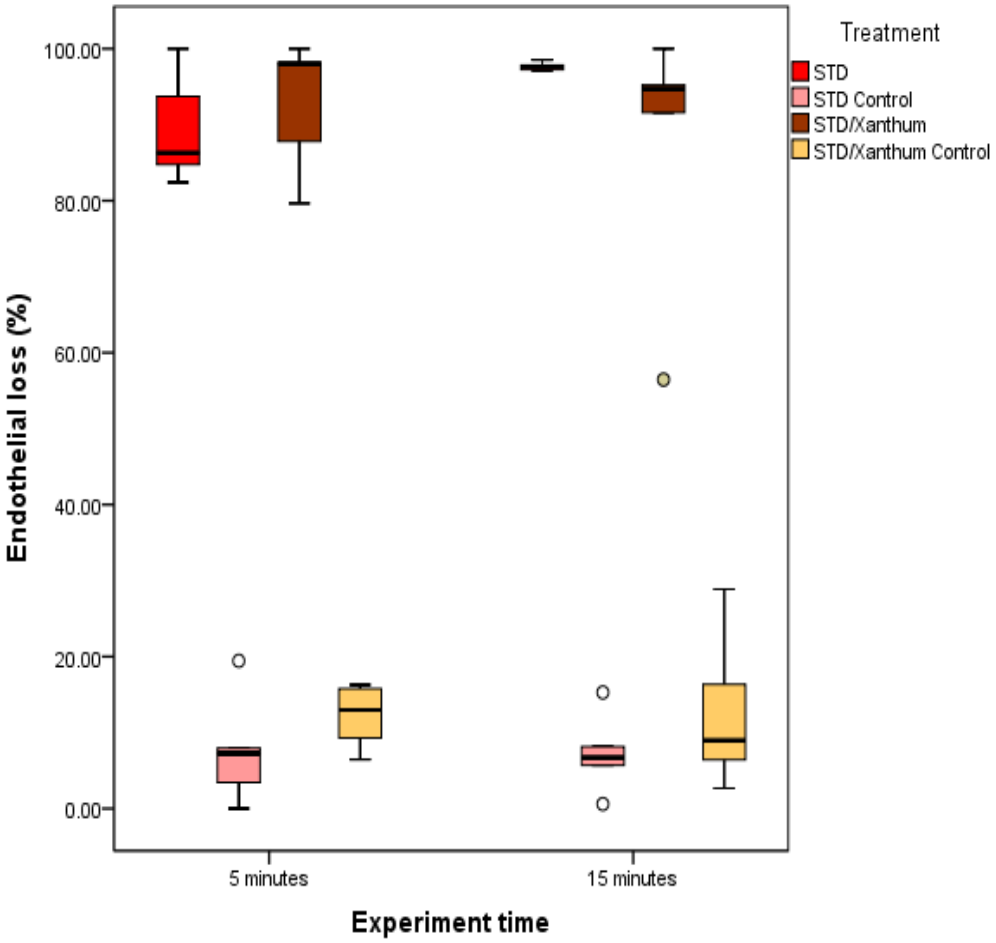


Figure 4.11: Percentage endothelial loss using 3% STD foam versus 3% STD + 0.15% xanthum foam and their respective heparinised blood controls at 5 and 15 minutes.

Comparing endothelial loss between 3% STD and STD/Xanthum revealed no differences after either 5 (p=0.548) or 15 minutes (p=0.151; Mann Whitney U).

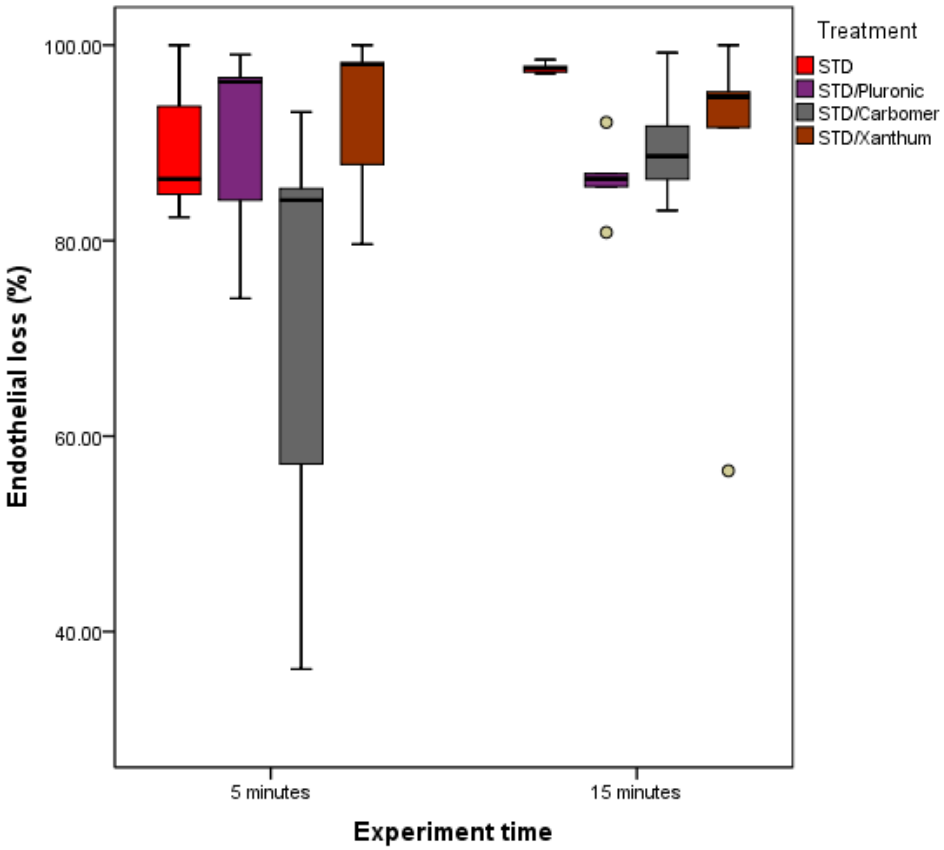


Figure 4.12: Summary chart of percentage endothelial cell loss at 5 and 15 minutes with 3% STD foam, 3% STD + 5% pluronic foam, 3% STD + 0.45% carbomer foam and 3% STD + 0.15% xanthum foam.

4.4.2 MEDIA INJURY WITH LONGER LASTING FOAMS.

This chapter displays results for the absolute depth of media injury in µM and the percentage depth of the media injured using longer lasting foams.

3% STD 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% STD + 5% Pluronic 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	37.35	3.45		Median	21.08	3.83
IQR1	35.31	3.12		IQR1	18.23	3.04
IQR3	45.76	3.59		IQR3	31.34	4.23

Table 4.22: Depth of media injury (median [µm] and depth of injury as a % of total depth of media [%]) using 3% STD foam versus 3% STD + 5% pluronic foam and their respective heparinised blood controls at 15 minutes.

3% STD 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% STD + 5% Pluronic 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	43.39	5.3		Median	25.89	2.97
IQR1	42.05	3.72		IQR1	24.2	1.94
IQR3	46.68	5.96		IQR3	26.45	3

Table 4.23: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using 3% STD foam versus 3% STD + 5% pluronic foam and their respective heparinised blood controls at 15 minutes.

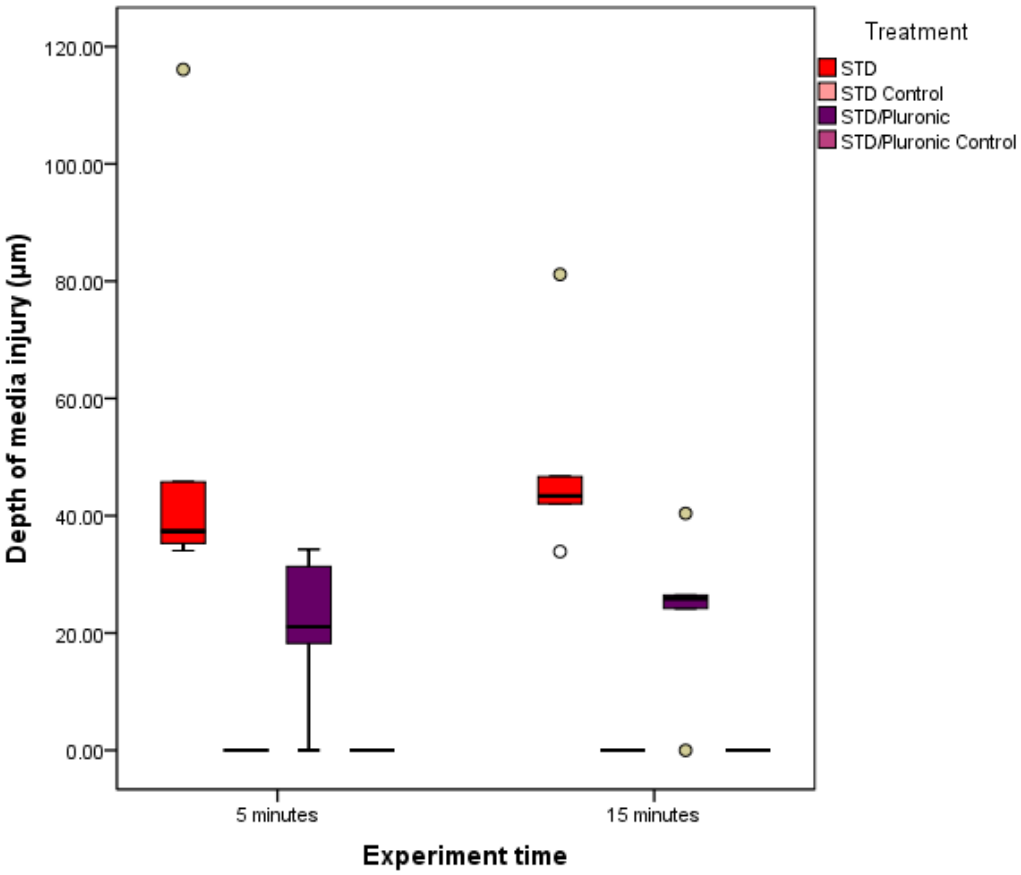


Figure 4.13: depth of media injury (µm) using 3% STD foam versus 3% STD + 5% pluronic foam and their respective heparinised blood controls at 5 and 15 minutes

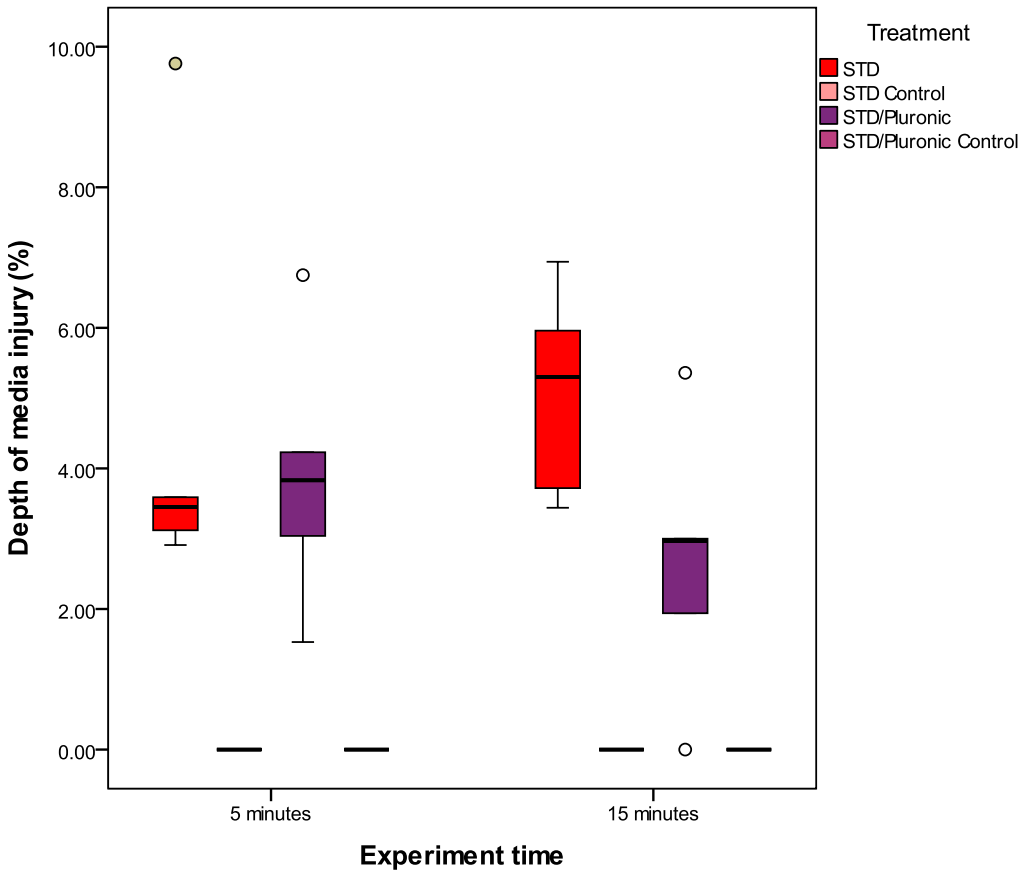


Figure 4.14: Depth of media injury (%) using 3% STD foam versus 3% STD + 5% pluronic foam and their respective heparinised blood controls at 5 and 15 minutes

5 minute experiments comparing depth of injury revealed a significant difference between 3% STD versus and STD/Pluronic when comparing absolute depth of injury [µm] p=0.016 although there was no difference when comparing the depth of injury as a percentage of the media depth (p=1.0; Mann Whitney U). After 15 minutes there was a significant difference between the two both for absolute depth of injury (p=0.016) and for depth of injury as a percentage of the media depth (p<0.01) with 3% STD causing greater injury than STD/Pluronic. Thus pluronic seems to reduce the effectiveness of STD in inflicting damage in the *in-vitro* vein wall media.

3% STD 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% STD + 0.45% Carbomer 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	37.35	3.45		Median	32.24	6.91
IQR1	35.31	3.12		IQR1	0	1.98
IQR3	45.76	3.59		IQR3	47.03	11.31

Table 4.24: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 5 minutes.

3% STD 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% STD + 0.45% Carbomer 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	43.39	5.3		Median	31.4	7.97
IQR1	42.05	3.72		IQR1	30.41	3.26
IQR3	46.68	5.96		IQR3	58.73	12.79

Table 4.25: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 15 minutes.

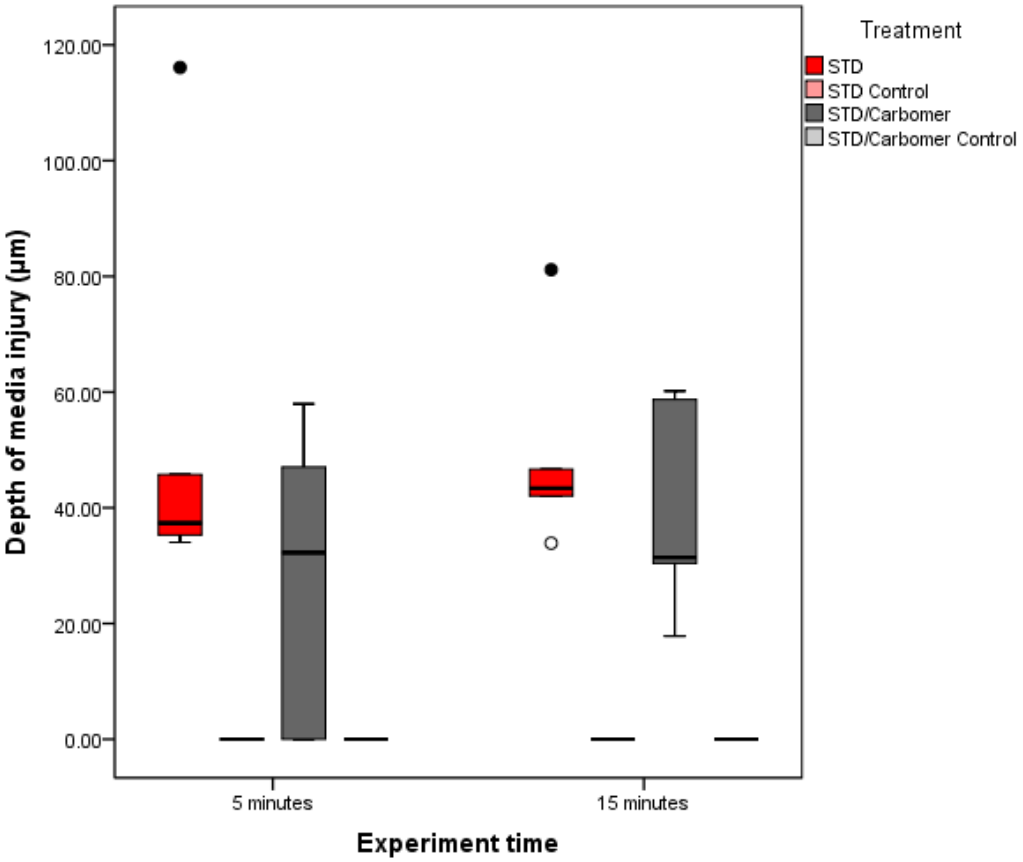


Figure 4.15: Depth of injury (µm) using 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 5 and 15 minutes

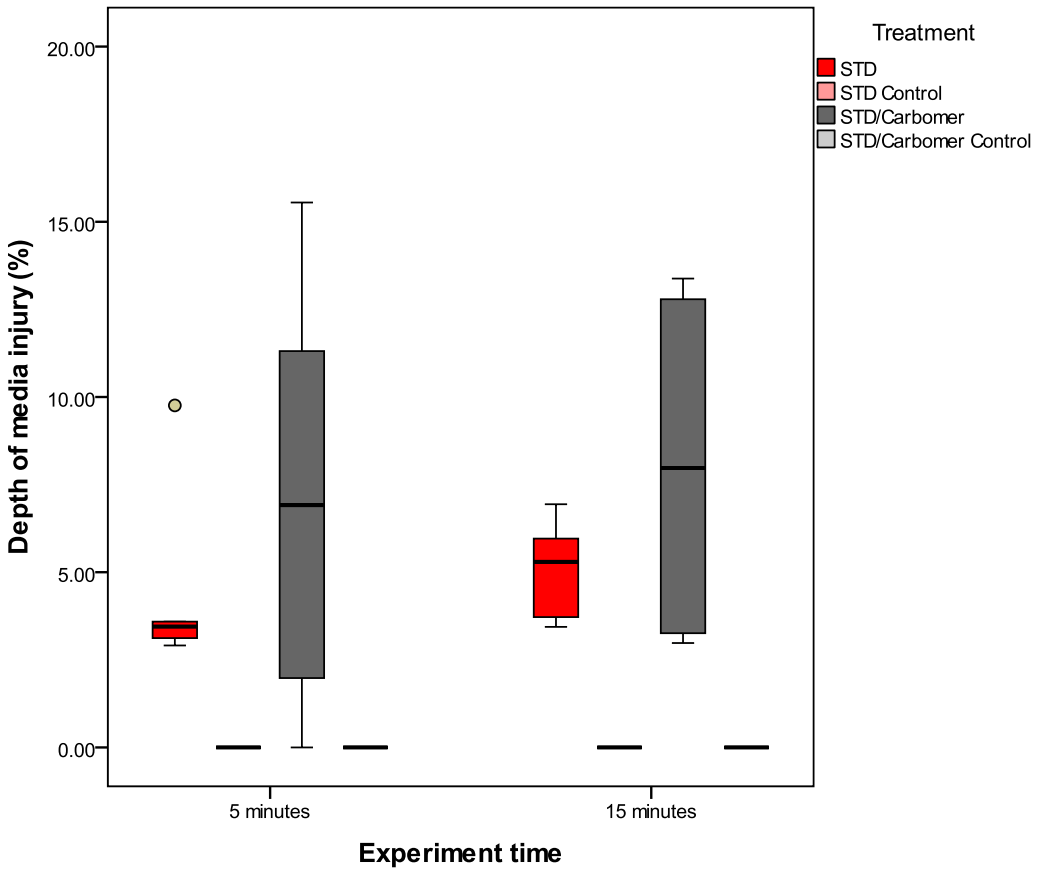


Figure 4.16: Depth of injury (% of total media depth) using 3% STD foam versus 3% STD + 0.45% carbomer foam and their respective heparinised blood controls at 5 and 15 minutes

5 minute experiments comparing 3% STD with STD/Carbomer revealed no difference in the absolute (p=0.421) or percentage depth of injury of the media (p=0.841). 15 minute experiments were similar (p=0.421 and p=0.690; Mann Whitney U) respectively.

3% STD 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% STD + 0.15% Xanthum 5 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	37.35	3.45		Median	57.87	9.51
IQR1	35.31	3.12		IQR1	36.78	5.44
IQR3	45.76	3.59		IQR3	58.11	9.58

Table 4.26: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using 3% STD foam versus 3% STD + 0.15% xanthum foam and their respective heparinised blood controls at 5 minutes.

3% STD 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %		3% STD + 0.15% Xanthum 15 min	Median depth of injury (µm)	Depth of injury vs media thickness %
Median	43.39	5.3		Median	32.48	4.32
IQR1	42.05	3.72		IQR1	25.95	3.79
IQR3	46.68	5.96		IQR3	38.31	11.25

Table 4.27: Depth of media injury (median [µm] and depth of injury vs media thickness [%]) using 3% STD foam versus 3% STD + 0.15% xanthum foam and their respective heparinised blood controls at 15 minutes.

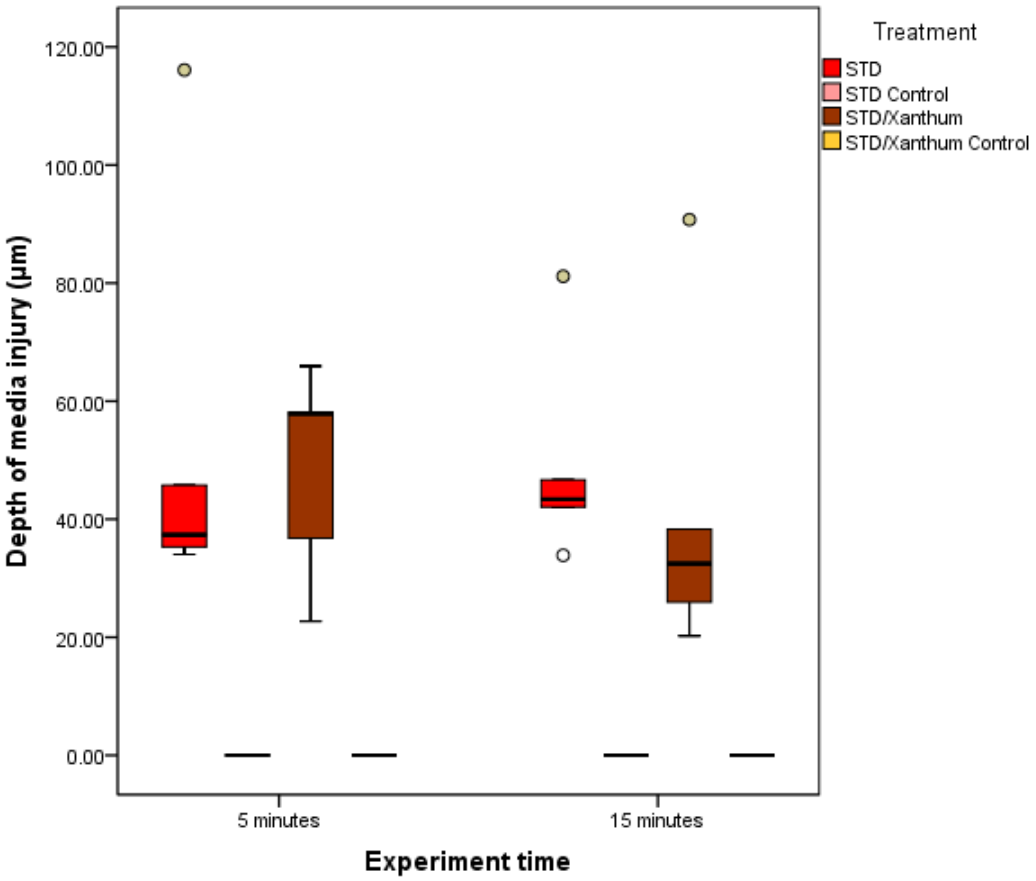


Figure 4.17: Depth of injury (µm) using 3% STD foam versus 3% STD + 0.15% xanthum foam and their respective heparinised blood controls at 5 and 15 minutes

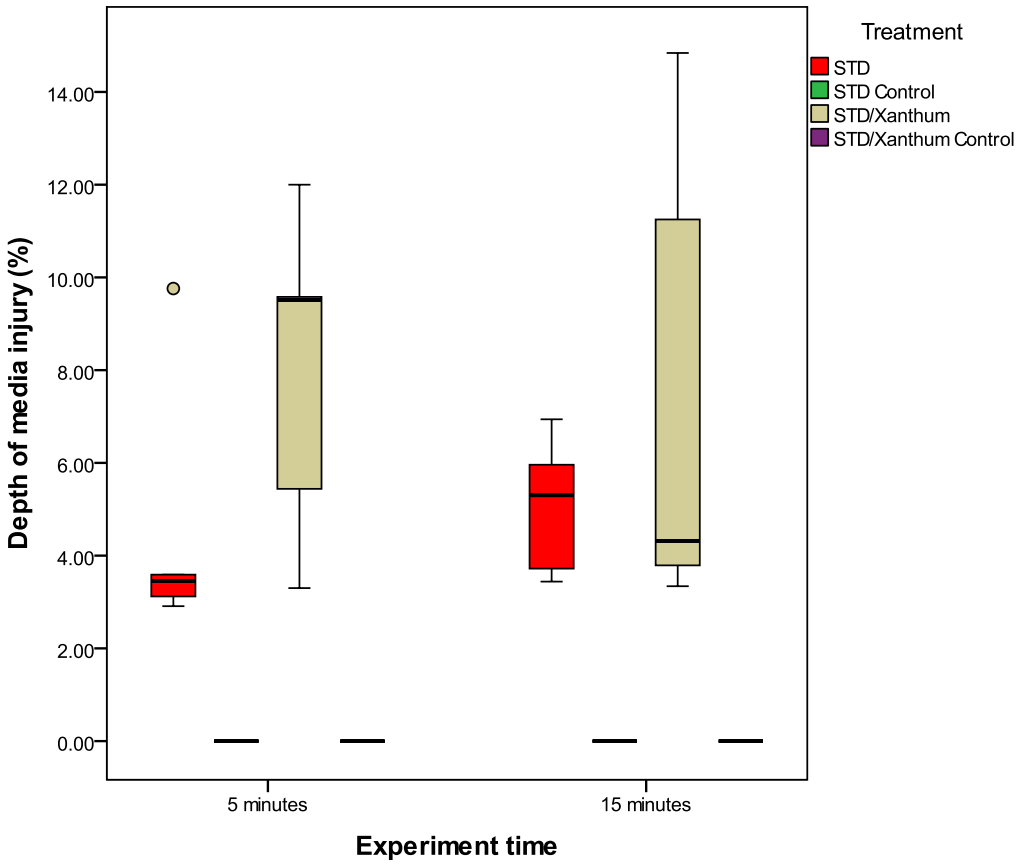


Figure 4.18: Depth of injury (%) using 3% STD foam versus 3% STD + 0.15% xanthum foam and their respective heparinised blood controls at 5 and 15 minutes

Experiments comparing depth of media injury over 5 (absolute depth p=0.841, % depth p=0.222) and 15 minutes (absolute depth p=0.222, % depth p=0.841) comparing 3% STD with STD/Xanthum showed no difference.

These results show that STD 3%/xanthum 0.15% caused a greater degree of injury than the other two longer-lasting foams hence its constituent parts were assessed individually.

These were phosphate buffer pH 7.6, normal saline 0.9% and buffered xanthum alone 5%. These had a minimal effect on the endothelium and none on the media.

The following are summary charts of the absolute and percentage depth of injury caused by longer lasting foams.

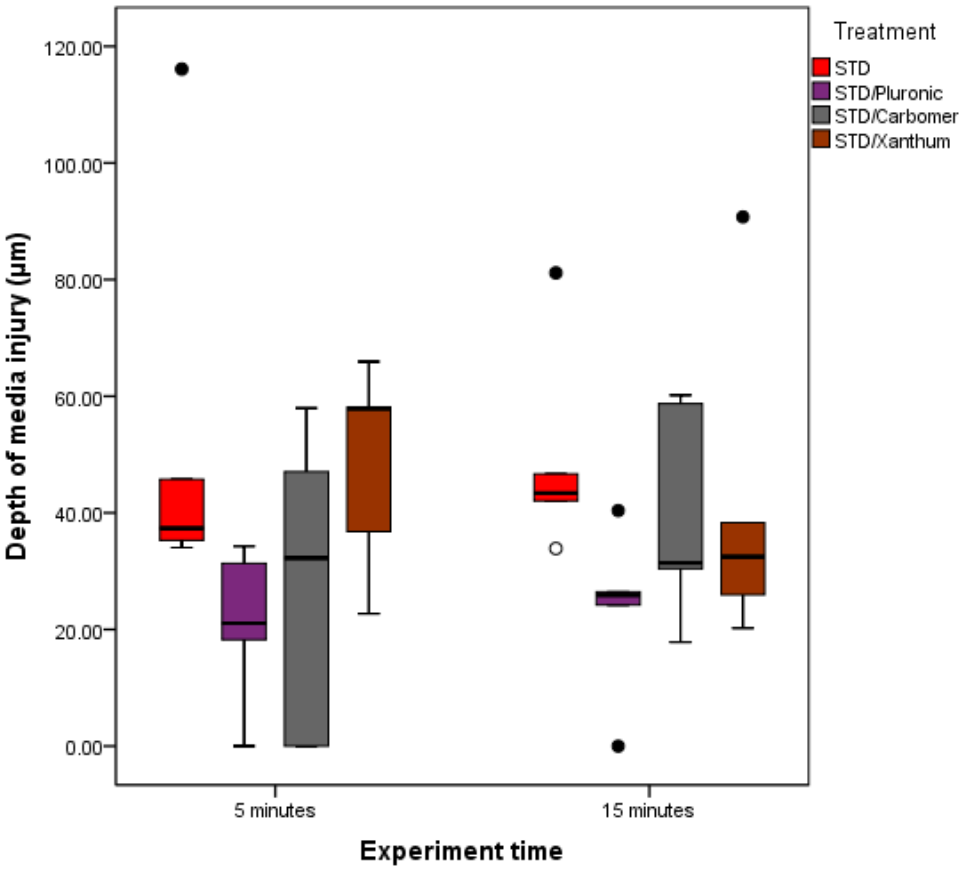


Figure 4.19: Depth of injury (µm) at 5 and 15 minutes with 3% STD foam, 3% STD + 5% pluronic foam, 3% STD + 0.45% carbomer foam and 3% STD + 0.15% xanthum foam.

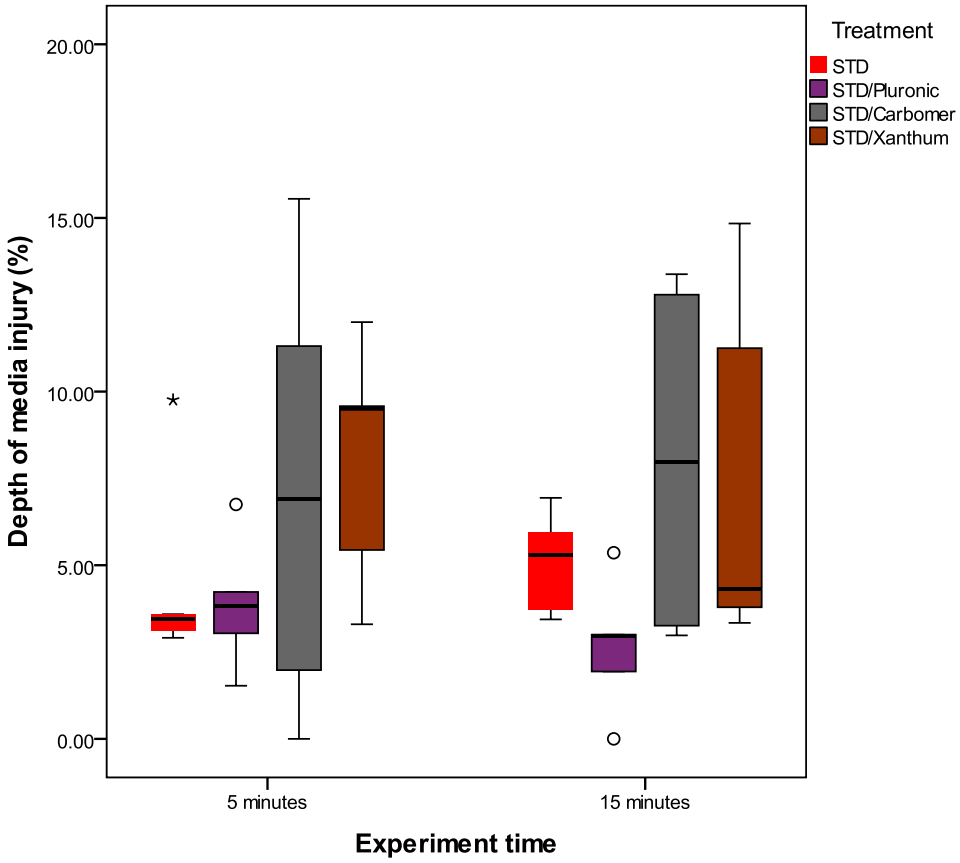


Figure 4.20: Depth of injury (%) at 5 and 15 minutes with 3% STD foam, 3% STD + 5% pluronic foam, 3% STD + 0.45% carbomer foam and 3% STD + 0.15% xanthum foam.

Compared to proprietary 3% STD foam, longer lasting 3% STD foams did not inflict greater injury on either the GSV endothelium or media. Indeed, the addition of pluronic had a negative impacted the efficacy of 3% STD in terms of both endothelial loss and damage to the vein media. Conversely, STD/Xanthum showed a trend towards greater foam efficacy sclerotherapy, at least in 5 minute experiments, but this still failed to reach significance.

CHAPTER V

ASSESSMENT OF CUTTING BALLOONS AS ADJUNCTS TO FOAM SCLEROTHERAPY

5 ASSESSMENT OF CUTTING BALLOONS AS ADJUNCTS TO FOAM SCLEROTHERAPY

5.1 ASSESSMENT OF CUTTING BALLOONS FOR USE AS MONO-THERAPY OR AS ADJUVANT TREATMENT WITH FOAM SCLEROTHERAPY FOR TREATMENT OF TRUNCAL VARICOSE VEINS

This chapter presents experiments performed using arterial cutting balloons. Their potential as mono-therapy or adjuvant therapy alongside foam sclerotherapy for treating truncal varicose veins is examined.

Each experiment in this group involved 5 GSV segments (each with test and control). The test segments were wrapped inside a saline soaked surgical swab held snuggly with finger thumb opposition beside the vein segment/balloon in order to mimic resistance of the subcutaneous fat as would be the case *in-vitro*. Cutting balloons utilised for these experiments had been previously used once in arterial treatments, hence they were used a maximum of twice more to prevent significant blunting of their blades. As vein segments are of differing dimension and so too are cutting balloons as recorded above, balloons were matched to vein segments according to vein diameter.

5.1.1 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF CUTTING BALLOONS ALONE ON IN-VITRO GSV WALL INTEGRITY

The first experiments here involved cutting balloons (matched to size of vein) inflated in the lumen of the test vein segment for 10 seconds then deflated and removed. Inflation was performed using an Encore™26 Inflation Device (Boston Scientific, USA; Figure 2.19) to a pressure of 6.0 Atmospheres (ATM) / 608 kilo Pascals (kPa) (pressure recommended by the manufacturers for each of the balloons).

The second batch of experiments involved cutting balloons (matched to size of vein) being inflated to a pressure of 6.0 ATM / 608 kPa in lumen of test vein segment for 10 seconds and then withdrawn (pulled out) from the test segment whilst inflated. deBakey (non-traumatic forceps) placed at one end of the specimen were used to anchor the vein to allow withdrawal of the cutting balloon whilst inflated.

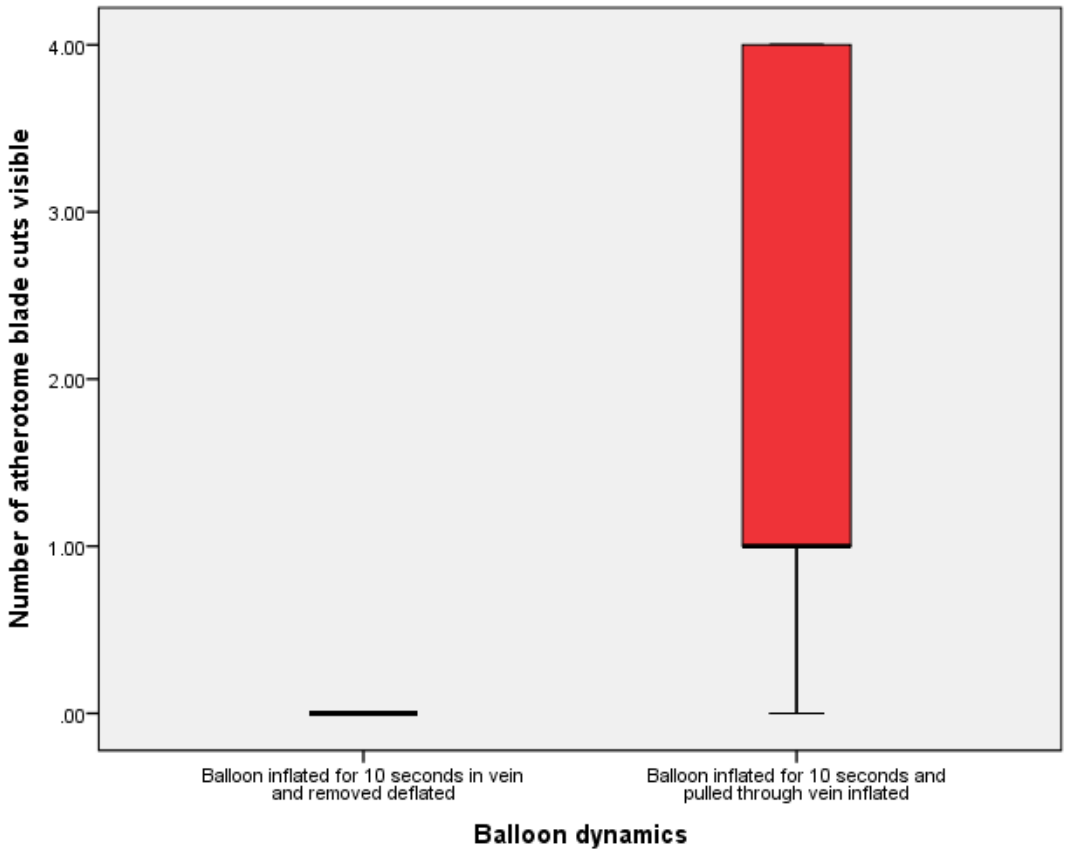


Figure 5.1: Number of cuts visible on histological analysis of GSV segments after cutting balloon inflated for 10 seconds versus cutting balloon inflated for 10 seconds then pulled out of vein segment whilst inflated.

These experiments revealed that cuts were only identified in vein segments where the cutting balloons were removed from the vein segment whilst fully inflated (number of incisions 1(1-4) versus 0(0-0); p=0.032; Mann-Whitney U) This is at variance to arterial work were the balloons are designed to be inflated and deflated without being pulled through the vessel lumen. This necessity to drag the exposed blade through the lumen in order to make it cut the luminal surface is likely due to high vein wall compliance compared to arteries.

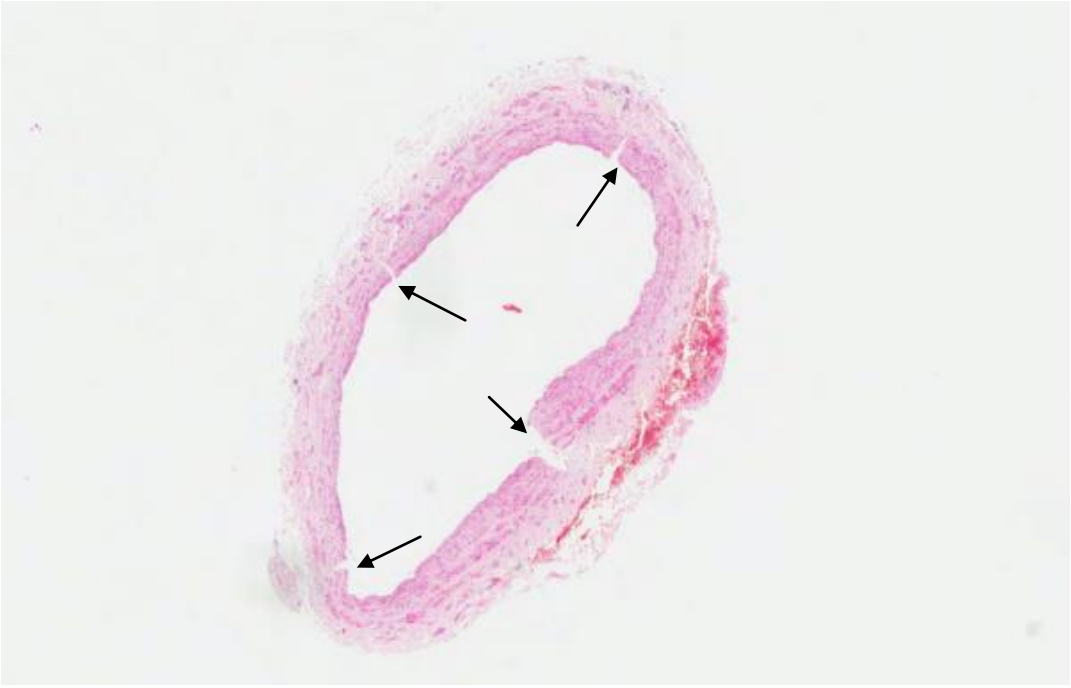


FIGURE 5.2: x1 cross-sectional image of *in-vitro* GSV damaged by withdrawing a cutting balloon through the vein lumen whilst fully inflated. Arrows highlight the damage caused by each of the four atherotomes.

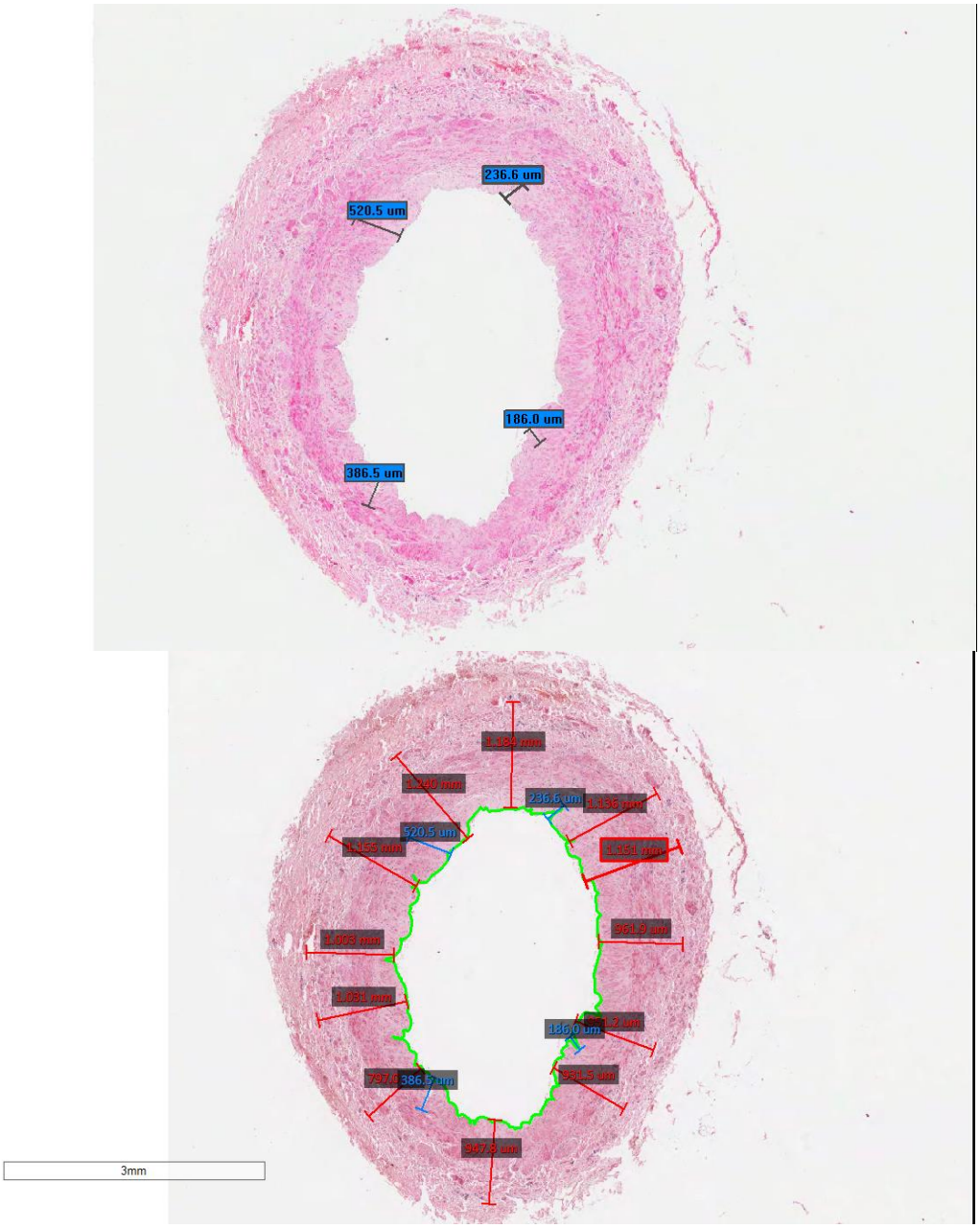


Figure 5.3: x1 cross-sectional image of *in-vitro* GSV damaged by withdrawing a cutting balloon through the vein lumen whilst fully inflated. Arrows highlight the damage caused by each of the four atherotomes and the measurements taken using Aperio Imagescope.

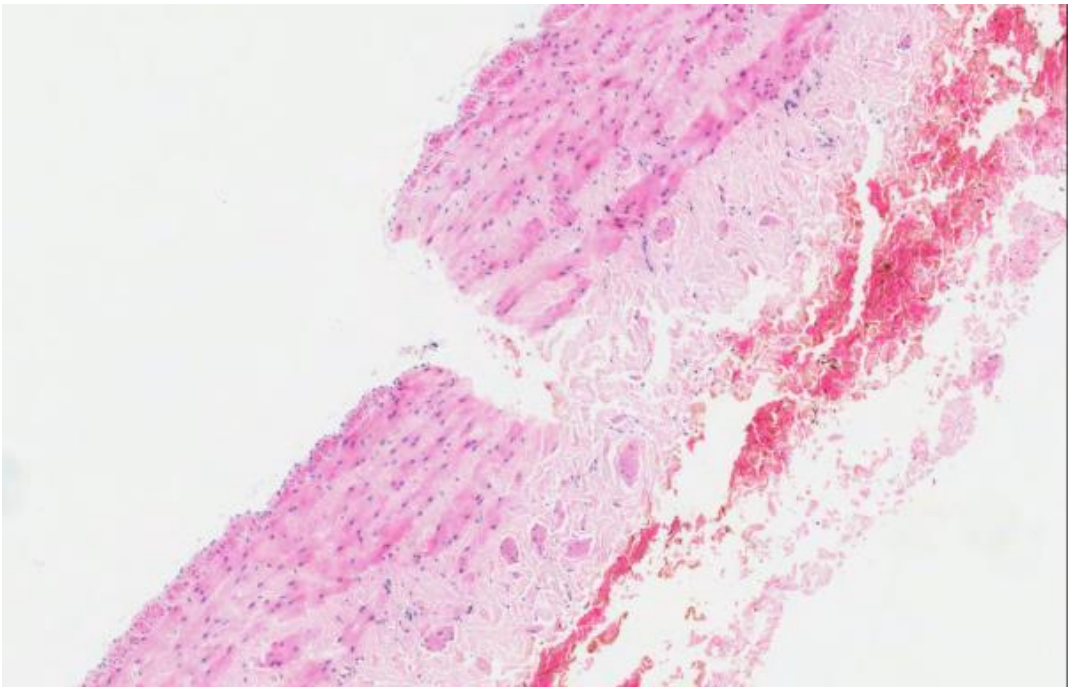


Figure 5.4: Magnified view of damaged caused to GSV media by an atherotome on a cutting balloon withdrawn whilst fully inflated. Injury is almost the full depth of the media

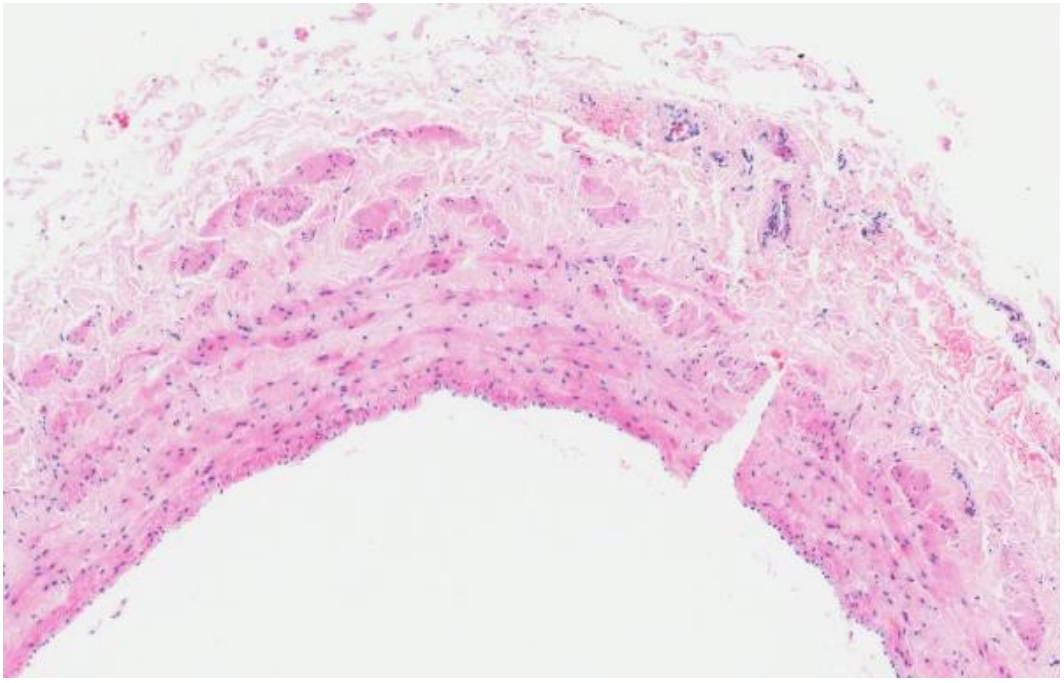


Figure 5.5: Further close-up of damaged caused to GSV media by an atherotome on a cutting balloon withdrawn whilst fully inflated. Injury is again almost the full depth of the media.

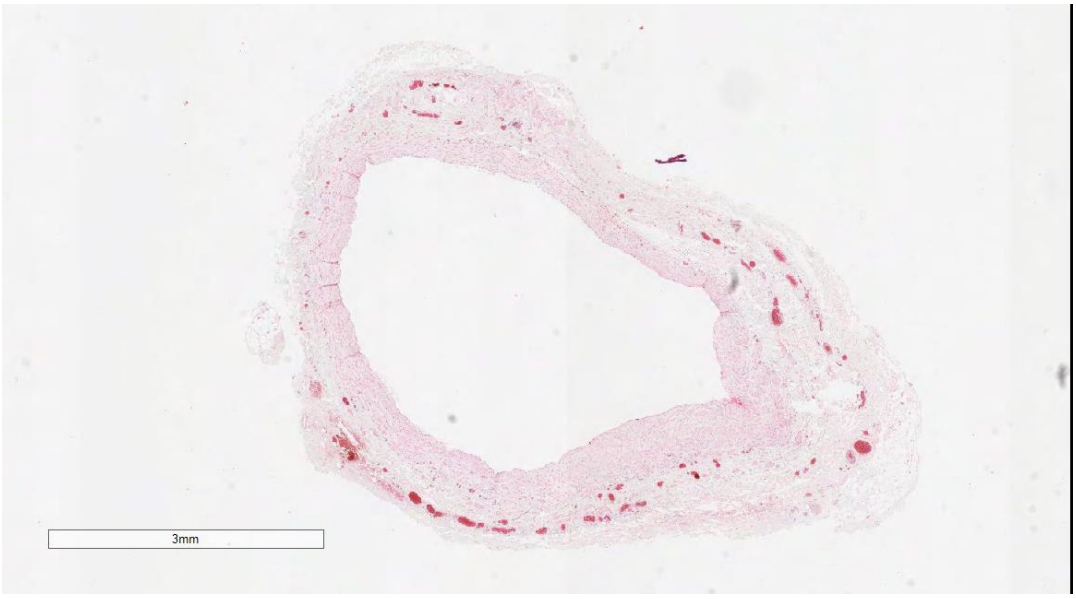


Figure 5.6: x1 cross-sectional image of *in-vitro* GSV. Cutting balloon was inflated and deflated prior to removal with no resultant atherotome blade cuts.

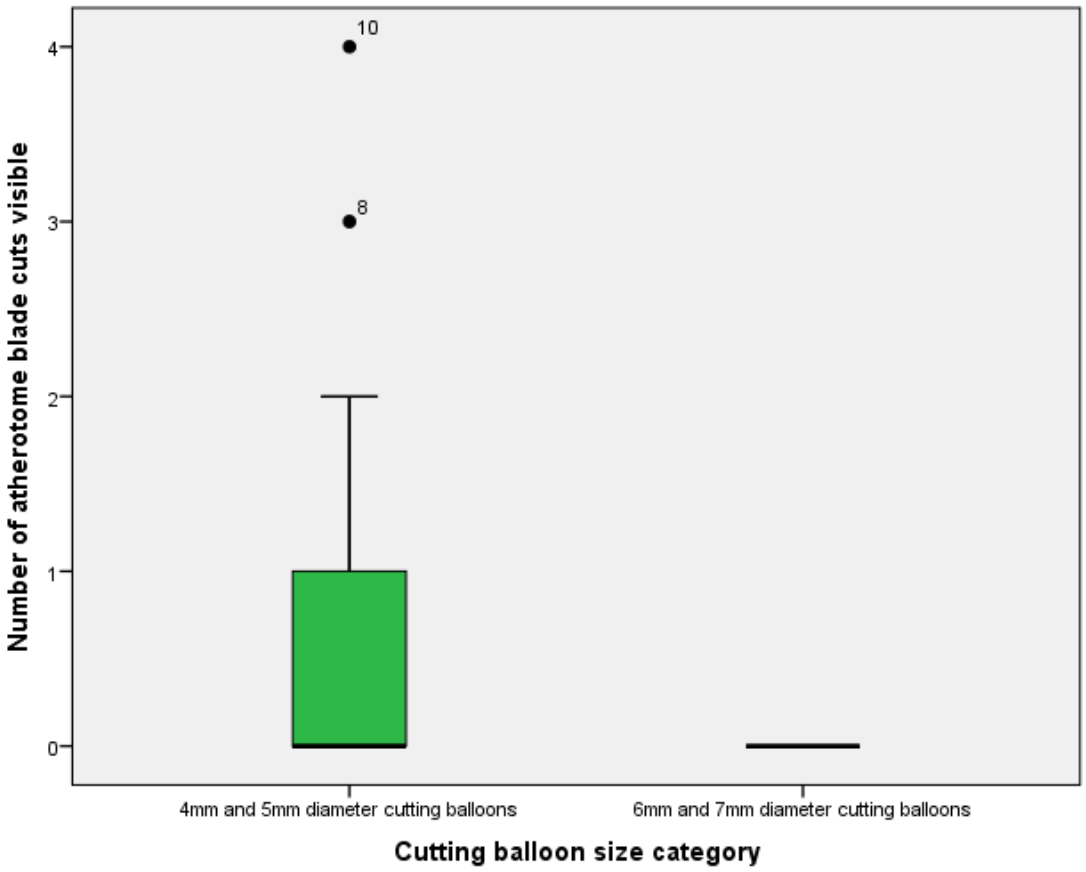


Figure 5.7: Cutting balloon size category versus number of atherotome cuts visible.

Balloon sizes available were 4mm diameter with a balloon circumference (circ.) 12.56mm, 5mm diameter and 15.7mm circumference, 6mm diameter and 18.85mm circumference, and 7mm diameter and 21.99mm circumference.. Balloons were matched 1:1 with vein diameter. Experiments revealed that there was a significant difference between the number of atherotome blade cuts made with smaller (4-5mm diameter) 0 (0-1) vs larger 6-7mm diameter cutting balloons 0 (0-0), p=0.03. Therefore despite attempts to match veins to balloon size, only the 4-5mm diameter cutting balloons created any cuts in the luminal surface of the vein. No-matter the presumed greater force created by larger balloons they appear to have been restricted in the deployment of their atherotomes. Hence where it may have been expected that in relatively compliant venous versus arterial tissue larger balloons would create more

luminal cuts, it appears that smaller cutting balloons are needed to allow atherotome deployment.

5.1.2 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF CUTTING BALLOONS IN COMBINATION WITH PROPRIETARY FOAM SCLEROTHERAPY ON IN-VITRO GSV WALL INTEGRITY

Experiments in this case involved cutting balloons being inflated as above, again to a pressure of 6.0 ATM / 608kPa in lumen of test vein segment for 10 seconds, with the balloon then withdrawn (pulled) from the test segment whilst inflated. Subsequently either 3% STD or 3% Polidocanol foam was placed (as per experiments in 2.2.2) into the vein segments for 5mins. Controls for this experiment had the same balloon treatment as test segments and were then filled with heparinised blood to control for pressure as per previous experiments.

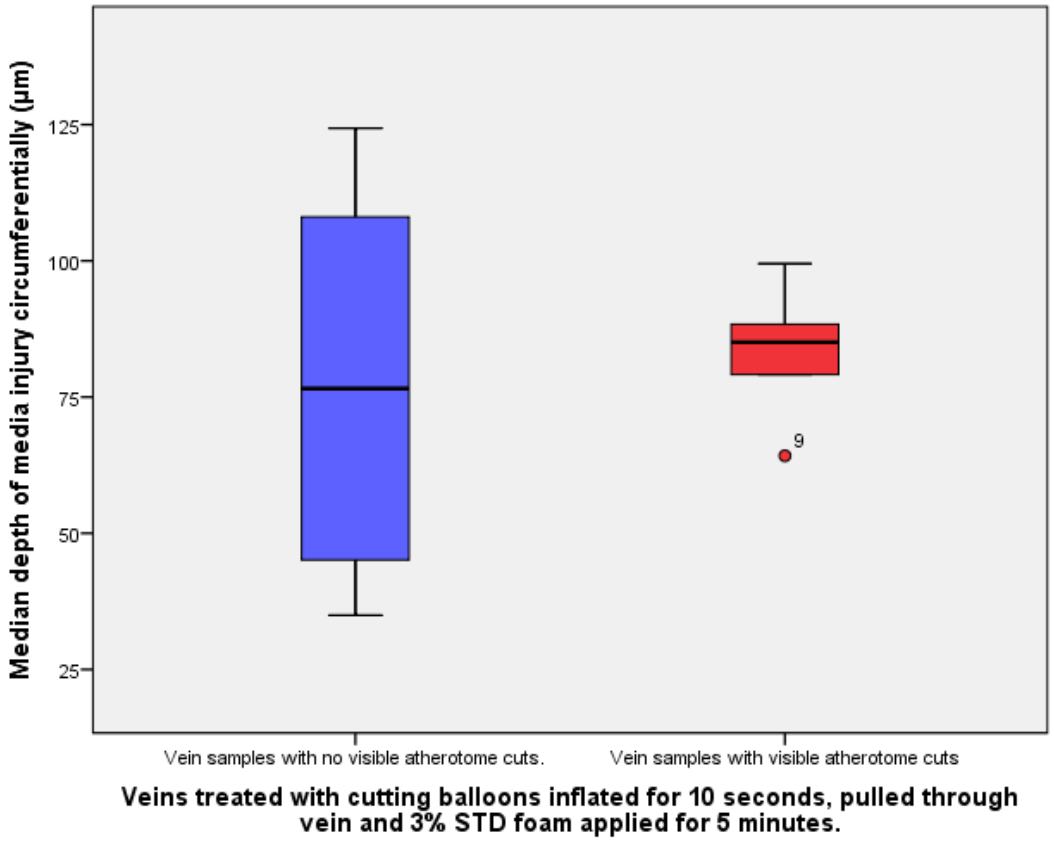


Figure 5.8: Depth of media injury caused by 3% STD with atherotome blade cuts versus no atherotome blade cuts.

Presence of atherotome cuts did not increase the depth of media injury caused by 3% STD. Controls treated with 3% STD had a median depth of injury of 76.57µm (45.1, 108.05) versus those with atherotome blade cuts 85.07µm (79.13, 88.4) p=1.00 Mann-Whitney U.

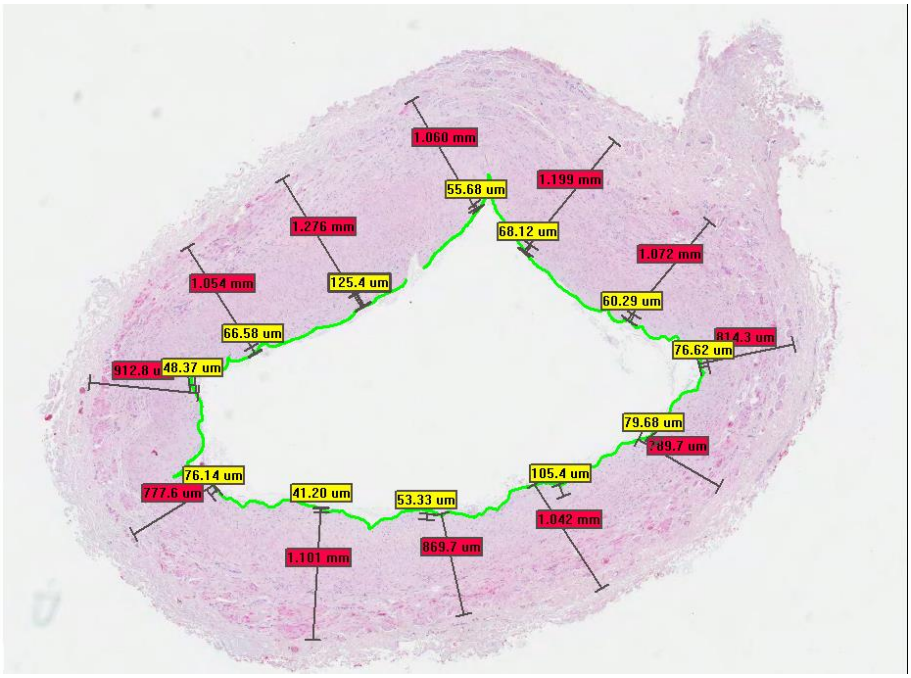


Figure 5.9: Vein segment demonstrating atherotome blade cuts with concurrent use of 3% STD. Measurements of luminal circumference, media depth and depth of injury by 3%STD (Aperio Imagescope) are displayed.

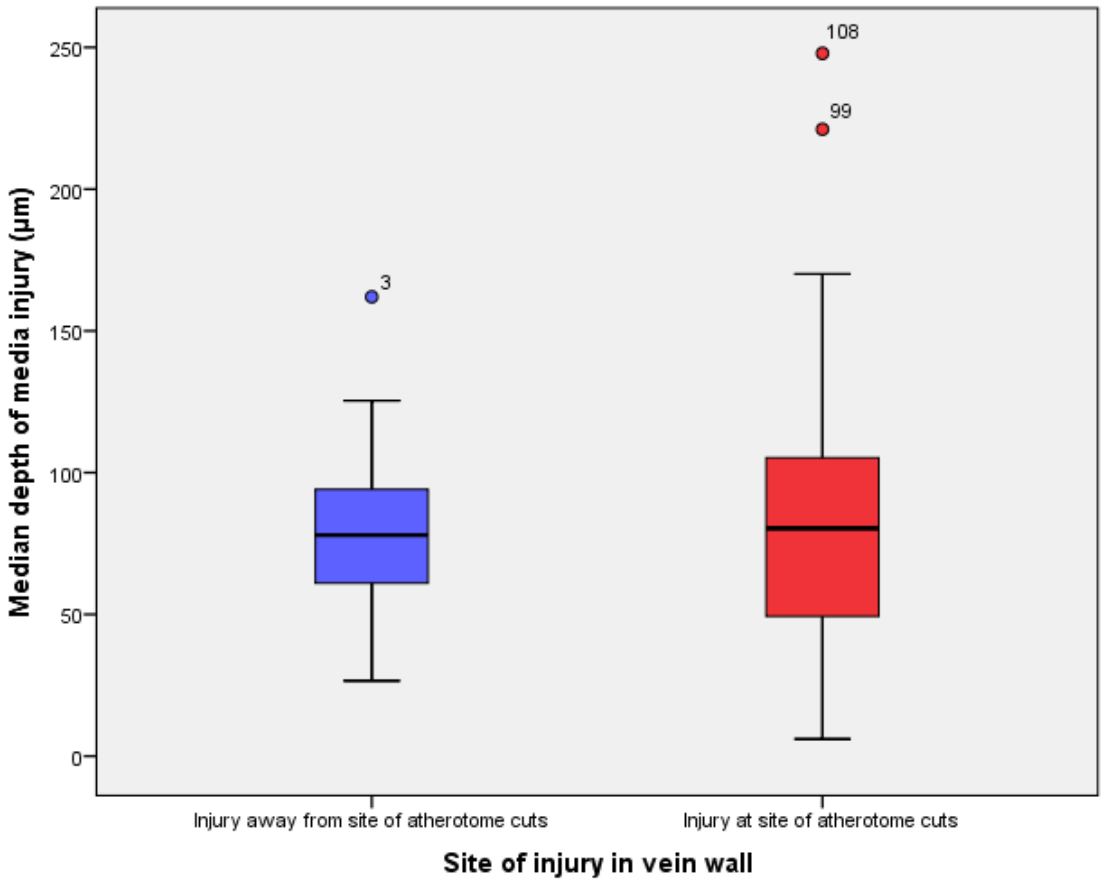


Figure 5.10: Effect of atherotome blade cuts on media damage caused by 3% STD: depth of injury perpendicular to atherotome blade cuts compared to depth of injury in non-cut luminal surface of vein.

Depth of injury perpendicular to atherotome blade cuts was no deeper than that created by 3% STD at the luminal surface of the same vein segments being 77.93µm (61.35, 94.09) vs 80.32µm (49.59, 104.63) respectively, p=0.837 Mann-Whitney U. Hence inflicting cuts on the vein lumen did not augment the media injury caused by STD alone.

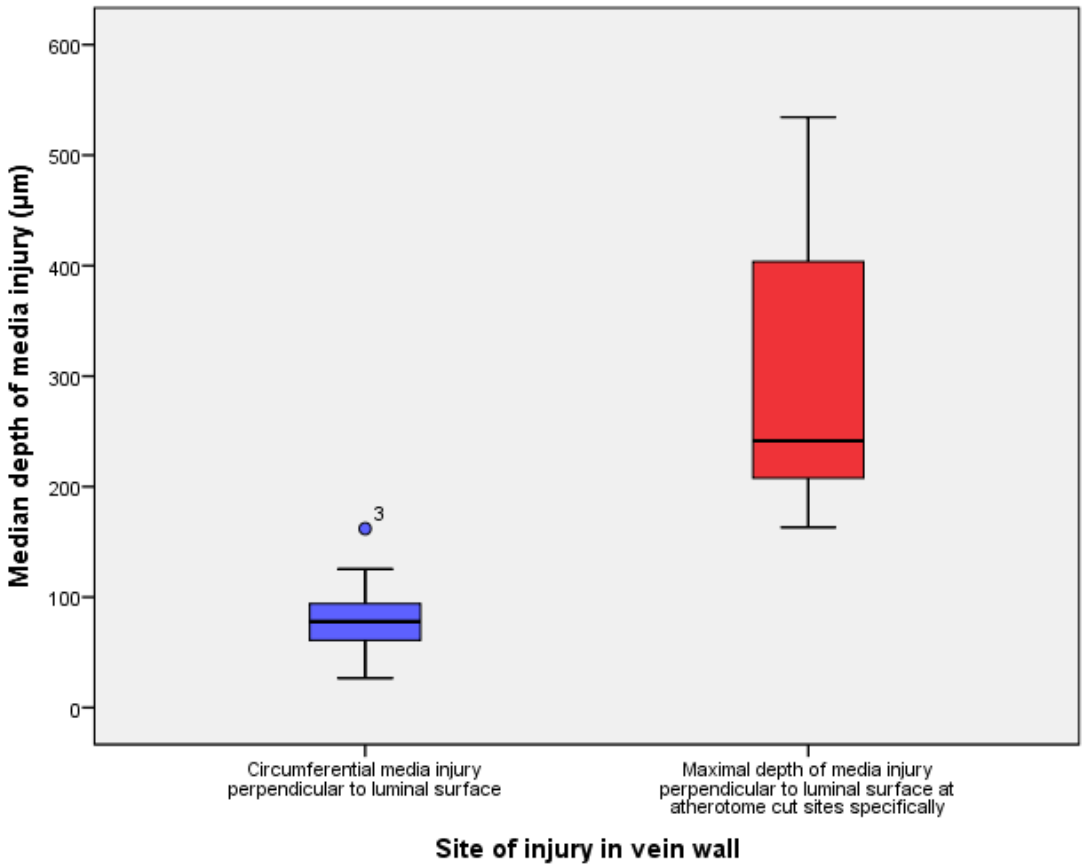


Figure 5.11: Depth of injury at atherotome blade cut sites, perpendicular to luminal surface versus elsewhere on same vein segments.

The depth of injury inflicted into the media perpendicular to the luminal surface was significantly deeper at the sites of atherotome blade cuts, 241.4µm (195.65, 410.60) compared to elsewhere on the vein luminal surface, 77.93µm (61.35, 94.09) p=0.001 Mann-Whitney U.

Hence it appears that though 3% STD damages the same depth of vein tissue no-matter it's surface of entry, luminal or cut media, the additional aid of a deeper starting point afforded by the atherotome blade cut allows for a significant enhancement of STD's depth of penetration into the media relative to the luminal surface.

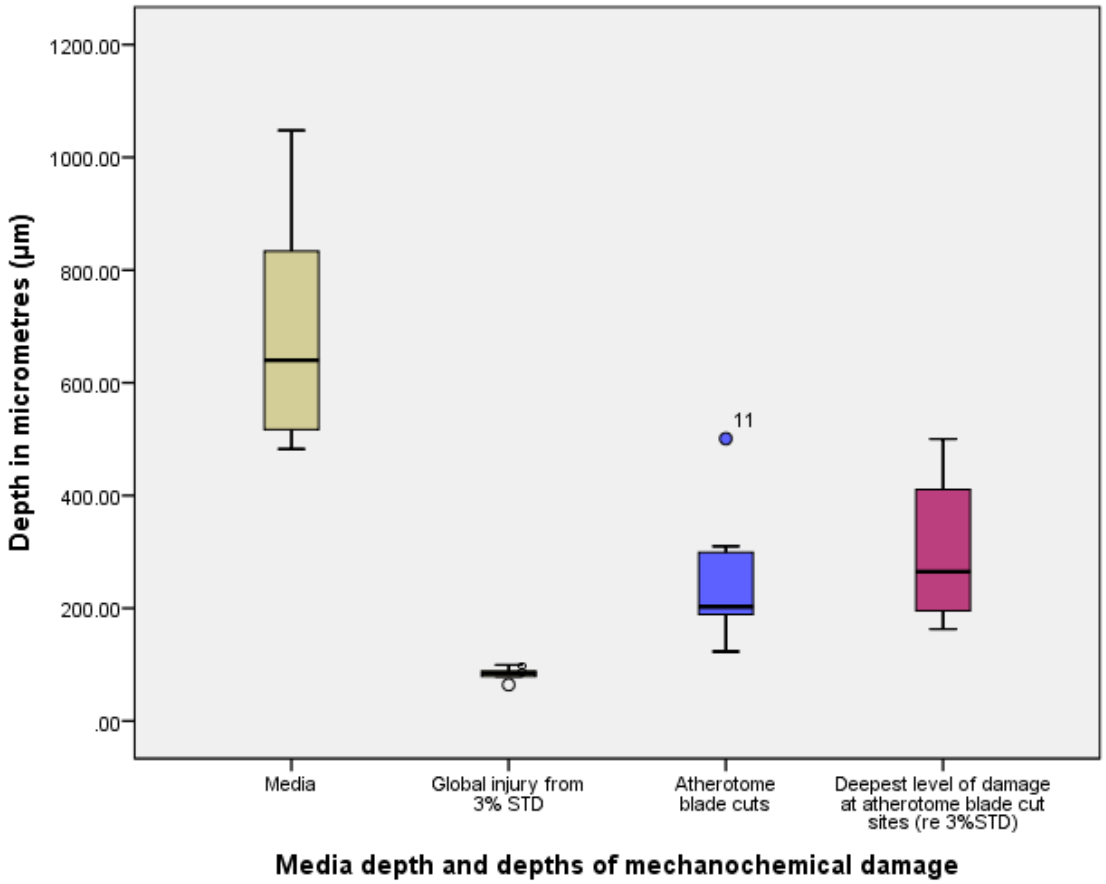


Figure 5.12: Media thickness compared to depth of mechanocemical injury.

Vein wall media had a median thickness of 640µm (516-833). Depth of injury inflicted upon it circumferentially by 3% STD was median 77.93µm (61.35-94.09), by atherotome blades 202.6µm (188.9-298.95) and the deepest level of damage at atherotome blade cut sites with additional 3% STD damage was 241.4µm (207.8-403.6) $p<0.01$.

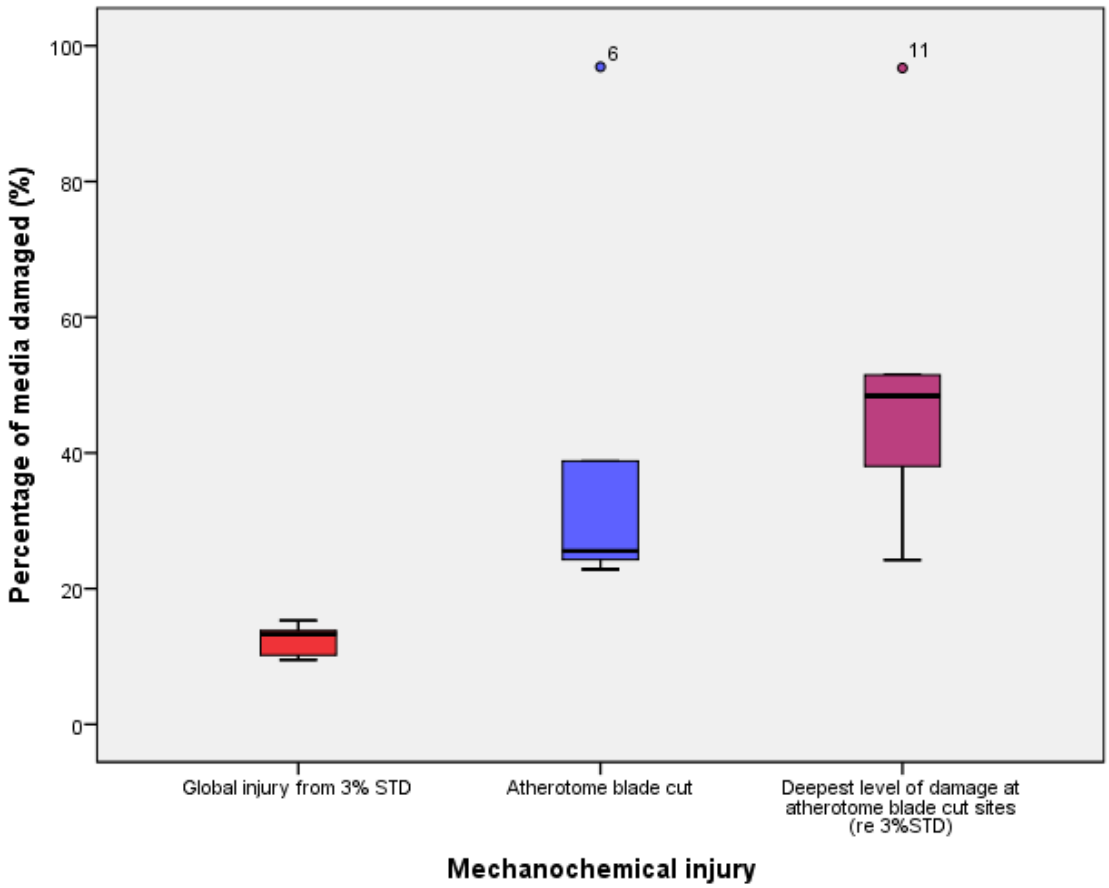


Figure 5.13: Percentage depth of injury seen in samples treated with cutting balloons and 3% STD.

With STD alone the median percentage depth of media injury circumferentially was 13.32% (10.21, 13.8), at atherotome blade cut sites 25.56% (24.3, 38.8) and at the deepest level of damage at atherotome blade cut sites from the effects of 3% STD 48.42% (38.04, 51.48). Comparing the percentage depth of media injury with 3% STD alone versus 3% STD with prior use of an atherotome cutting blade there was a significant increase in the percentage depth of media injured (13.32% to 48.42% p=0.01; Mann-Whitney U).

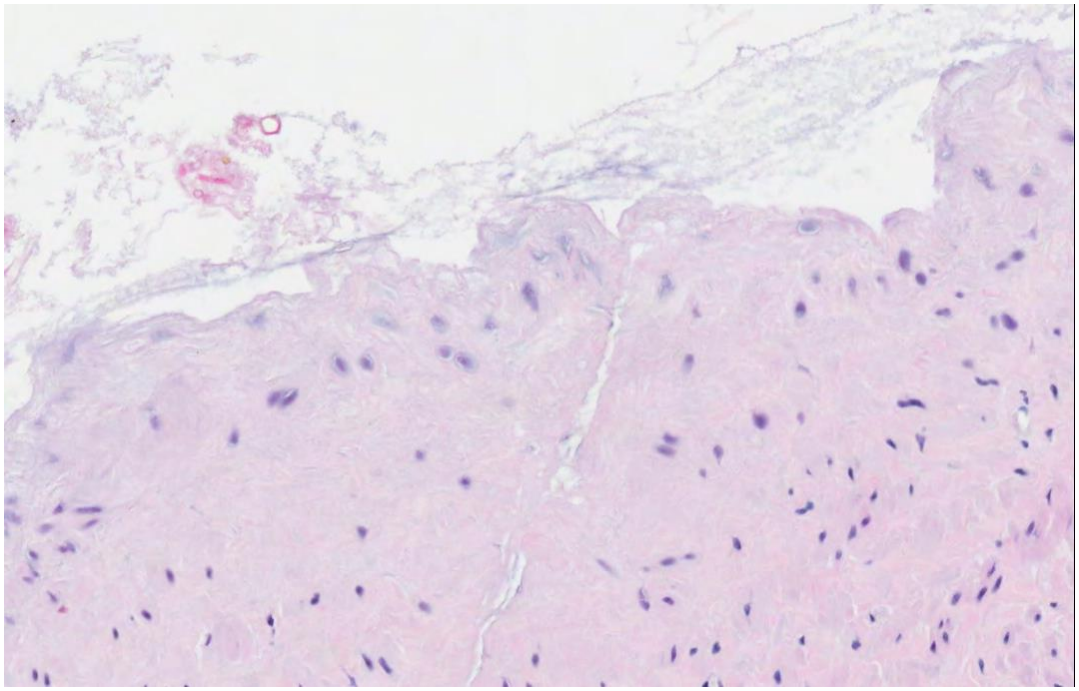


Figure 5.14: x20 example of nuclear vacuolation caused by deeper penetration of 3% STD into the vein wall media at the site of an atherotome blade cut. 3% STD foam is seen in-situ on the luminal surface.

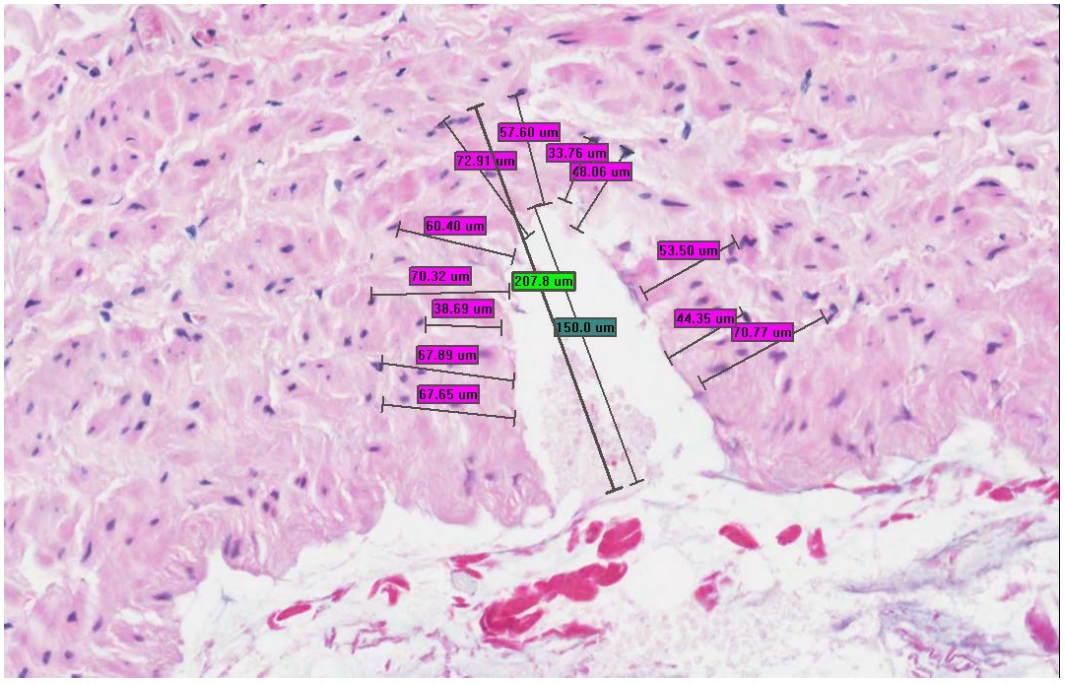


Figure 5.15: Example of measurements made at the site of an atherotome blade cut (green boxes) with addition of 3% STD showing depth of cut and the enhanced depth of penetration of 3% STD at this point (purple boxes: Aperio Imagescope)

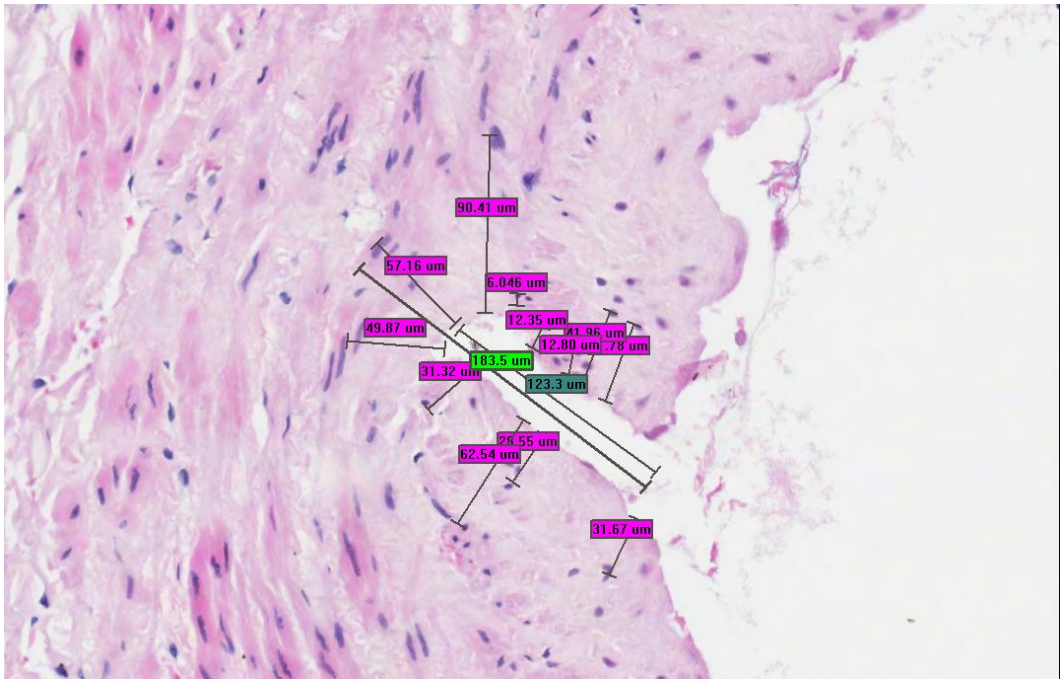


Figure 5.16: Further example of measurements made at the site of an atherotome blade cut with addition of 3% STD showing depth of cut and the enhanced depth of penetration of 3% STD at this point (Aperio Imagescope)

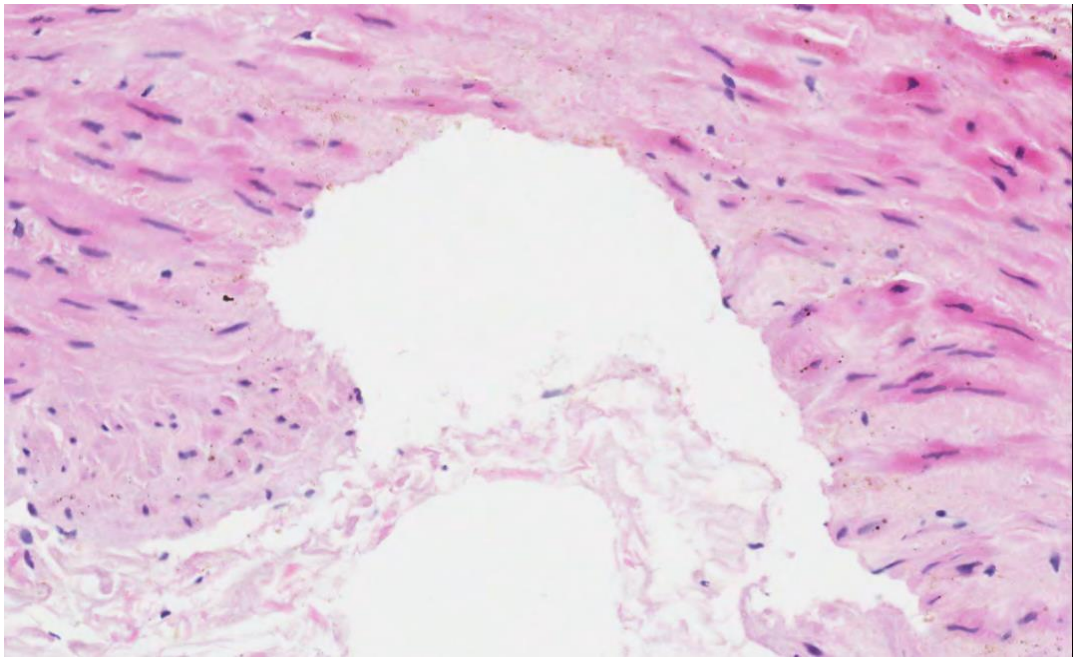


Figure 5.17: Example of an atherotome cut with concurrent 3% polidocanol foam. The media is not damaged at either the luminal surface nor at the deeper starting point afforded by the atherotome blade cut.

CHAPTER VI

IN-VIVO ANIMAL STUDIES

6 *IN-VIVO* ANIMAL STUDIES

6.1 ASSESSMENT OF THE THERAPEUTIC EFFECTS OF PROPRIETARY FOAM SCLEROTHERAPY, "LONGER-LASTING" FOAM SCLEROTHERAPY AND ENDOVENOUS CATHETER BALLOONS (FOGARTY AND CUTTING) IN PORCINE VEINS

This chapter presents the results from all animal experiments. All of the procedures were carried out by a licensed animal practitioner under the guidance of the lead researcher with details of experiments as per chapter two.

Fore-limb veins in both animals were treated with a 3Ch Fogarty balloon catheter and a cutting balloon with the right fore-limbs additionally treated with 3% STD foam. The proximally treated segment of vein was compared histologically to the distally untreated segment acting as control.

Hind-limb veins were treated with either 3% STD foam or 0.15% xanthum and 3% STD foam. The hind-veins are paired hence the paired vein acted as control.

The *in-vivo* tissue was harvested at three months during animal termination and processed as per chapter two.

Animal tissue was sectioned every 5mm, and every other section analysed. Initial qualitative results, examined blindly, were recorded by the lead researcher and consultant vascular pathologist and then in-order to more accurately assess samples the blinding was removed and the samples grouped together in the order in which they were both resected and hence sectioned.

This was necessary to decipher each section's relation to one another by allowing a representation of the full length of the specimen. The en-bloc nature of the tissue specimens allowed orientation and thereby assessment of the effects of each treatment.

Data were gained from a minimum of six and maximum of eight sections per treatment or control with two, of a maximum four cuts, analysed to derive both the qualitative score and quantitative results. Hence there are twelve to sixteen data points per treated segment for both qualitative and quantitative results.

Due to these experiments involving only two animals, statistical analysis is limited to examination of the differences within each animal individually.

6.1.1 QUALITATIVE DATA ANALYSIS

A scale of damage was developed according to what the desired outcome of the treatment on the veins would be i.e. obliteration/occlusion without damage to neighbouring tissue, through to what would be deemed treatment failure i.e. no change or worse still varicose vein formation. Thereby a qualitative scale was formed on this basis with the lowest score of 1 delineating the most effective treatment causing obliteration/occlusion, with a score of 7 for the least effective i.e. the treatment itself resulting in varicosities of the treated vein (TABLE 6.1). A score was given for each section with a minimum of two sections used per slide for every 5mm of tissue. The initial blindly gathered qualitative data for all samples was compared to the animal vein qualitative pathology score 2 showing no significant variance.

Effect	**Points**
Vein obliteration with no additional effects	1
Vein obliteration with additional effects	2
Recanalisation without additional effects	3
Recanalisation with additional effects	4
Vein patent without additional effects	5
Vein patent with additional effects	6
Vein varicosity	7

Table 6.1: Animal vein qualitative pathology score 2

Additional effects included acute inflammation, chronic inflammation, fibrosis and proliferative repair.

6.1.2 QUANTITATIVE DATA ANALYSIS

It was clear on initial blinded qualitative analysis of animal tissue which had been subjected to treatment three months previous, that as per preceding human vein experiments measuring the acute changes of endothelial loss and depth of media injury, that there was no such marked change in the media or endothelial loss that could be measured. The only useful measure that could be made was the patent luminal circumference or PLC in micrometres (μm). This was done by ensuring that the sections of vein in question were orientated correctly in relation to each other to ensure that the correct vein was being assessed. This was necessary more so with paired hind-limb veins with one acting as test and the other control. The PLC could be measured for both fully patent and partly occluded vein segments with fully occluded segments having a PLC measure of 0. PLC measurements were made using Aperio imagescope as per chapter two.

6.2 3% STD AND CUTTING BALLOON VERSUS CUTTING BALLOON ALONE

6.2.1 QUALITATIVE RESULTS: 3% STD AND CUTTING BALLOON VERSUS CUTTING BALLOON ALONE

Qualitative analysis of slides from both animals right fore-limb veins treated with Fogarty Ch3/Cutting Balloon/3%STD foam revealed widely patent veins with the majority of analysed sections being varicose. None of the test vein segments were occluded. Controls in animal 1, being distal parts of the same vein, were mostly normal vein segments but in animal 2 it appeared that there may have been some thrombosis with evidence of recanalisation.

		3% STD with Cutting balloon and fogarty pre-treatment	**Cutting balloon and fogarty**	
Animal 1	**Test**	6 (5,7)	7 (7,7)	p=0.101
	Control	6 (5,6)	5 (5,5)	
		p=0.639	**p=0.01**	
Animal 2	**Test**	7 (7,7)	7 (7,7)	p=0.730
	Control	4 (3.25,4)	5 (5,5)	
		p=0.036	**p=0.029**	

P values derived from Mann-Whitney U test

Table 6.2: Animal vein qualitative pathology score 2 results for 3% STD and cutting balloon treatment versus cutting balloon alone.

Qualitative scores for animal 1 (Fogarty Ch3/Cutting Balloon/3%STD foam) were 6(5,7) and its control segment, 6(5,6), p=0.639 Mann Whitney U revealing no difference, yet those for animal 2, 7(7,7) and its control, 4 (3.25,4) p=0.036 reached significance. It is difficult to draw any conclusions regarding the non-significant difference between this treatment and its control in animal 1 and the

significant difference in animal 2. This could be explained by a thrombus propagating in a retrograde fashion in animal 2.

Veins treated with Fogarty Ch3/Cutting Balloon alone in both animals were found to be varicose and their respective controls showed normal vein. Treatment with Fogarty and cutting balloons therefore appears to have altered the treated section of vein compared to control segments, but unfortunately to become varicose.

Comparing the two treatments, Fogarty Ch3/Cutting Balloon/3% STD foam versus Fogarty Ch3/Cutting Balloon alone within animal 1 [6(5,7) versus 7 (7,7)] showed no difference, p=0.101 with both causing varicosities. Similarly in animal 2 comparison of the same treatments [7 (7,7) versus 7 (7,7)] p=0.730 showed no difference. Hence it appears that the addition of 3% STD to the cutting balloon treatments made no difference.

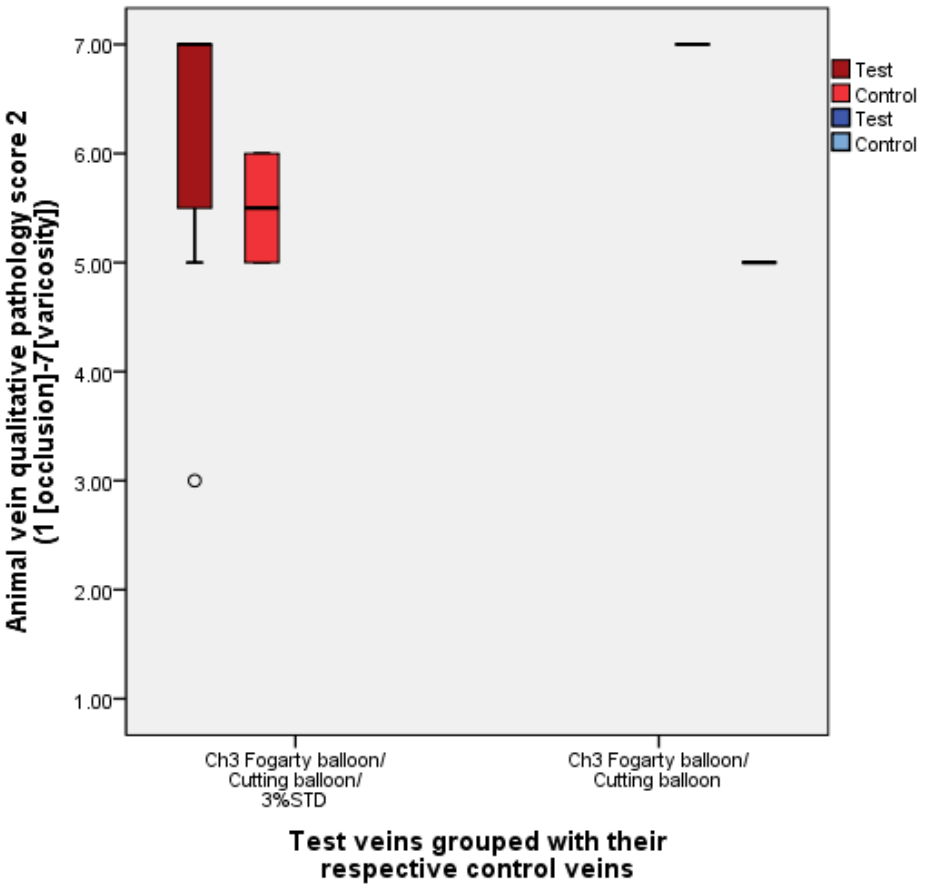


Figure 6.1: Animal vein qualitative pathology score 2 results for 3% STD and cutting balloon treatment versus cutting balloon alone (Animal 1 and 2 scores combined for graphical purposes only).

6.2.2 QUANTITATIVE RESULTS: % STD and cutting balloon versus cutting balloon alone

		3% STD with Cutting balloon and fogarty pre-treatment	**Cutting balloon and fogarty**	
Animal 1	**Test**	2654µm (2517, 3611)	3424µm (3105, 5164)	p=0.138
	Control	1481µm (1481, 1843)	1047µm (974, 1110)	
		p=0.01	**p=0.01**	
Animal 2	**Test**	4864µm (3438, 5844)	5802µm (5387, 6037)	p=0.730
	Control	1217µm (793, 2317)	432µm (424, 439)	
		p=0.016	**p=0.029**	

P values derived from Mann-Whitney U test

Table 6.3: Patent luminal circumference/PLC (µm) for Fogarty Ch3/cutting balloon/3%STD foam versus Fogarty Ch3/cutting balloon.

There was a statistically significant difference between all test segments PLC and their respective control segments highlighting that the treatment had indeed altered the vein. Overall however no difference was noted between the two therapies resultant PLC's when compared to each other within both animals.

These quantitative results reflect the qualitative results, showing that dilated or varicose veins resulted from treatment involving balloons, with control segments being of a smaller calibre.

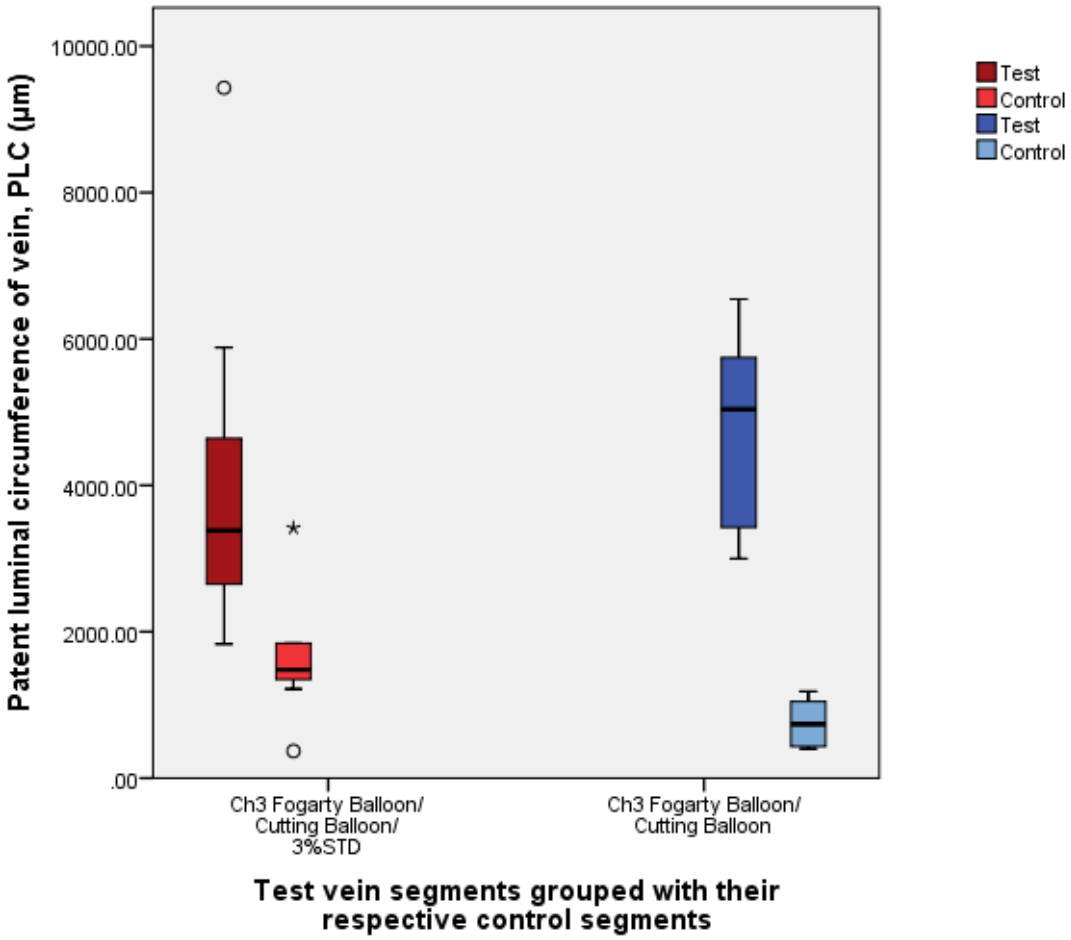


Figure 6.2: Patent luminal circumference (PLC) (µm) for 3% STD and cutting balloon treatment versus cutting balloon alone (Animal 1 and 2 results combined for graphical purposes only).

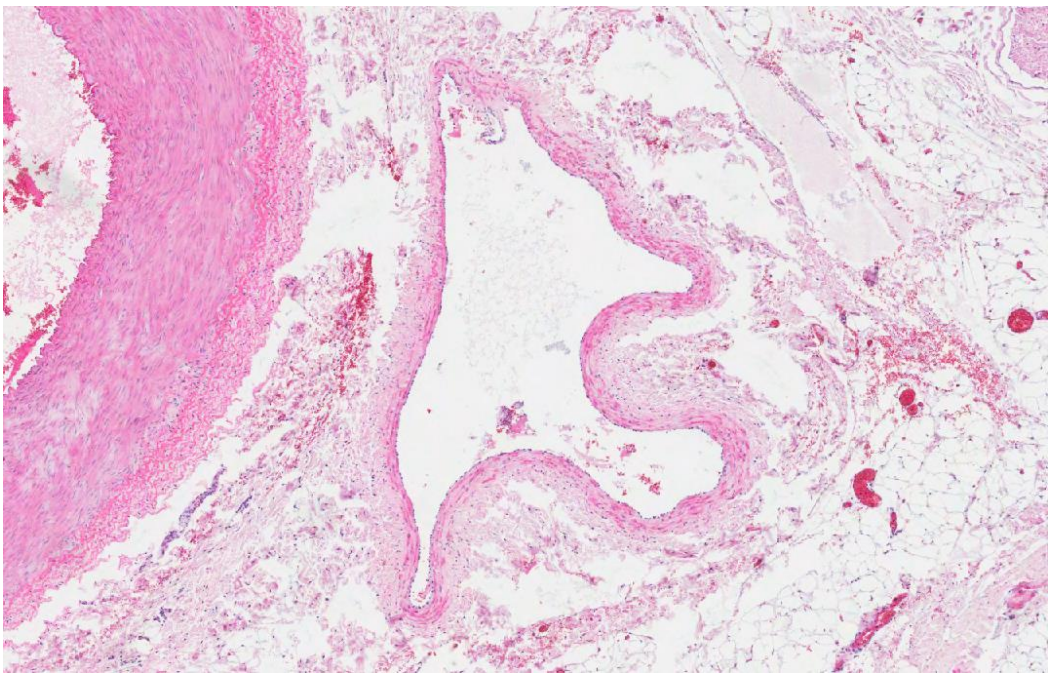


Figure 6.3: x3 Example of forelimb vein experiments with varicosity created by cutting balloon and 3%STD treatment

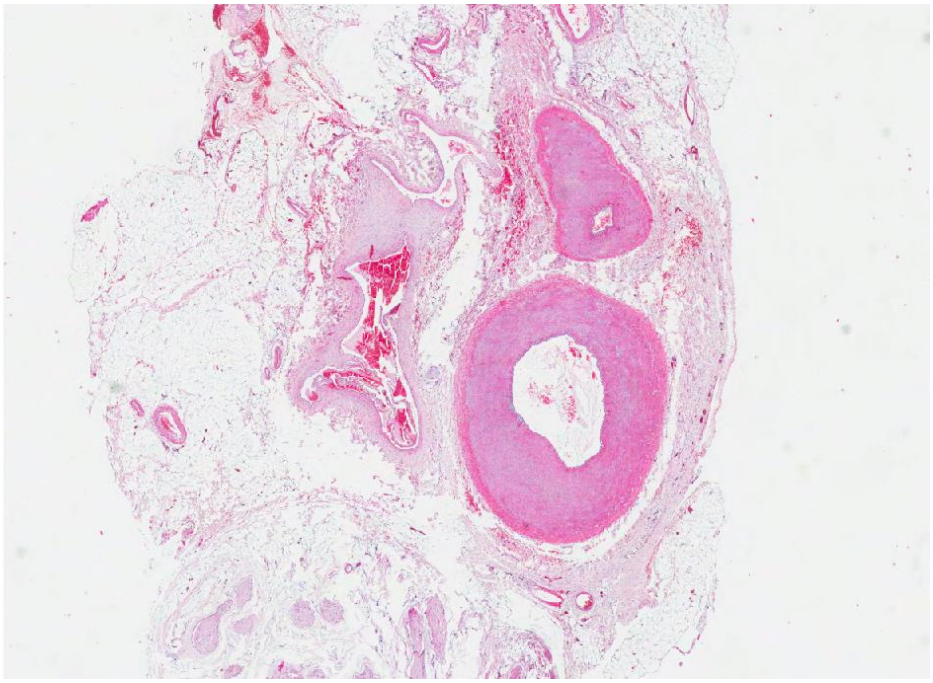


Figure 6.4: x1 Example of varicosity created by cutting balloon treatment and 3% STD treatment

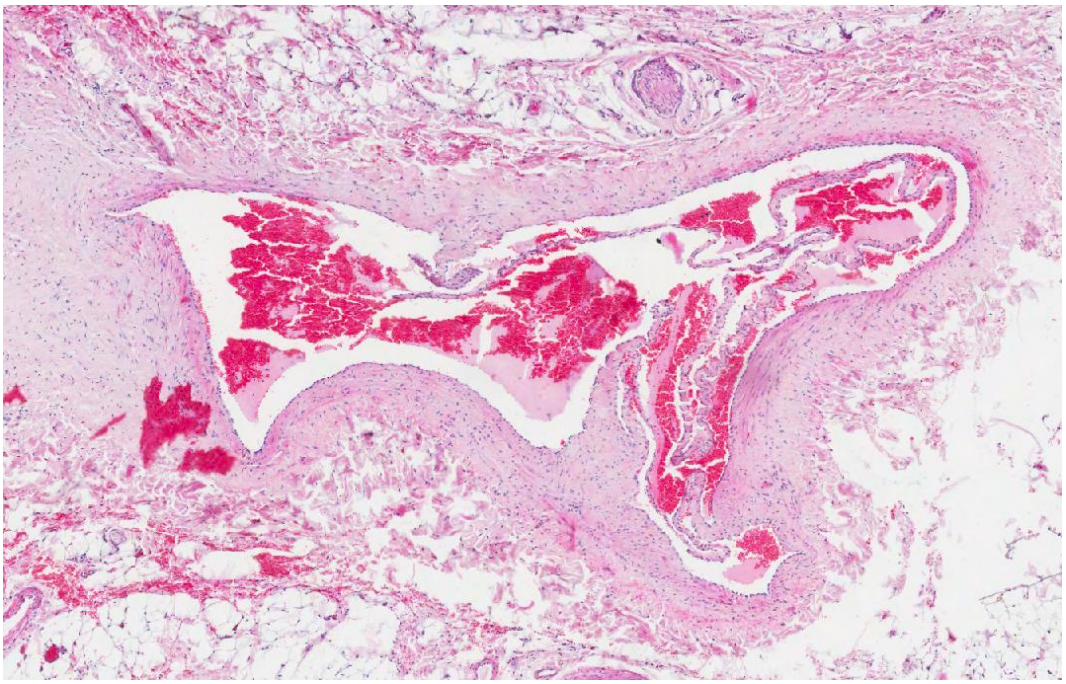


Figure 6.5:x4 Example varicosity created by 3% STD and cutting balloon treatment

6.3 0.15% XANTHUM AND 3% STD VERSUS 3% STD ALONE

6.3.1 QUALITATIVE RESULTS: 0.15% XANTHUM AND 3% STD VERSUS 3% STD ALONE

Qualitative analysis of hind-limb veins in animal 1 revealed that vein treated with 0.15% xanthum and 3% STD was obliterated with some sections having fibrosis and others with at worst some evidence of recanalisation. The qualitative pathological vein score was median 2(1,3) with paired vein control score being 5(5,5) i.e. normal vein. Analysis of animal 2's hind-limb vein similarly treated with 0.15% xanthum and 3% STD foam revealed similar changes to animal 1 in that the vein was obliterated with occasional areas of fibrosis and a qualitative pathological vein score of 1 (1,1.5) with paired vein control score again being 5(5,5) i.e. normal vein.

Animal 1's vein treated with 3% STD foam had recanalised with the additional effects of fibrosis noted. The qualitative pathological vein score using 3% STD in animal 1 was median 4 (3.5,4). Animal 2's vein treated with 3% STD was 3% STD again recanalised with an area of obliteration surrounding fibrosis with a qualitative pathological vein score of 4 (4.5,5.75). Qualitative pathological vein score for paired vein control segments for both animal's hind-limb vein 3% STD experiments were 5(5,5) i.e. normal vein.

		0.15% Xanthum & 3% STD	**3% STD**	
Animal 1	**Test**	2 (1,3)	4 (3.5,4)	**p=0.035**
	Control	5 (5,5)	5 (5,5)	
		p=0.01	p=0.365	
Animal 2	**Test**	1 (1,1.5)	4 (4.5,5.75)	**p=0.01**
	Control	5 (5,5)	5 (5,5)	
		p=0.01	p=0.485	

P values derived from Mann-Whitney U test

Table 6.4: Animal vein qualitative pathology score 2 results for 0.15% xanthum and 3% STD versus 3% STD alone

Hence there was a significant difference between Xanthum/3%STD's test segment scores versus its respective controls in both animals, but not for scores with 3 % STD versus its controls. In both animals a significant difference is found when results from Xanthum/3%STD are compared with those from 3 % STD with more favourable results in terms of vein occlusion at three months occurring with Xanthum/3%STD than with 3% STD.

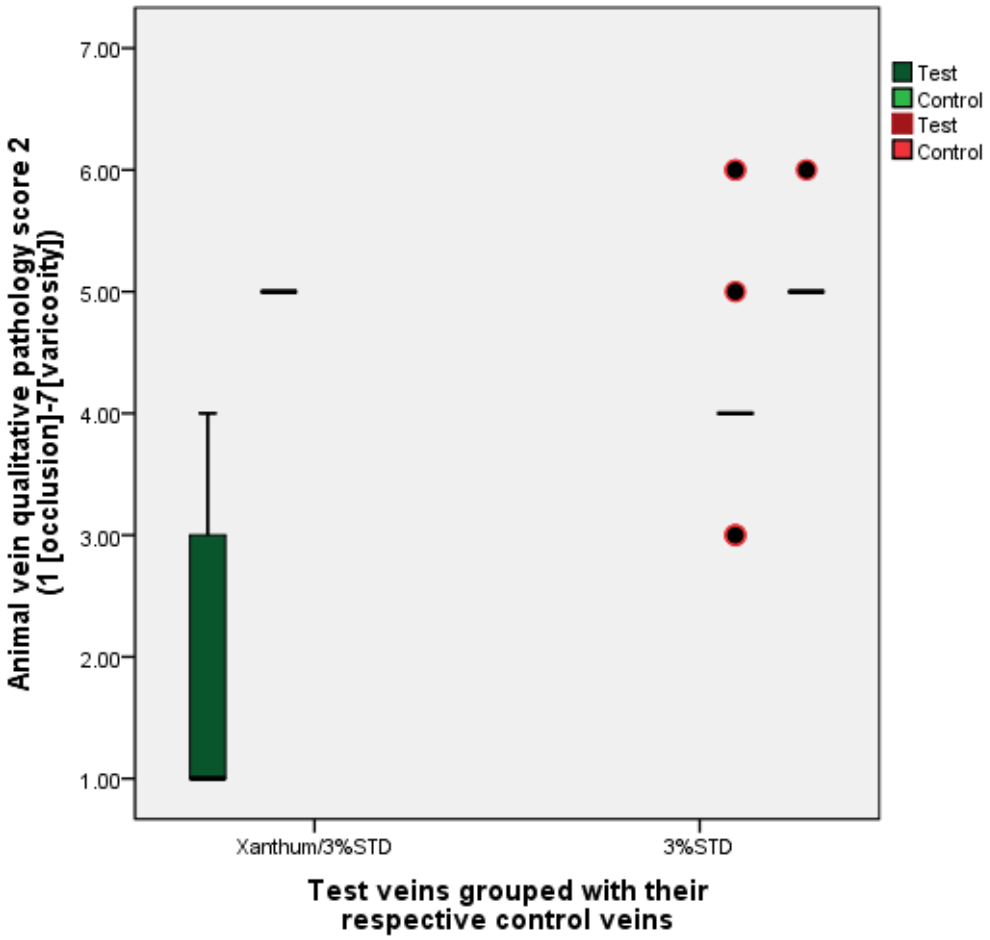


Figure 6.6: Animal vein qualitative pathology score 2 for 0.15% xanthum and 3% STD versus 3% STD alone (Animal 1 and 2 results combined for graphical purposes only)

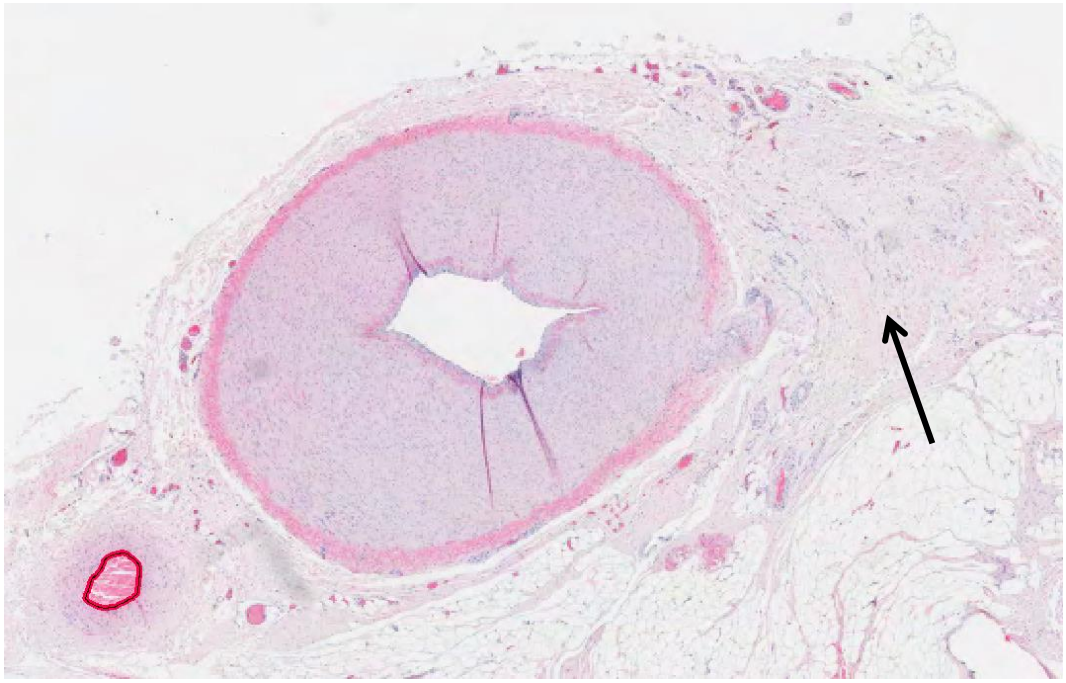


Figure 6.7: x2.4 Example of hind-limb experiments with control vein on left patent (lumen highlighted in red) and test vein on right (arrow) obliterated post treatment with 0.15% xanthum and 3% STD

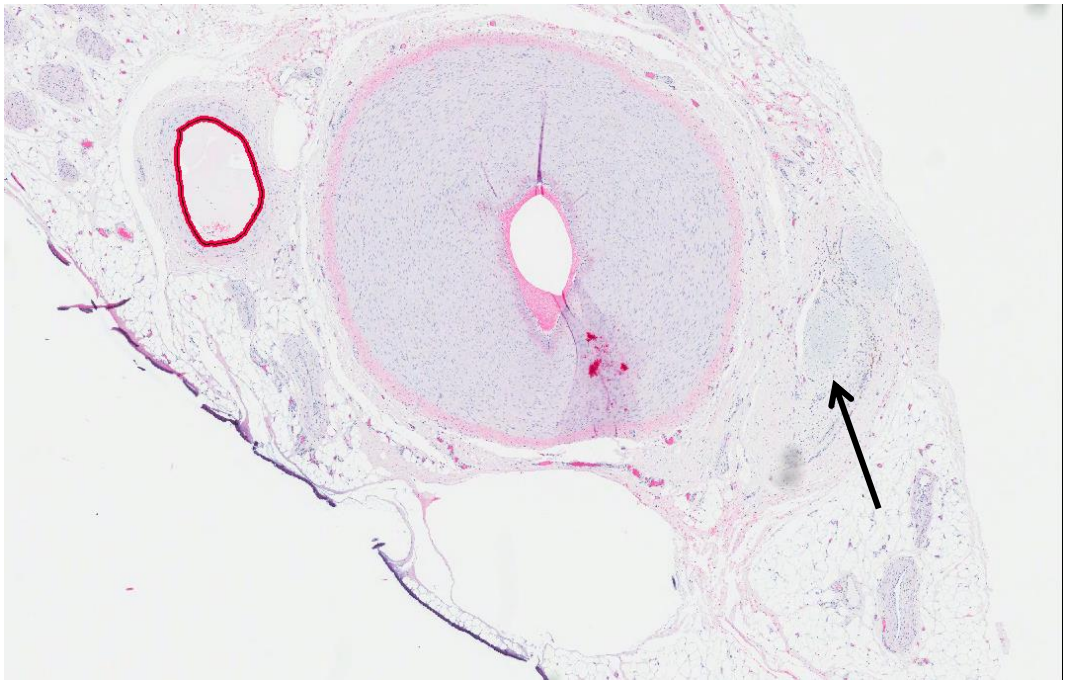


Figure 6.8: x2 Example of patent control vein and test vein (arrow) obliterated using with 0.15% xanthum and 3% STD

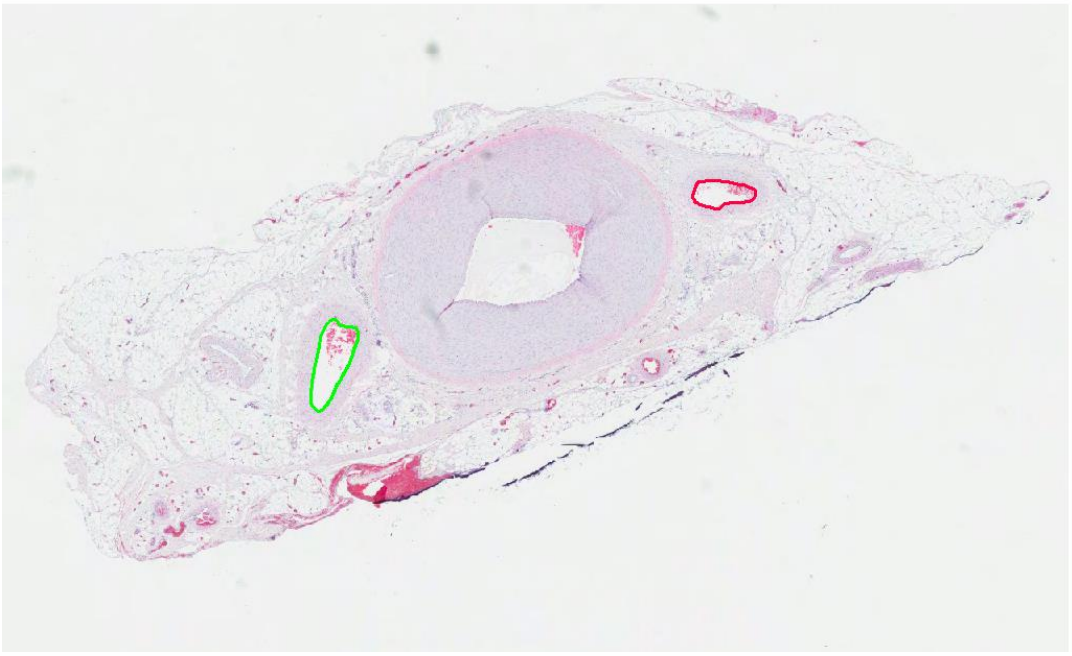


Figure 6.9: X1 Example of distal segment in hind-limb with patent test and control veins in limb tested with 3% STD foam into more proximal aspect of vein with lumen circled in red.

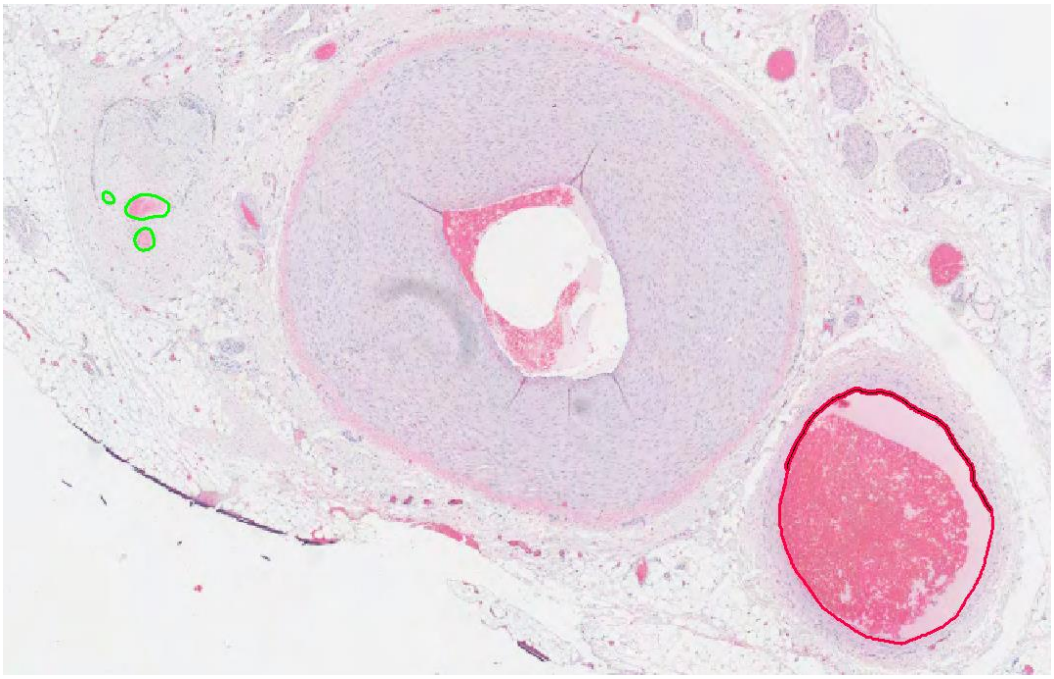


Figure 6.10: x2 Example of recanalisation of 3% STD treated test vein on left with neovascularised lumens marked in green. Control vein lumen is marked in red.

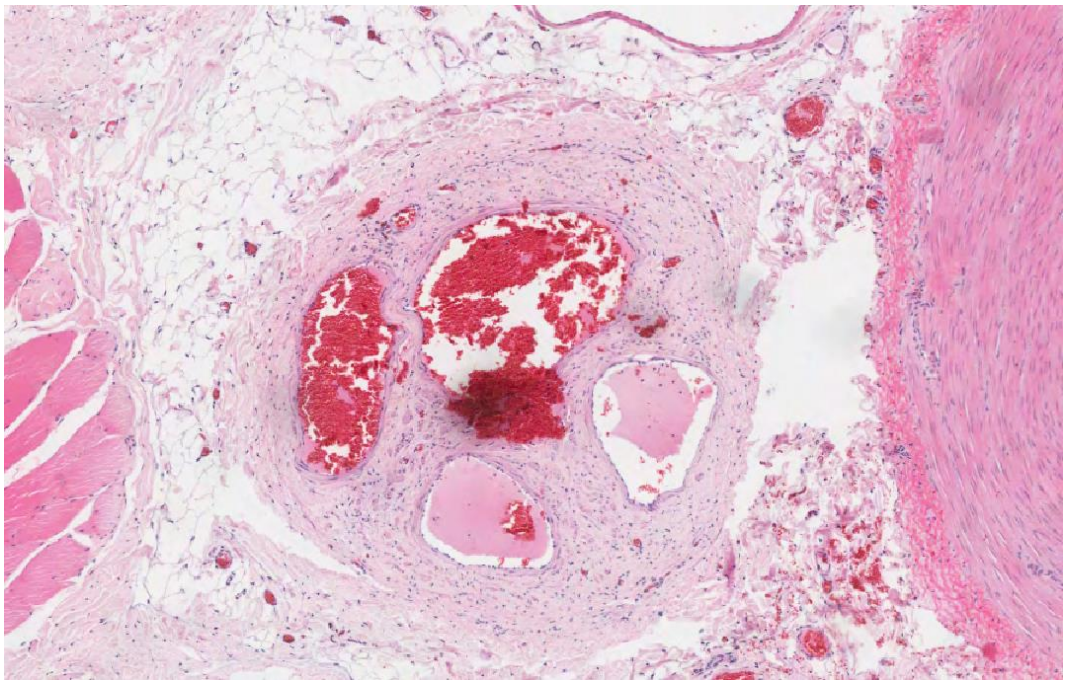


Figure 6.11: x6 Example of recanalisation of 3% STD treated test vein

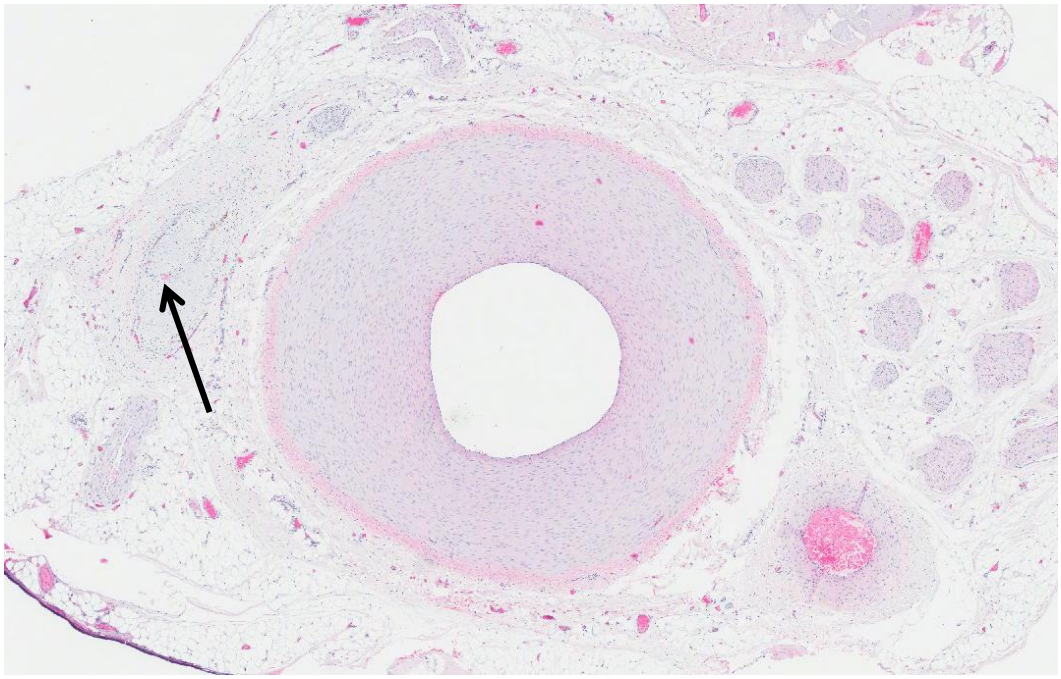


Figure 6.12: x2 Example of test vein obliteration using 3% STD (arrow) with patent control vein

6.3.2 QUANTITATIVE RESULTS: 0.15% XANTHUM AND 3% STD VERSUS 3% STD ALONE

		0.15% xanthum & 3% STD	**3% STD**	
Animal 1	**Test PLC**	268µm (0, 540)	1926µm (1495, 3102)	**p=0.01**
	Control PLC	1013µm (913, 1436)	1857µm (1160, 2510)	
		p=0.026	p=0.694	
Animal 2	**Test PLC**	0µm (0, 609)	1070µm (975, 1183)	**p=0.023**
	Control PLC	1367µm (919, 1701)	1704µm (1063, 2765)	
		p=0.035	p=0.24	

Table 6.5: Patent luminal circumference/PLC (µm) for Xanthum/3%STD foam versus 3%STD foam

Hence Xanthum/3%STD significantly reduced the patent luminal circumference of the treated veins in comparison to its controls in both animals whereas 3%STD did not. Moreover Xanthum/3%STD significantly reduced the patent luminal circumference in comparison to veins treated with 3% STD alone across both animals.

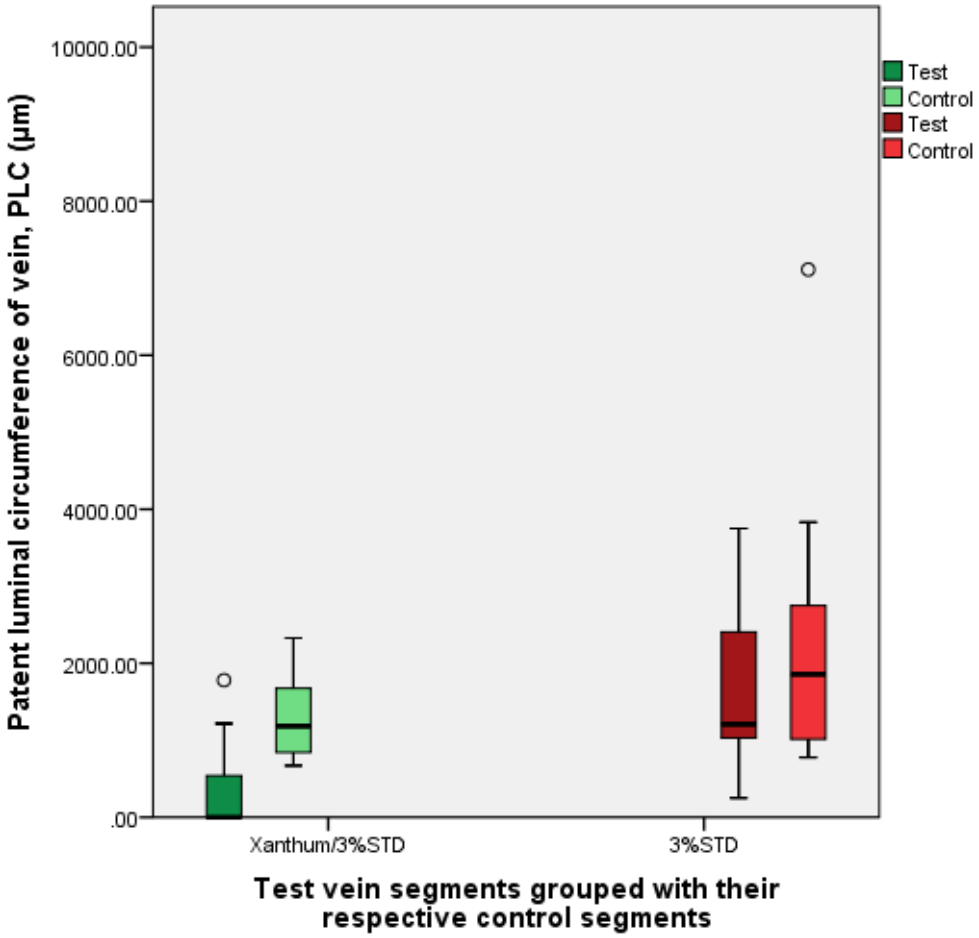


Figure 6.13: Patent luminal circumference/PLC (µm) for Xanthum/3%STD foam versus 3%STD foam (Animal 1 and 2 results combined for graphical purposes only)

6.4 CONCLUSIONS

6.4.1 3% STD AND CUTTING BALLOON VERSUS CUTTING BALLOON ALONE

Qualitative and quantitative results showed that in both animals the two cutting balloon related treatments resulted in making the veins varicose as opposed to occluding them. Regarding these varicose changes to test segments, it appears that the balloon treatments have in fact stretched the vein walls and made it less likely that they will occlude with or without subsequent use of 3% STD foam. All experiments involved trawling an inflated cutting balloon within the lumen after trawling with a Fogarty Ch3 balloon. The cutting balloon size was matched to the treated vein although the pig veins were narrower calibre than in previous human experiments. It is likely from analysis of veins in the human cutting balloon experiments that in instances where the vein diameter is too small in relation to the cutting balloon that in fact the atherotomes may not have deployed. However in this partially open experiment with part of the treated vein exposed, it was possible to see that the atherotomes were at least partially, if not fully deployed.

In summary Fogarty and cutting balloon treatment with or without 3% STD caused varicose changes in the treated vein instead of the desired obliteration/ occlusion. It appears that the addition of 3% STD to the cutting balloon treatments made no difference.

It would appear that either the balloons were too large compared to the treated veins and caused a significant structural deformity by overstretching the vein wall resulting in varicosities or the atherotomes rather than cause venous scarring and fibrotic occlusion heightened by an increased influx of sclerosant

into the vein wall, actually weakened the vein wall such that it was rendered structurally deficient therefore caused varicosities.

6.4.2 0.15% XANTHUM AND 3% STD VERSUS 3% STD ALONE

Xanthum/3%STD treated segments had a significantly lower qualitative score and PLC, both markers of treatment success, versus its respective controls in both animals, but this was not the case for 3 % STD versus its controls. Moreover Xanthum/3%STD had significantly lower qualitative scores and reduced the patent luminal circumference in comparison to veins treated with 3% STD alone across both animals.

From these preliminary animal tests it appears that Xanthum/3%STD shows promise as a treatment for varicose veins with more favourable results in terms of vein occlusion at three months occurring with Xanthum/3%STD than with 3% STD.

CHAPTER VII

DISCUSSION AND CONCLUSIONS

7 DISCUSSION AND CONCLUSIONS

Primary varicose veins are a common problem with a multitude of treatments which suggests that the search continues for the optimal treatment. Minimally invasive treatments show the most promise as they at least appear to match the recurrence rates of surgery but with lower morbidity. Minimally invasive treatments that have shown most success thus far are those that damage the GSV with thermal related injury. However, the device purchase and consumables and the requirement for tumescence all increase the cost. In contrast sclerotherapy is less expensive and requires little in the way of specialist equipment. Further, success rates may be greater when foam sclerotherapy is used. UGFS can be repeated but this increases the cost financially and in terms of time and man-power. It also relies on significant patient compliance. Despite 150 years of unregulated human experimentation the “perfect sclerosant,” free from complications and causing permanent vein occlusion has not been found. The desired functions of sclerosing solutions are the destruction of venous endothelial cells, exposure of subendothelial collagen fibres, and ultimately fibrotic occlusion of the treated vein[13]. Endothelial damage is enhanced by greater concentrations of sclerosant although side effects, particularly pigmentation are more common with stronger solutions of the commonly used sclerosants. The use of foam increases the surface area of vein wall that is treated and prolongs the contact time of the sclerosants. This ought to amplify the effect of the agent.

The hypothesis of this thesis is that current foam sclerotherapy can be modified or assisted via other means to improve its efficacy.

As a starting point this thesis examines current primary varicose veins treatments at the outset of this research. STD and polidocanol foams, which appear to have the best proven efficacy and are the most widely used worldwide, are examined in chapter three regarding their foam half-lives and in chapter four regarding their activity against a novel in-vitro GSV model to assist in answering the question, is foam longevity the most important factor in determining the efficacy of UGFS and which of the most widely used scleroant foams is most potent? In chapter three, the half-life of 3% polidocanol foam is shown to be much longer than that of 3% STD. In chapter four experiments show that 3% STD is the most active sclerosant agent in terms of damage to the vein wall compared with polidocanol. It therefore appears from the results obtained that the active ingredient is the most important factor and not the foam half-life.

The next set of experiments in chapter four again pose the question, is the longevity of foam a factor in its efficacy? This question is answered by examining the damage inflicted on vein wall by STD foams with prolonged half-lives. There are few studies detailing the pathological changes induced by sclerotherapy on varicose veins. An in-vitro GSV model was used for this research to allow testing of multiple agents in order to gain answers to the questions of this thesis. This model is used firstly to assess the constituent parts of proprietary STD to ensure that, as is proven the case, STD itself is in fact the most active agent against the vein wall. Experiments appear to show that the ingredients included in proprietary STD are synergistic in that the combination causes more damage compared with its separate parts. This

however could be due to a fault of experimental methodology e.g. the buffer used with STD. One of the key questions of this research is does prolonging foam longevity improve foam efficacy? Results in chapter four have shown that longer lasting STD foams do not enhance its activity against the vein wall, at least acutely. Therefore, it appears that the active agent in its current proprietary form is optimal, at least according to these in-vitro results.

The further question of whether adjuncts can improve the efficacy of STD foam is examined. Ikponmwosa *et al* formerly investigated this question in terms of Fogarty catheters used prior to foam sclerotherapy, which did not appear to improve its efficacy against the vein media. In this research cutting balloons are tested as a potential pre-treatment prior to the use of foam sclerotherapy. They appear to increase the depth of penetration of sclerosant foam into the vein wall media by affording the sclerosant a deeper starting point. If intermittent increased depth of penetration of sclerosant foam and resultant enhanced depth of STD vein wall injury were to translate to improved efficacy of foam sclerotherapy through subsequent fibrosis, then this adjunct does show promise. In order to ascertain whether cutting balloons used prior to foam does translate to improvement of foam sclerotherapy using STD alone, preliminary animal studies were carried out on a porcine model. Animal studies were performed on two pigs with test experiments performed and the vein samples collected and analysed three months later. These preliminary studies of cutting balloons used as adjuncts to foam sclerotherapy show that rather than assist in the occlusion/obliteration of the treated vein, the balloons appear to have damaged the structure of the vein wall leaving them varicose which is in effect the opposite of the desired treatment effect. 3% STD foam and the most promising of the longer lasting foams were also tested and results show that

counter to those of the in-vitro studies that in fact 0.15% xanthum and 3% STD actually outperformed proprietary 3% STD in causing vein occlusion. Discussion of each chapter is included below.

7.1 FOAM HALF-LIVES

Chapter three shows that 3% polidocanol foam lasts longer than 3% STD foam with a median difference of 21.3sec and 53.2sec for T_{90} and T_{50} respectively. Similar differences between the longevity of the respective foams have been found by others using different strengths of these sclerosants[35]. This may be related to the larger molecular size of polidocanol thus enhancing its surfactant and foaming properties. This ought to be beneficial given that *f*oam sclerotherapy is *considered* more efficacious than liquid sclerotherapy because of a longer contact time between the sclerosant and the endothelial cells[13] however subsequent results refute that.

Areas for improvement in these experiments include that varying initial volumes of foam were observed during these experiments. Although a graduated syringe was used to draw up the liquid to a volume of 2mL for foaming, there may have been small volume variation above or below this. This also applies to the volume of air drawn up in the second syringe to produce the foam. A smaller syringe, with smaller increment markings could have been used to draw up the initial volumes and then this transferred to a 10mL syringe. It would however have proved difficult to transfer the liquid completely as viscous liquids adhere to the syringe wall. The results however were reproducible.

7.2 ASSESSMENT OF THE HISTOLOGICAL EFFECTS OF TESTED FOAMS

Experiments in chapter four involve a novel *in-vitro* model for the assessment of foam sclerosants on human GSV samples from patients with primary varicose veins. Firstly the model was tested using normal saline compared with heparinised blood from the patient which acted as the control for future foam related experiments. The model proved effective in that endothelial loss was minimal and there was no media damage inflicted by normal saline or heparinised blood. Though this model is relatively simple and could be criticised mainly on the basis of being *in-vitro*, it gave consistent results over the whole period of this research and allowed multiple experiments to be carried out due to the high patient number, based on the simplicity of the model. *In-vivo* testing in humans though the ideal, is not realistic in this country from an ethical perspective and many of the questions from this chapter could be answered to an appropriate degree using *in-vitro* experimentation. Examining the most widely used proprietary foams revealed that though endothelial loss was similar using STD and polidocanol, the median depth of injury and the percentage of media injured were significantly higher with STD. Although there are no other histological studies with which to compare these findings they would support the clinical view that STD is a more potent sclerosant than polidocanol with higher concentrations of the latter necessary to produce the same effect[3-5, 35, 36]. Our model has shown that in fact the same effect clinically is possibly derived from very different effects at the cellular level, as it would seem unlikely that polidocanol has any propensity to damage the vein media in-vivo if it does not do so in-vitro. Of the two proprietary foams this research appears to show that STD foam is the only sclerosant likely to damage the media in-vivo, however, we cannot prove this.

The constituent parts of proprietary STD were examined in the same way showing as expected that STD is the most active ingredient as opposed to benzyl alcohol which is included in the proprietary product for its preservative function. Of note when broken down into its constituent parts STD foam did not damage the vein media as opposed to proprietary STD foam. This indicates that either the combination of STD and benzyl alcohol in proprietary STD is synergistic, or our buffering mechanism was at least interfering with the activity of STD against the media. This question could be answered by working in conjunction with the manufacturers of STD and using a more appropriate buffering agent simply extracting the benzylalcohol from proprietary STD foam.

Compared to proprietary 3% STD foam, longer lasting 3% STD foams did not inflict greater injury on either the GSV endothelium or media. Indeed, the addition of pluronic had a negative impact on the efficacy of 3% STD in terms of both endothelial loss and damage to the vein media. Conversely, 3% STD and xanthum showed a trend towards enhanced media damage compared to 3% STD at least in 5 minute experiments, but this still failed to reach statistical significance.

Combining results from chapters three and four it is evident that although polidocanol foam shows greater stability than STD foam and may thus remain in the treated segment of vein for longer (in-vivo), damage to the media is significantly greater with STD. Similarly it appears that modifying proprietary STD to increase the foam half-life does not improve its efficacy in damaging the vein wall. Parsi *et al*found that the therapeutic effect of a sclerosant appears to occur in the first few seconds after injection[19]. Thus it is possible that as long as the active drug is distributed to the target area, longer exposure may not significantly increase its efficacy. Certainly our results suggest that the longevity

of the foam matters less than the active ingredient. A question that arises from this is that, if the active ingredients damage a certain depth of media and this is all done upon the first contact between treated surface and sclerosant, is there a possibility that an immediate or planned early but delayed repeat treatment with foam could inflict a greater degree of damage on vein wall?

Furthermore on the basis this work it seems likely that the initial success of sclerotherapy is associated with simple thrombotic occlusion and that recanalisation is promoted by persisting islands of endothelial cells and the absence of significant fibrosis due to the failure to inflict a significant injury to the media. Although this hypothesis seems logical this study only assesses the immediate impact of sclerotherapy in an in vitro model. It is possible that these sclerosants may cause greater tissue damage in vivo.

Although the findings of this section of results appear robust in their being repeatable, it has a number of shortcomings. These include the previously discussed in-vivo versus in-vitro issue but also the relatively small number of veins tested per experiment. This research demanded a method that allowed throughput of a significant number of patients to allow the relevant questions to be answered, yet the defined research period coupled with the competing relative lack of patients having open surgical procedures during this age of minimally invasive therapies meant that this number of five patients per test was deemed an appropriate balance between these factors.

Furthermore whilst animal models could be used as the next best thing to in-vivo human studies, animal research expense would preclude large numbers. In addition there is no established animal model with SFJ and GSV incompetence. The advantage of the human in-vitro GSV model used in this research is that it

can easily be used in the initial assessment of longer lasting foams and novel sclerosants.

7.3 CUTTING BALLOONS AS ADJUNCTS TO FOAM SCLEROTHERAPY

Experiments assessing cutting balloons as a potential adjunct for foam sclerotherapy certainly showed promise for improving its efficacy by affording the foam a deeper starting point for sclerosant to damage the vein wall. The depth of injury inflicted into the media, perpendicular to the luminal surface, was significantly deeper at the sites of atherotome blade cuts, 241.4µm (195.65, 410.60) compared to elsewhere on the vein luminal surface, 77.93µm (61.35, 94.09) $p<0.01$ Mann-Whitney U. This translates to 13.32% (10.21, 13.8) depth of media injured using 3% STD alone versus 48.42% (38.04, 51.48) at atherotome blade cut sites, with the combined effects of a cutting balloon used prior to application of 3% STD foam $p<0.01$ Mann-Whitney U.

Potential problems with the experimental methods in these human GSV *in-vitro* cutting balloon studies include that firstly the balloons are designed for arterial work and not vein specific. This could not be overcome during the timeframe of this research as no such device exists and there is no precedent, therefore this was the most appropriate device in the circumstance. Secondly due to the expense of arterial cutting balloons those used were second hand and were used (maximally) twice further in these experiments. These cutting balloons were used despite these obvious issues as the experiments were designed to make initial assessments of the potential of these devices as adjuncts for foam sclerotherapy. The expense of new balloons for each experiment was not a viable alternative at this stage of research into such adjuncts. Notwithstanding

the above problems, results show that larger balloon sizes did not inflict atherotome blade cuts in the vein media. Matching the balloon to vein sizes was necessary to attempt to optimise the use of each balloon and not cause significant mechanical stretch of the vein wall. This was done as much as possible however matching was limited by the range of balloons available and occasionally by the range available at a given time. This limitation has however inadvertently allowed the question of the most appropriate arterial cutting balloon size required to cause atherotome blade damage to vein tissue to be answered. Smaller balloons inflict more atherotome blade cuts as the blade delivery mechanism of larger balloons is restricted in larger balloons, despite the increased compliance of veins over arteries. Thirdly, the moist swab held closed with finger pressure, used to mimic the extrinsic pressure of the subcutaneous component of the average upper thigh was basic. However other models considered were more expensive without any significant improvement in function.

Experiments in chapters four and five reveal that 3% STD damages the same depth of vein no-matter its surface of entry. Pre-treatment with a cutting balloon allows for a significant enhancement of STD's depth of penetration at the site of atherotome blade cut sites. Whether this translates to improved efficacy of 3% STD requires *in-vivo* studies which are conducted in preliminary animal studies in chapter six.

7.4 ANIMAL STUDIES

Results of animal fore-limb vein experiments revealed that Fogarty and cutting balloon treatment with or without 3% STD caused varicose changes in the treated vein instead of the desired obliteration/ occlusion. It appears that the addition of 3% STD to the cutting balloon treatments made no difference. It would appear that either the balloons were too large compared to the treated veins and caused a significant structural deformity by overstretching the vein wall resulting in varicosities or the atherotomes rather than cause venous scarring and fibrotic occlusion enhanced by an increased influx of sclerosant into the vein wall, rather weakened the vein wall such that it was rendered structurally deficient therefore caused varicosities. In hind-limb experiments Xanthum/3%STD had significantly lower qualitative histopathology animal vein scores and reduced the patent luminal circumference, both markers of treatment success, in comparison to veins treated with 3% STD alone within both animals. Results from these studies would suggest that Xanthum/3%STD has promise as a treatment for varicose veins with more favourable vein occlusion results at three months compared with 3% STD.

No other animal suffers from varicose veins hence the difficulties in obtaining a suitable model. Other animal models were researched and assessed and the advice of experts in the field was sought before the decision was made to use pigs as the test model. These preliminary experiments performed on a pig model presented difficulty due to the different anatomy and sizes of vessel. At the time of the experiments it was obvious that the hind-limb veins were not usable for catheter related experiments and the experiments could not be

conducted as planned. In order to utilise the animal to its fullest extent, hind limb vein experiments were adjusted to compare proprietary 3% STD foam to the best of the longer lasting foams. This resulted in no whole single leg vein acting as a control but this was felt justifiable on the basis of maximising use of the animals and the number of experiments performed. Beyond this difficulties with fore-limb vein experiments were encountered largely in the placement of balloon catheters into what were smaller veins compared to human GSV. Unfortunately the result was that the number of passes of respective balloon catheters was not uniform however was kept so as much as possible. Attempts to use the smallest available cutting balloon catheter (3mm) were thwarted by the fact that it is not usable with a guidewire and therefore would not advance with ease. A 4mm catheter was the smallest available that was passable with relative ease but was less well matched to the treated vein size. These practical issues were only raised when experimentation had begun and though adaptations were made they do lead to differences in the planned experimental methods hence affect interpretation of the results. Beyond this other issues such as the partial open nature of the procedures and resultant wound healing over the vein, the unlicensed use of xanthum with STD in the same animal, the lack of ability to place dressings on the hind legs and other variations and anomalies do effect interpretation of the results. As a preliminary model there are certainly positives to take from these learning points and the results which were achieved. The results highlight the need for any further research into mechanical adjuncts for foam sclerotherapy in the form of cutting balloons, to be conducted with balloons of a smaller maximal diameter than the treated vein. In fact it is likely for venous use that any such adjunct should not include a balloon at all, as it appears from both human and animal studies in this research and by

Ikponmwosa *et al* that balloons do not add mechanical benefit on their own and unless very small likely hinder the object of the treatment. Cutting balloons in their current form are very expensive, which would in itself, render them impossible to justify as an adjunct to foam sclerotherapy. The mechanisms involved in deploying the atherotomes in cutting balloons as well as the atherotomes themselves are the cause of their great expense and both faculties in relation to mechanically damaging the media of veins are likely unnecessary. It would appear from this research that an adjunct to foam sclerotherapy should be something more in keeping with a valvulotome with retractable blades. Experimentation with foams on hind-limb veins had the advantage of an inherent though not perfect control of the paired vein. The disadvantage with these veins is the transfer value of results to human GSV, in that the vein calibre was extremely small in comparison. Vein obliteration occurred significantly more with the use of 0.15% xanthum and 3% STD but it did occur with 3% STD alone. It is difficult to establish whether this is in fact as a result of the “strength” of the treatments or the “weaknesses” of the veins which were very thin walled. Results need to be interpreted with this in mind, though there is certainly more activity against the vein wall using the longer lasting STD foam in these preliminary animal experiments, which is certainly promising.

Occasional vein segments treated with either 0.15% xanthum and 3% STD as well as 3% STD alone showed evidence of recanalisation which is a marker for potential treatment failure. Of particular interest is that results here show that segments above and below obliterated segments are recanalised. It is known that even when complete endothelial loss is achieved re-endothelialisation may still occur via circulating endothelial progenitor cells. It would be thought much

less likely when the vein is obliterated but these results show that in this pig model where obliteration is segmental recanalization can occur even in a three month time frame. This certainly would prove ominous for any varicose vein treatment and is likely the downfall somewhere along their course for all treatments for this condition.

LIST OF ABBREVIATIONS

AOR Adjusted odds ratio

CEAP Clinical Etiological Anatomical Pathophysiological

CI Confidence intervals

CLASS Comparison of LAser, Surgery and foam Sclerotherapy

CVD Chronic venous disease

EVLT Endovenous laser therapy

GSV Great Saphenous vein

H&E Haematoxylin and eosin

HES Hospital episode statistics

ICER Incremental cost-effectiveness ratio

Min Minute

µm Micrometre

NICE National Institute for Clinical Excellence

RCT Randomised control trial

RFA Radiofrequency ablation

Sec Second

SFJ Sapheno-femoral junction

SPJ Sapheno-popliteal junction

SSV Short saphenous vein

STD Sodium tetradecyl sulphate

SVT Superficial venous thrombosis

APPENDIX I

PATIENT INFORMATION SHEET (VERSION 3) 12/01/2008

Study: “Development of an ideal model for foam sclerotherapy in the treatment of primary varicose veins”

Investigation of a new method in the treatment of varicose veins

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done. We will explain what it would involve for you. Please take time to read the information carefully.

Ask us if anything is unclear or if you would like more information. Take time to decide whether or not you wish to take part.

Purpose of the study

Varicose veins are very common and new ways of treating the condition are being looked at. We would like to look at ways of improving methods for treating varicose veins. In the future it may mean that treatment can be done as an outpatient.

It is completely up to you to decide to take part. We will describe the study and go through this information sheet, which we will then give to you. We will then ask you to sign a consent form to show you have agreed to take part.

You are free to withdraw at any time, without giving a reason.

This would not affect the standard of care you will be given.

If I decide to take part?

You have been offered surgery for varicose veins. This involves tying off the vein (faulty blood vessel) at the main link in the groin. Two small cuts (about 5mm long) are made. One in the groin at the top of the main faulty vein and one near your knee. The top end of the vein in your groin is tied. Then a flexible wire is passed through the vein. The wire, along with the vein, is carefully pulled out of the leg through the cut near your knee (see figure 1).

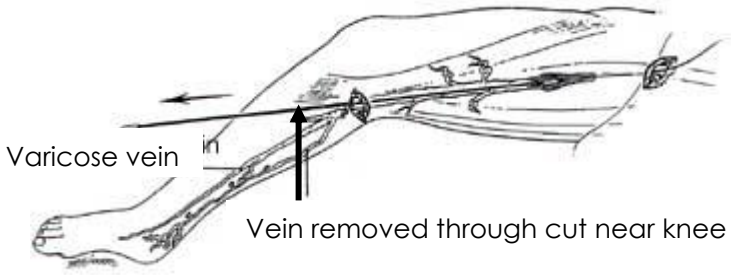


Figure 1.Inside view of right leg.

<u>To perform the research:</u>

We would like to do an experiment on the vein after collection. We will be adding a treatment called foam sclerotherapy. This is to see if it can be used to close veins. This could prevent an operation for varicose veins in the future. For the experiment to work, we need to wash the vein before we can look at the effects of the foam. We therefore need a small blood sample (5ml) to wash the vein. This can be taken when your drip line is placed before your anaesthetic or form the vein itself. You will not need any extra needles to take the blood

Are there are any risks?

No, there is no increase in the risk of the operation. **You will have the same treatment and there will not be any extra cuts on your leg. You will not need any extra needles to take the blood sample.**

You will not have any more pain or discomfort because of this extra procedure. Your operation will be longer by 2-3 minutes.

The information we get from this study will potentially help other people with varicose veins and provide a range of options for treatment of this condition.

Will my taking part in the study be kept confidential?

Yes. We will follow ethical and legal practice and all information about you will be handled in confidence. All information which is collected about you during the research will be kept strictly confidential. All tissue samples will have your name removed.

What will happen to my samples?

Once we have analysed data from the samples they will be destroyed. We will not store any samples after they have been used for this study.

What will happen if I don't want to carry on with the study?

If you withdraw from the study, we will destroy all your identifiable samples, but we will need to use the data collected up to your withdrawal.

Complaints

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions (Dr Barry McAree or Mr M J Gough 0113 2432799). If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the Leeds General Infirmary.

Further Information

For further information about the project please contact: Mr Barry McAree 01132432799. For independent advice regarding taking part please contact: Research and Development, Leeds General Infirmary 01132432799.

12/01/2008

Consent Form (Version 3)

Research study: Investigation of a new method in the treatment of varicose veins

Researcher: Barry McAree

I have read and understand the information sheet for the study. I have had the chance to think about the information and ask questions. YES/NO

I agree that left over tissue samples removed during my treatment may be used for research. The tissue samples will be discarded after research.

YES/NO

I agree that a blood sample can be taken. This will be discarded after research

YES/NO

I agree to my GP being told of my taking part in the study YES/NO

I agree to take part. This is voluntary and I am free to leave at any time without giving a reason. This will not affect my medical care or legal rights.

YES/NO

_____ _____ _____

Name of Patient Date Signature

_____ _____ _____

Name of Researcher Date Signature

APPENDIX II

ANIMAL RESEARCH PROTOCOL

Optimisation of medical/minimally invasive treatment of primary varicose veins: establishment of an animal (porcine) model to assess the therapeutic effects of current marketed foam sclerotherapy, optimised foam sclerotherapy and endovenous catheters versus control.

Version 4; 31/05/2011

SUMMARY

Title: Optimisation of medical/minimally invasive treatment of primary varicose veins: establishment of an animal model to assess the therapeutic effects of licensed foam sclerotherapy, optimised (unlicensed for human use) foam sclerotherapy and endovenous catheter therapy (unlicensed for human use), versus control on porcine superficial veins.

Objectives: to ascertain the haemodynamic, ultrasonographic and histopathological changes of superficial porcine veins treated with licensed foam sclerotherapy, optimised (unlicensed for human use) foam sclerotherapy and endovenous catheter therapy (unlicensed for human use), versus control.

Design

Study endpoints:

- Haemodynamic changes: patency, reflux and ultrasonographic changes
- Histopathological analysis - degree and extent of tunica intima and media damage

INTRODUCTION

Sclerotherapy has been used for several decades in the treatment of smaller varicose veins either alone or in combination with conventional ligation of the saphenofemoral junction (SFJ). Ultrasound-guided foam sclerotherapy involves a sclerosant solution forcibly mixed with air or another gas (CO_2) to produce foam. Compared with liquid, foam displaces blood from the vein lumen and thus has greater contact (both duration and extent) with the venous endothelium with the intention of increasing the efficacy of the procedure. This technique has further enabled the use of sclerotherapy in the treatment of great (GSV) [1,2] and small (SSV) saphenous vein incompetence. In man the procedure performed either without anaesthesia or after administration of local anaesthesia. The foam mixture is injected into the superficial vein and its distribution monitored with ultrasound. The upper end of the vein may be compressed to prevent the foam entering the deeper veins. Subsequent vein occlusion is initially the result of thrombosis following widespread disruption/destruction of the endothelium. However there is no evidence that significant injury is inflicted to the tunica media (in particular the collagen bundles) which is a dominant feature of the thermal minimally invasive techniques that are currently employed in the treatment of superficial venous incompetence.[3,4] The latter is believed to be central to promoting permanent venous occlusion and elimination of superficial venous incompetence. Further, evidence from our own laboratory has shown that with conventional foam sclerotherapy islands of endothelial cells persist and this may explain the relatively high incidence of re-canalisation (up to 30% at 3 years) with this treatment.[5]

Various sclerosants and aeration methods may be used to produce the foam for this procedure. However the most widely used technique in the UK is that described by Tessari [6,7]using air and sodium tetradecyl sulphate (STD) at a

ratio of 3:1. Early results of a randomised trial comparing foam sclerotherapy to conventional surgery have demonstrated quicker return to normal activities and similar short term occlusion rates, although as indicated above medium term recurrence rates (for superficial reflux) are disappointing.[1,2]

Although our previous studies have highlighted some of the reasons why foam sclerotherapy may have limited medium term success it is possible that if the foam was in contact with the vein for longer that deeper vessel injury may occur thus achieving denaturation and contraction of the collagen bundles in the tunica media.. To that end a method has been developed that increases the half-life of foam from around 160 seconds to greater than 15 minutes. As an alternative to chemical destruction of the endothelium and tunica media, mechanical (balloon) denudation of the endothelium and injury to the tunica media with an additional specialised catheter might prove effective on the basis that a combination of these two events appears important in achieving permanent vein ablation. Endovenous catheter balloon therapy might provide a possible alternative minimally invasive therapy for the treatment of varicose veins either alone or in combination with foam sclerotherapy. This hypothesis is currently being assessed in a series of ex-vivo experiments using GSV harvested from patients with GSV incompetence undergoing conventional surgery for their varicose veins.

STUDY AIM

The aim of this study is to ascertain the haemodynamic, ultrasonographic and histopathological changes in porcine superficial veins treated with licensed foam sclerotherapy, optimised (unlicensed for human use) foam sclerotherapy and endovenous catheter therapy (unlicensed for human use), versus controls (no intervention).

STUDY POPULATION/SAMPLE SIZE/RANDOMISATION

In the first instance two animals (4 veins: one per limb) will be studied over 12 weeks to confirm that the model is feasible. On the assumption this proves to be the case it is likely that further animals will be studied.

STUDY PROCEDURE

Porcine superficial veins are similar to superficial human veins in that they are of comparable size both before and after proximal occlusion. It should be possible to treat a 10cm length of superficial vein in each of the four limbs without a significant risk of sclerosant entering the deep venous in great volume. Porcine superficial veins are reasonably easily accessible for treatment and ultrasound assessment and they converge with the deep veins in a similar fashion to human superficial lower limb veins. In addition pigs have suitable limbs for comparable bandaging post-procedure as would be performed following foam sclerotherapy in humans.

This study will be undertaken in a licensed Home Office facility, with all aspects of the following procedures undertaken by licensed staff.

Animals of a suitable size and weight (50-60kg) will be selected on the basis that they have 3mm diameter foreleg superficial veins and c. 4mm diameter hind leg superficial veins. The animals will be given anaesthetic pre-medication and will then be fully anaesthetised in a theatre environment. The animal will be given relevant prophylactic antibiotics and long-acting analgesic medication. The veins will be scanned by ultrasound to ensure they are patent.

The following procedure will then be carried out by a licensed animal practitioner under the guidance of research staff:

1. Fogarty balloon x5 passes (control)
2. Fogarty balloon x5 passes; STD foam
3. Fogarty balloon x5 passes; cutting balloon x3 passes
4. Fogarty balloon x5 passes; cutting balloon x3 passes; STD foam

The length of vein treated will be measured by ultrasound (aiming to treat 10cm segments in all limbs) and the proximal and distal points of treatment marked on

the skin by tattooing with Indian Ink to facilitate the subsequent harvesting of the relevant segment of vein. Post procedure the limbs will be dressed with compression bandaging and swabs overlying the superficial veins to apply increased pressure in these areas hence mimicking the post-procedure dressing of humans treated with foam sclerotherapy for great saphenous varicose veins;

The veins will be scanned by ultrasound at pre-termination to assess their patency and ultrasonographic appearance. The ultrasound assessment of the pre-and post-treated veins will be recorded as - compressibility of the superficial veins will be assessed and classified as compressible (implies vein is patent) or non-compressible (implies occlusion), iso-echoic (no longer visible) or hypo-echoic (consistent with patency).

At the end of the 12 week period the animal would again be anaesthetised as previously and each 10cm length of superficial vein removed surgically, as atraumatically as possible. The segments will then be labelled and processed in formalin to allow histopathological preparation and analysis in an appropriate laboratory facility. The animal will then be slaughtered in accordance with Home Office regulations.

DATA RECORDING

Data will be recorded by study doctors and will be the responsibility of the chief investigator.

STATISTICAL CONSIDERATIONS

The sample size was chosen to prove concept and usefulness of an animal model.

SOURCE DATA/ DOCUMENTS/ CONFIDENTIALITY

Data will be recorded on encrypted files on NHS computers in concordance with the Data Protection Act 1998. Data will be accessible to study doctors and research nurses.

QUALITY CONTROL

Quality control/assurance will be maintained by the Home Office.

PUBLICATION POLICY

Upon completion of the study results and conclusions will be published in relevant peer reviewed vascular surgical journals.

REFERENCES

1. Bountouroglou DG, Azzam M, Kakkos SK, Pathmarajah M, Young P, Geroulakos G. Ultrasound-guided Foam Sclerotherapy Combined with Saphenofemoral Ligation Compared to Surgical Treatment of Varicose Veins: Early Results of a Randomised Controlled Trial. *Eur J Vasc Endovasc Surg* 2006;**31**: 93–100

2. Belcaro G, Cesarone MR, Di Renzo A, Brandolini R, Coen L, Acerbi G, Marelli C, Errichi BM, Malouf M, Myers K, Christopoulos D, Nicholaides A, Geroulakos G, Vasdekis S, Simeone E, Ricci A, Ruffini I, Stuard S, Ippolito E, Bavera P,Georgiev M, Corsi M, Scoccianti M,Cornelli U, Caizzi N, Dugall M, Veller M, Venniker R, Cazaubon M, Griffin M. Foam sclerotherapy, Surgery, Sclerotherapy and a combined treatment for varicose veins: a 10 year prospective, randomised trial (VEDICO Trial). *Angiology* 2003;**54**:307-315

3. Markovic JN, Shortell CK. Update on radiofrequency ablation. *Perspect Vasc Surg Endovasc Ther.* 2009;**21**(2):82-90

4. Min RJ. Khilnani NM. Endovenous laser treatment of saphenous vein reflux. Techniques in Vascular & Interventional Radiology. 2003;**6**(3):125-31

5. Ikponmwosa A, Abbott C, Graham A, Homer-Vanniasinkam S, Gough MJ. The impact of different concentrations of sodium tetradecyl sulphate and initial balloon denudation on endothelial cell loss and tunica media injury in a model of foam sclerotherapy. *Eur J Vasc Endovasc Surg.* 2010 Mar;**39**(3):366-71

6. Tessari L, Cavezzi A, Frullini A. Preliminary experience with a new sclerosing foam in the treatment of varicose veins. Dermatol Surg. 2001 Jan;27(1):58-60.

7. Cavezzi A, Tessari L. Foam sclerotherapy techniques: different gases and methods of preparation, catheter versus direct injection. Phlebology. 2009 Dec;24(6):247-51.

REFERENCES

1. Lim CS, Davies AH. Pathogenesis of primary varicose veins. *Br J Surg* 2009;96(11):1231-42.
2. Tisi PV. Varicose veins. *Clin Evid (Online)* 2011;2011.
3. Bradbury A, Evans C, Allan P, Lee A, Ruckley CV, Fowkes FG. What are the symptoms of varicose veins? Edinburgh vein study cross sectional population survey. *BMJ* 1999;318(7180):353-6.
4. Nicolaides AN. Investigation of chronic venous insufficiency: A consensus statement (France, March 5-9, 1997). *Circulation* 2000;102(20):E126-63.
5. Laing W. Chronic Venous Diseases of the Leg. *Office of Health Economics: London* 1992.
6. Xiao Y, Huang Z, Yin H, Lin Y, Wang S. In vitro differences between smooth muscle cells derived from varicose veins and normal veins. *J Vasc Surg* 2009;50(5):1149-54.
7. Sakurai T, Gupta PC, Matsushita M, Nishikimi N, Nimura Y. Correlation of the anatomical distribution of venous reflux with clinical symptoms and venous haemodynamics in primary varicose veins. *Br J Surg* 1998;85(2):213-6.
8. Labropoulos N, Delis K, Nicolaides AN, Leon M, Ramaswami G. The role of the distribution and anatomic extent of reflux in the development of signs and symptoms in chronic venous insufficiency. *J Vasc Surg* 1996;23(3):504-10.
9. Pittaluga P, Chastane S, Rea B, Barbe R. Classification of saphenous refluxes: implications for treatment. *Phlebology* 2008;23(1):2-9.
10. Labropoulos N, Kokkosis AA, Spentzouris G, Gasparis AP, Tassiopoulos AK. The distribution and significance of varicosities in the saphenous trunks. *J Vasc Surg* 2010;51(1):96-103.
11. DoH. Hospital Episodes Statistics: http://www.dh.gov.uk/en/Publicationsandstatistics/Statistics/HospitalEpisodeStatistics/index.htm. Last accessed 06/08/2011

2009-2010.

12. Frullini A, Cavezzi A. Sclerosing foam in the treatment of varicose veins and telangiectases: history and analysis of safety and complications. *Dermatol Surg* 2002;28(1):11-5.
13. Gohel MS, Epstein DM, Davies AH. Cost-effectiveness of traditional and endovenous treatments for varicose veins. *Br J Surg* 2010;97(12):1815-23.
14. Wright D, Gobin, J.P, Bradbury, A.W, Coleridge-Smith, P, Spoelstra, H, Berridge, D, Wittens, C.H.A, Sommer, A, Nelzen, O, Chanter, D, and The Varisolve® European Phase III Investigators Group Varisolve® polidocanol microfoam compared with surgery or sclerotherapy in the management of varicose veins in the presence of trunk vein incompetence: European randomized controlled trial *Phlebology* 2006;21(4):180-90.
15. Orsini C, Brotto M. Immediate pathologic effects on the vein wall of foam sclerotherapy. *Dermatol Surg* 2007;33(10):1250-4.
16. Ikponmwosa A, Abbott C, Graham A, Homer-Vanniasinkam S, Gough MJ. The impact of different concentrations of sodium tetradecyl sulphate and

initial balloon denudation on endothelial cell loss and tunica media injury in a model of foam sclerotherapy. *Eur J Vasc Endovasc Surg* 2010;39(3):366-71.

17. Robertson L, Evans C, Fowkes FG. Epidemiology of chronic venous disease. *Phlebology* 2008;23(3):103-11.
18. Carpentier PH, Maricq HR, Biro C, Poncot-Makinen CO, Franco A. Prevalence, risk factors, and clinical patterns of chronic venous disorders of lower limbs: a population-based study in France. *J Vasc Surg* 2004;40(4):650-9.
19. Beebe-Dimmer JL, Pfeifer JR, Engle JS, Schottenfeld D. The epidemiology of chronic venous insufficiency and varicose veins. *Ann Epidemiol* 2005;15(3):175-84.
20. Mekky S, Schilling RS, Walford J. Varicose veins in women cotton workers. An epidemiological study in England and Egypt. *Br Med J* 1969;2(5657):591-5.
21. Abramson JH, Hopp C, Epstein LM. The epidemiology of varicose veins. A survey in western Jerusalem. *J Epidemiol Community Health* 1981;35(3):213-7.
22. Sisto T, Reunanen A, Laurikka J, Impivaara O, Heliovaara M, Knekt P, et al. Prevalence and risk factors of varicose veins in lower extremities: mini-Finland health survey. *Eur J Surg* 1995;161(6):405-14.
23. Criqui MH, Jamosmos M, Fronek A, Denenberg JO, Langer RD, Bergan J, et al. Chronic venous disease in an ethnically diverse population: the San Diego Population Study. *Am J Epidemiol* 2003;158(5):448-56.
24. Brand FN, Dannenberg AL, Abbott RD, Kannel WB. The epidemiology of varicose veins: the Framingham Study. *Am J Prev Med* 1988;4(2):96-101.
25. Evans CJ, Fowkes FG, Ruckley CV, Lee AJ. Prevalence of varicose veins and chronic venous insufficiency in men and women in the general population: Edinburgh Vein Study. *J Epidemiol Community Health* 1999;53(3):149-53.
26. Hirai M, Naiki K, Nakayama R. Prevalence and risk factors of varicose veins in Japanese women. *Angiology* 1990;41(3):228-32.
27. Scott TE, LaMorte WW, Gorin DR, Menzoian JO. Risk factors for chronic venous insufficiency: a dual case-control study. *J Vasc Surg* 1995;22(5):622-8.
28. Franks PJ, Wright DD, Moffatt CJ, Stirling J, Fletcher AE, Bulpitt CJ, et al. Prevalence of venous disease: a community study in west London. *Eur J Surg* 1992;158(3):143-7.
29. Laurikka JO, Sisto T, Tarkka MR, Auvinen O, Hakama M. Risk indicators for varicose veins in forty- to sixty-year-olds in the Tampere varicose vein study. *World J Surg* 2002;26(6):648-51.
30. Komsuoglu B, Goldeli O, Kulan K, Cetinarslan B, Komsuoglu SS. Prevalence and risk factors of varicose veins in an elderly population. *Gerontology* 1994;40(1):25-31.
31. Callam MJ. Epidemiology of varicose veins. *Br J Surg* 1994;81(2):167-73.
32. Kaplan RM, Criqui MH, Denenberg JO, Bergan J, Fronek A. Quality of life in patients with chronic venous disease: San Diego population study. *J Vasc Surg* 2003;37(5):1047-53.
33. Campbell B. Varicose veins and their management. *BMJ* 2006;333(7562):287-92.

34. Bradbury A, W. Varicose Veins. In: Beard J, Gaines P, editors. *A companion to specialist surgical practice; vascular and endovascular surgery*. 4th ed: Saunders, Elsevier, 2009:303-22.
35. Bernstein IM, Ziegler W, Badger GJ. Plasma volume expansion in early pregnancy. *Obstet Gynecol* 2001;97(5 Pt 1):669-72.
36. Kristiansson P, Wang JX. Reproductive hormones and blood pressure during pregnancy. *Hum Reprod* 2001;16(1):13-17.
37. Ciardullo AV, Panico S, Bellati C, Rubba P, Rinaldi S, Iannuzzi A, et al. High endogenous estradiol is associated with increased venous distensibility and clinical evidence of varicose veins in menopausal women. *J Vasc Surg* 2000;32(3):544-9.
38. Naoum JJ, Hunter GC, Woodside KJ, Chen C. Current advances in the pathogenesis of varicose veins. *J Surg Res* 2007;141(2):311-6.
39. Vin F, Allaert FA, Levardon M. Influence of estrogens and progesterone on the venous system of the lower limbs in women. *J Dermatol Surg Oncol* 1992;18(10):888-92.
40. Canonico S, Gallo C, Paolisso G, Pacifico F, Signoriello G, Sciaudone G, et al. Prevalence of varicose veins in an Italian elderly population. *Angiology* 1998;49(2):129-35.
41. Seidell JC, de Groot LC, van Sonsbeek JL, Deurenberg P, Hautvast JG. Associations of moderate and severe overweight with self-reported illness and medical care in Dutch adults. *Am J Public Health* 1986;76(3):264-9.
42. Seidell JC, Bakx KC, Deurenberg P, van den Hoogen HJ, Hautvast JG, Stijnen T. Overweight and chronic illness--a retrospective cohort study, with a follow-up of 6-17 years, in men and women of initially 20-50 years of age. *J Chronic Dis* 1986;39(8):585-93.
43. van Noord PA, Seidell JC, den Tonkelaar I, Baanders-van Halewijn EA, Ouwehand IJ. The relationship between fat distribution and some chronic diseases in 11,825 women participating in the DOM-project. *Int J Epidemiol* 1990;19(3):564-70.
44. Iannuzzi A, Panico S, Ciardullo AV, Bellati C, Cioffi V, Iannuzzo G, et al. Varicose veins of the lower limbs and venous capacitance in postmenopausal women: relationship with obesity. *J Vasc Surg* 2002;36(5):965-8.
45. Danielsson G, Eklof B, Grandinetti A, Kistner RL. The influence of obesity on chronic venous disease. *Vasc Endovascular Surg* 2002;36(4):271-6.
46. Stanhope JM. Varicose veins in a population of lowland New Guinea. *Int J Epidemiol* 1975;4(3):221-5.
47. Malhotra SL. An epidemiological study of varicose veins in Indian railroad workers from the South and North of India, with special reference to the causation and prevention of varicose veins. *Int J Epidemiol* 1972;1(2):177-83.
48. Ducimetiere P, Richard JL, Pequignot G, Warnet JM. Varicose veins: a risk factor for atherosclerotic disease in middle-aged men? *Int J Epidemiol* 1981;10(4):329-35.
49. Dindelli M, Parazzini F, Basellini A, Rabaiotti E, Corsi G, Ferrari A. Risk factors for varicose disease before and during pregnancy. *Angiology* 1993;44(5):361-7.
50. Fowkes FG, Lee AJ, Evans CJ, Allan PL, Bradbury AW, Ruckley CV. Lifestyle risk factors for lower limb venous reflux in the general population: Edinburgh Vein Study. *Int J Epidemiol* 2001;30(4):846-52.

51. Tuchsen F, Krause N, Hannerz H, Burr H, Kristensen TS. Standing at work and varicose veins. *Scand J Work Environ Health* 2000;26(5):414-20.
52. Krijnen RM, de Boer EM, Ader HJ, Bruynzeel DP. Venous insufficiency in male workers with a standing profession. Part 1: epidemiology. *Dermatology* 1997;194(2):111-20.
53. Stvrtinova V, Kolesar J, Wimmer G. Prevalence of varicose veins of the lower limbs in the women working at a department store. *Int Angiol* 1991;10(1):2-5.
54. Maffei FH, Magaldi C, Pinho SZ, Lastoria S, Pinho W, Yoshida WB, et al. Varicose veins and chronic venous insufficiency in Brazil: prevalence among 1755 inhabitants of a country town. *Int J Epidemiol* 1986;15(2):210-7.
55. Lee AJ, Evans CJ, Allan PL, Ruckley CV, Fowkes FG. Lifestyle factors and the risk of varicose veins: Edinburgh Vein Study. *J Clin Epidemiol* 2003;56(2):171-9.
56. Gourgou S, Dedieu F, Sancho-Garnier H. Lower limb venous insufficiency and tobacco smoking: a case-control study. *Am J Epidemiol* 2002;155(11):1007-15.
57. Lee AJ, Evans CJ, Hau CM, Fowkes FG. Fiber intake, constipation, and risk of varicose veins in the general population: Edinburgh Vein Study. *J Clin Epidemiol* 2001;54(4):423-9.
58. Raffetto JD, Khalil RA. Mechanisms of varicose vein formation: valve dysfunction and wall dilation. *Phlebology* 2008;23(2):85-98.
59. Lim CS, Gohel MS, Shepherd AC, Paleolog E, Davies AH. Venous hypoxia: a poorly studied etiological factor of varicose veins. *J Vasc Res* 2011;48(3):185-94.
60. Somers P, Knaapen M. The histopathology of varicose vein disease. *Angiology* 2006;57(5):546-55.
61. Corcos L, De Anna D, Dini M, Macchi C, Ferrari PA, Dini S. Proximal long saphenous vein valves in primary venous insufficiency. *J Mal Vasc* 2000;25(1):27-36.
62. Van Cleef JF, Hugentobler JP, Desvaux P, Griton P, Cloarec M. [Endoscopic study of reflux of the saphenous valve]. *J Mal Vasc* 1992;17 Suppl B:113-6.
63. Blanchemaison P. [Significance of venous endoscopy in the exploration and the treatment of venous insufficiency of the legs]. *J Mal Vasc* 1992;17 Suppl B:109-12.
64. Ono T, Bergan JJ, Schmid-Schonbein GW, Takase S. Monocyte infiltration into venous valves. *J Vasc Surg* 1998;27(1):158-66.
65. Corcos L, Procacci T, Peruzzi G, Dini M, De Anna D. Sapheno-femoral valves. Histopathological observations and diagnostic approach before surgery. *Dermatol Surg* 1996;22(10):873-80.
66. Psaila JV, Melhuish J. Viscoelastic properties and collagen content of the long saphenous vein in normal and varicose veins. *Br J Surg* 1989;76(1):37-40.
67. Labropoulos N, Giannoukas AD, Delis K, Mansour MA, Kang SS, Nicolaides AN, et al. Where does venous reflux start? *J Vasc Surg* 1997;26(5):736-42.
68. Meissner MH, Gloviczki P, Bergan J, Kistner RL, Morrison N, Pannier F, et al. Primary chronic venous disorders. *J Vasc Surg* 2007;46 Suppl S:54S-67S.

69. Vanhoutte PM, Corcaud S, de Montrion C. Venous disease: from pathophysiology to quality of life. *Angiology* 1997;48(7):559-67.
70. Michiels C, Bouaziz N, Remacle J. Role of the endothelium and blood stasis in the appearance of varicose veins. *Int Angiol* 2002;21(1):1-8.
71. Lim CS, Shalhoub J, Gohel MS, Shepherd AC, Davies AH. Matrix metalloproteinases in vascular disease--a potential therapeutic target? *Curr Vasc Pharmacol* 2010;8(1):75-85.
72. Travers JP, Brookes CE, Evans J, Baker DM, Kent C, Makin GS, et al. Assessment of wall structure and composition of varicose veins with reference to collagen, elastin and smooth muscle content. *Eur J Vasc Endovasc Surg* 1996;11(2):230-7.
73. Taccoen A, Lebard C, Borie H, Poullain JC, Zuccarelli F, Gerentes I, et al. [Measurement of oxygen tension in normal and varicose vein walls]. *J Mal Vasc* 1996;21 Suppl C:259-65.
74. Malone PC, Agutter PS. To what extent might deep venous thrombosis and chronic venous insufficiency share a common etiology? *Int Angiol* 2009;28(4):254-68.
75. Michiels C, Arnould T, Remacle J. Endothelial cell responses to hypoxia: initiation of a cascade of cellular interactions. *Biochim Biophys Acta* 2000;1497(1):1-10.
76. Knaapen MW, Somers P, Bortier H, De Meyer GR, Kockx MM. Smooth muscle cell hypertrophy in varicose veins is associated with expression of estrogen receptor-beta. *J Vasc Res* 2005;42(1):8-12.
77. Moosman DA, Hartwell SW, Jr. The Surgical Significance of the Subfascial Course of the Lesser Saphenous Vein. *Surg Gynecol Obstet* 1964;118:761-6.
78. Vasdekis SN, Clarke GH, Hobbs JT, Nicolaides AN. Evaluation of non-invasive and invasive methods in the assessment of short saphenous vein termination. *Br J Surg* 1989;76(9):929-32.
79. Labropoulos N, Leon M, Nicolaides AN, Giannoukas AD, Volteas N, Chan P. Superficial venous insufficiency: correlation of anatomic extent of reflux with clinical symptoms and signs. *J Vasc Surg* 1994;20(6):953-8.
80. Delis KT, Knaggs AL, Khodabakhsh P. Prevalence, anatomic patterns, valvular competence, and clinical significance of the Giacomini vein. *J Vasc Surg* 2004;40(6):1174-83.
81. Burnand KG, Senapati A, Thomas ML, Browse NL. A comparison of preoperative long saphenous phlebography with operative dissection in assessing the suitability of long saphenous vein for use as a bypass graft. *Ann R Coll Surg Engl* 1985;67(3):183-6.
82. Shah DM, Chang BB, Leopold PW, Corson JD, Leather RP, Karmody AM. The anatomy of the greater saphenous venous system. *J Vasc Surg* 1986;3(2):273-83.
83. Kupinski AM, Evans SM, Khan AM, Zorn TJ, Darling RC, 3rd, Chang BB, et al. Ultrasonic characterization of the saphenous vein. *Cardiovasc Surg* 1993;1(5):513-7.
84. Ricci S, Ciaggiati, A. . Does a double long saphenous vein exist? *Phlebology* 1999;14:59-64.
85. Donnelly M, Tierney S, Feeley TM. Anatomical variation at the saphenofemoral junction. *Br J Surg* 2005;92(3):322-5.
86. Cavezzi A, Labropoulos N, Partsch H, Ricci S, Caggiati A, Myers K, et al. Duplex ultrasound investigation of the veins in chronic venous disease of

the lower limbs--UIP consensus document. Part II. Anatomy. *Eur J Vasc Endovasc Surg* 2006;31(3):288-99.
87. Zamboni P, Cappelli, M, marcellino, M.G, Murgia, A.P, Pisano, L, Fabi, P,. Does a saphenous varicose vein exist? *Phlebology* 1997;12:74-7.
88. Caggiati A, Rosi C, Heyn R, Franceschini M, Acconcia MC. Age-related variations of varicose veins anatomy. *J Vasc Surg* 2006;44(6):1291-5.
89. Labropoulos N, Kang SS, Mansour MA, Giannoukas AD, Buckman J, Baker WH. Primary superficial vein reflux with competent saphenous trunk. *Eur J Vasc Endovasc Surg* 1999;18(3):201-6.
90. Cohn JD, Korver KF. Selection of saphenous vein conduit in varicose vein disease. *Ann Thorac Surg* 2006;81(4):1269-74.
91. Negus D C-SR. Leg ulcers diagnosis and management. Third ed: Hodder Arnold 2005.
92. Junqueria LC CJ, Keeley RO. , editor. *Basic Histology*. 8th ed. London: Prentice-Hall international, 1995.
93. Bradbury AW, Evans CJ, Allan PL, Lee AJ, Ruckley CV, Fowkes FG. Vascular surgical society of great britain and ireland: symptoms of varicose veins. *Br J Surg* 1999;86(5):700.
94. Labropoulos N, Delis KT, Nicolaides AN. Venous reflux in symptom-free vascular surgeons. *J Vasc Surg* 1995;22(2):150-4.
95. Evans CJ, Allan PL, Lee AJ, Bradbury AW, Ruckley CV, Fowkes FG. Prevalence of venous reflux in the general population on duplex scanning: the Edinburgh vein study. *J Vasc Surg* 1998;28(5):767-76.
96. Hamahata A, Yamaki T, Osada A, Fujisawa D, Sakurai H. Foam sclerotherapy for spouting haemorrhage in patients with varicose veins. *Eur J Vasc Endovasc Surg* 2011;41(6):856-8.
97. Byard RW, Gilbert JD. The incidence and characteristic features of fatal hemorrhage due to ruptured varicose veins: a 10-year autopsy study. *Am J Forensic Med Pathol* 2007;28(4):299-302.
98. Marchiori A, Mosena L, Prandoni P. Superficial vein thrombosis: risk factors, diagnosis, and treatment. *Semin Thromb Hemost* 2006;32(7):737-43.
99. Belcaro G, Nicolaides AN, Errichi BM, Cesarone MR, De Sanctis MT, Incandela L, et al. Superficial thrombophlebitis of the legs: a randomized, controlled, follow-up study. *Angiology* 1999;50(7):523-9.
100. Gloviczki P, Bergan JJ, Menawat SS, Hobson RW, 2nd, Kistner RL, Lawrence PF, et al. Safety, feasibility, and early efficacy of subfascial endoscopic perforator surgery: a preliminary report from the North American registry. *J Vasc Surg* 1997;25(1):94-105.
101. van Langevelde K, Lijfering WM, Rosendaal FR, Cannegieter SC. Increased risk of venous thrombosis in persons with clinically diagnosed superficial vein thrombosis: results from the MEGA study. *Blood* 2011.
102. Decousus H, Quere I, Presles E, Becker F, Barrellier MT, Chanut M, et al. Superficial venous thrombosis and venous thromboembolism: a large, prospective epidemiologic study. *Ann Intern Med* 2010;152(4):218-24.
103. Decousus H, Prandoni P, Mismetti P, Bauersachs RM, Boda Z, Brenner B, et al. Fondaparinux for the treatment of superficial-vein thrombosis in the legs. *N Engl J Med* 2010;363(13):1222-32.
104. Gloviczki P, Comerota AJ, Dalsing MC, Eklof BG, Gillespie DL, Gloviczki ML, et al. 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. *J Vasc Surg* 2011;53(5 Suppl):2S-48S.

105. Eklof B, Perrin M, Delis KT, Rutherford RB, Gloviczki P. Updated terminology of chronic venous disorders: the VEIN-TERM transatlantic interdisciplinary consensus document. *J Vasc Surg* 2009;49(2):498-501.
106. Caggiati A, Bergan JJ, Gloviczki P, Eklof B, Allegra C, Partsch H. Nomenclature of the veins of the lower limb: extensions, refinements, and clinical application. *J Vasc Surg* 2005;41(4):719-24.
107. Raju S, Neglen P. Clinical practice. Chronic venous insufficiency and varicose veins. *N Engl J Med* 2009;360(22):2319-27.
108. Eberhardt RT, Raffetto JD. Chronic venous insufficiency. *Circulation* 2005;111(18):2398-409.
109. O'Meara S, Cullum NA, Nelson EA. Compression for venous leg ulcers. *Cochrane Database Syst Rev* 2009(1):CD000265.
110. Beale RJ, Gough MJ. Treatment options for primary varicose veins--a review. *Eur J Vasc Endovasc Surg* 2005;30(1):83-95.
111. Johnson S. Compression hosiery in the prevention and

treatment of venous leg ulcers., 2002.
112. Walker L, Lamont S. Use and application of graduated elastic compression stockings. *Nurs Stand* 2007;21(42):41-5.
113. Shingler S, Robertson L, Boghossian S, Stewart M. Compression stockings for the initial treatment of varicose veins in patients without venous ulceration. *Cochrane Database Syst Rev* 2011;11:CD008819.
114. Palfreyman SJ, Michaels JA. A systematic review of compression hosiery for uncomplicated varicose veins. *Phlebology* 2009;24 Suppl 1:13-33.
115. Labropoulos N, Leon M, Volteas N, Nicolaides AN. Acute and long-term effect of elastic stockings in patients with varicose veins. *Int Angiol* 1994;13(2):119-23.
116. Kiev J, Noyes LD, Rice JC, Kerstein MD. Patient compliance with fitted compression hosiery monitored by photoplethysmography. *Arch Phys Med Rehabil* 1990;71(6):376-9.
117. Samson RH, Showalter DP. Stockings and the prevention of recurrent venous ulcers. *Dermatol Surg* 1996;22(4):373-6.
118. Raju S, Hollis K, Neglen P. Use of compression stockings in chronic venous disease: patient compliance and efficacy. *Ann Vasc Surg* 2007;21(6):790-5.
119. Garreau C, Pibourdin JM, Nguyen Le C, Boisseau MR. [Elastic compression in golf competition]. *J Mal Vasc* 2008;33(4-5):250-1.
120. Couzan S, Assante C, Laporte S, Mismetti P, Pouget JF. [Booster study: comparative evaluation of a new concept of elastic stockings in mild venous insufficiency]. *Presse Med* 2009;38(3):355-61.
121. Michaels JA, Campbell WB, Brazier JE, Macintyre JB, Palfreyman SJ, Ratcliffe J, et al. Randomised clinical trial, observational study and assessment of cost-effectiveness of the treatment of varicose veins (REACTIV trial). *Health Technol Assess* 2006;10(13):1-196, iii-iv.
122. http://www.cks.nhs.uk/compression_stockings/drugs_in_this_topic/scenario_compression_stockings/compression_hosiery_class_ii_thigh_length_stocking_circular_knit_made_to_measure#. last accessed 10/06/2012.
123. Shepherd AC, Gohel MS, Lim CS, Hamish M, Davies AH. The treatment of varicose veins: an investigation of patient preferences and expectations. *Phlebology* 2010;25(2):54-65.

124. van den Bos R, Arends L, Kockaert M, Neumann M, Nijsten T. Endovenous therapies of lower extremity varicosities: a meta-analysis. *J Vasc Surg* 2009;49(1):230-9.
125. Shepherd AC, Gohel MS, Brown LC, Metcalfe MJ, Hamish M, Davies AH. Randomized clinical trial of VNUS ClosureFAST radiofrequency ablation versus laser for varicose veins. *Br J Surg* 2010;97(6):810-8.
126. O'Hare JL, Earnshaw JJ. Varicose veins today. *Br J Surg* 2009;96(11):1229-30.
127. Darvall KA, Bate GR, Silverman SH, Adam DJ, Bradbury AW. Medium-term results of ultrasound-guided foam sclerotherapy for small saphenous varicose veins. *Br J Surg* 2009;96(11):1268-73.
128. Darvall KA, Bate GR, Adam DJ, Silverman SH, Bradbury AW. Duplex ultrasound outcomes following ultrasound-guided foam sclerotherapy of symptomatic primary great saphenous varicose veins. *Eur J Vasc Endovasc Surg* 2010;40(4):534-9.
129. van den Bos RR, Milleret R, Neumann M, Nijsten T. Proof-of-principle study of steam ablation as novel thermal therapy for saphenous varicose veins. *J Vasc Surg* 2011;53(1):181-6.
130. Almeida JI, Min RJ, Raabe R, McLean DJ, Madsen M. Cyanoacrylate adhesive for the closure of truncal veins: 60-day swine model results. *Vasc Endovascular Surg* 2011;45(7):631-5.
131. Elias S, Raines JK. Mechanochemical tumescentless endovenous ablation: final results of the initial clinical trial. *Phlebology* 2011.
132. Tisi PV, Beverley C, Rees A. Injection sclerotherapy for varicose veins. *Cochrane Database Syst Rev* 2006(4):CD001732.
133. Fegan WG. Continuous compression technique of injecting varicose veins. *Lancet* 1963;2(7299):109-12.
134. McAusland S. The modern treatment of varicose veins. *Med Press Circular* 1939;201:404-10.
135. Orbach E, J. . Sclerotherapy of varicose veins: utilization of intravenous air block. *Am J Surg* 1944:362-6.
136. Orbach EJ. Contributions to the therapy of the varicose complex. *J Int Coll Surg* 1950;13(6):765-71.
137. Cabrera Garido J, Cabrera Garcia Olmedo, JR, Garcia Olmedo, D, . Nuevo metodo de esclerosis en las varices tronculares *Patologia Vasculares* 1995;1:55-72.
138. Breu FX, Guggenbichler S, Wollmann JC. 2nd European Consensus Meeting on Foam Sclerotherapy 2006, Tegernsee, Germany. *Vasa* 2008;37 Suppl 71:1-29.
139. Gibson KD, Ferris BL, Pepper D. Foam sclerotherapy for the treatment of superficial venous insufficiency. *Surg Clin North Am* 2007;87(5):1285-95, xii-xiii.
140. Ouvry P, Allaert FA, Desnos P, Hamel-Desnos C. Efficacy of polidocanol foam versus liquid in sclerotherapy of the great saphenous vein: a multicentre randomised controlled trial with a 2-year follow-up. *Eur J Vasc Endovasc Surg* 2008;36(3):366-70.
141. Coleridge Smith P. Sclerotherapy and foam sclerotherapy for varicose veins. *Phlebology* 2009;24(6):260-9.
142. Jia X, Mowatt G, Burr JM, Cassar K, Cook J, Fraser C. Systematic review of foam sclerotherapy for varicose veins. *Br J Surg* 2007;94(8):925-36.
143. Bountouroglou DG, Azzam M, Kakkos SK, Pathmarajah M, Young P, Geroulakos G. Ultrasound-guided foam sclerotherapy combined with

sapheno-femoral ligation compared to surgical treatment of varicose veins: early results of a randomised controlled trial. *Eur J Vasc Endovasc Surg* 2006;31(1):93-100.

144. Barrett JM, Allen B, Ockelford A, Goldman MP. Microfoam ultrasound-guided sclerotherapy of varicose veins in 100 legs. *Dermatol Surg* 2004;30(1):6-12.
145. Forlee MV, Grouden M, Moore DJ, Shanik G. Stroke after varicose vein foam injection sclerotherapy. *J Vasc Surg* 2006;43(1):162-4.
146. Gillet JL, Guedes JM, Guex JJ, Hamel-Desnos C, Schadeck M, Lauseker M, et al. Side-effects and complications of foam sclerotherapy of the great and small saphenous veins: a controlled multicentre prospective study including 1,025 patients. *Phlebology* 2009;24(3):131-8.
147. Sarvananthan T, Shepherd AC, Willenberg T, Davies AH. Neurological complications of sclerotherapy for varicose veins. *J Vasc Surg* 2012;55(1):243-51.
148. NICE. http://guidance.nice.org.uk/IPG314/Guidance/pdf/English last accessed 10/06/2012, 2009.
149. Duffy DM. Sclerosants: a comparative review. *Dermatol Surg* 2010;36 Suppl 2:1010-25.
150. Stucker M, Kobus S, Altmeyer P, Reich-Schupke S. Review of published information on foam sclerotherapy. *Dermatol Surg* 2010;36 Suppl 2:983-92.
151. Redondo P, Cabrera J. Microfoam sclerotherapy. *Semin Cutan Med Surg* 2005;24(4):175-83.
152. Kobayashi S, Crooks S, Eckmann DM. Dose- and time-dependent liquid sclerosant effects on endothelial cell death. *Dermatol Surg* 2006;32(12):1444-52.
153. Goldman MP. Treatment of varicose and telangiectatic leg veins: double-blind prospective comparative trial between aethoxyskerol and sotradecol. *Dermatol Surg* 2002;28(1):52-5.
154. Rao J, Wildemore JK, Goldman MP. Double-blind prospective comparative trial between foamed and liquid polidocanol and sodium tetradecyl sulfate in the treatment of varicose and telangiectatic leg veins. *Dermatol Surg* 2005;31(6):631-5; discussion 35.
155. DoH. NHS reference costs. *http://www.dh.gov.uk/en/Publicationsandstatistics/Publications/PublicationsPolicyAndGuidance/DH_098945* 2007-08.
156. Smith PC. Chronic venous disease treated by ultrasound guided foam sclerotherapy. *Eur J Vasc Endovasc Surg* 2006;32(5):577-83.
157. Darvall KA, Bate GR, Adam DJ, Bradbury AW. Recovery after ultrasound-guided foam sclerotherapy compared with conventional surgery for varicose veins. *Br J Surg* 2009;96(11):1262-7.
158. Nesbitt C, Eifell RK, Coyne P, Badri H, Bhattacharya V, Stansby G. Endovenous ablation (radiofrequency and laser) and foam sclerotherapy versus conventional surgery for great saphenous vein varices. *Cochrane Database Syst Rev* 2011(10):CD005624.
159. http://www.thelancet.com/protocol-reviews/09PRT-2275. last accessed 10/06/2012
160. Parsi K. Catheter-directed sclerotherapy. *Phlebology* 2009;24(3):98-107.
161. Williamsson C, Danielsson P, Smith L. Catheter-directed foam sclerotherapy for insufficiency of the great saphenous vein: occlusion rates and patient satisfaction after one year. *Phlebology* 2012.

162. Bidwai A, Beresford T, Dialynas M, Prionidis J, Panayiotopoulos Y, Browne TF. Balloon control of the saphenofemoral junction during foam sclerotherapy: proposed innovation. *J Vasc Surg* 2007;46(1):145-7.
163. Brodersen JP, Geismar U. Catheter-assisted vein sclerotherapy: a new approach for sclerotherapy of the greater saphenous vein with a double-lumen balloon catheter. *Dermatol Surg* 2007;33(4):469-75.
164. Kolbel T, Hinchliffe RJ, Lindblad B. Catheter-directed foam sclerotherapy of axial saphenous reflux: early results. *Phlebology* 2007;22(5):219-22.
165. Reich-Schupke S, Altmeyer P, Stucker M. Triple-lumen double-balloon catheter for foam sclerotherapy of the great saphenous vein: critical review on preliminary results. *Phlebology* 2010;25(5):241-5.
166. Ceulen RP, Jagtman EA, Sommer A, Teule GJ, Schurink GW, Kemerink GJ. Blocking the saphenofemoral junction during ultrasound-guided foam sclerotherapy-- assessment of a presumed safety-measure procedure. *Eur J Vasc Endovasc Surg* 2010;40(6):772-6.
167. Parsi K. Venous gas embolism during foam sclerotherapy of saphenous veins despite recommended treatment modifications. *Phlebology* 2011;26(4):140-7.
168. Kendler M, Averbeck M, Simon JC, Ziemer M. Histology of saphenous veins after treatment with the ClariVein(R) device - an ex-vivo experiment. *J Dtsch Dermatol Ges* 2013;11(4):348-52.
169. Elias S, Raines JK. Mechanochemical tumescentless endovenous ablation: final results of the initial clinical trial. *Phlebology* 2012;27(2):67-72.
170. van Eekeren RR, Boersma D, Elias S, Holewijn S, Werson DA, de Vries JP, et al. Endovenous mechanochemical ablation of great saphenous vein incompetence using the ClariVein device: a safety study. *J Endovasc Ther* 2011;18(3):328-34.
171. Barath P, Fishbein MC, Vari S, Forrester JS. Cutting balloon: a novel approach to percutaneous angioplasty. *Am J Cardiol* 1991;68(11):1249-52.
172. Ajani AE, Kim HS, Castagna M, Satler LF, Kent KM, Pichard AD, et al. Clinical utility of the cutting balloon. *J Invasive Cardiol* 2001;13(7):554-7.
173. Gohel MS, Davies AH. Radiofrequency ablation for uncomplicated varicose veins. *Phlebology* 2009;24 Suppl 1:42-9.
174. Darwood RJ, Gough MJ. Endovenous laser treatment for uncomplicated varicose veins. *Phlebology* 2009;24 Suppl 1:50-61.
175. Pichot O, Kabnick LS, Creton D, Merchant RF, Schuller-Petroviae S, Chandler JG. Duplex ultrasound scan findings two years after great saphenous vein radiofrequency endovenous obliteration. *J Vasc Surg* 2004;39(1):189-95.
176. Min RJ, Khilnani N, Zimmet SE. Endovenous laser treatment of saphenous vein reflux: long-term results. *J Vasc Interv Radiol* 2003;14(8):991-6.
177. Navarro L, Min RJ, Bone C. Endovenous laser: a new minimally invasive method of treatment for varicose veins--preliminary observations using an 810 nm diode laser. *Dermatol Surg* 2001;27(2):117-22.
178. Corcos L, Dini S, De Anna D, Marangoni O, Ferlaino E, Procacci T, et al. The immediate effects of endovenous diode 808-nm laser in the greater saphenous vein: morphologic study and clinical implications. *J Vasc Surg* 2005;41(6):1018-24; discussion 25.
179. Proebstle TM, Lehr HA, Kargl A, Espinola-Klein C, Rother W, Bethge S, et al. Endovenous treatment of the greater saphenous vein with a 940-nm

diode laser: thrombotic occlusion after endoluminal thermal damage by laser-generated steam bubbles. *J Vasc Surg* 2002;35(4):729-36.

180. Theivacumar NS, Dellagrammaticas D, Mavor AI, Gough MJ. Endovenous laser ablation: does standard above-knee great saphenous vein ablation provide optimum results in patients with both above- and below-knee reflux? A randomized controlled trial. *J Vasc Surg* 2008;48(1):173-8.
181. Luebke T, Gawenda M, Heckenkamp J, Brunkwall J. Meta-analysis of endovenous radiofrequency obliteration of the great saphenous vein in primary varicosis. *J Endovasc Ther* 2008;15(2):213-23.
182. Goldman MP. Closure of the greater saphenous vein with endoluminal radiofrequency thermal heating of the vein wall in combination with ambulatory phlebectomy: preliminary 6-month follow-up. *Dermatol Surg* 2000;26(5):452-6.
183. Schmedt CG, Sroka R, Steckmeier S, Meissner OA, Babaryka G, Hunger K, et al. Investigation on radiofrequency and laser (980 nm) effects after endoluminal treatment of saphenous vein insufficiency in an ex-vivo model. *Eur J Vasc Endovasc Surg* 2006;32(3):318-25.
184. Reich-Schupke S, Mumme A, Stucker M. Histopathological findings in varicose veins following bipolar radiofrequency-induced thermotherapy--results of an ex vivo experiment. *Phlebology* 2011;26(2):69-74.
185. Vuylsteke ME, Mordon SR. Endovenous laser ablation: a review of mechanisms of action. *Ann Vasc Surg* 2012;26(3):424-33.
186. van Ruijven PW, van den Bos RR, Alazard LM, van der Geld CW, Nijsten T. Temperature measurements for dose-finding in steam ablation. *J Vasc Surg* 2011;53(5):1454-6.
187. Saatci I, Geyik S, Yavuz K, Cekirge HS. Endovascular treatment of brain arteriovenous malformations with prolonged intranidal Onyx injection technique: long-term results in 350 consecutive patients with completed endovascular treatment course. *J Neurosurg* 2011;115(1):78-88.
188. Paramasivam S, Toma N, Niimi Y, Berenstein A. Development, clinical presentation and endovascular management of congenital intracranial pial arteriovenous fistulas. *J Neurointerv Surg* 2012.
189. Vanlangenhove P, De Keukeleire K, Everaert K, Van Maele G, Defreyne L. Efficacy and Safety of Two Different n-Butyl-2-Cyanoacrylates for the Embolization of Varicoceles: A Prospective, Randomized, Blinded Study. *Cardiovasc Intervent Radiol* 2012;35(3):598-606.
190. Sze DY, Kao JS, Frisoli JK, McCallum SW, Kennedy WA, 2nd, Razavi MK. Persistent and recurrent postsurgical varicoceles: venographic anatomy and treatment with N-butyl cyanoacrylate embolization. *J Vasc Interv Radiol* 2008;19(4):539-45.
191. Levrier O, Mekkaoui C, Rolland PH, Murphy K, Cabrol P, Moulin G, et al. Efficacy and low vascular toxicity of embolization with radical versus anionic polymerization of n-butyl-2-cyanoacrylate (NBCA). An experimental study in the swine. *J Neuroradiol* 2003;30(2):95-102.
192. Vinters HV, Galil KA, Lundie MJ, Kaufmann JC. The histotoxicity of cyanoacrylates. A selective review. *Neuroradiology* 1985;27(4):279-91.
193. Spiegel SM, Vinuela F, Goldwasser JM, Fox AJ, Pelz DM. Adjusting the polymerization time of isobutyl-2 cyanoacrylate. *AJNR Am J Neuroradiol* 1986;7(1):109-12.
194. Almeida J, I, . Nonthermal ablation for the treatment of varicose veins. *Endovascular Today* 2011;April:34-38.

195. Proebstle T. http://www.iupcongress2011.cz/docs/final-programme.pdf, 2011.
196. Hartmann K, Klode J, Pfister R, Toussaint M, Weingart I, Waldermann F, et al. Recurrent varicose veins: sonography-based re-examination of 210 patients 14 years after ligation and saphenous vein stripping. *Vasa* 2006;35(1):21-6.
197. Winterborn RJ, Foy C, Earnshaw JJ. Causes of varicose vein recurrence: late results of a randomized controlled trial of stripping the long saphenous vein. *J Vasc Surg* 2004;40(4):634-9.
198. Campbell WB, Vijay Kumar A, Collin TW, Allington KL, Michaels JA. The outcome of varicose vein surgery at 10 years: clinical findings, symptoms and patient satisfaction. *Ann R Coll Surg Engl* 2003;85(1):52-7.
199. Fligelstone LJ, Salaman RA, Oshodi TO, Wright I, Pugh N, Shandall AA, et al. Flush saphenofemoral ligation and multiple stab phlebectomy preserve a useful greater saphenous vein four years after surgery. *J Vasc Surg* 1995;22(5):588-92.
200. Hammarsten J, Pedersen P, Cederlund CG, Campanello M. Long saphenous vein saving surgery for varicose veins. A long-term follow-up. *Eur J Vasc Surg* 1990;4(4):361-4.
201. Carandina S, Mari C, De Palma M, Marcellino MG, Cisno C, Legnaro A, et al. Varicose vein stripping vs haemodynamic correction (CHIVA): a long term randomised trial. *Eur J Vasc Endovasc Surg* 2008;35(2):230-7.
202. Dwerryhouse S, Davies B, Harradine K, Earnshaw JJ. Stripping the long saphenous vein reduces the rate of reoperation for recurrent varicose veins: five-year results of a randomized trial. *J Vasc Surg* 1999;29(4):589-92.
203. Franceschi C. *Theorie et practique de la cure conservatrice de l'insuffisance veineuse en ambulatoire*. Precy-sous-Thil: Editions de l'Armancpn ed: Precy-sous-Thil: Editions de l'Armancpn, 1988.
204. Maeso J, Juan J, Escribano J, Allegue NM, Di Matteo A, Gonzalez E, et al. Comparison of clinical outcome of stripping and CHIVA for treatment of varicose veins in the lower extremities. *Ann Vasc Surg* 2001;15(6):661-5.
205. Zamboni P, Cisno C, Marchetti F, Quaglio D, Mazza P, Liboni A. Reflux elimination without any ablation or disconnection of the saphenous vein. A haemodynamic model for venous surgery. *Eur J Vasc Endovasc Surg* 2001;21(4):361-9.
206. Escribano JM, Juan J, Bofill R, Maeso J, Rodriguez-Mori A, Matas M. Durability of reflux-elimination by a minimal invasive CHIVA procedure on patients with varicose veins. A 3-year prospective case study. *Eur J Vasc Endovasc Surg* 2003;25(2):159-63.
207. Zamboni P, Marcellino MG, Cappelli M, Feo CV, Bresadola V, Vasquez G, et al. Saphenous vein sparing surgery: principles, techniques and results. *J Cardiovasc Surg (Torino)* 1998;39(2):151-62.
208. Cappelli M, Lova RM, Ermini S, Turchi A, Bono G, Bahnini A, et al. Ambulatory conservative hemodynamic management of varicose veins: critical analysis of results at 3 years. *Ann Vasc Surg* 2000;14(4):376-84.
209. Creton D. A nondraining saphenous system is a factor of poor prognosis for long-term results in surgery of great saphenous vein recurrences. *Dermatol Surg* 2004;30(5):744-9; discussion 49.
210. Perrin M, Guidicelli H, Rastel D. [Surgical techniques used for the treatment of varicose veins: survey of practice in France]. *J Mal Vasc* 2003;28(5):277-86.

211. Porto LC, da Silveira PR, de Carvalho JJ, Panico MD. Connective tissue accumulation in the muscle layer in normal and varicose saphenous veins. *Angiology* 1995;46(3):243-9.
212. Gandhi RH, Irizarry E, Nackman GB, Halpern VJ, Mulcare RJ, Tilson MD. Analysis of the connective tissue matrix and proteolytic activity of primary varicose veins. *J Vasc Surg* 1993;18(5):814-20.
213. Lengyel I, Acsady G. Histomorphological and pathobiochemical changes of varicose veins. A possible explanation of the development of varicosis. *Acta Morphol Hung* 1990;38(3-4):259-67.
214. Abu-Own A, Scurr JH, Coleridge Smith PD. Saphenous vein reflux without incompetence at the saphenofemoral junction. *Br J Surg* 1994;81(10):1452-4.
215. Cooper DG, Hillman-Cooper CS, Barker SG, Hollingsworth SJ. Primary varicose veins: the sapheno-femoral junction, distribution of varicosities and patterns of incompetence. *Eur J Vasc Endovasc Surg* 2003;25(1):53-9.
216. Engelhorn CA, Engelhorn AL, Cassou MF, Salles-Cunha SX. Patterns of saphenous reflux in women with primary varicose veins. *J Vasc Surg* 2005;41(4):645-51.
217. Myers KA, Ziegenbein RW, Zeng GH, Matthews PG. Duplex ultrasonography scanning for chronic venous disease: patterns of venous reflux. *J Vasc Surg* 1995;21(4):605-12.
218. Labropoulos N, Leon L, Kwon S, Tassiopoulos A, Gonzalez-Fajardo JA, Kang SS, et al. Study of the venous reflux progression. *J Vasc Surg* 2005;41(2):291-5.
219. Pittaluga P, Chastanet S, Rea B, Barbe R. Midterm results of the surgical treatment of varices by phlebectomy with conservation of a refluxing saphenous vein. *J Vasc Surg* 2009;50(1):107-18.
220. Rutgers PH, Kitslaar PJ. Randomized trial of stripping versus high ligation combined with sclerotherapy in the treatment of the incompetent greater saphenous vein. *Am J Surg* 1994;168(4):311-5.
221. Jones L, Braithwaite BD, Selwyn D, Cooke S, Earnshaw JJ. Neovascularisation is the principal cause of varicose vein recurrence: results of a randomised trial of stripping the long saphenous vein. *Eur J Vasc Endovasc Surg* 1996;12(4):442-5.
222. Nicolini P. Treatment of primary varicose veins by endovenous obliteration with the VNUS closure system: results of a prospective multicentre study. *Eur J Vasc Endovasc Surg* 2005;29(4):433-9.
223. Merchant RF, Pichot O. Long-term outcomes of endovenous radiofrequency obliteration of saphenous reflux as a treatment for superficial venous insufficiency. *J Vasc Surg* 2005;42(3):502-9; discussion 09.
224. Luebke T, Brunkwall J. Systematic review and meta-analysis of endovenous radiofrequency obliteration, endovenous laser therapy, and foam sclerotherapy for primary varicosis. *Journal of Cardiovascular Surgery* 2008;49(2):213-33.
225. Rasmussen LH, Lawaetz M, Bjoern L, Vennits B, Blemings A, Eklof B. Randomized clinical trial comparing endovenous laser ablation, radiofrequency ablation, foam sclerotherapy and surgical stripping for great saphenous varicose veins. *Br J Surg* 2011;98(8):1079-87.
226. Rasmussen LH, Bjoern L, Lawaetz M, Lawaetz B, Blemings A, Eklof B. Randomised clinical trial comparing endovenous laser ablation with

stripping of the great saphenous vein: clinical outcome and recurrence after 2 years. *European Journal of Vascular & Endovascular Surgery* 2010;39(5):630-5.

227. Belcaro G, Cesarone MR, Di Renzo A, Brandolini R, Coen L, Acerbi G, et al. Foam-sclerotherapy, surgery, sclerotherapy, and combined treatment for varicose veins: a 10-year, prospective, randomized, controlled, trial (VEDICO trial). *Angiology* 2003;54(3):307-15.
228. Lemole GM, Anderson, R.R, DeCosta, S, . Preliminary evaluation of collagen as a component in the thermally-induced weld. *SPIE Lasers Dermatol Tissue Welding* 1991;1422:116-21.
229. Weiss RA. Comparison of endovenous radiofrequency versus 810 nm diode laser occlusion of large veins in an animal model. *Dermatol Surg* 2002;28(1):56-61.
230. Vuylsteke M, Van Dorpe J, Roelens J, De Bo T, Mordon S. Endovenous laser treatment: a morphological study in an animal model. *Phlebology* 2009;24(4):166-75.
231. Rabe E, Pannier F. Sclerotherapy of varicose veins with polidocanol based on the guidelines of the German Society of Phlebology. *Dermatol Surg* 2010;36 Suppl 2:968-75.
232. Rao J, Goldman MP. Stability of foam in sclerotherapy: differences between sodium tetradecyl sulfate and polidocanol and the type of connector used in the double-syringe system technique. *Dermatol Surg* 2005;31(1):19-22.
233. http://www.fibro-vein.co.uk/what_con_2.asp. last accessed 10/06/2012.
234. http://www.angiodynamics.com/products/sotradecol. last accessed 10/06/2012.
235. O'Hare JL, Earnshaw JJ. The use of foam sclerotherapy for varicose veins: a survey of the members of the Vascular Society of Great Britain and Ireland. *Eur J Vasc Endovasc Surg* 2007;34(2):232-5.
236. Parsi K, Exner T, Connor DE, Herbert A, Ma DD, Joseph JE. The lytic effects of detergent sclerosants on erythrocytes, platelets, endothelial cells and microparticles are attenuated by albumin and other plasma components in vitro. *Eur J Vasc Endovasc Surg* 2008;36(2):216-23.
237. Parsi K, Exner T, Low J, Fung Ma DD, Joseph JE. In vitro effects of detergent sclerosants on clot formation and fibrinolysis. *Eur J Vasc Endovasc Surg* 2011;41(2):267-77.
238. Rotter SM, Weiss RA. Human saphenous vein in vitro model for studying the action of sclerosing solutions. *J Dermatol Surg Oncol* 1993;19(1):59-62.
239. Crosby JR, Kaminski WE, Schatteman G, Martin PJ, Raines EW, Seifert RA, et al. Endothelial cells of hematopoietic origin make a significant contribution to adult blood vessel formation. *Circ Res* 2000;87(9):728-30.
240. Modarai B, Burnand KG, Sawyer B, Smith A. Endothelial progenitor cells are recruited into resolving venous thrombi. *Circulation* 2005;111(20):2645-53.
241. AG R. Sclerovein *Product Insert*, Last updated 2007.
242. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/EnforcementActivitiesbyFDA/WarningLettersandNoticeofViolationLetterstoPharmaceuticalCcompanies/ucm054378.pdf. Last accessed 31/08/2011.

243. http://www.mydr.com.au/cmis/ReducedPDFs/CMR02646.pdf. Last accessed 31/08/2011

244. http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=5248&loc=ec rcs. Last accessed 30/08/2011.

245. Nijsten T, van den Bos RR, Goldman MP, Kockaert MA, Proebstle TM, Rabe E, et al. Minimally invasive techniques in the treatment of saphenous varicose veins. *J Am Acad Dermatol* 2009;60(1):110-9.

246. Peterson JD, Goldman MP. An investigation into the influence of various gases and concentrations of sclerosants on foam stability. *Dermatol Surg* 2011;37(1):12-7.

247. Breu FX, Guggenbichler S. European Consensus Meeting on Foam Sclerotherapy, April, 4-6, 2003, Tegernsee, Germany. *Dermatol Surg* 2004;30(5):709-17; discussion 17.

248. Wollmann JC. The history of sclerosing foams. *Dermatol Surg* 2004;30(5):694-703; discussion 03.

249. Wollmann JC. Sclerosant foans: stabilities, physical properties and rheological behaviour. *Phlebologie* 2010;39:208-17.

250. Van Deurzen B, Ceulen RP, Tellings SS, C VDG, Nijsten T. Polidocanol Concentration and Time Affect the Properties of Foam Used for Sclerotherapy. *Dermatol Surg* 2011.

251. McMaster S. Sodium tetradecyl sulphate foam stability prior to injection: factors affecting liquid reformation. *Phlebology* 2011;26(6):222-6.

252. Blomqvist B, R. Ridout, M,J. Mackie, A, R. Warnheim, T. Claesson, P, M. Wilde, P. . Disruption of viscoelastic beta-lactoglobulin surface layers at the air-water interface by nonionic polymeric surfactants.: Langmuir, 2004.

253. Exerowa DK, P,M. *Foam and foam films: theory, experiment, application.*: Elsevier, 1998.

254. Furie B, Furie BC. Mechanisms of thrombus formation. *N Engl J Med* 2008;359(9):938-49.

255. Vuylsteke M, Van Dorpe J, Roelens J, De Bo T, Mordon S, Fourneau I. Intraluminal fibre-tip centring can improve endovenous laser ablation: a histological study. *Eur J Vasc Endovasc Surg* 2010;40(1):110-6.

256. Vuylsteke ME, Vandekerckhove PJ, De Bo T, Moons P, Mordon S. Use of a new endovenous laser device: results of the 1,500 nm laser. *Ann Vasc Surg* 2010;24(2):205-11.

257. Vuylsteke ME, Martinelli T, Van Dorpe J, Roelens J, Mordon S, Fourneau I. Endovenous laser ablation: the role of intraluminal blood. *Eur J Vasc Endovasc Surg* 2011;42(1):120-6.

258. Lurie F, Creton D, Eklof B, Kabnick LS, Kistner RL, Pichot O, et al. Prospective randomised study of endovenous radiofrequency obliteration (closure) versus ligation and vein stripping (EVOLVeS): two-year follow-up. *Eur J Vasc Endovasc Surg* 2005;29(1):67-73.

259. Rasmussen LH, Bjoern L, Lawaetz M, Lawaetz B, Blemings A, Eklof B. Randomised clinical trial comparing endovenous laser ablation with stripping of the great saphenous vein: clinical outcome and recurrence after 2 years. *Eur J Vasc Endovasc Surg* 2010;39(5):630-5.

260. Darwood RJ, Theivacumar N, Dellagrammaticas D, Mavor AI, Gough MJ. Randomized clinical trial comparing endovenous laser ablation with surgery for the treatment of primary great saphenous varicose veins. *Br J Surg* 2008;95(3):294-301.

261. Parsi K, Exner T, Ma DD, Joseph JE. In vitro effects of detergent sclerosants on fibrinolytic enzymes and inhibitors. *Thromb Res* 2010;126(4):328-36.
262. Martin DE, Goldman MP. A comparison of sclerosing agents: clinical and histologic effects of intravascular sodium tetradecyl sulfate and chromated glycerin in the dorsal rabbit ear vein. *J Dermatol Surg Oncol* 1990;16(1):18-22.
263. MacGowen WAL, Jolland, P.D.J, Browne, H.I, Byrnes, D.P. The local effects of intraarterial injections of sodium tetradecyl sulphate (STD) 3 per cent: an experimental study. *Br J Surg* 1972;59:101-4.
264. Jones GT, Grant MW, Thomson IA, Hill BG, van Rij AM. Characterization of a porcine model of chronic superficial varicose veins. *J Vasc Surg* 2009;49(6):1554-61.
265. Subwongcharoen S, Praditphol N, Chitwiset S. Endovenous microwave ablation of varicose veins: in vitro, live swine model, and clinical study. *Surg Laparosc Endosc Percutan Tech* 2009;19(2):170-4.
266. Beier JP, Horch RE, Arkudas A, Polykandriotis E, Bleiziffer O, Adamek E, et al. De novo generation of axially vascularized tissue in a large animal model. *Microsurgery* 2009;29(1):42-51.