CLINICAL AND MOLECULAR EFFECTS OF ENDOSCOPIC VEIN HARVESTING

A thesis submitted to the University of Manchester for the degree of MPhil in the Faculty of Medical and Human Sciences.

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ABSTRACT

Coronary artery bypass grafting (CABG) is the most commonly performed surgical cardiac procedure. Despite a worldwide increase in the use of arterial grafts, the long saphenous vein (LSV) still remains the most frequently used conduit in coronary artery bypass grafts since its introduction in 1968. Traditionally, the LSV is harvested by a continuous long incision in the donor leg, which can result in major wound complications and various studies have reported an incidence of wound infection ranging from 20% to 40%. Newer minimally invasive techniques (MIVH) such as bridging and endoscopic vein harvesting (EVH) can reduce the incidence of wound infection and significantly improve healing. Although the effect of MIVH vein techniques on wound related problems has been extensively investigated in the literature, its effect on the quality of the harvested conduit remains unclear.

More recently, a major article has been published which suggests EVH is associated with increased sudden death and >75% graft occlusion (within a 12 month period) compared to open vein harvesting (OVH). This has resulted in many centres stopping their EVH programmes. However, a potential issue with this article is that 2 endoscopic vein harvesting systems were utilised. One system requires 1kPa pressure of CO_2, which compresses the long saphenous vein. Potentially this may result in endothelial dysfunction and denudation, leading to graft failure. The second system utilises an open tunnel dissection method with no venal collapse.

The aim of this study was to characterise the effects of the two EVH techniques on endothelial damage and to observe post-clinical outcome at 6 months. One hundred and forty vein samples were allocated non-randomly into EVH1 (closed CO_2 tunnel - 70 samples) and EVH2 (open CO_2 tunnel - 70 samples). Vein specimens were stained using immunohistochemistry (to detect the expression of CD34), and then blindly scored by three independent assessors. The student's t-test was used to evaluate statistical differences between the groups. In this study, significantly greater conduit integrity was observed in EVH2 compared to EVH1 (mean 65.0% vs. 11.4%, $p<0.001$). However, the clinical follow-up of patients (until 6 months) offered no statistically significant difference in the incidence of major adverse cardiac events. Potential reasons for this outcome were a lack of randomisation, an extended learning curve for the surgical procedure and the collection of samples over different time

periods. Importantly, vein graft failure post CABG surgery is a multifarious process, meaning that histological evidence cannot be utilised as the sole indicator of vein graft failure, unless demonstrated alongside poor clinical outcome.

DECLARATION

No portion of the work referred to in this thesis has been submitted in support of an application for another degree or qualification at this or any other University or learning institute.

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LIST OF ABBREVIATIONS

ACC :	**American College of Cardiology**
ADP :	**Adenosine Di-Phosphate**
AHA :	**American Heart Association**
CABG :	**Coronary Artery Bypass Surgery**
CAD:	**Coronary Artery Disease**
CCS :	**Canadian Classification System**
CI :	**Confidence Interval**
CO_2 :	**Carbon Dioxide**
ECG :	**Electrocardiography**
ECHO :	**Echocardiography**
EDRF :	**Endothelial Derived Relaxing Factors**
EVH :	**Endoscopic Vein Harvesting**
HCO_3 :	**Bicarbonate**
LAD :	**Left Anterior Descending Artery**
LIMA :	**Left Internal Mammary Artery**
LMS :	**Left Main Stem**
LSV :	**Long Saphenous Vein**
LVEF :	**Left Ventricular Ejection Fraction**
MACE :	**Major Adverse Cardiac Events**
MIVH :	**Minimally Invasive Vein Harvesting**
MRI :	**Magnetic Cardiac Resonance Imaging**
NREC :	**National Research Ethics Committee**
NO :	**Nitric Oxide**
NYHA :	**New York Heart Association Scoring System**
OVH :	**Open Vein Harvesting**
$PaCO_2$:	**Partial Pressure of Carbon dioxide**
pH :	**Concentration of Hydrogen Ions**
PTCA :	**Percutaneous Transluminal Coronary Angioplasty**
SPSS :	**Statistical Package for the Social Sciences**
UHSM :	**University Hospital of South Manchester**

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

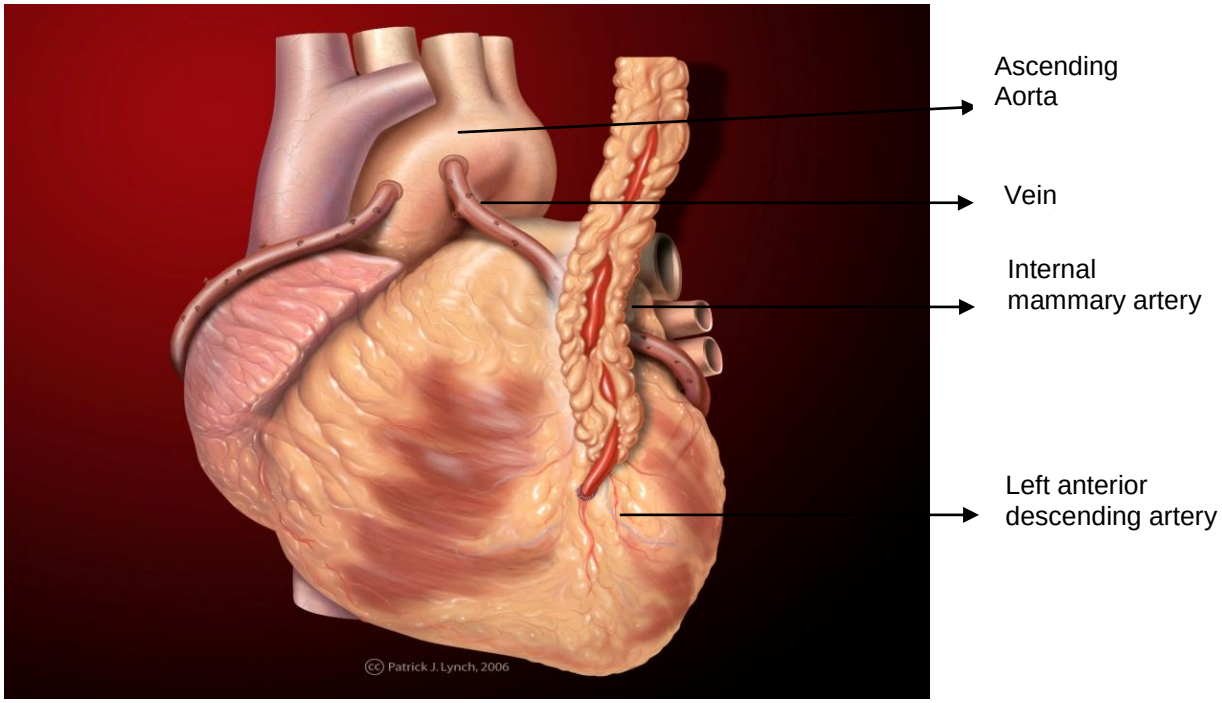
Coronary artery disease (CAD) is the most common heart disease worldwide, with a mortality rate exceeding 4.5 million deaths per annum [1]. CAD is caused by the gradual deposition of atherosclerotic plaques in the vessel wall of the coronary arteries, which prevents blood flow and leads to myocardial infarction (MI) and ischaemia [2]. This presents clinically as angina (severe chest pain radiating to arms, jaws and shoulders), shortness of breath during exertion and sweating. Despite improvements in medical therapies (i.e. nitroglycerin, aspirin, beta-adrenergic blockers, calcium channel blockers and angiotension-converting enzyme inhibitors) and drug-eluting stents, the management of CAD still remains medically challenging [2-4]. The current management of CAD involves coronary angioplasty and coronary artery bypass graft surgery (CABG).

The first CABG procedure was performed in 1960 [5]. Since this time, CABG has become one of the most frequent cardiac surgical procedures in the world, and is a highly effective method of relieving symptoms of coronary artery disease [6]. The conduits used for bypassing blocked coronary arteries are arterial and venous blood vessels. Venous conduit harvesting is traditionally performed by an open vein harvesting (OVH) technique, which has been associated with a high incidence of post-operative wound complications ranging from 5% to 44% [7]. Minimally invasive vein harvesting techniques (MIVH) may be associated with a reduction in post-operative wound complications. However, MIVH may also be associated with endothelial vein damage [8-10]. The endothelial vein layer plays an essential role in graft patency following CABG surgery [8, 9, 11, 12]. Injury to this layer can aggregate platelets and induce endothelial denudation, promoting intimal proliferation and hyperplasia leading to subsequent graft occlusion [13].

1.1. Coronary Artery Bypass Grafting

It is estimated that more than 800,000 CABG surgeries are performed worldwide per annum. CABG surgery is performed via median sternotomy, whilst arterial and venous conduits are harvested for the procedure [14]. The main conduits used for CABG surgery are arterial (internal mammary artery and radial artery) and saphenous veins (long and short) [15] (Fig 1).

Figure 1: Diagrammatic representation of the heart with coronary artery bypass grafts



This diagram illustrates how both the internal mammary artery and the saphenous vein can be utilised as conduits to bypass the coronary arteries. The internal mammary artery is surgically attached to the left anterior descending artery, allowing oxygenated blood to be directed into the heart. This can also be achieved by grafting the proximal end of the saphenous vein conduit onto the ascending aorta and attaching the distal end of the vein to the posterior cardiac vessels.

1.2. Internal mammary artery (IMA)

The left internal mammary artery (LIMA) (fig 1) was first introduced for coronary anastomosis in 1964 [16, 17]. In 1970 [18] it was used as a pedicle graft to the left anterior descending artery (LAD) and provided a 10 year patency rate greater than 90% [19]. The internal mammary artery (IMA) is considered to be a gold-standard in CABG surgery because of its significantly improved short and long-term survival [20, 21].

A study carried out by Edwards *et al* with n=38,578 patients undergoing CABG (between 1981 and 1991) observed that patients receiving an IMA graft exhibited significantly reduced postoperative mortality, when compared to patients receiving sole venous conduits [22]. In contrast, another study reported that the IMA should not be performed on older female patients with a low body mass index (BMI), and emergency cases with decreased left ventricular ejection fractions, due to the surgical risks involved [23]. The pedicle IMA can be used only to replace anterior coronary arteries, such as the left anterior descending artery and diagonal arteries [24]. The posterior coronary arteries such as the circumflex, obtuse marginal and posterior descending arteries, require longer length conduits, such as the radial artery and long saphenous vein; situations where LIMA length limits clinical efficacy [20].

1.3. Radial artery

The radial artery was first used for CABG surgery as a conduit in 1973 and abandoned 2 years later due to a 35% graft failure rate [25]. In 1989, it was reintroduced after a review of early angiogram data, which demonstrated the radial artery went into early spasm (not graft failure) [26]; those patients survived for 15 years with patent radial artery grafts, confirmed by angiography [27]. The radial artery is now widely used in some cardiac centres as a free-graft to posterior vessels in CABG surgery and appears to offer a higher patency rate compared to the long saphenous vein, but is more prone to spasm than LIMA [28]. However, due to its location and functional properties, the complications connected to its harvest can be significant and include spasm, nerve damage (superficial radial, median nerve) and compartment syndrome.

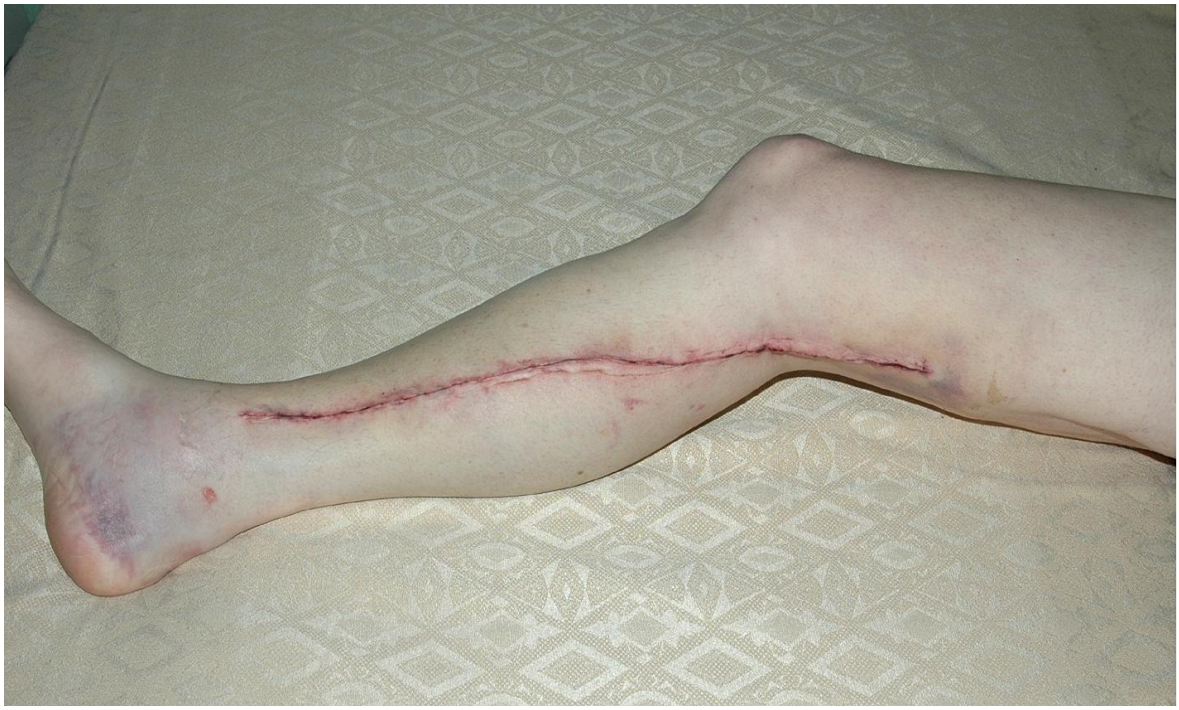
A meta-analysis was performed by Athanasiou *et al* [29] using 35 studies including 11184 angiographic assessments (n=3678 radial artery and n=7506 saphenous vein grafts). This study concluded that the radial artery conduit is better than the LSV conduit. In contrast, other reports suggest that bypass using the radial artery is associated with ischaemic, neurological and sudden

cardiac arrest [30, 31]. Although arterial conduits such as IMA and the radial artery are being increasingly utilised, the long saphenous vein remains the most common conduit in multi-vessel CABG procedures.

1.4. Long saphenous vein (LSV)

The LSV was first used as a conduit for coronary artery bypass surgery in 1967 [16, 32]. The LSV is now the most commonly used conduit due to its long length and lack of invasiveness [33]. Harvesting the LSV is relatively simple due to its location and anatomical position in the leg. A variety of surgical techniques are utilised to harvest the LSV, such as open, bridging and endoscopic vein harvesting[34]. However, problems with wound healing are commonly observed after open vein harvesting and subsequently minimally invasive techniques (bridging and endoscopic) have been used in surgical practice for the past 10 years [35].

Figure 2: Open vein harvesting.



This image demonstrates the typical length of incision required to retrieve LSV conduits via the traditional open vein harvesting technique, and is adapted from a Society of Cardiothoracic Surgery conference abstract[46].

1.4.1. Open vein harvesting technique (OVH)

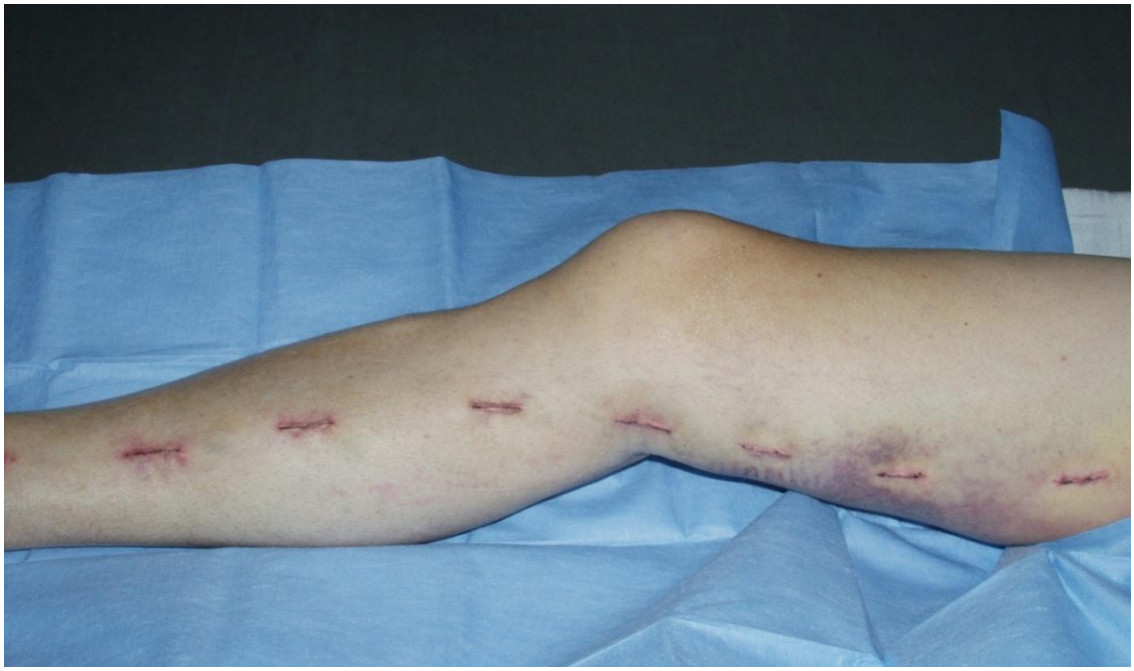
Open vein harvesting (fig 2) is the traditional method of harvesting the LSV, which entails a longitudinal incision from the ankle up to the groin. The length of the skin incision depends on the number of vein conduits required for surgery [36]. The vein is carefully dissected using Metzenbaum scissors and forceps with the aim of minimising vein/branch trauma. This technique has been associated with postoperative complications such as wound infections, which if experienced, require a course of antibiotics to complete wound dehiscence and plastic surgery/skin grafting [37]. Common complications include postoperative pain, leg oedema, cellulitis, serous drainage, subcutaneous fat tissue necrosis and delayed healing [7, 38-41]. These complications can delay post-operative recovery and increase the length of hospital stay.

More recently, reports suggest that a decrease in wound complications and morbidity can be achieved by harvesting the LSV using MIVH techniques [42-45].

1.4.2. Bridging vein harvesting technique

The bridging technique (fig 3) uses multiple short incisions to harvest the LSV and is associated with a reduction in post-operative pain and wound infection [47]. This technique involves a number of 2-3cm incisions with 5-6cm gaps from ankle to groin, depending on the length of conduit required for surgery [47]. Although bridging is considered a minimally invasive vein harvesting technique, complications such as haematoma, leg wound pain, bruising and seroma formation can occur due to the multiple incisions [24]. However, a prospective non-randomised study performed by Hovarth *et al* comparing MIVH techniques recorded a lower incidence of wound complications when the LSV was harvested via the bridging method compared to endoscopic vein harvesting (EVH) ($p=0.0048$) [48]. Moreover, our group performed a randomised prospective study comparing OVH (n=50) vs. bridging technique (n=50). We demonstrated significantly improved patient satisfaction ($p<0.001$), reduced leg wound pain ($p<0.001$) and reduced wound complications ($p<0.001$)[49]. As a result of these findings, we concluded that the bridging method of vein harvesting is a safe and effective technique.

Figure 3: Bridging vein harvesting

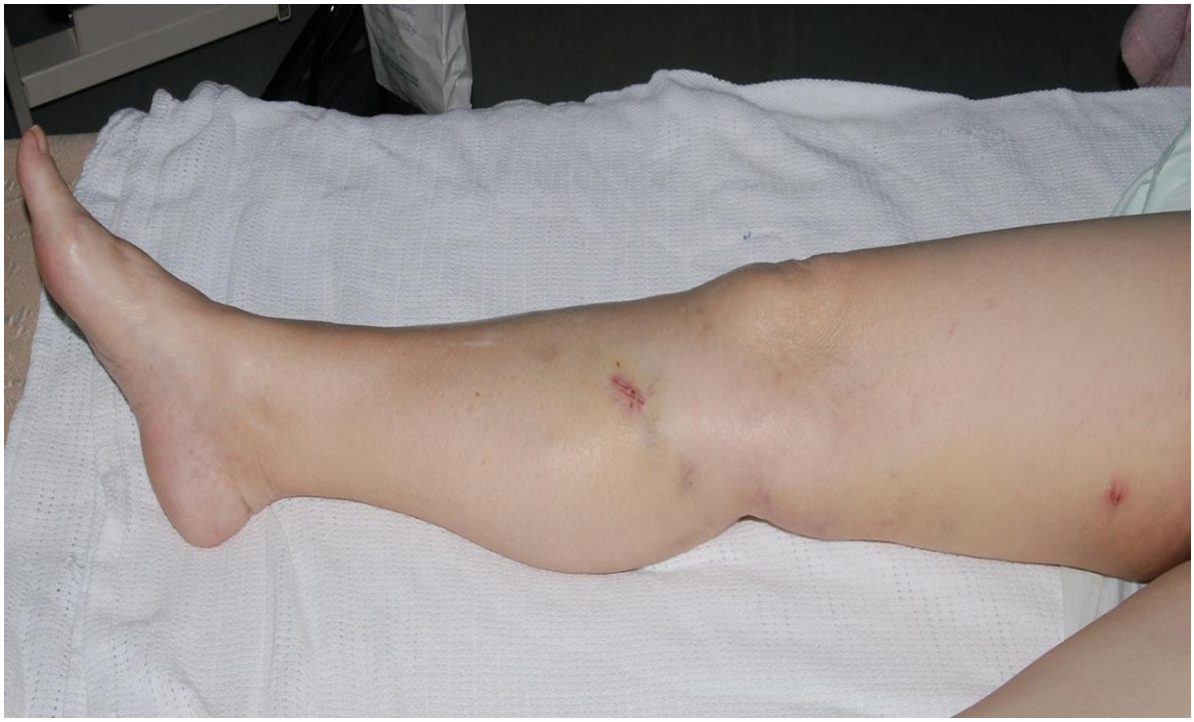


This image illustrates the multiple incisions that typify the bridging method of vein harvesting and is adapted from a Society of Cardiothoracic Surgery conference abstract[46].

1.4.3. Endoscopic vein harvesting technique

A thin endoscope is inserted through a small 2cm skin incision below the knee and the LSV is harvested under visual guidance. It is well established that the EVH technique is efficacious in reducing leg wound infections, especially in high risk groups such as diabetic and obese patients [50]. Previous studies have reported that EVH significantly lowers the wound infection rate to 4 to 6.3% compared to 14.8 to 28.3% in the OVH group [42, 50-52]. Puskas *et al* [53] compared OVH (n=50) with EVH (n=47) and determined that wound infection rates were significantly lower in the EVH group (p=0.001). They concluded that EVH is safe and reduces the incidence of infection with improved cosmetic results. Two different methods of EVH can be used to harvest the LSV which includes closed or open CO_2 tunnel system.

Figure 4: Endoscopic vein harvesting.



This image demonstrates the two small incisions that are required in order to retrieve the LSV using the endoscopic vein harvesting method. This image is adapted from a Society of Cardiothoracic Surgery conference abstract [46].

1.4.3.1. Closed tunnel CO_2 EVH system

This procedure involves making a 2cm longitudinal incision below the knee. The vein is identified on the medial tibial border and with the aid of CO_2 insufflation (12 – 15mmHg and flow rate of 3 litres per minute), the incision site is sealed completely with a port balloon containing 15cc of air. A dissection tip is introduced into the tunnel to isolate the vein and adjoining branches from the surrounding tissue. Once the vein isolation is complete, a second endoscopic instrument incorporating a cautery device is inserted into the same port to cut and cauterise the tributaries. A 1cm skin incision near the groin crease is made to ligate the distal part of the LSV and free the vein graft. The vein is carefully removed from the tunnel under camera (live-view) guidance. The vein is inflated and observed for any leaks before quality assessment with 20ml of heparinised blood.

1.4.3.2. Open tunnel CO_2 EVH system

In a similar manner to the closed tunnel CO_2 system, an incision is made above the knee, however, this procedure differs as the skin port is unsealed and a pressure of 0mmHg is set on the insufflator. The vein is manually dissected anteriorly, posteriorly and laterally without any pressure on the vein and side branches. Once the vein is isolated from surrounding tissue, the endoscopic instrument incorporating bipolar cautery is inserted to cut and seal the tributaries. A 1cm skin incision is made near the groin crease to ligate the distal part of the LSV. The vein is carefully removed through a proximal 2cm skin incision. The inflated vein is again checked for any subsequent leaks before quality assessment using 20ml of heparinised blood.

1.4.3.3. Closed tunnel CO_2 vs. open tunnel CO_2 EVH system

A retrospective closed tunnel CO_2 vs. open tunnel CO_2 EVH study performed by Chavanon *et al* recruited n=40 patients (closed CO_2 n=25 and open CO_2 n=15). They concluded that both EVH techniques are effective in reducing the risk of wound infection, but the incidence of vein trauma and wound complications was greater in the open tunnel CO_2 technique[54]. They compared the initial learning experiences of the two techniques but did not analyse the effects of CO_2 on the veins. Unfortunately, this is the only study comparing closed tunnel CO_2 vs. open tunnel CO_2. A number of studies assessing practitioner learning curves propose a minimum of 30 cases as a proficiency threshold when performing EVH [50, 55, 56], although they have not compared data regarding long term morbidity, mortality or graft failure rates following CABG.

1.5. Clinical outcome

More recently, EVH has increased in popularity due to a significant reduction in postoperative pain and wound related complications. The Society of Thoracic Surgeons' National Cardiac Database reported that 70% of CABG procedures performed in 2008 used EVH techniques [57]. Many studies comparing EVH vs. OVH have reported significantly reduced short-term complications such as leg wound infection, postoperative wound pain and early mortality following EVH (Table 1). A meta-analysis of 27 studies conducted by Athanasiou *et al* observed a reduction in wound-related complications amongst patients undergoing EVH, in comparison with OVH [35]. In this meta-analysis, 12 randomised trials demonstrated that EVH was associated with a significant reduction in wound complications such as haematoma, drainage, dehiscence, necrosis and seroma formation (including surgical debridement) (4% vs. 13%). A randomised study comparing EVH vs. OVH by Yun *et al* involved the recruitment of n=200 patients (n=100 in each group) and aimed to assess clinical outcomes, which included graft patency and wound infection at 6 months [58]. The researchers observed EVH was associated with a reduction in leg wound infection when compared with OVH (7.4% vs. 19.4%; p=0.014). Furthermore, there was no significant difference in the incidence of graft failure (21.7% EVH vs. 17.6% OVH, p=0.584). Similarly, Allen *et al* [59] conducted a randomised trial of 112 patients and reported no significant difference in 5 year survival or the incidence of recurrent angina or MI during follow-up (EVH 75% vs. OVH 74%; p=0.85).

Another large study published by Dacey *et al* involving the recruitment of 8542 patients, (n=4480 EVH and n=4062 OVH) reported that the number of patients returning to theatre for post-surgical bleeding and wound infections was significantly lower in the EVH group compared to OVH (p=0.03 and p<0.001 respectively)[60]. The researchers also found that EVH significantly reduced short-term mortality (p=0.007).

Lopes *et al* recently questioned the use of EVH as the standard surgical vein harvesting procedure and raised concerns regarding the risk of vein graft failure, death, MI and repeat revascularisation in post CABG patients [61]. This large randomised study was designed to assess the efficacy of pre-treating vein grafts with the aim of decreasing neo-intimal hyperplasia. The data for this study was collected from the database project of PREVENT IV which was a multicentre, randomised, double blind, placebo-controlled trial of vein grafts treated *ex vivo* with E2F transcription factor decoy and edifoligide (107 CABG patients).

A secondary analysis within this study involving n=1753 EVH and n=1247 OVH patients, demonstrated that those undergoing an EVH procedure experienced higher rates of vein graft failure (stenosis of ≥75% of the diameter of the graft observed with angiography) at 12 – 18 months, compared with OVH (p<0.001; 46.7% vs. 38.0%). However, this study was not designed to evaluate the different vein harvesting techniques. Patients underwent both on and off-pump CABG surgery, whilst different groups were administered drugs to prevent graft failure. In addition, two different types of endoscopic vein harvesting systems were used. These factors have significant implications on study outcome. Therefore, the conclusion that EVH elicits increased graft occlusion may be inappropriate.

CABG surgery can be performed with two primary techniques: on-pump or off-pump. During on-pump surgery, a cardiopulmonary bypass machine (CPB) is used to take over the role of the heart and lungs, and the myocardium is protected by cardioplegia [62]. However, increasing evidence indicates that cardiopulmonary bypass may be responsible for a percentage of the morbidity associated with CABG surgery [4, 6]. Complications such as systemic inflammatory response syndrome may be initiated by the extracorporeal circuit, resulting in mechanical trauma to blood. Furthermore, activation of various immunological cascades (i.e. complement and cytokines), impaired haemostasis and impaired neurological, renal and gastrointestinal function may also occur [15, 63, 64]. In contrast, off-pump surgery utilises an octopus stabilizer to support the beating heart until coronary anastomosis is carried out. Whilst off-pump surgery avoids the potential complications described above [65], the quality of the anastomosis can be comprised due to the beating heart, which could subsequently contribute to graft failure post-coronary surgery [64, 65]. The Lopes study did not take into consideration the use of on and off pump coronary artery bypass surgery while comparing EVH with OVH. A further retrospective analysis of the PREVENT IV trials by Magee *et al* [66] compared off-pump (n=637) with on-pump (n=2377) CABG techniques and found that the incidence of graft failure was significantly higher in the closed tunnel CO_2 EVH group when using an off-pump procedure (p=0.05).

Another important factor to consider is whether the EVH devices may have affected clinical outcome in the Prevent IV trial. Two different EVH devices were used (Guidant – closed CO_2 tunnel and Ethicon – open CO_2 tunnel). This information was not disclosed in the Lopes paper but was later revealed by Cheng *et al* [67] in a letter to the editor suggesting that there may be device-related problems in regards to clinical outcomes. These findings remain unclear, and at the time of writing, no

clinical trial evaluating the effects of both EVH devices has been performed. Unfortunately, in the PREVENT IV trial, randomisation was not based on harvesting technique. The design of a major randomised study must take into consideration the type of device used as this may significantly influence clinical outcome.

There is significant concern that excessive vein manipulation when using EVH techniques can cause vessel trauma, leading to early graft failure and stenosis [7, 44, 68-70]. We believe that the use of CO_2 during closed tunnel CO_2 EVH can affect the endothelium of the LSV. In our opinion, it is therefore crucial to delineate the effects of CO_2 pressure on vessel integrity and clinical outcome following CABG.

1.6. Structure of the long saphenous vein

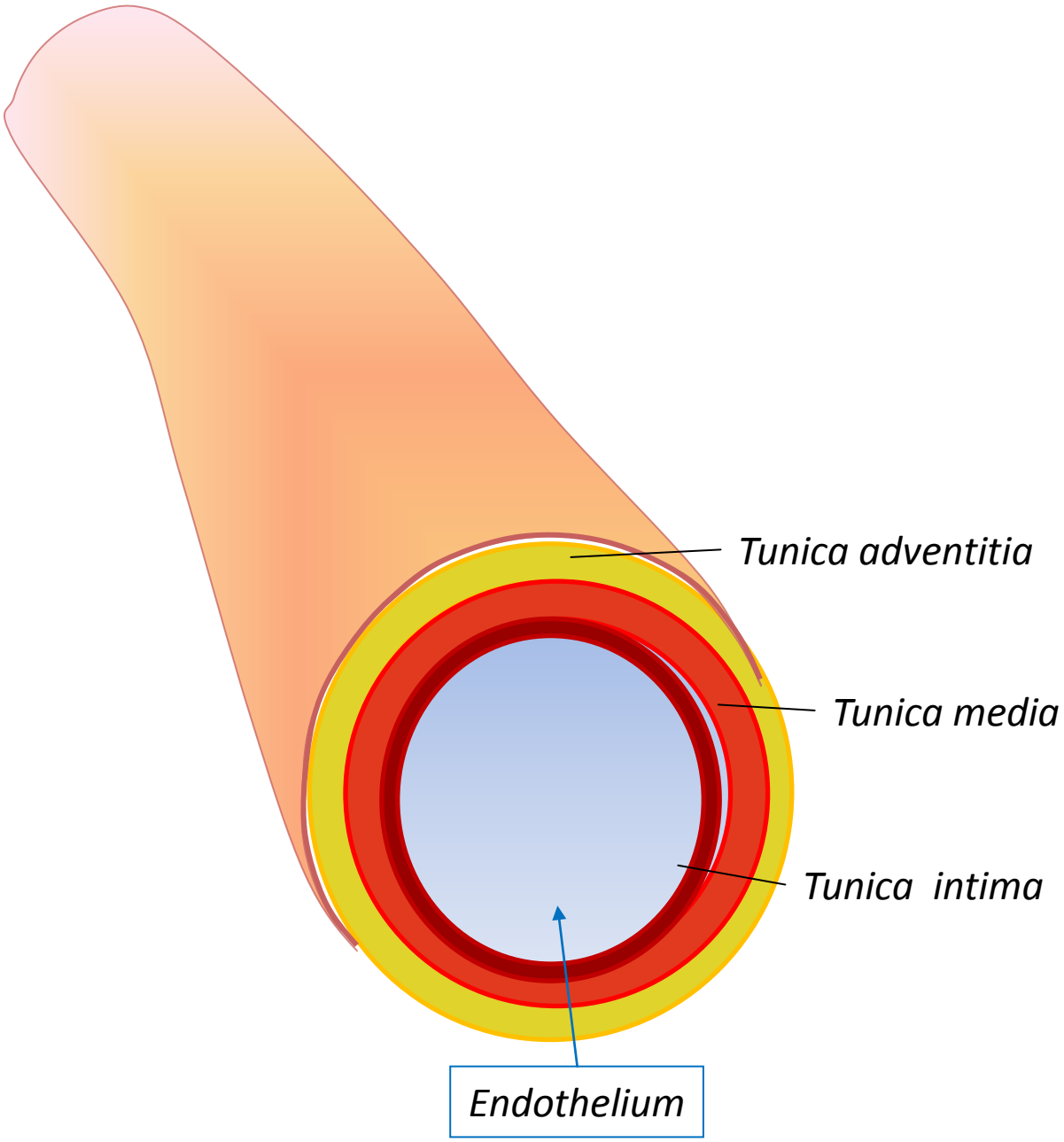
The vein wall is made up of three anatomic layers (Fig 5); 1) the tunica intima or innermost layer, 2) tunica media or mid-muscle layer and 3) the tunica adventitia or outer layer [83]. The intima consists of a thin layer of endothelial cells which is separated from the middle layer by a thin layer of basal elastic lamina. The tunica media is made up of longitudinal smooth muscle cells with collagen and elastic fibres. At the site of the valves, the tunica media layer is thick, thus preventing back flow of blood to the leg. The adventitia is the outermost layer and consists of a loose network of longitudinal collagen bundles and scattered fibroblasts.

The vasa vasorum which lies within the adventitia provides blood supply and protects the vein from ischaemic injury in the presence of localised vein stress [84, 85]. The vasa vasorum is a micro vascular network which is responsible for the exchange of gases and supply of nutrients to the vein wall [86]. A canine study performed to identify the role of the vasa vasorum blood supply and intraluminal arterial blood flow in maintaining endothelial integrity in vein bypass surgery (n=15) has demonstrated that significant endothelial changes (with fibrin platelet deposition) occur in dogs lacking vein-wall vasa vasorum [87]. This observation was also supported by the Krupski *et al*, who reported that veins mobilised or stripped of adventitia display a loss of endothelial cells (25% to 50%) [88]. In addition to preventing ischaemic injury to the vein wall, preservation of vasa vasorum blood supply also maintains the antithrombotic properties of the endothelial monolayer [89].

Table 1: An overview of the existing studies that compare vein harvesting techniques.

S.no	Study author	Sample size	Study findings	P values
1.	Hovarth *et al* [41]	EVH (n=31) Bridging (n=29)	Lower incidence of wound infections in EVH group.	P=0.0048
2.	Puskas *et al* [42]	EVH (n=47) OVH (n=50)	Lower incidence of wound infections in EVH group.	P=0.001
3.	Haward *et al* [46]	EVH (n=50) OVH (n=50)	No significant difference in leg wound infections in both groups.	P=0.75
4.	Kiaii *et al* [45] Randomised trial	EVH (n=72) OVH (n=72)	a. Leg wound infection reduced at discharge in EVH. b. Leg wound infection reduced at 6 weeks in EVH group.	P=0.12 P=.0006
5.	Athanasiou *et al*[28] Meta analysis	12 randomised trials	EVH group had reduced wound complications. 4% vs. 13%.	
6.	Yun *et al* [47] Randomised trial	EVH (n=100) OVH (n=100)	a. Wound infections were reduced in EVH group. b. Graft patency	P=0.014 P=0.584
7.	Allen *et al* [48]	n=112 (EVH + OVH)	No significant differences in 5 year follow up (MI, angina and death).	P=0.85
8.	Lopes *et al* [97] Randomised, double blinded.	EVH (n=1753) OVH (n=1247)	75% graft failure in EVH group during 12-18 month follow up.	P<0.001
9.	Magee *et al* [99]	Off pump (n=637) On pump (n= 2377)	EVH group display higher graft failure when performed off-pump	P=0.05
10.	Dacey *et al* [101]	OVH (n= 4480) EVH (n=4062)	a. Bleeding b. Reduced wound infections in EVH. c. Long-term mortality	P=0.03 P<0.001 P=0.007
11.	Chou *et al* [71]	EVH (n=270) OVH (n=78) All cases performed with off-pump CABG surgery.	a. Reduced wound infection in EVH. b. Acute graft failure. c. One year follow up for graft failure.	P=0.0002 P=0.9999 P=0.3985
12.	Chavanon *et al*[54]	EVH - closed tunnel CO_2 (n=25) EVH –open tunnel CO_2 (n=15)	a. Wound complications. b. Vein trauma.	P=0.243 P=0.05

Figure 5: Cross sectional view of the long saphenous vein



This figure provides a cross sectional view of the long saphenous vein and demonstrates the individual layers that constitute its structure.

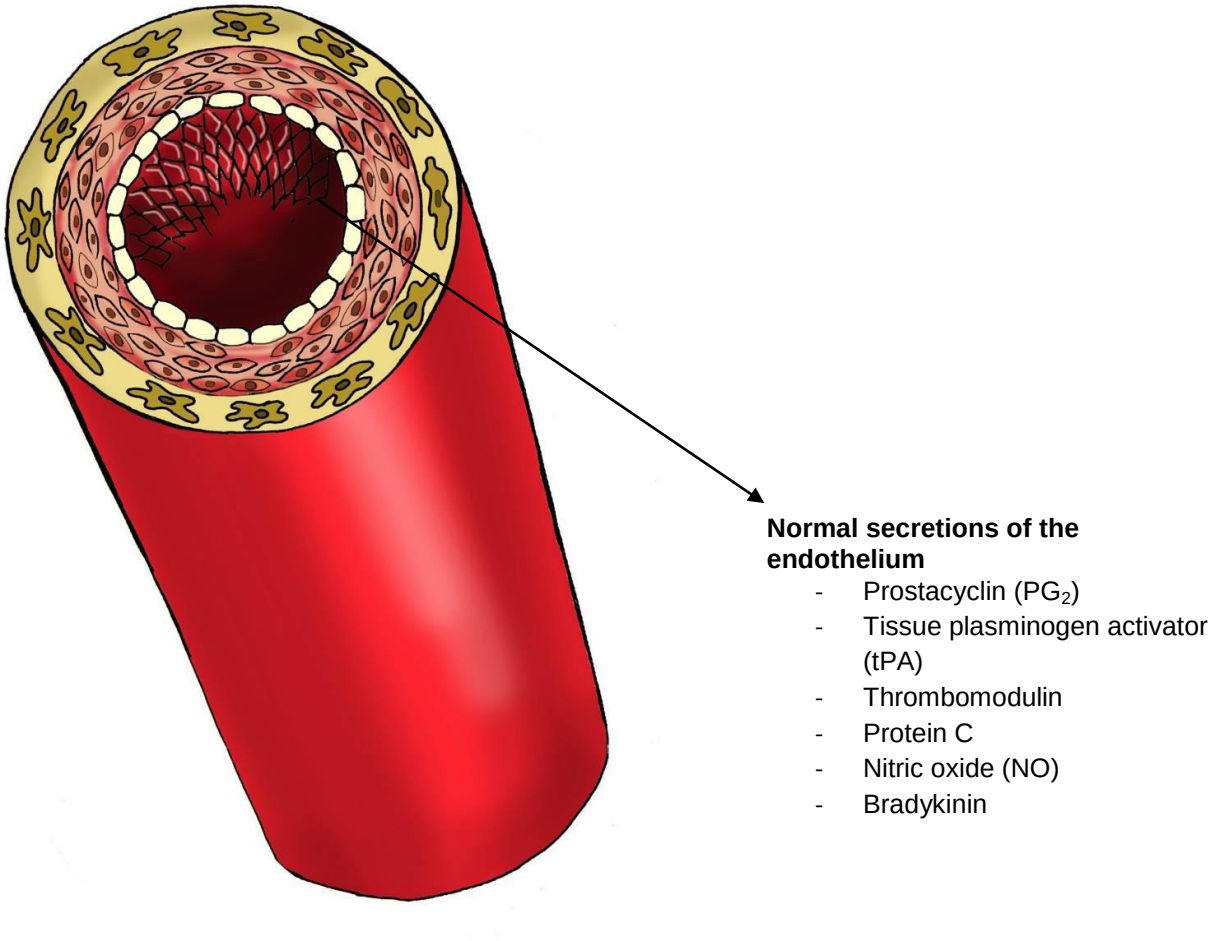
1.7. Endothelium

Endothelial cells are flat polygonal cells with large nuclei in the centre, surrounded by cytoplasm and plasmalemmal vesicles [90]. Numerous microvilli are situated on the luminal surface [90, 91]. The vascular endothelium harbours a complex structure with various biological properties. It also constitutes the smooth inner lining of the blood vessel and provides a vital role in haemostasis, vascular tone, inflammation and angiogenesis [92]. The endothelial layer maintains these functions by secreting a variety of products (Fig 6).

1.7.1. Endothelial function

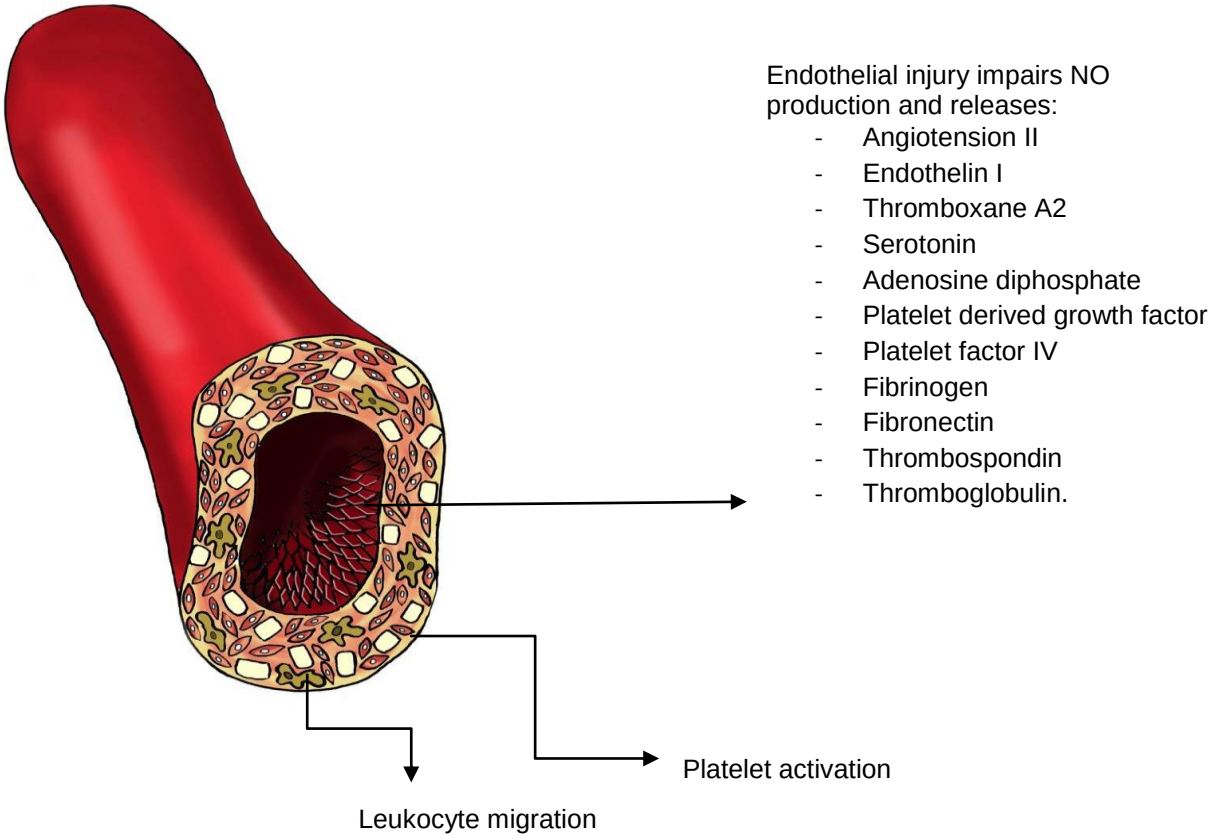
Endothelial cells secrete a range of molecules such as prostacyclin (PG_2), tissue plasminogen activator (tPA) and associated inhibitor PAI-1, thromobomodulin and protein C. These factors each play an important role in the maintenance of smooth blood flow and in preventing intravascular clotting [93]. One of the functions of a normal endothelium is to prevent vasoconstriction and thrombi development within the vessels [94]. The endothelium is also a primary site for the production of nitric oxide (NO), which is a potent vasodilator. Prostacyclin and bradykinin control vasodilation, inhibit platelet aggregation and prevent smooth muscle cell proliferation and migration within the vessel [94, 95]. The injured endothelium is also a site for the production of angiotensin II, endothelin I, thromboxane A2, serotonin, adenosine diphosphate (ADP), platelet derived growth factor, platelet factor IV, fibrinogen, fibronectin, thrombospondin and thromboglobulin. These induce vasoconstriction, platelet aggregation and thrombus formation, as well as smooth muscle cell proliferation and migration [96, 97]. When endothelial cells undergo surgical trauma, the production of NO is impaired, whilst angiotensin II, endothelin I and thromboxane A2 increase, resulting in intimal thickening and atherosclerosis [95, 98]. This cycle of events usually occurs within arterial circulation, but has also been demonstrated in the pathophysiology of LSV graft failure post CABG [98, 99].

Figure 6: Healthy endothelial layer and their secretions



This diagram highlights the major factors secreted by the healthy endothelium.

Figure 7: Secretions from the injured endothelial layer



This diagram highlights the major factors secreted from the damaged endothelium.

Endothelial cells play a vital role, not only when modulating haemostasis, cell proliferation, inflammatory and immunological processes in the vascular wall, but also in regulating underlying vascular smooth muscle tone [100-103]. The various secretions released by the endothelium (NO, PGI_2 and hyperpolarising factors) are collectively called endothelium-derived relaxing factors (EDRFs); these are able to protect vessels from pathological changes [104, 105]. If endothelial dysfunction or denudation occurs due to surgical trauma [101], excessive manipulation [100], or pressurised CO_2 tunnel use in closed EVH, this can in turn reduce synthesis and release of EDRFs, consequently leading to vascular spasm, thrombosis and atherosclerosis [106].

1.7.2. Endothelial injury

Endothelial trauma initiates platelet activation and the migration of leukocytes such as neutrophils, and monocytes (Fig 7) [107, 108]. Endothelial repair and the consequent cessation of platelet activation do not stop the process of intimal proliferation and medial muscle migration in vein grafts [99]. Injured endothelial platelet formation is inevitable because of the manipulation and distension of the vein during surgery [96].

The underlying aetiological factors surrounding intimal proliferation in vein grafts are less well defined when compared to arterial grafts [109]. Arterial grafts offer superior smooth muscle function by contracting and dilating according to pressure of the blood flow, thus preventing ongoing intimal proliferation when compared with vein grafts. This major functional difference enables arterial conduits to survive for longer periods than vein grafts [18, 19]. The major causes of vein graft failure were identified as excessive manipulation, ischaemia during the harvesting procedure and increased intraluminal pressure applied during grafting [110]. The progression of medial and neointimal thickening are continuous in the vein wall and may be primary causes of late graft failure post-CABG [111, 112]. However, certain types of venous diseases (i.e. phlebitis and varicosities) can also significantly affect the quality of vein conduits [113].

Intra-operative vein manipulation prior to grafting has been shown to inflict significant tissue damage [114]. This damage may lead to endothelial dysfunction, injury, denudation and smooth muscle cell injury, ultimately leading to intimal hyperplasia. It follows that surgeons must minimise the degree of manipulation during vein harvesting [115-118]. Many studies have advised adherence to the principles of “no touch or minimal handling techniques” [115-119]. This includes reducing the risk of local damage

caused by instrumentation or the surgical operator during preparation of the LSV. Numerous studies discuss the importance of limiting distension pressures to approximately 100mmHg during vein assessment following LSV harvesting [120-124].

1.7.3. Functional Integrity of the LSV

In addition to structural and morphological changes occurring in the harvested LSV, endothelial functional integrity is equally important [125, 126]. The quality of the endothelium in LSV conduits used for CABG can impact early or late graft occlusion [125, 126]. Vein harvesting techniques can potentially cause structural damage to the vessel wall leading to graft failure as shown in angiographic and ultra-structural studies, which reveal mural thinning and endothelial cell damage [13].

A histological study comparing EVH (n=88) with OVH (n=82) used hematoxylin-eosin staining to assess endothelial cellular continuity. Mason's trichrome staining was used to assess connective tissue and smooth muscle uniformity and elastin staining allowed examination of elastic lamina continuity. The researchers report that mild histological disruption occurred in all layers, but there was no significant difference between the two groups [127]. Moreover, a porcine histological study compared the endothelial integrity of conduits obtained by EVH (n=5) and OVH (n=5). Using light and electron microscopy, the researchers demonstrate no significant difference in endothelial integrity between groups [128]. The controversial argument surrounding this study is that performing EVH on a porcine leg is very difficult when compared to a human leg, and carries an increased incidence of vein trauma [128]. A further histological study comparing EVH (n=9) with OVH (n=5), using CD34 and factor VIII:vWF, detected a continuous endothelial layer and mild separation of medial smooth muscle fibres in EVH, yet no significant difference was observed when compared with OVH [129].

A prospectively randomised study [130] compared three different vein harvesting techniques (Group A - EVH, n=31; Group B - light coupled retractor, n=31 and Group C - OVH, n=33). All non-distended vein samples were stained using hematoxylin-eosin and Giemsa dyes to assess endothelial intima quality, cell dissociation and collagen fibre bundles. There was no significant difference in endothelial layer denudation of groups A and B when compared with group C. Several vein studies concentrating on the biological properties of impaired endothelial layers demonstrated that myointimal proliferation

affects short and long-term graft performance [125, 126]. Impairment of the endothelial layer in OVH conduits can occur as a result of pre or post-surgical preparation practices when determining vein quality [131, 132] or result from inappropriate manual handling of the vein [12, 133, 134]. Manderson *et al* [135] suggest that histological vein studies harvested using different minimally invasive techniques should be performed periodically at various times to assess endothelial integrity, since endothelial denudation leads to intimal and medial layer repair with neointimal thickening.

In contrast, a prospective study comparing the structural and functional viability of both EVH (n=5) and OVH (n=5) conduits demonstrate endothelial viability is significantly higher in OVH samples ($p<0.001$). Similar tests were performed with vascular endothelial cells using a fluorescence-based super vitality Live-Dead assay. The researchers concluded that CO_2 insufflation used in EVH may be responsible for impaired endothelial function [10]. The major concerns of this study are that the vein was harvested in the same leg using the closed tunnel CO_2 EVH technique performed above the knee, whereas the OVH technique was performed below the knee. This raises the issue of whether the CO_2 pressure affected the entire length of the LSV during EVH harvesting.

1.8. Benefits of CO2 insufflation in EVH

Certain EVH systems utilising CO_2 insufflation possess a number of technical advantages, such as maintaining an open working space free of subcutaneous tissues. This allows freedom of both hands to perform tissue dissection, isolation of the vein and surrounding branches[51]. CO_2 was first used in 1926 as an insufflating agent and its use soon became firmly established among all surgical specialities after the development of the automated insufflator system [137].

Table 2: An overview of the existing histological studies comparing EVH vs. OVH.

Author of the study	Sample size	Methods	Study findings	P values
Rousou *et al* [10]	n=5 (EVH) n=5 (OVH)	Immunofluorescence (caveloin, endothelial nitric oxide synthase, von Willebrand factor and cadherin) and Western blot techniques.	Esterase activity (cell viability) was significantly higher in OVH group.	p<0.001
Cable *et al* [128]	n=5 (EVH) n=5 (OVH)	Light and electron microscopy. Verhoeff-van Gieson stain and hematoxylin-eosin stain	No significant loss of endothelial cell or connective tissue in both groups.	p=0.68
Meyer *et al*[129]	n=9 (EVH) n=5 (OVH)	Histological appearance (Hematoxylin, eosin, Verhoeff's elastic, Gomori's one-step trichrome) and immunohistochemical studies (factor VIII:vWF (von Willebrand factor protein)) and CD34 stain used.	No difference in the intima, media and adventitia layer between both groups.	No statistical analysis was performed in this study.
Griffith *et al* [127]	n=88 (EVH) n=82 (OVH)	Hematoxylin-eosin (endothelial), Mason's trichrome (smooth muscle) and elastin (elastic lamina) staining	Minor histological alterations but more significantly, no differences between both groups.	p=0.88
Crouch *et al* [50]	n=4 (EVH) n=4 (OVH)	Immunoperoxidase stains (vimentin, Factor VIII and CD31).	No traumatic effect on the vein wall following hematoxylin-eosin staining No significant difference in vein structural integrity between both groups.	No statistical analysis of histological data was performed in this study.
Fabricius *et al* [130]	a. n=25 (EVH) b. n=29 (light coupled retractor) c. n=30 (OVH)	Electron microscopy, Hematoxylin-eosin and Giemsa stains.	No significant difference found in all groups. The endothelial layer is preserved.	Endothelial denudation (>90%) A. 10.7%(3) B. 6.8%(2) C. 13.0%(4).
Rinia-Feenstra *et al* [136]	n=6 (OVH) n=4 (mediastinoscopy) n=5 (EVH)	8ml organ bath filled with oxygenated Krebs-Henseleit solution of 37°C (pH 7.4).	No significant differences in the vascular integrity between these groups.	p=0.46

1.8.1. Effects of CO_2 on endothelial tissue during minimally invasive surgical procedures.

A major concern regarding the use of CO_2 insufflation is gas embolism. Lin *et al* [138] reported a 4% incidence of CO_2 embolism following EVH, although this can now be monitored carefully with intra-operative trans-oesophageal echocardiography (TOE) [139]. Studies have reported a sudden cardiac arrest rate of 1% as a result of these complications [140]. The problem of gas embolism can now be identified earlier and the high CO_2 level in the blood corrected by making significant changes in ventilatory and haemodynamic parameters. There are no cardiovascular studies focusing directly on CO_2-induced morphological and structural changes in the LSV, yet there are various CO_2 studies in general surgery. Recent reports suggest that the use of CO_2 in laparoscopic surgery is associated with a number of adverse structural, metabolic and immune derangements [141, 142]. The adverse changes include structural alterations in the mesothelial lining, pH disturbances and alterations in peritoneal macrophage responsiveness [141, 142].

Cardiopulmonary physiology during laparoscopic surgery has been studied extensively in recent years with no major life threatening problems observed [143-145]. Interestingly, certain studies have found that after CO_2 insufflation induced pneumoperitoneum and mesothelial cell swelling. Furthermore the basal lamina was exposed on scanning electron microscopy [146]. The exposure of the basal laminal layer can cause conversion of β-lipoprotein into a modified form, subsequently generating chemoattractant, inflammatory molecules and triggering endothelial cell dysfunction. This plays an important role in vascular endothelial atherosclerosis [147]. CO_2 insufflation can also alter intracellular and extracellular pH and calcium levels [148], which regulate cellular functions such as ATP production, cell cycle progression, intracellular signalling and apoptosis [148-150]. Elevations in pH, $PaCO_2$ and HCO_3 level promptly normalise following desufflation, and can be controlled by mechanical ventilation during the procedure [151].

Volz *et al* [152] clearly stated that characteristic ultra-structural changes to the peritoneal surface following CO_2 insufflation cause morphological alterations to the peritoneum, which were evident via scanning electron microscopy 2 hours post-insufflation. The morphological changes included swelling of mesothelial cells and widening of the intercellular junctions with subsequent exposure of the basement membrane. It is not clear whether these changes are attributable to an inherent property of the direct pressure effect of CO_2 or the temporary stretching and expansion of the peritoneal surface

area by pressure created in the pneumoperitoneum. The mesothelial monolayer and human peritoneal mesothelial cells prevent the infiltration of cancer cells [153] and peritoneal metastasis [154], therefore, retaining their integrity is of paramount importance. Injury to these layers can induce adhesion formation, TGF Beta 1, IL-1B and IL-8 production and release, which promotes peritoneal carcinomatosis [154, 155].

A murine study comparing insufflation of air (n=3), helium (n=3), CO_2 (n=9), and laparotomy (n=9), alongside a control group receiving sole anaesthesia (n=9) demonstrated that CO_2 induces distinct morphological changes to the hepatic vascular endothelium [156]. This included dilatation of intercellular clefts, irregular endothelial cell arrangement, increased platelet adherence and free tumour cells, which may lead to liver metastasis. The study demonstrated that these morphological changes were caused not only by the reduction in portal blood flow induced by increased intra-abdominal pressure, but also by CO_2.

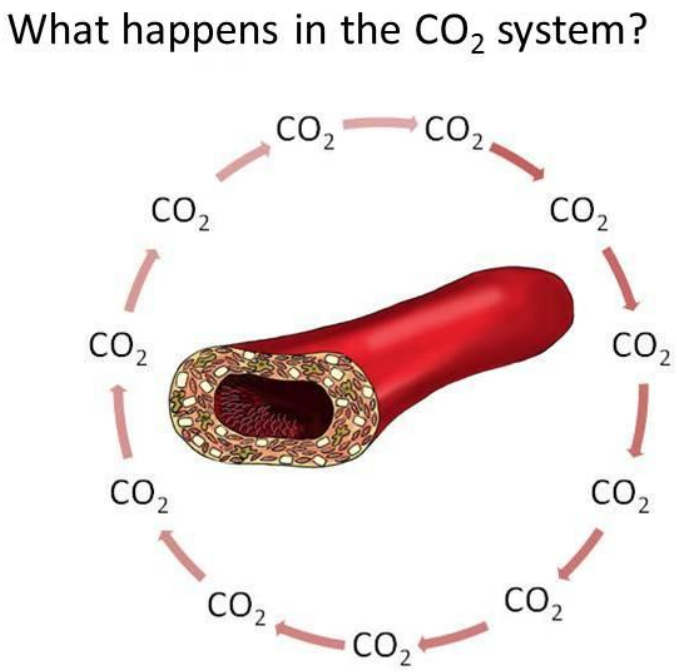
1.9. Hypothesis

There is a paucity of histological studies examining the effects of CO_2 pressure on the LSV for CABG surgery. It is not clear from general surgical studies whether the morphological and structural changes are due to the effect of direct pressure of CO_2 or temporary stretching of the abdomen. The effect of CO_2 on endoscopic LSV surgery is still unknown and more studies are required to demonstrate the mechanism of graft failure by various EVH techniques. To test this hypothesis, we designed a prospective, non-randomised study of patients undergoing open tunnel CO_2 and closed tunnel CO_2 endoscopic vein harvesting for CABG.

1.10. Aim of the project

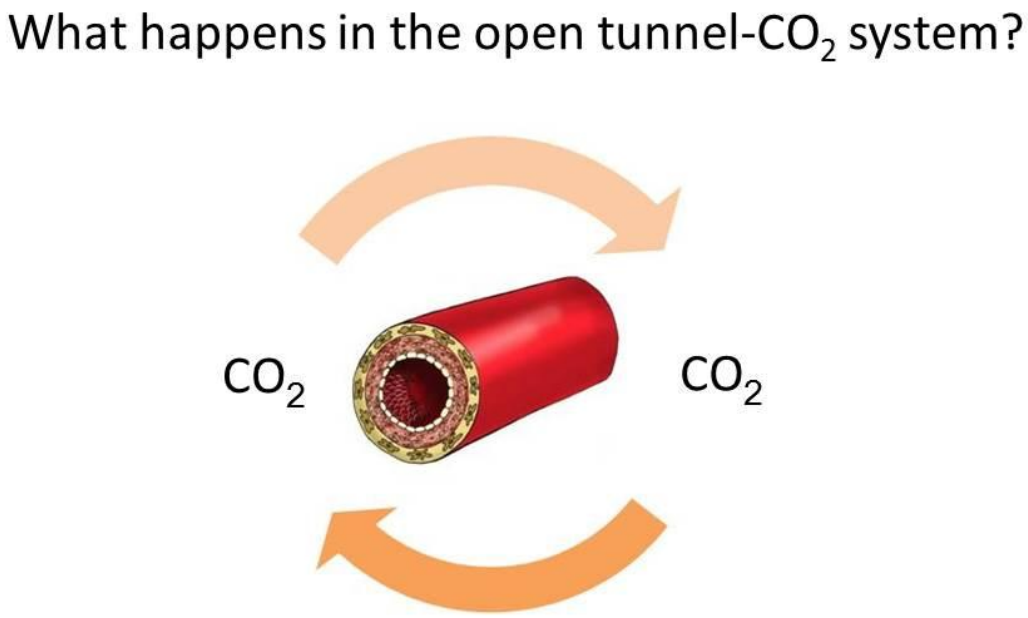
The primary aim of this research project (appendix 1) is to characterise the effect of CO_2 pressure on the LSV (figs 8 & 9) by comparing the results of harvesting with open tunnel CO_2 and closed tunnel CO_2 EVH methods. The primary outcome measure of this study is to assess and compare the incidence of endothelial dysfunction and denudation between the EVH groups, which can in turn lead to graft failure (fig 10). The secondary aim of this project is to clinically follow up these patients for 6 months following surgery to assess mortality and the incidence of repeat angina and myocardial ischaemia.

Figure 7: Pressurisation of the vein during the closed tunnel CO_2 EVH technique



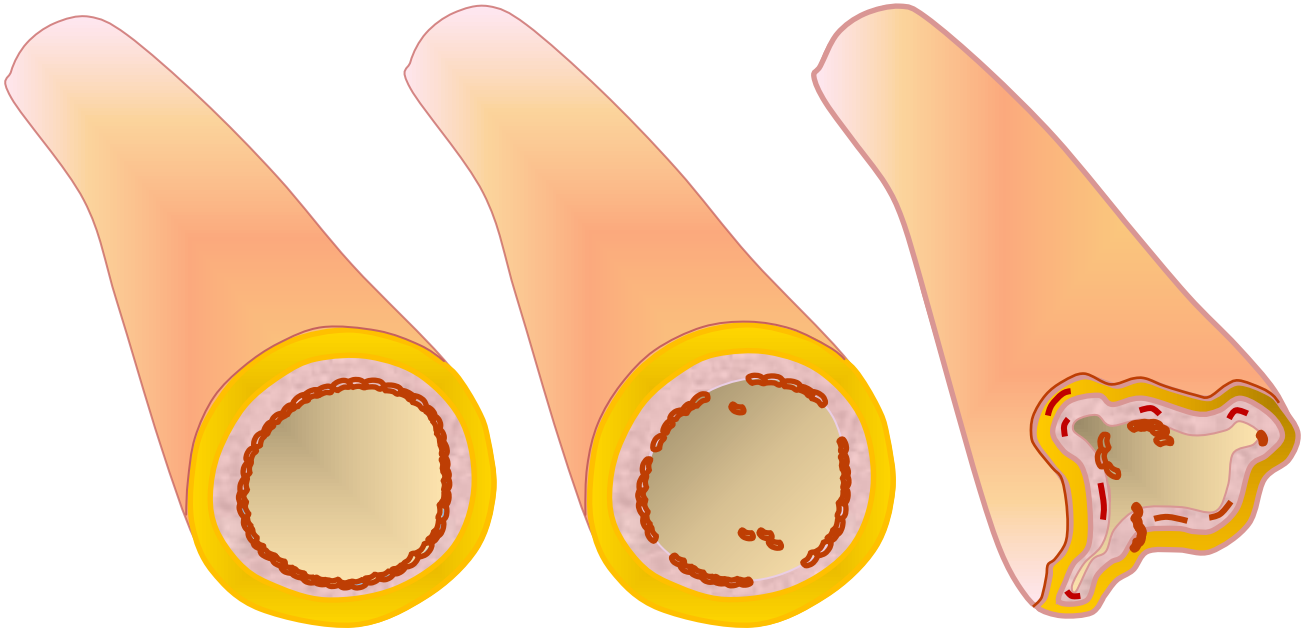
This figure illustrates the effect of the high pressure CO_2 tunnel in the closed EVH technique. The force applied to the LSV subsequently induces vessel collapse.

Figure 8: The non-pressurised vein in open tunnel CO_2 EVH technique.



This figure illustrates that the non-pressurised open EVH system does not exert significant force on the LSV. As a result, no vessel collapse occurs in this system.

Figure 9: A cross sectional view of the long saphenous vein obtained by three different vein harvesting methods.



a. LSV harvested by OVH
Continuous endothelial layer

b. LSV harvested by open CO_2
Intermittent endothelial layer

c. LSV harvested by closed CO_2 - Denuded endothelial layer

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

2.1. Ethics and Informed Consent

This research has been approved by the National Research Ethics Committee (NREC). All patients recruited in this study provided written informed consent.

2.2. Samples and Recruitment (Appendix 2)

From a consecutive group of 820 CABG patients, 140 patients were prospectively recruited into this non-randomised study into two groups (70 in each arm). These patients were identified from the cardiac annual waiting list after their initial pre-operative clinic visit. All procedures were performed by a surgical care practitioner in a single centre. Exclusion criteria included emergency CABG surgery, clinically diagnosed varicose veins and surgically removed varicose veins. Inclusion criteria: all other patients were included, unless they refused to participate in the study.

2.2.1. Recruitment

Total fellowship period is 24 months (part time).

Approximately 16 CABG surgeries carried out every 3 days at UHSM, from which 3 patients were recruited for the study each week. This allowed almost 154 patients to be recruited in a thirteen month period (which accounted for a 10% drop out rate from the study due to loss of contact, change of GP and death). This enabled the follow-up period to be completed for the final patient after 1 year and 9 months. Consequently, this allowed 6 months to finalise the analysis.

2.2.2. Sample size, power calculation and justification

With 70 patients in each of the two groups (EVH_1 and EVH_2), i.e. 140 in total, the study would have 80% power to detect differences in the percentage of conduits with zero endothelial integrity of 20% or more (for example 20% vs. 40%). This calculation is based on a comparison of just two groups using a simple chi-square test with continuity correction at the 5% level of significance, and thus no allowance is made for testing differences between the two groups in pairs, using three pair-wise comparisons.

In order to incorporate a 10% drop-out, approximately 155 patients in total would need to be recruited, excluding an allowance for multiple comparisons.

2.2.3. Duration of treatment and justification

All patients will be followed up at 3 and 6 months post-cardiac surgery. We selected these time points on the basis of results gained from our own research studies and those of other centres. Any early histological aberration, such as graft occlusion, caused by trauma to the grafted vein typically manifests clinically within three months following CABG. The downstream effect of these structural changes can present within a year of surgery as MI, recurrent angina pain and graft failure. We will be using the validated Major Adverse Cardiac Events (MACE) questionnaire to collect clinical and patient reported outcome data.

2.2.4. Funding for this study

The Heart Ticker Club charity at Wythenshawe hospital provided financial support for laboratory consumables. The Cardiothoracic Transplant Research fund provided support to cover clinical consumables costs associated with endoscopic vein harvesting.

2.2.5. Group allocation

Group EVH1: This group consisted of 70 patients and utilised the closed CO_2 tunnel EVH system (Vasoview Haemopro (VH-3000) Maquet Cardiovascular, LLC, Wayne, NJ).

Group EVH2: This group consisted of 70 patients but instead utilised the open CO_2 tunnel EVH system (Clear Glide (KTV-15), Sorin, Milano, Italy).

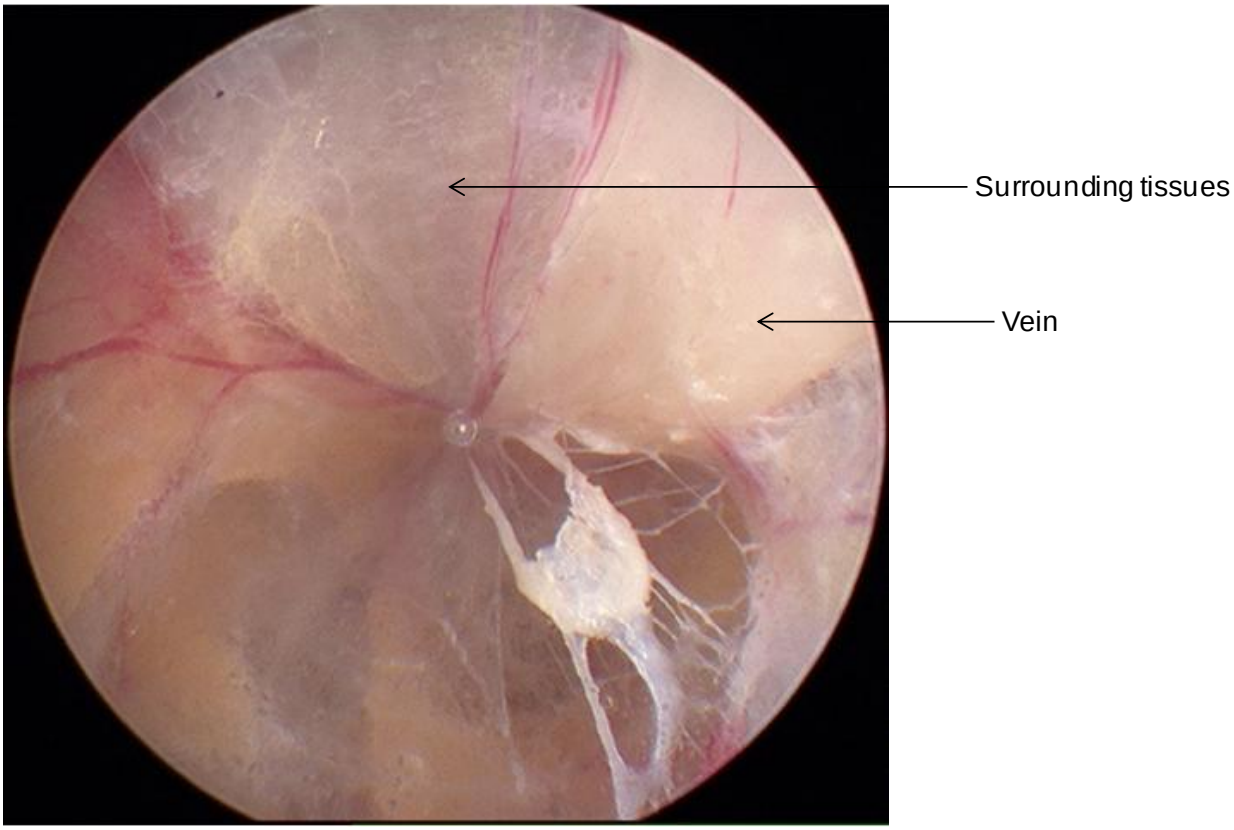
2.2.6. Surgical techniques

2.2.6.1. Closed CO_2 tunnel: (Group EVH1)

A 2-3cm skin incision was made just below the knee joint. The long saphenous vein was exposed and dissected using a West retractor and Langenbeck retractor. An intravenous bolus of 5000 IU heparin was administered systemically just before sealing the skin incision port, to reduce the intraluminal clot strand formation inside the vein during CO_2 insufflation [157]. A 30mm, 0° endoscope tipped with a sharp, clear dissecting cone, was inserted through the skin incision. After a few centimetres of anterior dissection, the balloon was inflated to seal the incision port. The CO_2 was

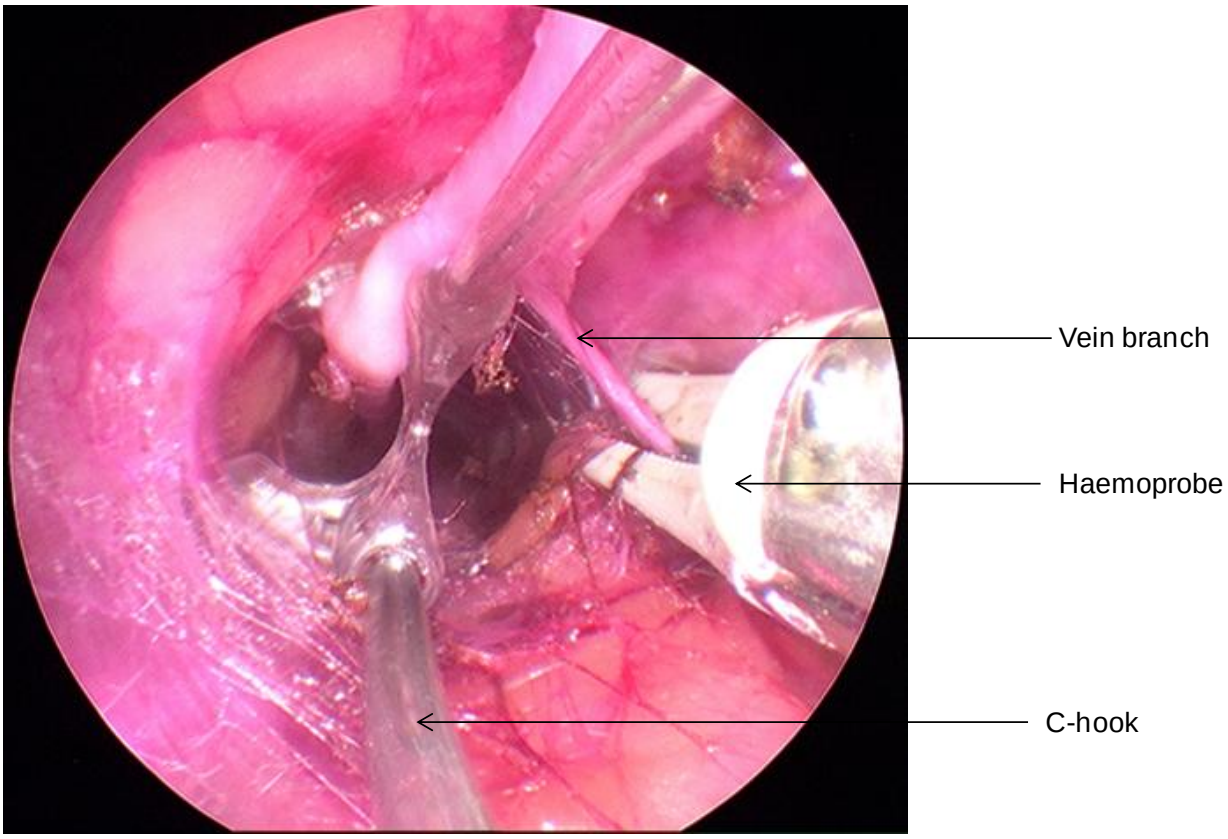
insufflated using an insufflator machine set at a pressure of 12mmHg and a flow rate of 3 litres per minute, to create a pressurised tunnel (Figure 11). The vein was dissected from the surrounding tissues, anteriorly and posteriorly, until reaching the sapheno-femoral junction in the groin. The length of dissection depends upon the length of vein required for surgery. The vein side branches were divided and coagulated using a haemopro diathermy probe (Figure 12). The completed vein graft was then disconnected from the main branch using a stab and grab technique which involves a 3-4mm skin incision just below the groin crease using mosquito artery forceps (Figure 13). The vein was checked for tears and avulsion using 30ml of heparinised venous blood at a pressure of 10-20mmHg. The side branches were tied and secured using 4/0 vicryl ties and titanium ligaclips. The vein was handed over to the surgeon to check again for any leakages using the same technique. Any tears were sutured with 8/0 prolene sutures and veins with excessively large tears were discarded, whilst an additional vein conduit was harvested from the opposite leg using the bridging technique. The vacuum wound drain (size 10) was inserted into the leg tunnel to stop hematoma formation. The leg wound was closed using 2/0 vicryl and 3/0 skin sutures. A Tegaderm™ dressing and pressure bandage was applied for 48 hours as per local wound care policy. Prophylactic antibiotics were administered pre and post-operatively using a single dose of either Gentamycin or Flucloxacillin. Allergic patients were given Vancomycin as per departmental protocol.

Figure 10: Pressurised CO_2 tunnel and venal collapse.



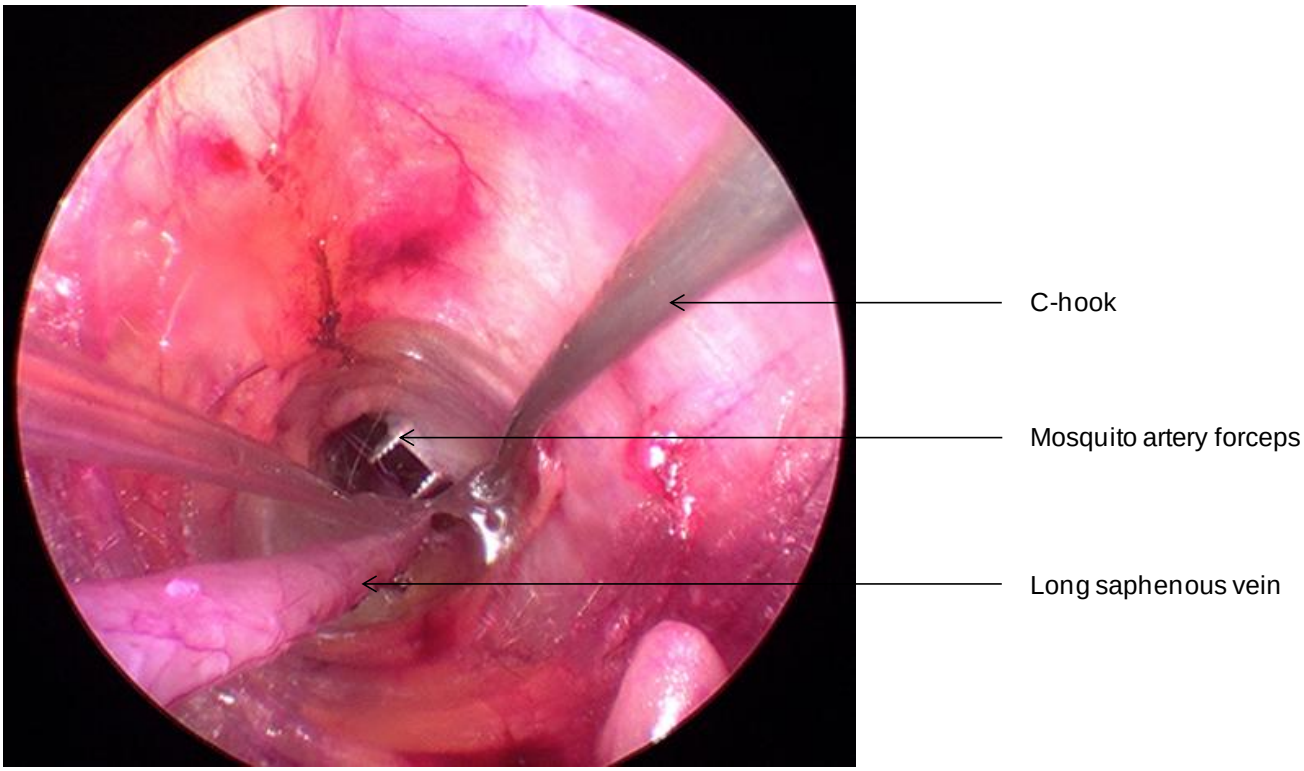
This image demonstrates the view within the closed tunnel CO_2 system. The high pressure gas collapses the vein and forces it to one side.

Figure 11: Vein branch division and coagulation using the haemoprobe.



This image illustrates the view inside a closed CO_2 tunnel and the traction applied to the vein during side branch dissection.

Figure 12: The stab and grab technique using mosquito artery forceps

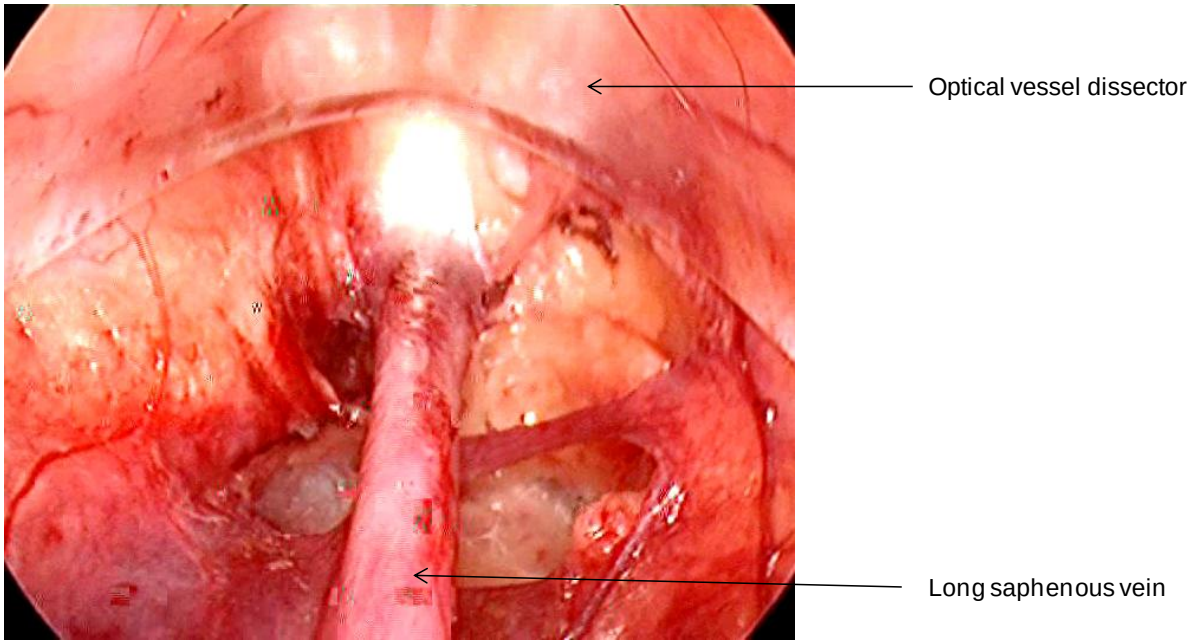


The image illustrates the use of mosquito artery forceps to cut the vein inside the pressurised tunnel.

2.2.7. Open CO2 tunnel: (Group EVH2)

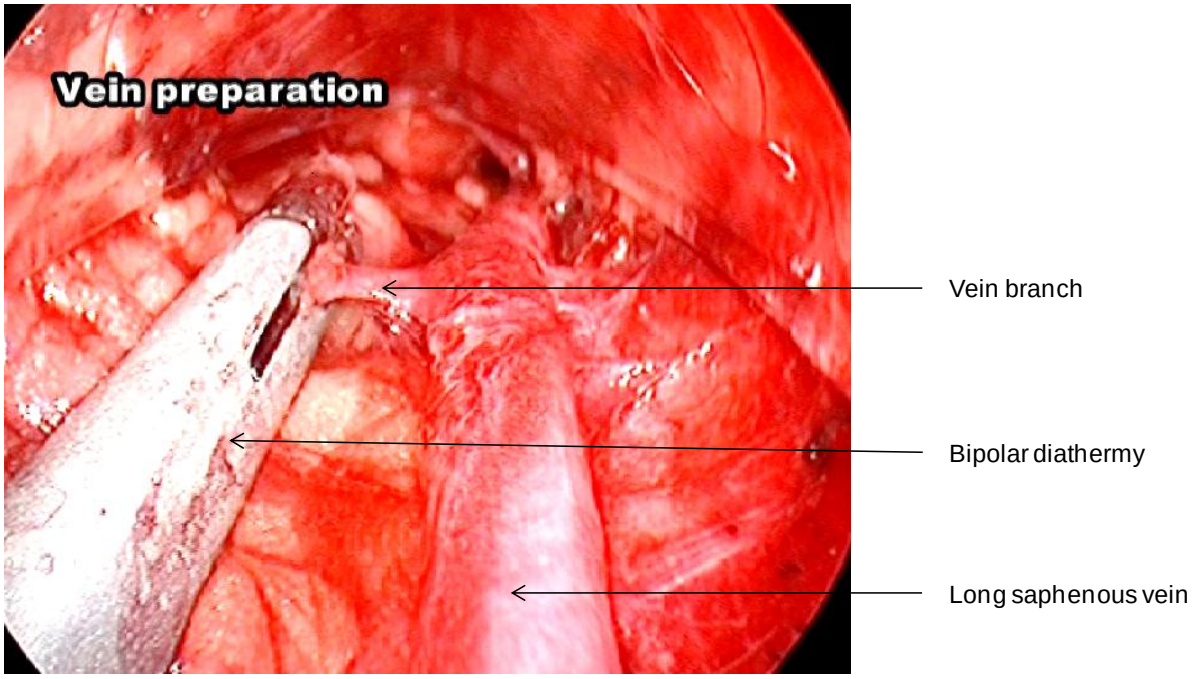
A 2-3 cm skin incision was made just above the knee joint. The long saphenous vein was exposed and dissected using a West retractor and a Langenbeck retractor. A 30mm (0°) telescope with a Clearglide dissecting retractor was introduced through the skin incision. The CO_2 insufflator was set at a continuous flow rate of 3 litres per minute and a pressure of 0mmHg. The vein was dissected from the surrounding tissue anteriorly and posteriorly until reaching the sapheno-femoral junction in the groin (Figure 14). The length of dissection depends upon the length of vein required for surgery. The vein side branches were divided and coagulated using a bipolar diathermy probe (Figure 15). The vein graft was then disconnected from the main branch via the stab and grab technique, which involves a 3-4mm skin incision just below the groin crease using mosquito artery forceps. The vein was checked for any tears and avulsion using 30 ml of heparinised venous blood with a pressure of 10-20mmHg. The side branches were tied and secured using 4/0 vicryl ties and titanium ligaclips. The vein was handed over to the surgeon to examine for any leakages using the same technique. Any tears were sutured with 8/0 prolene suture and veins with excessively large tears were discarded and an additional vein conduit harvested from the opposite leg using the bridging technique. The vacuum wound drain (size 10) was inserted into the leg tunnel to stop hematoma formation. The leg wound was closed using 2/0 vicryl and 3/0 skin sutures. A Tegaderm™ dressing and pressure bandage was applied for 48 hours as per local wound care policy. Prophylactic antibiotics were administered pre and post-operatively using a single dose of either Gentamycin or Flucloxacillin. Allergic patients were given Vancomycin as per departmental protocol.

Figure 13: Long saphenous vein dissection with optical vessel dissector in non-pressurised CO_2 tunnel EVH



This image illustrates the view inside the open CO_2 tunnel system. The vein lies in the normal plane and is not pushed against the tunnel wall. An optical vessel dissector is used to harvest the vein.

Figure 14: Bipolar vein dissection in non-pressurised CO_2 tunnel EVH



This image demonstrates the use of a bipolar cautery probe to cut the vein side branch within the non-pressured tunnel.

2.2.8. Vein handling and mechanical stress

Group EVH1: The vein was exposed to a 12mmHg pressurised CO_2 tunnel for a minimum of 30 minutes and was handled manually by both the harvester and surgeon. In addition, the vein was also subjected to a 30mmHg insufflation pressure to examine the vein quality prior to grafting. Cardioplegia pressure ranged from 0mmHg to 150mmHg during bypass grafting.

Group EVH2: Rather than using a CO_2 pressurised tunnel, the vein was dissected manually by a harvester. In addition to the manual dissection, the vein sustained a 30mmHg insufflation pressure during examination of the vein. Cardioplegia pressure ranged from 0mmHg to 150mmHg during bypass grafting.

These aforementioned factors were recorded and measured to assess for any difference between the groups. Numerous scientific publications have suggested that manual handling and mechanical stress during harvesting can change the biochemical properties of the LSV, which subsequently leads to graft occlusion [157-159].

2.3. Clinical Methodology

2.3.1. Data collection

All general demographics including gender, age, race and body mass index were collected from patient notes. Pre-operative risk factors such as diabetes, peripheral vascular disease, hypertension, hypercholesterolaemia, previous MI, previous percutaneous transluminal coronary angioplasty (PTCA), Parsonnet score (a simplified Canadian risk scoring system to estimate the cardiac surgical mortality risk) and European system for Cardiac Operative Risk Evaluation (Euroscore) were recorded. Intra-operative data including the number of grafts planned pre-surgery, number of coronary vessels grafted, type of conduits used and details of the cardioplegia were recorded. All this data was prospectively collected into a relational database. In-hospital mortality and community mortality outcomes were obtained from validated registry data and post-mortem reports.

Long-term Major Adverse Cardiac Events (MACE) outcomes were measured for this study at 6 months post-surgery. MACE was defined as post-CABG recurrent angina, MI, target vessel revascularisation, coronary/vein graft stenting (for the partially occluded grafts) and death [160]. Repeat

angina was classified using Canadian Cardiovascular society grading system score (CCS) [161]. This is a validated scoring system for standardisation of angina grade ranging from I-IV. Class I indicates angina with sustained, strenuous exertion, class II represents slight limitation with angina upon vigorous action, class III represents moderate limitation with symptoms during everyday activity and class IV, which indicates severe limitation and the inability to perform any activity with angina even at rest (4).

Breathlessness was assessed using the New York Heart Association (NYHA) scaling system [162]. This is a validated scoring system for standardisation of breathlessness ranging from I-IV. Class I indicates no limitation of physical activity, class II represents a mild shortness of breath and slight limitation of physical activity, class III indicates marked limitation of physical activity and class IV indicates severe limitation, with the inability to carry out any physical activities.

Magnetic Cardiac Resonance Imaging (MRI), repeat angiogram and echocardiogram (ECHO) results were obtained via the UHSM cardiology database. The American College of Cardiology (ACC) and American Heart Association coronary lesion scoring system was used to identify the quality of coronary vessels in pre and post-operative angiographic pictures. This system is based on parameters such as length of the lesion, eccentricity, angulation, calcification, side branch involvement and severity of stenosis. The lesions are classified as Type A (discrete, <10mm), Type B (tubular, 10-20mm) and Type C (diffuse, >2cm) [163].

2.3.2. Telephone data collection

Patients were followed up after 6 months following surgery using a modified validated scoring system, which includes the MACE questionnaire. The quality of life scoring system included patient involvement in physical activities post-surgery such as housework, lifestyle, shopping, driving and gardening. Emotional and social activities were also taken into consideration to compare their level of improvement following surgery.

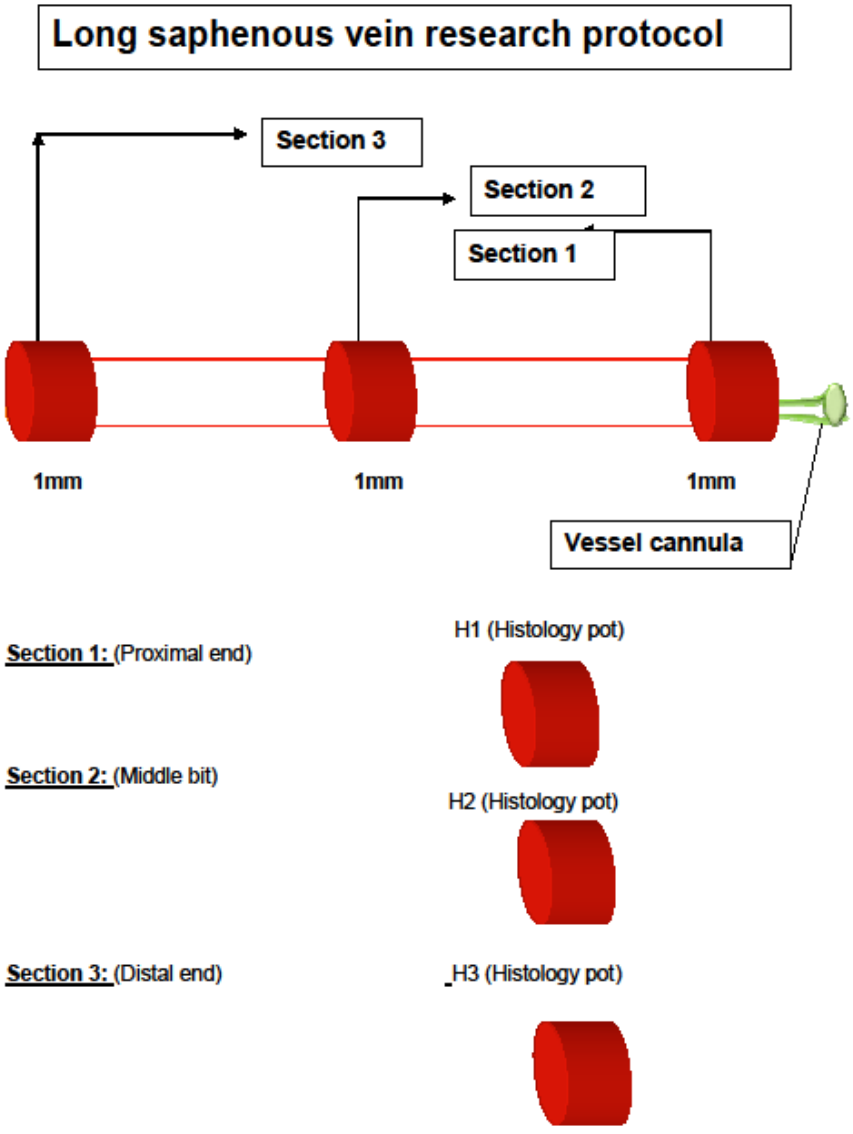
2.4. Immunohistochemistry methodology

All immunohistochemical preparation and analysis protocols were provided by the Histology Department at the UHSM.

2.4.1. Sample collection

Once the LSV was harvested from the donor leg, the first specimens were taken from the proximal end (H_1) and distal end (H_3) of the vein to represent the damage caused by the endoscopic technique alone (Figure 15). The remaining LSV was prepared for grafting using cardioplegia solution (0-150mmHg) and was manually manipulated during surgery. At the end of surgery, a H_2 sample was taken to be utilised in the study to assess for endothelial damage caused by the entire surgical procedure (including endoscopic technique, surgical handling and the pressure of cardioplegic solutions).

Figure 15: Long saphenous vein tissue research protocol



2.4.2. Sample storage

The 1cm vein samples were cut and placed directly into a 4% formalin solution (1:10 ratio of formalin and distilled water pH-7.4). Storing the samples in formalin prevents alteration of the tissue structures through decomposition by chemical cross-linking of proteins and the removal of water.

2.4.3. Dehydration

All traces of water were removed by embedding the samples in paraffin. The samples were washed through a series of alcohol concentrations, ranging from 30%, 50%, 70%, 80%, 90%, 95% and 100% for two hours in each solution. Finally, the samples were placed in a 100% ethanol solution to ensure complete water removal.

2.4.4. Paraffin embedding

Once the dehydration procedure was completed, the samples were washed in xylene to clean the tissues in preparation for immersion in paraffin wax. Initially, the samples were soaked in a 50:50 solution of absolute ethanol and xylene for 3 hours. The samples were transferred to 100% xylene and then into a 50:50 solution of xylene and paraffin. Finally, the samples were immersed in 100% paraffin at 56°C for three hours to allow infiltration of the samples. The samples were transferred to an embedding mould (small plastic cassette) and melted paraffin was poured over the mould to form a block. These blocks were allowed to cool before sectioning.

2.4.5. Sectioning

A paraffin microtome was used to section the sample blocks. The samples were cut into 4µm thin cross sections, moved to a warm water bath (50°C) and placed on to poly-l-lysine histology slides. These special histology slides were used instead of standard histology slides to reduce the loss of tissue during microwave preparation for antigen retrieval. These slides are highly recommended for immunohistochemistry staining.

2.4.6. De-waxing and rehydration of slides

Each slide was soaked and de-waxed twice in 100% xylene for 10 minutes each, then rehydrated in 100% ethanol solution for 6 minutes and 95%, 70%, 50% solutions for 3 minutes respectively. All

slides were soaked and rinsed under running tap water for 5 minutes to remove any residual alcohol from the tissues.

2.4.7. Incubation and washing

To block endogenous peroxidase activity, all the slides were incubated in a freshly prepared 3% solution of hydrogen peroxide (H_2O_2) and industrial methylated spirit (IMS) (ratio of 1:10- H_2O_2:IMS) for 30 minutes. Finally, the slides were washed gently under running tap water for 5 minutes.

2.4.8. Antigen retrieval

The slides were placed in a metal rack and soaked in a pre-warmed 0.01M citrate buffer (pH6.0) and heated in a microwave at 800W for 30 minutes. After cooling, the slides were removed from the buffer and flicked to remove excess solution. The sample sections were circled with a PAP pen (liquid repellent slide marker pen), to ensure the blocking agent remained within the circle. The samples were incubated in 2.5% ready-to-use normal horse serum (VectorLabs Immpress) for antigen retrieval at room temperature for 30 minutes.

2.4.9. Primary antibody incubation

After removing any excess horse serum, the CD34 antibody (LEICA™) was diluted in a ratio of 1:30, using the DAKO™ antibody diluent and vortexed to thoroughly mix the solutions. The primary antibody was added to the samples and incubated for 1 hour 10 minutes. All slides were washed in 0.05M TRIS buffer (pH 7.6) solution and placed on a mechanical shaker for 15 minutes to remove excess primary antibodies. Few *in vivo* studies have been published with the intention of systematically comparing the expression and distribution of endothelial cell (EC) markers found in different vascular beds in normal human tissues [164]. These studies also found that the strongest expression of CD34 was found in EC of arteries, veins, arterioles and venules.

2.4.10. Secondary antibody incubation

One to three drops of ImmPRESS™ universal anti-mouse/rabbit IgG reagent (polymerised reporter enzyme) were added to the samples and incubated for 30 minutes in a moist box. The slides were then washed in TRIS buffer for a further 15 minutes.

2.4.11. Substrate incubation, counterstaining and mounting

The slides were incubated in an Immpact DAB (3, 3'-diaminobenzidine, peroxidase substrate solution - Vector Labs, Cat#SK-4100) to bind the secondary antibody. This DAB substrate solution was prepared by combining 1ml chromogen substrate and 1 drop peroxidase (enzyme). This solution was vortexed to ensure proper mixing. These slides were then incubated for 5 minutes to attain adequate sample staining intensity. The slides were counterstained by dipping in haematoxylin for 1 minute and immediately washed under running tap water.

The slides were left to dry for 4-6 hours and mounted using a mixture of distyrene, plasticiser and xylene (DPX), a colourless synthetic resin mounting medium. The slides were finally left to dry overnight.

2.4.12. Scoring

Each slide was allocated a random number before any assessors assigned a score. Three independent assessors, who were not involved in this research project, blindly evaluated the outcomes. The slides were placed under microscopy and assessed for endothelial integrity (variability was <15%). A validated scoring system proposed by Fischlein *et al* [165] was adopted and modified using the following criteria: 0 – no endothelium, 1 – islands of endothelium, 2 – loosely netted endothelium, 3 – partially confluent endothelium and 4 – completely confluent endothelium.

2.5. Statistical analysis

2.5.1. Immunohistochemistry

All data was expressed as mean percentage, with differences between the two sets of results determined using the independent student's T test. P-values of <0.05 were considered statistically significant. SPSS 19.0 software was used for all calculations (SPSS, Inc., Chicago, IL).

2.5.2. Clinical data analysis

Categorical parameters were summarised using frequencies, percentages and cross tabulations. Categorical data was compared using Fisher's exact tests or Linear Trend tests. The normality of continuous data was assessed and normally distributed data were summarised showing means and

standard deviations. Continuous variables were analysed using the Student's t-test. Non-parametric data were summarised using medians and compared using Mann-Whitney U tests.

All statistical analysis was performed using SPSS version 15. All analysis used the conventional two-sided 5% significance level.

The patients who required conversion to OVH have been excluded from this study. There were a total of 15 patients across the two groups who were excluded from the clinical and sample collection to reduce the bias.

CHAPTER 3

RESULTS

3. RESULTS

3.1. Clinical Results

Baseline demographic characteristics and the presence of important risk factors were compared between closed CO_2 tunnel and open CO_2 tunnel groups. This was performed in order to determine whether the lack of randomisation resulted in significant demographic variation between the groups. A statistically significant difference between the groups was observed regarding the reason for surgery (Table 3).

Table 3: A comparison of categorical demographics and risk factors between surgical groups

Procedure	**Categories**	**Closed CO_2 tunnel (EVH1, n=70)**	**Open CO_2 tunnel (EVH2, n=70)**	**p-value**
Gender	Male Female	59 (84.3%) 11 (15.7%)	55 (78.6%) 15 (21.4%)	0.392 @
Post-menopausal	No Yes	0 (0.0%) 11 (100.0%)	3 (20.0%) 12 (80.0%)	0.238 @
Smoking	No smoking Ex-smoker Current	21 (30%) 44 (62.9%) 5 (7.1%)	15 (21.4%) 48 (68.6%) 7 (10.0%)	0.180 @
Surgery	CABG CABG + valve Redo CABG	65 (92.9%) 4 (5.7%) 1 (1.4%)	61 (87.1%) 8 (11.4%) 1 (1.4%)	0.350 @
Reason for Operation	Elective Urgent	56 (80.0%) 14 (20.0%)	42 (60.0%) 28 (40.0%)	0.010* @
@ - p-value from Fisher's Exact test * Statistical significance at 5% level				

This table illustrates the results of comparing categorical demographic data between EVH groups 1 and 2. The Fisher's exact test was used to calculate statistical significance.

Table 4: A comparison of continuous demographics and risk factors between surgical groups.

Categorical basic demographics		**Closed CO_2 tunnel (EVH1, n=70)**	**Open CO_2 tunnel (EVH2, n=70)**	**p-value**
Age at the time of surgery (years)	Mean	66	68	
	Standard Deviation	10	9	
	Minimum	45	48	0.169 (+)
	Maximum	85	86	
Height (cm)	Mean	168.8	169	
	Standard Deviation	9.0	9	
	Minimum	148	150	0.681 (+)
	Maximum	200	185	
Weight (kg)	Mean	80.70	79.97	
	Standard Deviation	13.22	15.14	
	Minimum	54.0	49.0	0.743 (+)
	Maximum	121.0	126.0	
Body Mass Index (kg/m^2)	Mean	28.255	27.752	
	Standard Deviation	3.711	4.284	
	Minimum	19.72	19.88	0.430 (+)
	Maximum	38.12	38.89	
Parsonnet Score	Mean	8.5	10.0	
	Minimum	0	0	0.691 (&)
	Maximum	38	31	
Logistic Score	Median	2.30	3.10	
	Minimum	0.9	0.9	0.162 (&)
	Maximum	30.3	25.8	
+ p-value from Student's T-test & - p-value from Mann-Whitney U test				
* Statistical significance at 5% level				

This table illustrates the results of comparing continuous demographic data between EVH groups. The Student's T-test and the Mann-Whitney U test were used to determine significance.

Table 5: A comparison of basic cardiac risk factors between EVH groups.

Categories		**Closed CO_2 tunnel (EVH1, n=70)**	**Open CO_2 tunnel (EVH2, n=70)**	**p-value**
Diabetes	No	56 (80.8%)	51 (72.9%)	0.426 (+)
	Yes	14 (20.8%)	19 (27.1%)	
Angina (Canadian Classification System)	None	3 (4.3%)	4 (5.7%)	0.369 (#)
	Class I	16 (22.9%)	10 (14.3%)	
	Class II	16 (22.9%)	16 (22.9%)	
	Class III	34 (48.6%)	37 (52.9%)	
	Class IV	1 (1.4%)	3 (4.3%)	
Dyspnoea (New York Heart Association)	Class I	19 (27.1%)	19 (27.1 %)	0.733 (#)
	Class II	33 (47.1%)	30 (42.9%)	
	Class III	18 (25.7%)	21 (30.0%)	
	Class IV	0 (0.0%)	0 (0.0%)	
Previous PTCA	0	66 (94.3%)	55 (78.6%)	0.004 (#) *
	1	4 (5.7%)	11 (15.7%)	
	2	0 (0.0%)	4 (5.7%)	
	3	0 (0.0%)	0 (0.0%)	
Previous Myocardial Infarction	No	43 (61.4%)	34 (48.6%)	0.174 (+)
	Yes	27 (38.6%)	36 (51.4%)	

+ - p-value from Fisher's Exact test # - p-value from Linear Trend test

* Statistical significance at 5% level

This table illustrates the results of comparing the incidence of basic cardiac risk factors in each group. The Fisher's exact test and the linear trend test were utilised to determine any significant differences between the groups.

Table 6: A comparison of additional cardiac risk factors between EVH groups.

Cardiac risk factor		**Closed CO_2 tunnel (EVH1, n=70)**	**Open CO_2 tunnel (EVH2, n=70)**	**p-value**
Cardiac family history	No Yes	26 (37.1%) 44 (62.9%)	32 (45.7%) 38 (54.3%)	0.391 (=)
Multi-vessel disease	No Yes	16 (22.9%) 54 (77.1%)	24 (34.3%) 46 (65.7%)	0.190 (=)
Hypertension	No Yes	12 (17.1%) 58 (82.9%)	14 (20.0%) 56 (80.0%)	0.828 (=)
Hypercholesterolaemia	No Yes	10 (14.3%) 60 (85.7%)	11 (15.7%) 59 (84.3%)	1.000 (=)
Neurological problems	No Yes	67 (95.7%) 3 (4.3%)	66 (94.3%) 4 (5.7%)	1.000 (=)
Transient Ischaemic attack (TIA)	No Yes	67 (95.7%) 3 (4.3%)	66 (94.3%) 4 (5.7%)	1.000 (=)
Gastro Intestinal / Renal problem	No Yes	57 (81.4%) 13 (18.6%)	62 (88.6%) 8 (11.4%)	0.344 (=)
Peripheral Vascular Disease	No Yes	47 (67.1%) 23 (32.9%)	57 (81.4%) 13 (18.6%)	0.081 ^ (=)
(=) – p-value from Fisher's Exact test * Statistical significance at 5% level ^ Statistical significance at 10% level				

This table illustrates the results of comparing the incidence of cardiac risk factors between the EVH groups. The Fisher's exact test was used to calculate statistical significance.

Table 7: Peri-operative procedural risk factors analyses between EVH groups.

Risk factor		Closed CO_2 tunnel (EVH1, n=70)	Open CO_2 tunnel (EVH2, n=70)	p-value
Left ventricular ejection fraction (LVEF)	Good (>50%) Fair (30-50%) Poor (<30%)	59 (84.3%) 10 (14.3%) 1 (1.4%)	57 (81.4%) 11 (15.7%) 2 (2.9%)	0.571 (#)
Left Main Stem (LMS) diseased	No Yes	58 (82.9%) 12 (17.1%)	47 (67.1%) 23 (32.9%)	0.050 * (@)
No of Grafts	1 2 3 4	0 (0.0%) 17 (24.3%) 43 (61.4%) 10 (14.3%)	4 (5.7%) 25 (35.7%) 37 (52.9%) 4 (5.7%)	0.006 (#)
No. of vein grafts	1 2 3	16 (22.9%) 42 (60.0%) 12 (17.1%)	28 (40.0%) 38 (54.3%) 4 (5.7%)	0.007 * (#)
@ p-value from Fisher's Exact test * Statistical significance at 5% level			# - p-value from Linear Trend test	

This table illustrates the difference in peri-operative risk factor incidence between EVH groups. The Fisher's exact test and the linear trend test were used to calculate statistical significance.

Table 8: Analysis of intra-operative procedural risk factors in each EVH group.

		Closed CO_2 tunnel (EVH1, n=70)	**Open CO_2 tunnel (EVH2, n=70)**	**p-value**
Leg wound vacuum drain inserted	No Yes	22 (31.4%) 48 (68.6%)	0 (0.0%) 70 (100.0%)	<0.001* (@)
No. of vein tears	0 1 2 3	44 (62.9%) 24 (34.3%) 2 (2.9%) 0 (0.0%)	44 (62.9%) 20 (28.6%) 4 (5.7%) 2 (2.9%)	0.435 (#)
Pump	On pump Off pump	66 (94.3%) 4 (5.7%)	67 (95.7%) 3 (4.3%)	1.000 (@)
Cardioplegia Inflation pressure on the vein	0 mm 70 mm 150 mm	26 (37.1%) 14 (20.0%) 30 (42.9%)	14 (20.0%) 20 (28.6%) 37 (52.9%)	0.067 ^ (#)
Ventilator setting changed	No Yes	28 (40.0%) 42 (60.0%)	68 (96.8%) 2 (3.2%)	<0.001 * (@)

@ - p-value from Fisher's Exact test
# - p-value from Linear Trend test.
* Statistical significance at 5% level
^ - Statistical significance at 10% level.

This table illustrates the difference in intra-operative risk factor incidence between EVH groups. The Fisher's exact test and the linear trend test were used to calculate statistical significance.

Table 9: A comparison of CO_2 absorption at serial time points between EVH groups.

CO_2 partial pressures		**Closed CO_2 tunnel (EVH1)**	**Open CO_2 tunnel (EVH2)**	**p-value**
Pre-inflation pressure	Number of patients Mean Standard Deviation (SD) Minimum Maximum	69 4.89 0.76 3.2 6.8	62 5.39 0.76 4.0 7.66	<0.001* (+)
Partial pressure at 30 minutes post-inflation	Number of patients Mean Standard Deviation (SD) Minimum Maximum	69 5.48 0.79 3.4 7.8	62 5.38 0.73 4.0 7.44	0.442 (+)
Partial pressure at 10 minutes post-deflation	Number of patients Mean Standard Deviation (SD) Minimum Maximum	69 5.82 0.83 3.8 8.69	62 5.35 0.73 4.0 7.44	0.001 * (+)
Pre-inflation endotracheal pressure of CO_2	Number of patients Mean Minimum Maximum	69 3.40 2.8 5.2	60 3.50 2.8 5.5	0.074 ^ (&)
Endotracheal pressure of CO_2 at 30 minutes post-inflation	Number of patients Mean Minimum Maximum	69 3.60 2.9 5.5	60 3.50 2.7 5.5	0.153 (&)
Endotracheal pressure of CO_2 at 10 minutes post-deflation	Number of patients Mean Minimum Maximum	69 3.60 3.1 6.8	60 3.50 2.8 4.9	0.041 * (&)
+ - p-value from Student's t test. & - p-value from Mann-Whitney U-test. * Statistical significance at 5% level. ^ Statistical significance at 10% level.				

This table illustrates the difference in CO_2 absorption between EVH groups. Importantly, there is a discrepancy in sample size due to a lack of data recording by the anaesthetist. The Student's T-test and the Mann-Whitney U test were used to calculate statistical significance.

3.2. Clinical analyses post-operative outcomes

Table 10: Post-operative clinical outcomes comparison between two EVH groups.

Clinical parameter		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Leg wound healed	No Yes	0 (0.0%) 69 (100.0%)	1 (1.5%) 68 (98.5%)	0.496 (@)
Leg wound numbness	No Yes	59 (85.5%) 10 (14.5%)	59 (85.5%) 10 (14.5%)	1.000 (@)
Leg wound tender	No Yes	58 (84.1%) 11 (15.9%)	62 (89.9%) 7 (10.1%)	0.449 (@)
@ - p-value from Fisher's Exact test * Statistical significance at 5% level ^ Statistical significance at 10% level				

This table illustrates the difference in post-operative clinical outcomes between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

Table 11: Incidence of repeat angina following CABG surgery in each EVH group.

Repeat angina observed		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH1, n=69)**	**p-value**
Repeat angina at 3 months	No Yes	66 (95.7%) 3 (4.3%)	68 (98.6%) 1 (1.4%)	0.619 @
Repeat angina at 6 months	No Yes	62 (89.9%) 7 (10.1%)	67 (97.1%) 2 (2.9%)	0.615 @
@ - p-value from Fisher's Exact test * - Statistical significance at 5% level ^ - Statistical significance at 10% level				

This table illustrates the difference in the incidence of repeat angina between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

Table 12: Proportion of patients experiencing breathlessness following CABG in each EVH group.

This table illustrates the difference in the incidence of breathlessness at 3 and 6 months post-CABG between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

Breathlessness observed		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Breathlessness at 3 months	No Yes	62 (89.9%) 7 (10.1%)	61 (88.4%) 8 (11.6%)	0.791 @
Breathlessness at 6 months	No Yes	61 (88.4%) 8 (11.6%)	60 (87.0%) 9 (13.0%)	0.801 @
@ - p-value from Fisher's Exact test * - Statistical significance at 5% level ^ - Statistical significance at 10% level				

Table 13: A comparison of the incidence of re-intervention in each EVH group.

Re-intervention necessary		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Re-intervention at 3 months	No Yes	66 (95.7%) 3 (4.3%)	68 (98.6%) 1 (1.4%)	0.619 @
Re-intervention at 6 months	No Yes	66 (95.7%) 3 (4.3%)	68 (98.5%) 1 (1.5%)	0.619 @
@ - p-value from Fisher's Exact test * - Statistical significance at 5% level ^ - Statistical significance at 10% level				

This table illustrates the difference in the number of patients requiring re-intervention at 3 and 6 months post-CABG between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

Table 14: Incidence of myocardial ischaemia following CABG in each EVH group.

Myocardial ischaemia experienced		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Myocardial ischaemia at 3 months	No Yes	66 (95.7%) 3 (4.3%)	69 (100.0%) 0	0.245 @
Myocardial ischaemia at 6 months	No Yes	65 (94.2%) 4 (5.8%)	68 (98.6%) 1 (1.4%)	0.366 @
@ - p-value from Fisher's Exact test * - Statistical significance at 5% level ^ - Statistical significance at 10% level				

This table illustrates the difference in the number of patients experiencing myocardial ischaemia at 3 and 6 months post-CABG between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

Table 15: Incidence of cardiac arrhythmias following CABG in each EVH group.

Cardiac arrhythmias		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Arrhythmia	No Yes	61 (88.4%) 8 (11.6%)	65 (94.2%) 4 (5.8%)	0.366 @
Atrial fibrillation	No Yes	66 (95.7%) 3 (4.3%)	65 (94.2%) 4 (5.8%)	0.718 @
Ventricular fibrillation / Ventricular tachycardia	No Yes	69 (100.0%) 0 (0.0%)	69 (100.0%) 0 (0.0%)	1.000 @
Pacemaker required	No Yes	67 (97.1%) 2 (2.9%)	68 (98.6%) 1 (1.4%)	1.000 @
@ - p-value from Fisher's Exact test * - Statistical significance at 5% level ^ - Statistical significance at 10% level				

This table illustrates the difference in the number of patients experiencing cardiac arrhythmias post-CABG between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

Table 16: Incidence of patients requiring cardiac investigations post-surgery.

Cardiac investigation		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Electrocardiography (ECG)	No Yes	65 (94.2%) 4 (5.8%)	63 (91.3%) 6 (8.7%)	0.21* @
Chest X-ray	No Yes	59 (85.5%) 10 (14.5%)	64 (92.8%) 5 (7.2%)	0.274 @
ECHO	No Yes	65 (94.2%) 4 (5.8%)	63 (91.3%) 6 (8.7%)	0.528 @
MRI scan	No Yes	63 (91.3%) 6 (8.7%)	66 (95.7%) 3 (4.3%)	0.493 @
Angiogram	No Yes	63 (91.3%) 6 (8.7%)	67 (97.1%) 2 (2.9%)	0.274 @
@ - p-value from Fisher's Exact test * - Statistical significance at 5% level ^ - Statistical significance at 10% level				

This table illustrates the difference in the number of patients requiring cardiac investigation post-CABG between EVH groups. Investigations were only performed for patients experiencing cardiac symptoms post-surgery. The Fisher's exact test was used to calculate statistical significance.

Table 17: A comparison of post-surgical re-intervention and mortality rates between the two EVH groups.

Parameter		Closed CO_2 tunnel (EVH1)	Open CO_2 tunnel (EVH2)	p-value
ACC/AHA scoring system for coronary arteries	Type A (Discrete <10mm) Type B (Tubular 10-20mm) Type C (Diffuse >2cm)	1 (9.1%) 3 (27.3%) 7 (63.6%)	0 0 4 (100%)	-
Vein graft occlusion scale	Patent: No flow limiting Patent : Flow limiting Completely Blocked	5 (55.6%) 1 (11.1%) 3 (33.3%)	3 (100%) 0 0	-
Complete revascularisation carried done during the CABG surgery	No Yes	7 (63.6%) 4 (36.4%)	1 (25%) 3 (75.0%)	-
Mortality (number of deceased patients post-surgery)	Alive Deceased	68 (97.1%) 2 (2.9%)	68(97.1%) 2 (2.9%)	1.000 @
Survival – 3 months	Yes	69	68	-
Survival – 6 months	Yes	69	68	-

There was insufficient data to perform statistical analysis between coronary artery scoring, vein graft occlusion and complete revascularisation. Analysis was only performed for symptomatic patients.

3.2.1. Health related quality of life analyses post cardiac surgery

Table 18: A comparison of health-related patient's quality of life post-cardiac surgery between the two groups.

Parameter		**Closed CO_2 tunnel (EVH1, n=69)**	**Open CO_2 tunnel (EVH2, n=69)**	**p-value**
Able to carry out every day physical activities	**No**	**9 (11.6%)**	**9 (13.0%)**	**1.000** @
	Yes	61 (88.4%)	60 (87.0%)	
Emotional Feeling	**No**	**41 (58.6%)**	**40 (58.0%)**	1.000 @
	Yes	28 (41.4%)	29 (42.0%)	
In employment	**No**	**54 (77.6%)**	**55 (79.7%)**	1.000 @
	Yes	15 (22.4%)	14 (20.3%)	
Able to fly abroad without any breathlessness	**No**	**36 (52.2%)**	**46 (66.7%)**	0.117 @
	Yes	33 (47.8%)	23 (33.3%)	
Tiredness throughout the day.	**No**	**44 (62.7%)**	**38 (55.1%)**	0.384 @
	Yes	25 (37.3%)	31 (44.9%)	

@ - p-value from Fisher's Exact test

*** - Statistical significance at 5% level** **^ - Statistical significance at 10% level**

This table illustrates the difference in patients' health-related quality of life following cardiac surgery between EVH groups. One patient in the EVH1 group died and the data could not be collected for one patient in the EVH2 group. The Fisher's exact test was used to calculate statistical significance.

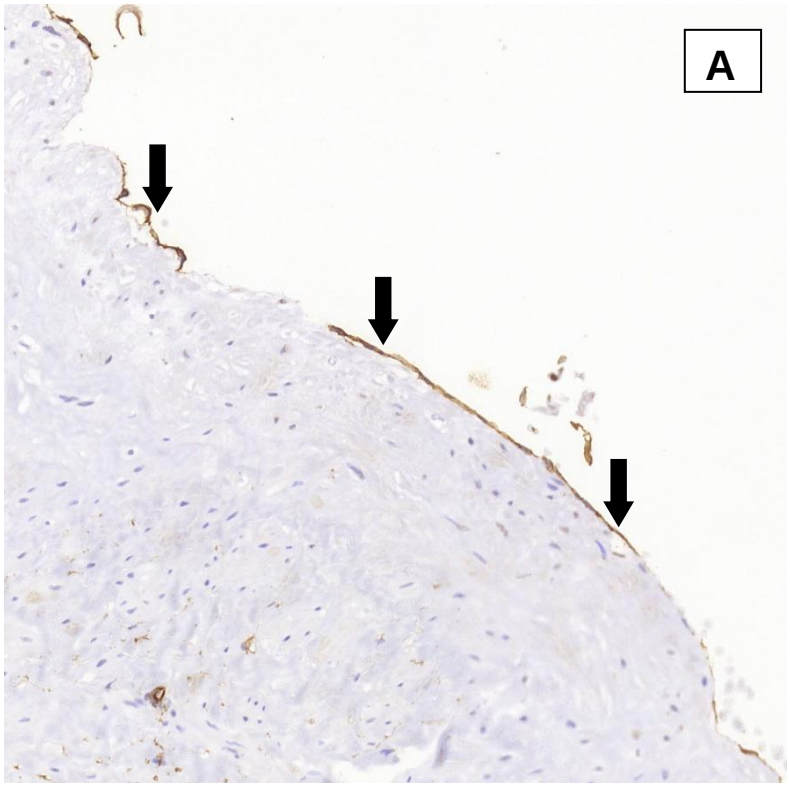
3.3. Histological assessment results

Samples taken from 140 patients were allocated into closed tunnel CO_2 (n=70) and open tunnel CO_2 (n=70) groups. The patient demographics and clinical characteristics of patients in both groups were summarised in tables 3, 4 & 5. There were no statistically significant differences between the groups.

The 140 vein specimens (H_2 code) were sufficiently stained using the study protocol. The stained specimens were blindly scored by individual assessors. The open tunnel CO_2 specimen had superior endothelial integrity (Figure 16a) compared to closed tunnel CO_2 specimen (Figure 16b).

The student's T-test was used to perform statistical analysis between the groups. The open tunnel CO_2 group had greater endothelial integrity compared with closed the tunnel CO_2 group (mean 65% (95% confidence interval (CI)), 56.1 to 73.8; mean 11.4% (95% CI, 3.6 to 10.1; $p<0.001$) (Figure 24). The majority of the vein specimens from the closed tunnel CO_2 group had areas of endothelial denudation compared to open tunnel technique (Figure 16 to 23).

Figure 16: Endothelial integrity of vein specimens stained using CD34; (a) Illustrates the partial continual layer of endothelium on the vein specimens obtained via the open CO_2 tunnel system. Figure (b) illustrates the endothelial denudation in vein specimens obtained via the closed CO_2 tunnel system. The red arrows indicate the areas of endothelial denudation and black arrows indicate the areas of continual endothelium. (A x 300; B x 200 magnification).



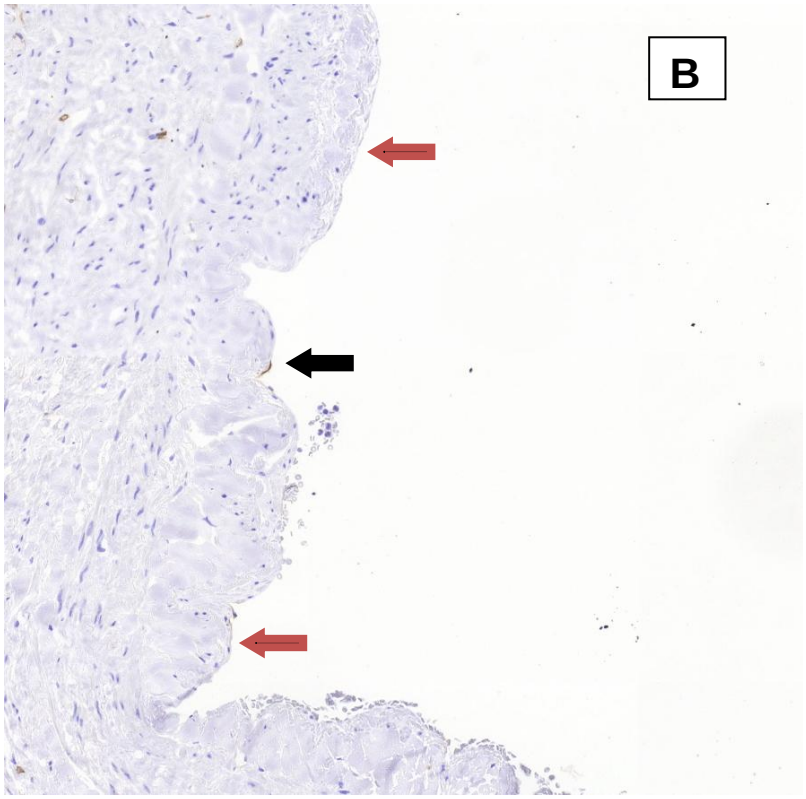
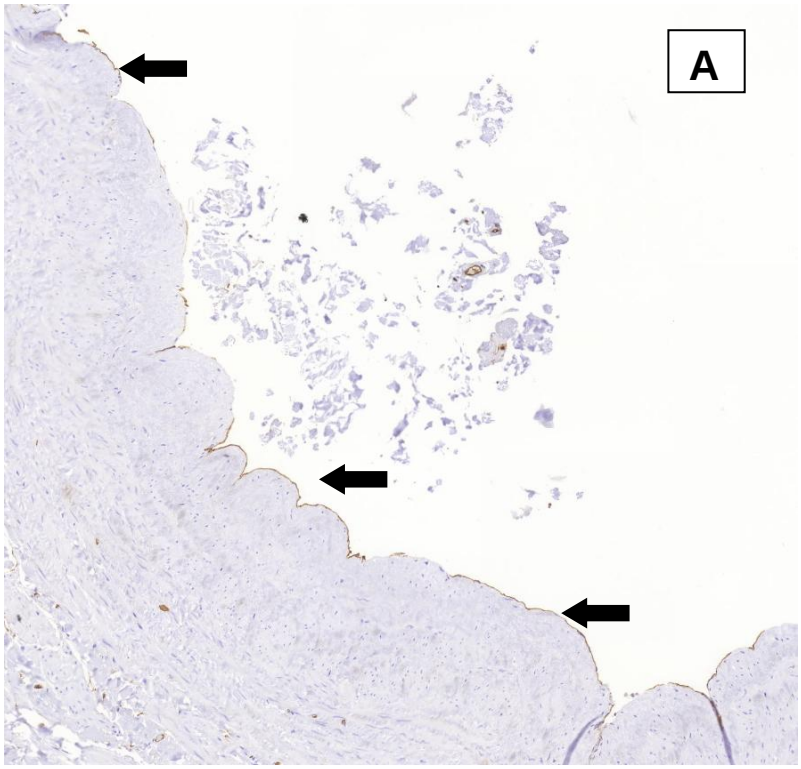


Figure 17: a). The black arrow indicates the luminal endothelial layer (x200) on open CO_2 technique b). Red arrows indicate no endothelial layer on closed CO_2 technique (x200).



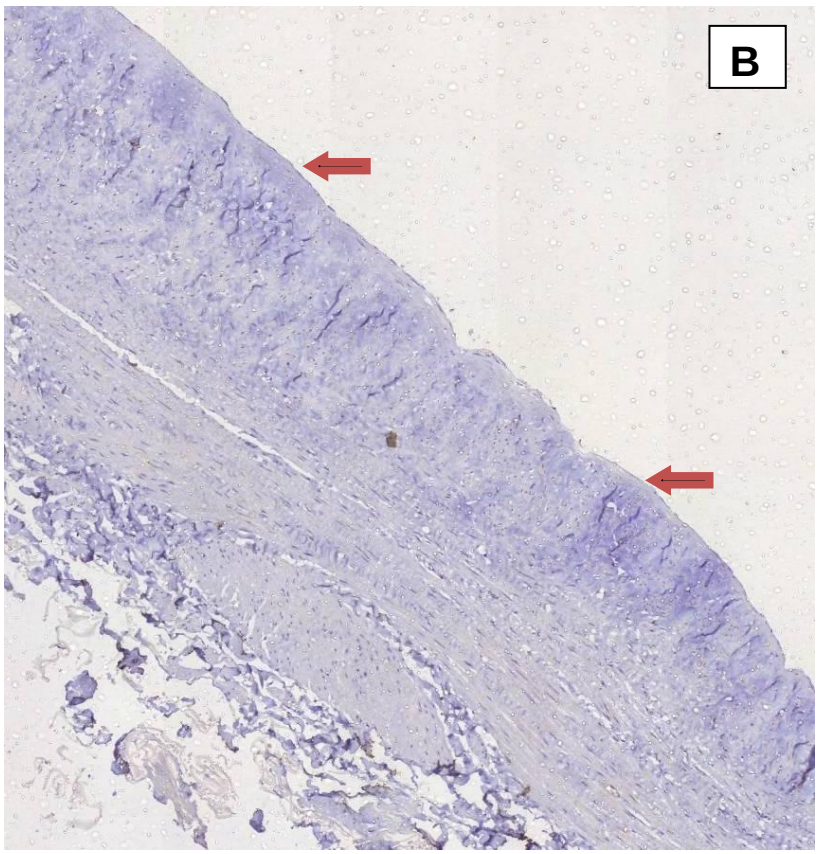


Figure 18: A). Luminal endothelial disruption of the long saphenous vein indicated on red arrows on closed tunnel technique (x100). B). Thin endothelial layer of brown stained using CD34 indicated on black arrows (x200).

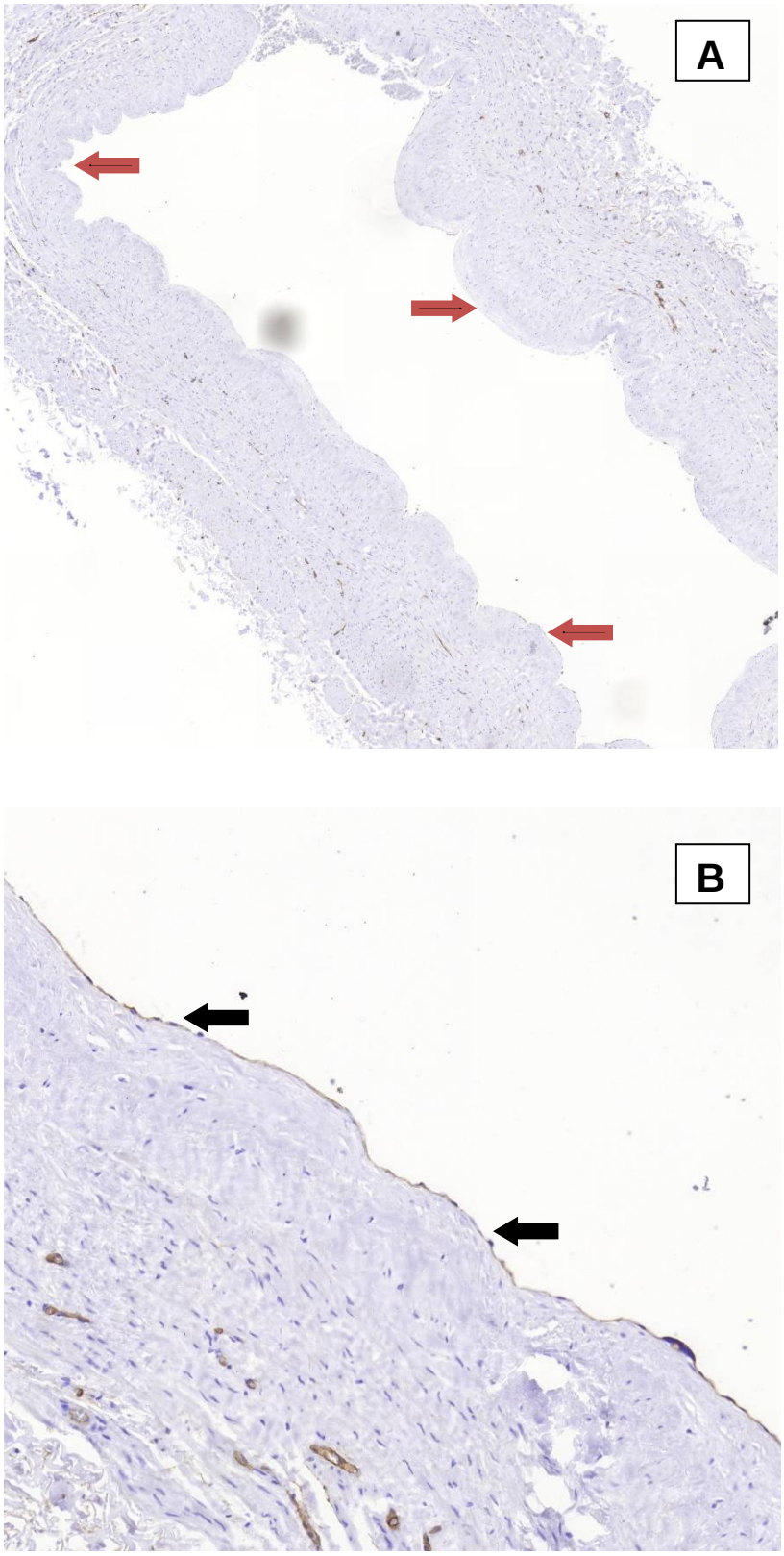
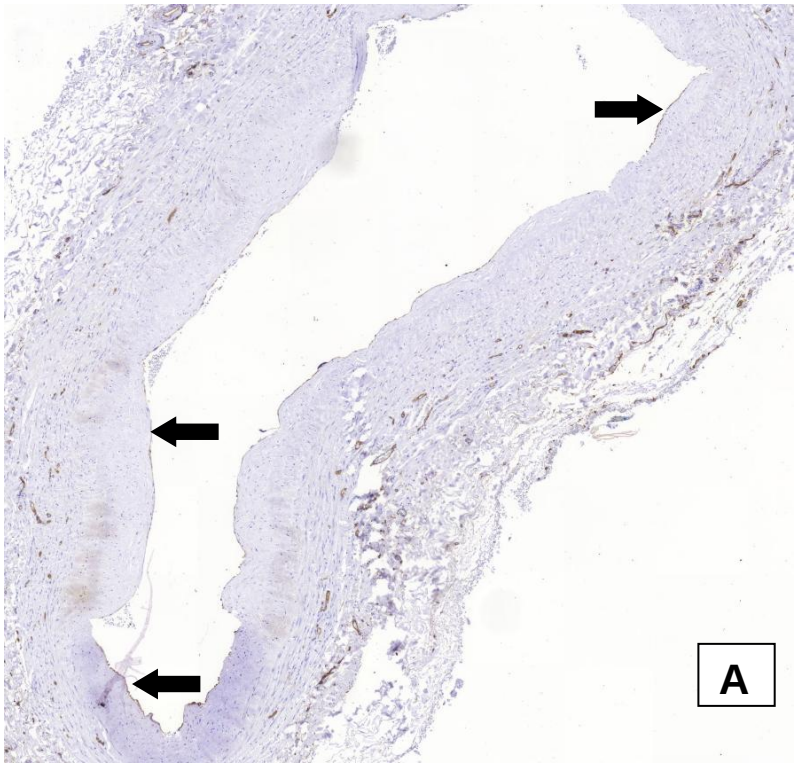


Figure 19: a). Luminal endothelium of the long saphenous vein (A) Open tunnel technique(x100) and (B) Closed tunnel technique (x100), CD34 was stained as a brown colour. Note the defects in staining (red arrows) and continual layer in staining (black arrows) in both groups.



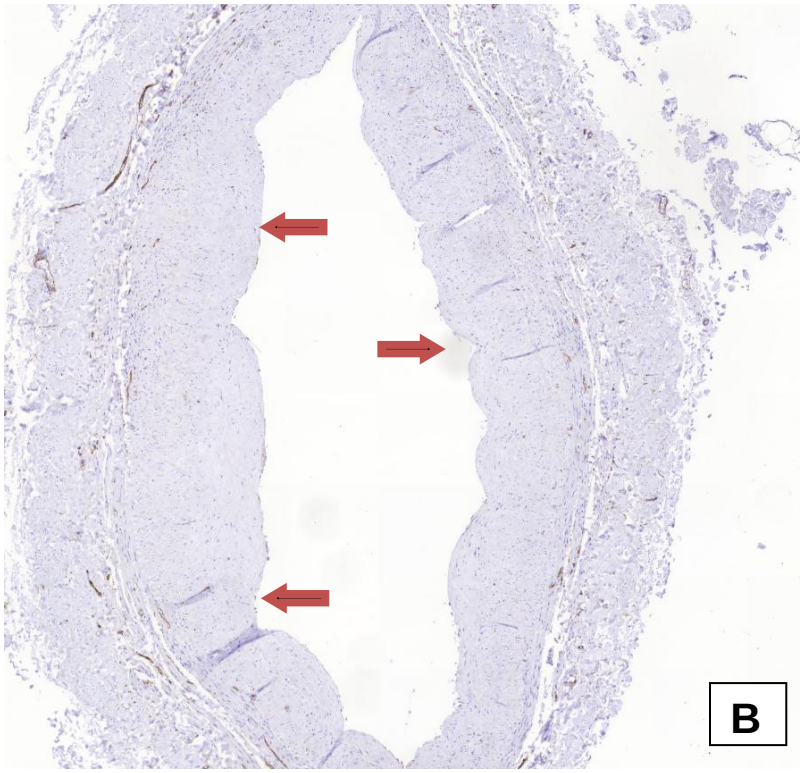
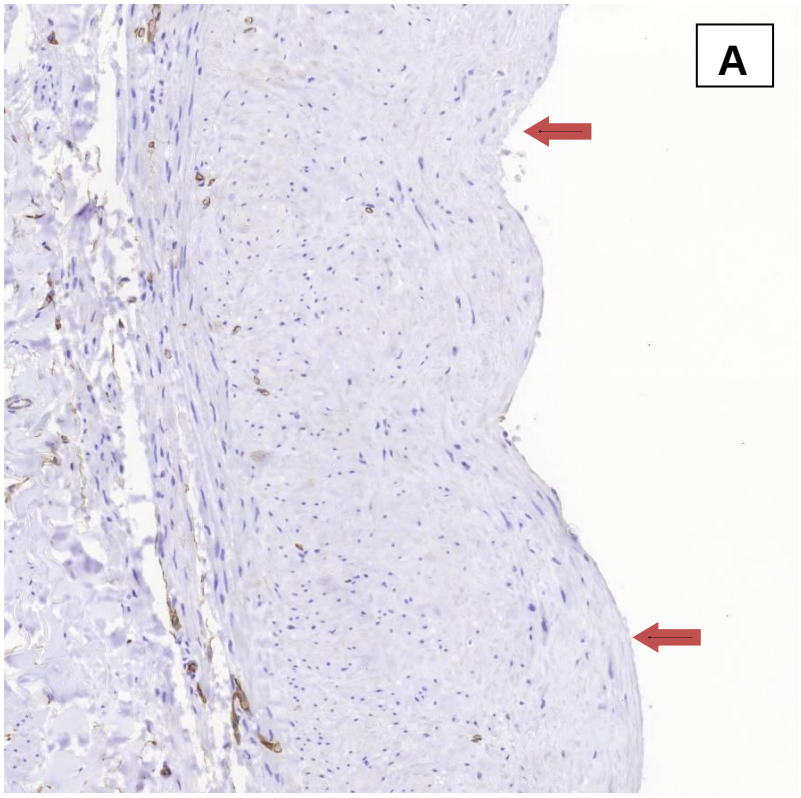


Figure 20: a). Hematoxylin-eosin and CD34 staining of luminal endothelium of the LSV in the Closed tunnel (A), Open tunnel (B) (x200). Note the loss of endothelium indicates by red arrows and continual layer of endothelium by black arrows.



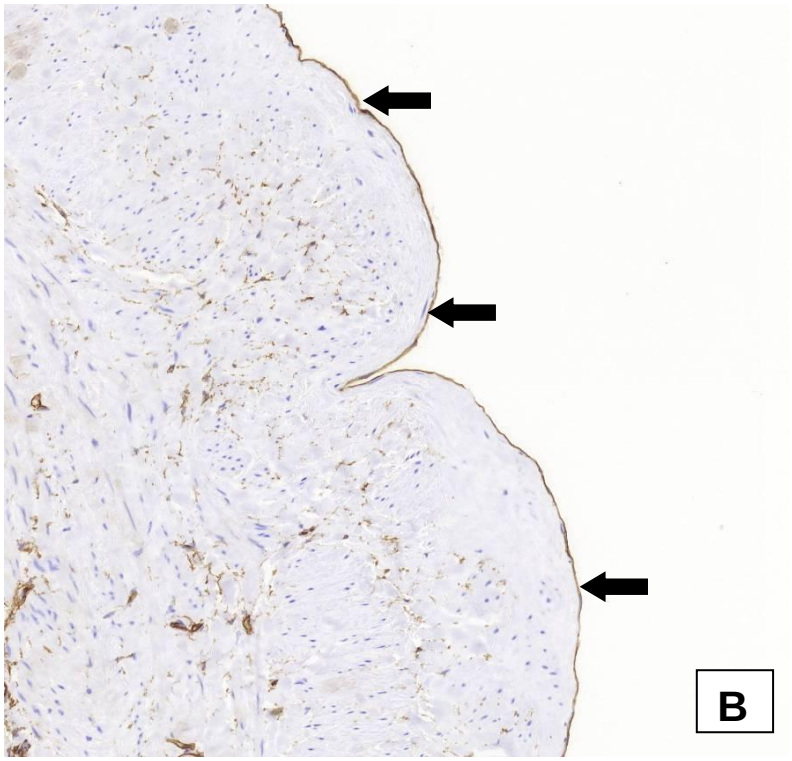
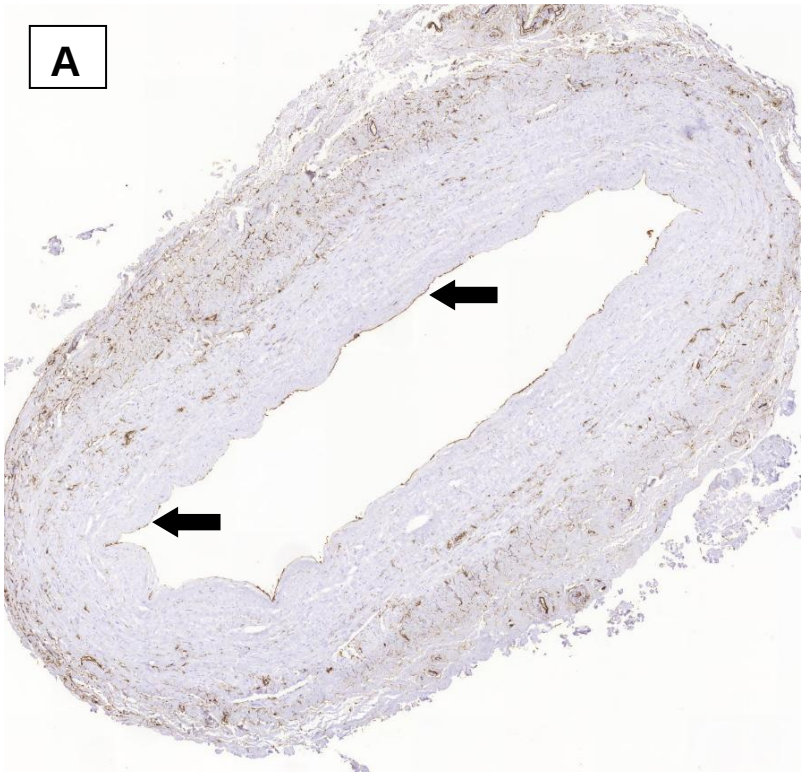


Figure 21: Luminal slight interrupted endothelium of the LSV on Closed tunnel CO_2 technique (A X 100) and (Bx200). The black arrows indicates the layer of endothelium in contrast red arrows indicates endothelial denudation.



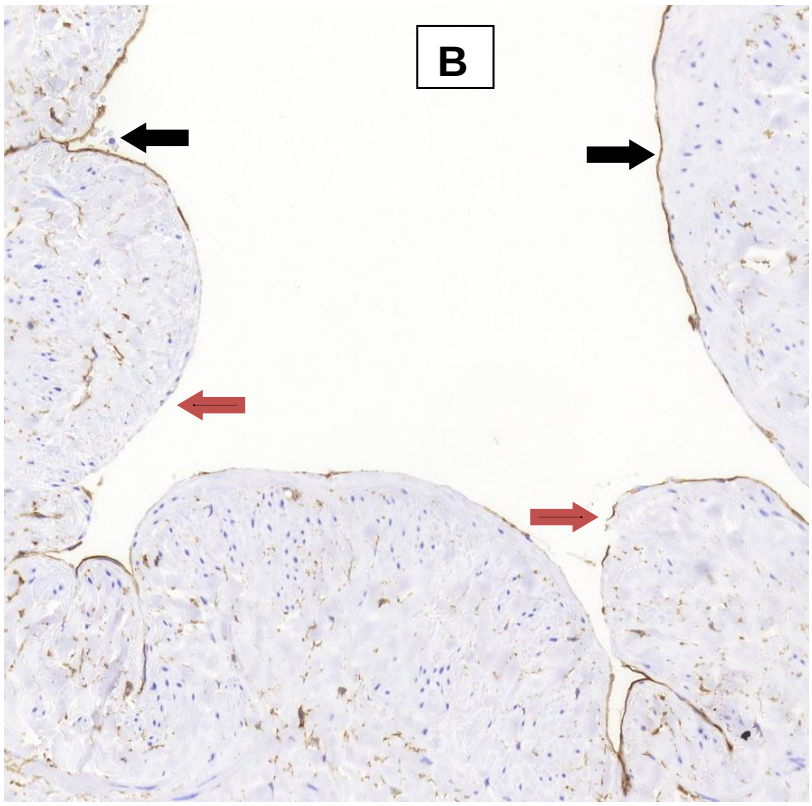


Figure 22: Luminal slight interrupted endothelium of the LSV on Open tunnel CO_2 technique (A X 100) and (Bx100). The black arrows indicates the layer of endothelium.

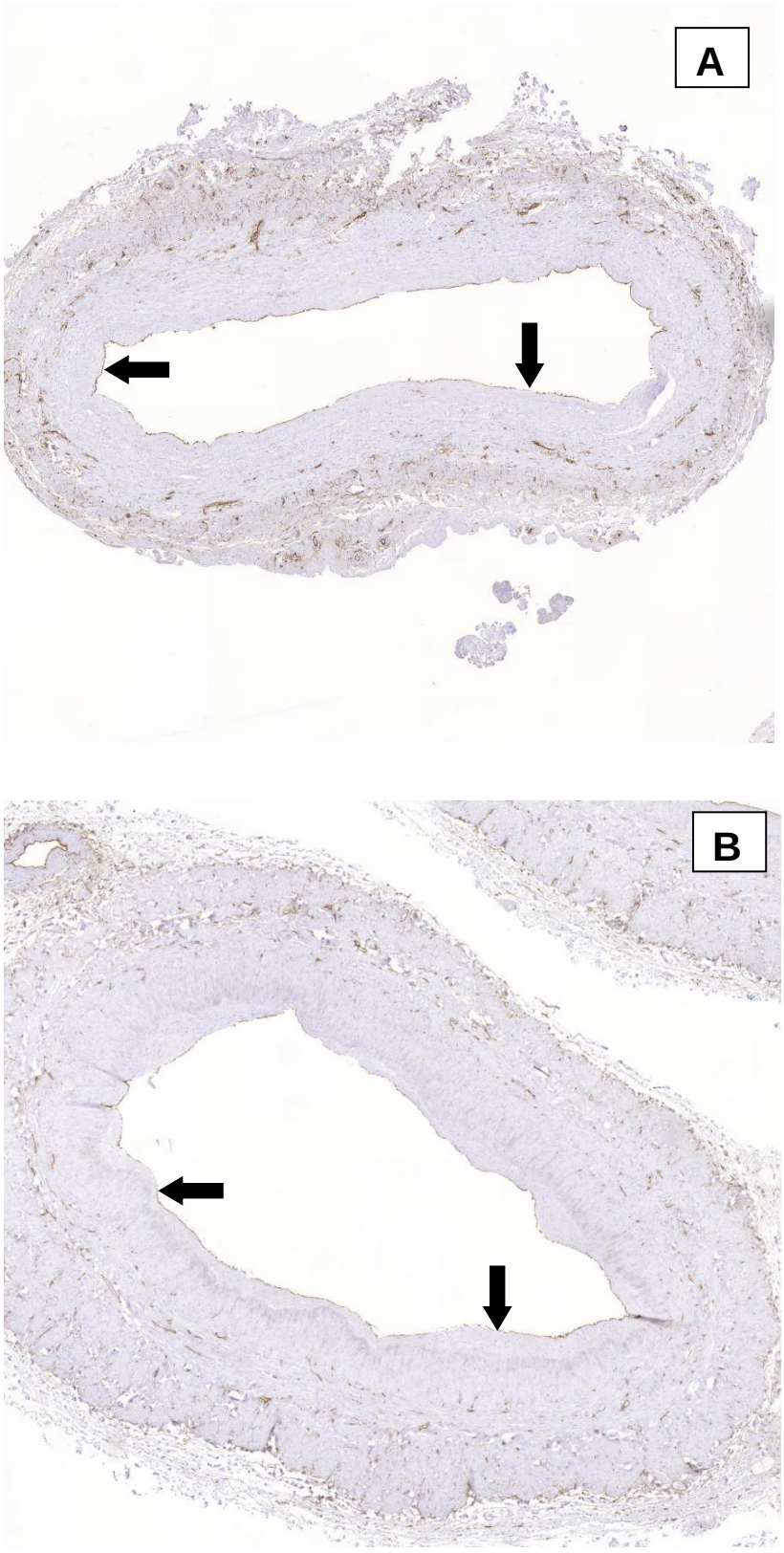


Figure 23: Luminal endothelium of the LSV on Open tunnel CO_2 technique (A X 100) and (Bx200). The black arrows indicate the continual layer of endothelium stained by using CD34 and Haematoxylin-eosin.

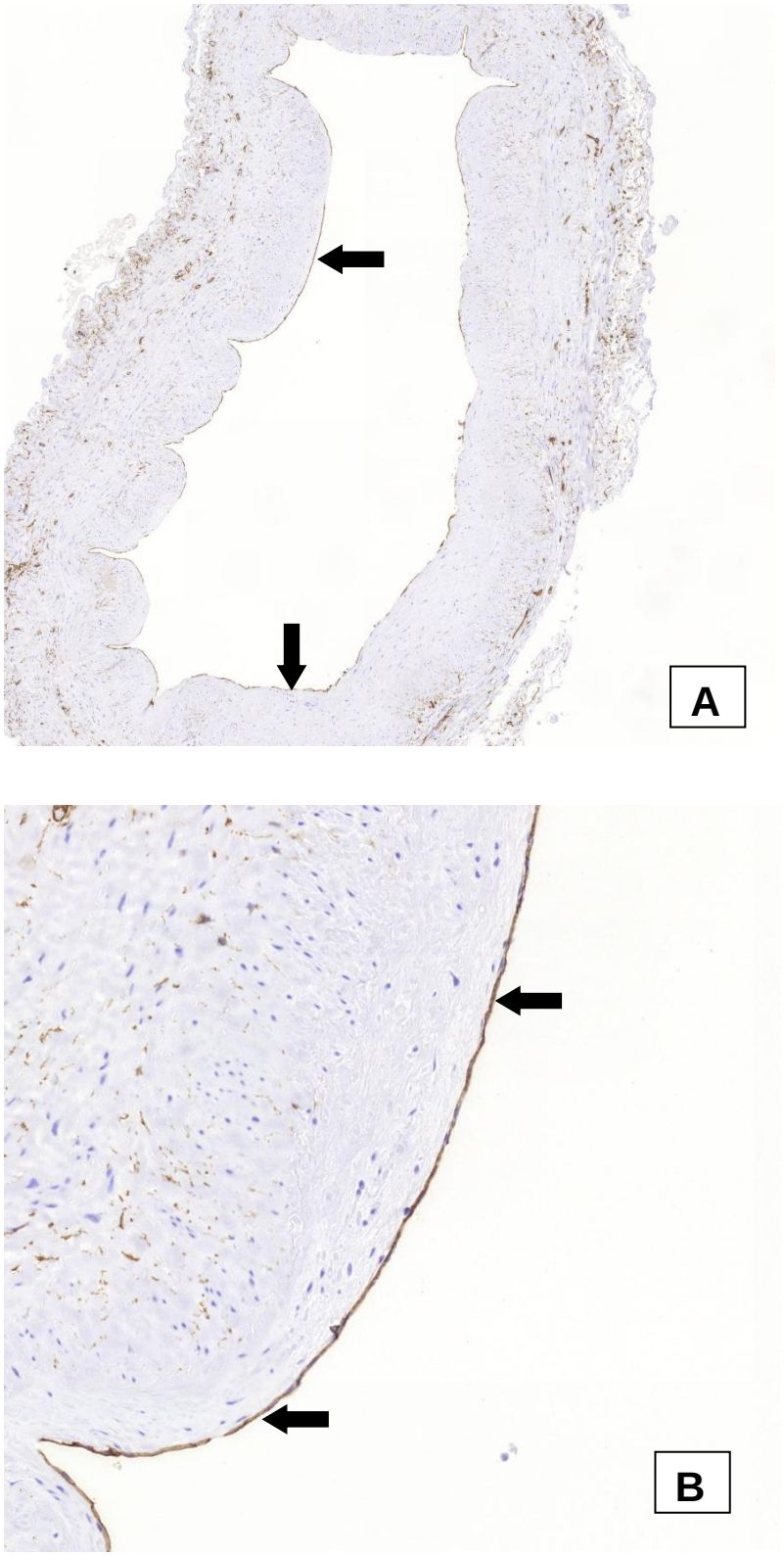
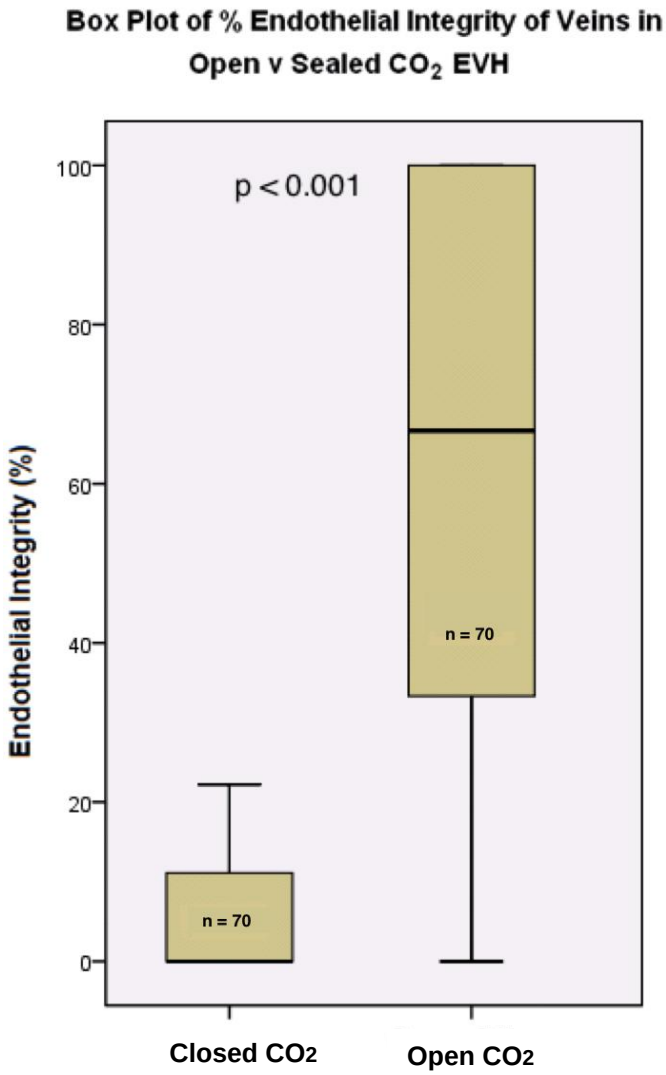


Figure 24: The endothelial integrity of vein specimens in closed (sealed) and open CO_2 EVH groups.



This box plot represents a comparison of the endothelial integrity of veins obtained via closed tunnel CO_2 and open tunnel CO_2 EVH systems. The bold line indicates the median value, the box represents the lower and upper quartiles and the whiskers indicate the highest and lowest data points.

CHAPTER 4

DISCUSSION

4. DISCUSSION

The histological findings of this study demonstrate that disruption of the endothelial layer occurs to a significantly greater extent in the closed tunnel CO_2 group ($p<0.001$) compared to the open tunnel CO_2 group. In contrast, the clinical findings of this study indicate that there is no statistically significant difference in overall mortality ($p=1.000$) or incidence of vein graft failure between these groups. Vein graft failure is one of the major complications post CABG surgery. There are numerous aetiological factors for vein graft failure or occlusion. There are well reported prospective and retrospective studies suggesting that the quality of conduit [166, 167], graft diameter [168], type of the graft [169, 170], grafting site [171], handling of the conduit [172], surgical conduit preparation [173-175], grafting technique [171, 172, 176], patients' pre-existing risk factors [177, 178] and technical error [45, 172, 175] contribute to graft failure. The detailed histological and clinical results of this study are discussed in the following sections.

4.1. Histological results discussion

The histological results from our non-randomised study demonstrate that the severity of disruption of the endothelium is increased in the closed tunnel CO_2 compared to the open tunnel CO_2 system. The closed system requires a constant CO_2 pressure of 12mmHg at the rate of 3 litres per minute. The pressurised tunnel in the closed insufflation method causes the vein to collapse. This abrupt venal collapse causes the endothelial layers to adhere to each other for a time period dependent upon the duration of the surgical procedure. Once the vein is harvested and the pressure is released in the tunnel, the compensatory vein dilation can cause detachment of the endothelial layer and significantly ameliorate production of nitric oxide (NO). Endothelial absence and the loss of protective mechanisms in the harvested vein can lead to early graft failure, which supports the findings of the Lopes study [61].

The saphenous vein graft consists of three layers, with the outermost adventitial layer playing a vital role in preservation of the vasa vasorum which provides blood supply to the vessel wall [181-183]. The innermost intimal layer (endothelium) secretes various factors (Figure 6) and also exerts anti-thrombotic, anti-spasmodic and anti-atherosclerotic effects (Figure 25). In the OVH method, current evidence suggests that preserving the adventitia, peri-vascular tissues and endothelial layers inhibits

the positive remodelling of the vein graft [184, 185] which prevents vein graft failure. The traditional OVH technique also better preserves the functional, structural and mechanical integrity of the vein wall compared to the endoscopic method of vein harvesting [128]. The current literature suggests that the learning curve of the practitioner has a high impact on the quality of the vein, which may therefore contribute to endothelial disruption and graft patency [179, 186]. This is due to the nature of the endoscopic harvesting technique, which requires more manipulation and handling of the vein compared to the traditional non-touch OVH method [187].

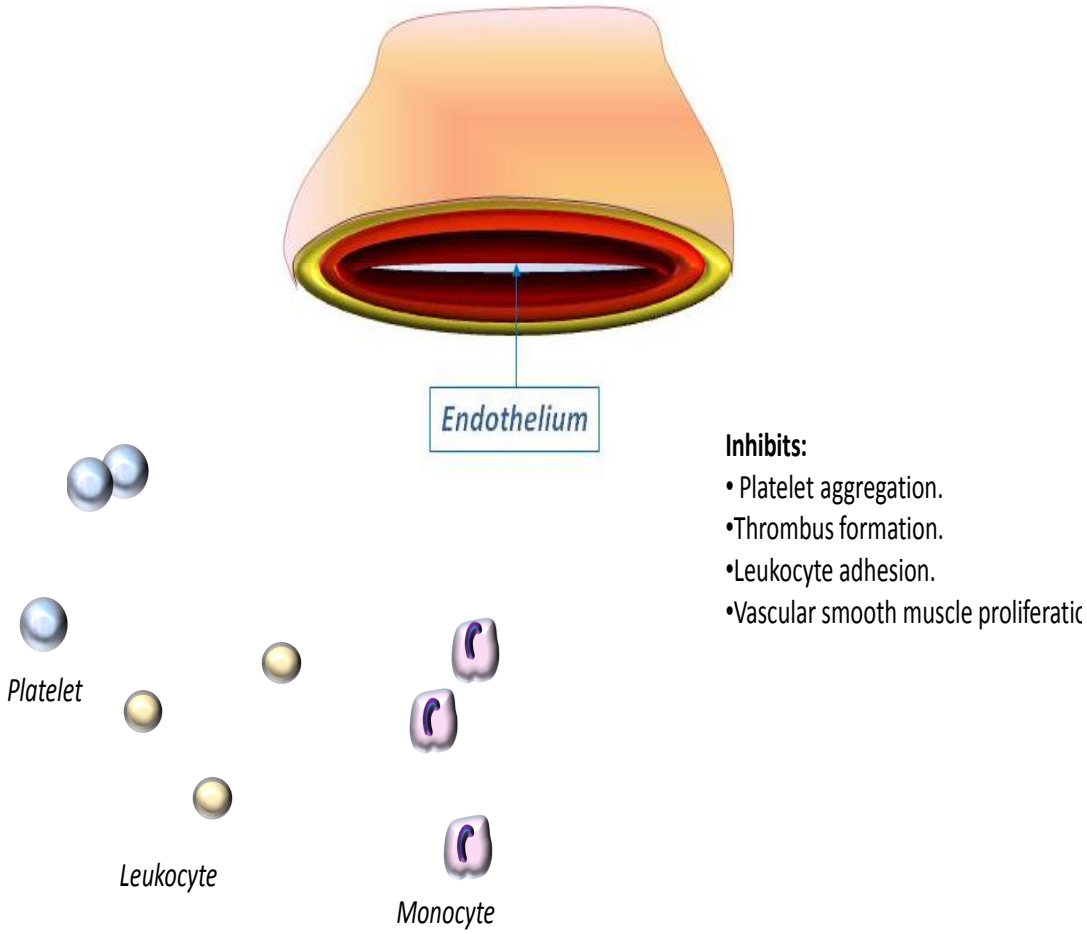
The important difference between these two techniques is the trauma involved in the vein harvesting method. This study demonstrates that when samples are compared, significantly greater endothelial damage is incurred when using a closed tunnel CO_2 technique versus the open tunnel CO_2 system. However, the samples of these two groups were collected almost 4 months apart due to the learning curve involved in the procedure. Although patients were recruited prospectively for this pilot work, the histological samples were stored and stained at the same time at the end of the study period. Therefore, veins from the closed tunnel CO_2 group were stored longer in 4% formalin at room temperature than the open tunnel CO_2 group. It remains to be explored whether fresh open tunnel CO_2 samples retain a more continual endothelium due to the shorter storage period.

The findings of our study also suggest that endothelial disruption in the closed CO_2 tunnel may be due to problems associated with the practitioner learning curve. Recent evidence also suggests that the learning curve [179] and harvesting method [58, 180, 181] may play a major role in graft failure. Further research in this area must be performed to analyse the extent to which practitioner inexperience contributes to endothelial damage. This is important for delineating the true effect of the technique itself.

Graft failure is the major complication following coronary artery bypass surgery. Thrombus formation within the vein is the major cause of early graft occlusion [188]. Pathologic changes occur in the vein once it has been grafted into the coronary arteries, leading to the failure of 10-20% of LSV grafts within one year [189]. At 10 years post-surgery, 50% of vein grafts are occluded and the remaining 50% show atherosclerotic vein changes [173]. Atherosclerotic changes to the vein occurring as a result of endothelial injury are well reported [190]. There are many factors affecting the endothelial layer of the vein and one of the main contributors is the technique use to harvest the conduit [190]. The uppermost endothelial layer acts as a physiological, electrical and mechanical barrier between the blood and the

sub-endothelial layer causing thrombogenic changes to the vein graft [190, 191]. The traumatic damage to this layer induced by CO_2-induced compression of the vein, prior to grafting on the coronaries, may promote early graft occlusion. This needs to be verified by long-term clinical follow up of the patients from this study.

Figure 25: The effect of nitric oxide produced by a healthy endothelium.



This diagram illustrates the effect of endothelium derived nitric oxide. Nitric oxide inhibits the aggregation of platelets, thereby reducing thrombus formation, leukocyte adhesion and the proliferation of cells between the layers of the vein.

4.2. Clinical results discussion

Acute graft failure and long-term graft patency are the major issues following coronary artery bypass surgery. Vein graft failure post-surgery can be due to many factors, such as technical errors, thrombosis and intimal hyperplasia [173]. The quality of the vein differs from patient to patient according to their age, gender, pre-disposing factors such as diabetes, hypertension, hypercholesterolemia and peripheral vascular disease [193].

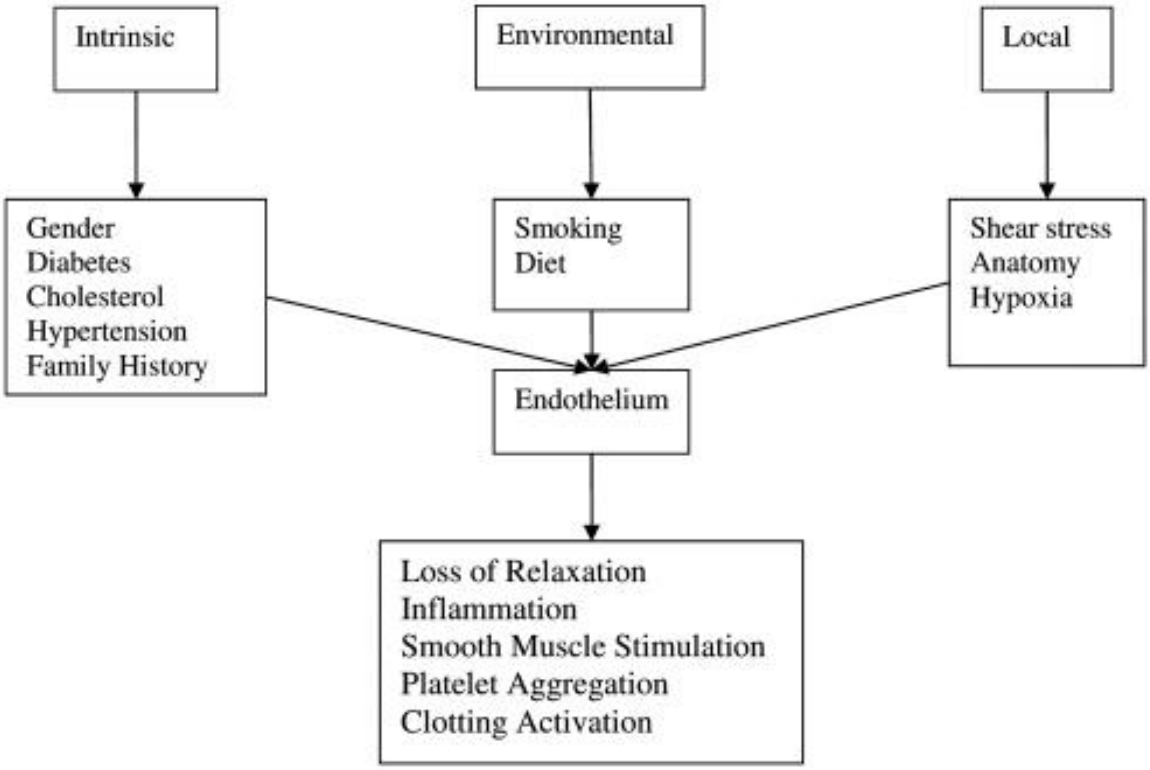
The two endoscopic groups: closed CO_2 tunnel (EVH1) and open CO_2 tunnel (EVH2), were compared for basic demographics between the groups. There was no statistical difference observed between the groups. There was also no statistical difference in existing risk factors that predispose to cardiac disease between the groups. However, patients in the EVH1 group were given 5000 units of heparin before starting the procedure. This is essentially to avoid any intraluminal clot strand formation during pressurised CO_2 tunnel vein harvesting. This was supported by the paper published by Brown *et al*, which concluded that the risk of clot formation is attributable to venous blood stasis induring pressurised CO_2 endoscopic vein harvesting and poses significant risk [157]. There was a disparity in the number of pre-op PTCA on the coronary arteries between EVH groups. The evidence from the literature suggests that patients who have previous percutaneous coronary intervention (PCI) experience a more aggressive atherosclerotic process post-CABG surgery than stand-alone CABG surgery [194, 195].

The left main stem stenosis and number of grafts required also differed between EVH groups. Stenosis of the critically important left main stem, and multi-vessel disease, plays an key role in repeat revascularisation incidence, which is 3 to 4 times higher due to the progressive atherosclerotic process in coronary artery disease [196].

Coronary artery disease is one of the major causes of death in patients with diabetes mellitus (DM). Disease progression is diffuse and requires complete revascularisation during the first CABG surgery [193]. The incomplete revascularisation of patients with DM results in early recurrence of angina, increased morbidity and mortality in post-CABG surgery [197]. The endothelium plays an important role in controlling vascular tone and producing a wide range of vasoactive compounds [198]. Various factors influence vascular endothelial health (Figure 26 [199]). Diabetic patients produce higher levels of superoxide which affects the function of the venous conduit vessels [199] and results in saphenous vein graft failure. Additional endothelial damage caused by using a pressurised CO_2 tunnel increases the

risk of acute graft failure following endoscopic vein harvesting in diabetic patients, although large randomised trials are required to explore this in more depth.

Figure 26: The major factors that influence the vascular endothelium.



This schematic diagram illustrates the wide range of factors that affect the quality of the endothelial layer, which in turn can lead to histological changes in the vein.

Six months' worth of clinical data were collected to assess the MACE score; these include, repeat angina, breathlessness, myocardial ischaemia and myocardial ischaemia re-interventions such as MRI scan, the fitment of a pacemaker and angiogram, CT scan, ECG and ECHO. No statistically significant difference in the MACE score was observed between groups. Our results show that the MACE score had many discrepancies due to the lack of randomisation between the groups.

Angiogram was carried out only for symptomatic patients with severe angina and breathlessness. In the EVH1 group, 6 patients out of 63 underwent angiogram. Other patients who had experienced repeat angina were excluded due to symptomatic relief from medical treatment. In the EVH2 group, 2 out of 67 patients underwent an angiogram. The main predictor of the MACE post-CABG surgery is graft blockage leading to repeat angina. In the EVH1 group, 4 out of 6 patients who underwent repeat angiography exhibited blocked grafts. The results demonstrated that vein grafts were completely blocked in 3 patients, and patent with limited flow in 1 patient. No graft blockage was observed in either patient from the EVH2 group. All post-surgical vein graft surgery was assessed using the American College of Cardiology /American Heart Association (ACC/AHA) scoring system to identify the vein graft and coronary arteries. The number of graft blockages was minimal, and a lack of data in most categories made it difficult to demonstrate a statistical difference between the groups.

We made the decision to reanalyse these patients to find out the root cause of their repeat angina. The data was re-filtered to assess whether complete revascularisation was performed during the initial CABG. In the symptomatic EVH1 group, 7 out of 11 symptomatic patients were not completely re-vascularised due to small coronary arteries. Furthermore, the coronary arteries were diffusely diseased. However, in the EVH2 group, only 1 patient was not completely re-vascularised, and this may explain the incidence of repeat angina pain. Many studies demonstrate that incomplete revascularisation during first-time CABG has a negative influence on several post-operative clinical outcomes [200, 201] and also aggregates the risk of myocardial damage, which leads to repeat angina [202, 203].

4.3. Limitations

Unfortunately this study was not powered optimally, owing to its exploratory pilot design, and the paucity of existing data comparing between EVH groups. Additional limiting factors include sample collection during the clinicians' learning curve period, longer sample storage in one group and a lack of randomisation between all groups. Additionally, we explored whether prolonged sample storage in 4% formaldehyde destroys tissue structure. The fixation of samples in formaldehyde plays four vital roles in immunohistochemistry by stabilising cell morphology and tissue structure, whilst also disabling proteolytic enzymes. This process also strengthens the samples, helping them to withstand further processing and staining, and finally, protecting against microbial contamination and decomposition [192]. We conclude that endothelial denudation in the closed CO_2 tunnel group is not due to prolonged formaldehyde storage. The lack of randomisation between groups limits the strength of our conclusions, as endothelial damage in the LSV may be due to pathological changes occurring in the coronary arteries and could be associated with advancing age.

Our study has several limitations. Firstly, it is not a randomised trial and evaluating two different EVH systems would require a controlled marker (such as traditional open vein harvesting) to compare how the vein graft should appear histologically with different baseline characteristics. Patient baseline characteristics such as diabetes [204], peripheral vascular disease, smoking, age [199] and hypertension [204] play a vital role in vein wall morphology, and endothelium-dependent relaxant vasomotion [199]. This could have resulted in a bias due to the lack of stratified randomisation, which cannot be fully eliminated by multivariable analysis, although no significant disparities in baseline demographics were observed upon analysis. The second limitation was the lack of experience in the EVH technique, which can increase the likelihood of structural damage to the vessel wall, thereby promoting early graft occlusion [179, 181]. The third limitation was that not all patients received an angiogram to confirm the absence of vessel damage or vein graft failure. The collection of pre-surgical angiographic reports is still required, along with complete revascularisation details for all other non-symptomatic patients. This will enable us to compare all patients' results and ascertain the level of coronary disease and vessel health.

4.4. Future Work

The use of pressurised closed CO_2 tunnel and non-pressurised open CO_2 tunnel for EVH needs to be examined further using several additional experiments and data collection.

Experiment 1: Randomised study comparing both EVH groups with OVH as control:

The EVH patients in both groups require randomisation and need to include an OVH control group to assess vessel wall integrity following coronary artery grafting. To minimise bias, statistical analysis would be performed with the help of a medical statistician.

Collection of clinical data at time of surgery:

All general demographics need to be included such as gender, age, race and BMI and we will use the exact data collection method which was used for this pilot work as follows: Pre-operative risk factors include diabetes, peripheral vascular disease, hypertension, family/smoking history, hypercholesterolemia, previous MI and percutaneous trans-coronary angioplasty (PTCA), gastrointestinal, lung, neurological and renal dysfunction, stroke, Parsonnet score and EuroSCORE, LV function and medication. Intra-operative data consists of a planned number of grafts and the type of conduit and choice of cardioplegia. All this data would be prospectively collected from a relational database. In addition, in-hospital mortality and community mortality need to be obtained from validated registry data, along with post mortem reports to assure data validity.

Long-term post-operative Major Adverse Cardiac Event (MACE) outcomes would be measured for this study. MACE was defined as recurrent angina, MI, target vessel revascularization , coronary / vein graft stenting and death [160]. MACE data includes repeat angina using Canadian Cardiovascular society grading system score (CCS) [161]. CCS is a validated scoring system that enables standardisation of angina grades and ranges from class I to class IV - inability to perform any activity (even at rest). Breathlessness will be assessed using the New York Heart Association (NYHA) scaling system [162]. This is a validated scoring system for standardising breathlessness and ranging from class I to class IV. Magnetic Cardiac Resonance Imaging (MRI), repeat angiogram, ECHO in-house and community hospital results will be obtained through the University Hospital of South Manchester (UHSM) cardiology database. The American College of Cardiology (ACC) and American Heart Association coronary lesion scoring system will be used to identify the quality of coronary vessels in

pre and post-operative angiographic pictures. This system is based on parameters such as lesion length, eccentricity, angulations, calcification, side branches involvement, thrombus formation and stenosis severity. The lesions are classified as Type A - discrete - <10mm, Type B - Tubular - 10-20mm or Type C - diffuse >2cm involvement [163].

Telephone data collection:

Patients will require follow up contact every 3 months for 24 months using a modified validated scoring system, which includes a MACE questionnaire. The quality of life scoring systems assess patient involvement in post-surgical physical and lifestyle activities, such as housework, shopping, driving and gardening. Emotional and social activities have also been taken into consideration and will compare the level of improvement following surgery. Health related quality of life will also be observed every three months using SF-36 and EQ-5D. A structured resource use data collection form will be used to identify each patient's use of NHS resources. This will be conducted every 3 months (total of 12 months) following hospital discharge.

Laboratory based endothelium assessment of collected samples:

Endothelial integrity must be determined with standard streptavidin/peroxidase techniques. Briefly, samples will be dehydrated using xylene/alcohol, before embedding in paraffin and sectioning to 4um with the aid of a microstat. Each section will be placed on poly-l-lysine coated histology slides, rehydrated, and endogenous peroxidase activity inhibited using hydrogen peroxide. Samples will be incubated with endothelial specific antibodies, including CD31 and CD34, then localised and visualised on a section of vessel. CD31, or PECAM-1, which is a 130 kDa member of the immunoglobulin super family required for cell-to-cell adhesion. CD31 is expressed constitutively on the surface of adult endothelial cells. CD34 is a single-chain transmembrane protein of approximately 116 kDa that is also expressed on vascular endothelial cells. Following antibody incubation, samples will be washed and incubated with a secondary antibody conjugated with biotin. This induces a colourimetric reaction. Following this, samples will be counter-stained using haematoxylin and eosin, and endothelial integrity will be visualised using microscopy. All samples will be initially assessed by the Principal Histopathologist at UHSM, and then graded by three independent assessors using a previously reported scale system, where 0 represents no staining and 4 represents intense staining [165].

Health economic analysis:

The primary aim of the economic analysis is to compare the cost of the two approaches to EVH and OVH. Unit cost data must be attached to the resource use data collected during surgery, in-patient readmission and 12 months follow up. Descriptive statistics will help summarise the mean costs and associated variations. The mean cost per patient, and total cost for each approach, will be calculated then analysed alongside the data on health status to define the relative costs and outcomes of the two EVH approaches. Appropriate statistical methods will compare the cost and health status data, taking into account the skewed nature of the data (for example, bootstrapping methods will help analyse the cost data). A key aspect of the economic analysis will be to understand the key drivers of cost effectiveness.

Experiment 2:

To analyse the absorption and true physiological effect of each CO_2 technique, a sample will be taken from each end of the vein. Samples must be obtained before receiving any surgical stress, such as manual handling, vein inflation and cardioplegia pressure. Further analysis will entail examination for endothelial denudation using the immunohistochemistry protocol used in our pilot work.

Experiment 3: Endothelial microparticle analysis

Endothelial denudation leads to vein graft failure and may contribute to negative clinical outcomes in EVH patients. In order to capture these changes, we aim to quantify the generation and release of endothelial microparticles (EMPs). EMPs have gained recent attention as they are released during the activation and apoptosis of endothelial cells. Exploring the production of EMPs in different vein harvesting techniques may be of significant clinical benefit.

CHAPTER 5

CONCLUSION AND SUMMARY

5. CONCLUSION AND SUMMARY

The greater endothelial denudation observed histologically in the closed tunnel CO_2 EVH system compared to open tunnel CO_2 represents an important finding in this pilot study. However, further prospective, randomised studies involving significantly greater numbers of patients are required to definitively confirm these findings. Additionally, it remains unclear what effect endothelial denudation has on long-term clinical outcomes, as our study was unable to demonstrate a significant difference between the groups over the time period assessed. Therefore, in our opinion, further exploration is required, with the aid of a randomised trial, to help determine the most favourable vein harvesting technique with the aim of improving patient care and clinical outcomes.

CHAPTER 6

REFERENCES

6. REFERENCES

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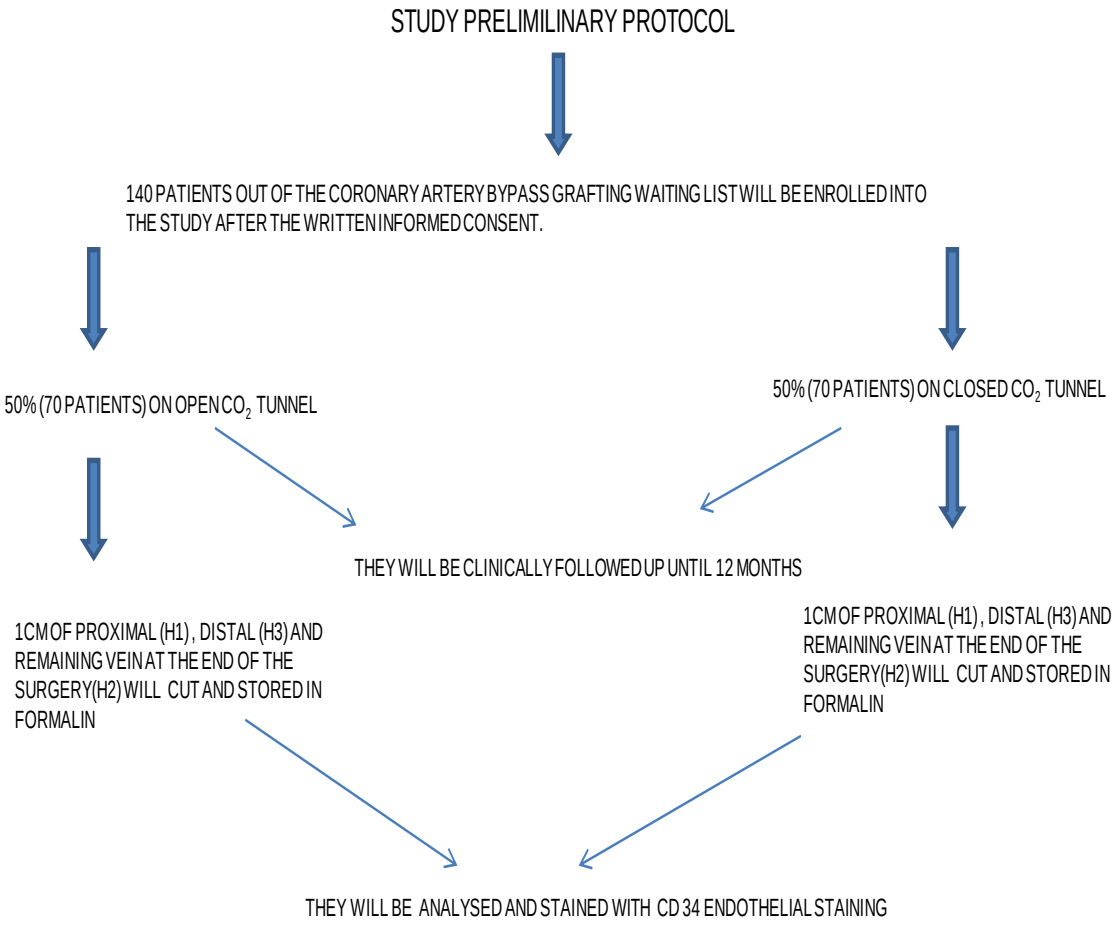
APPENDIX

Appendix 1: Preliminary study protocol



Histological end point: To assess the endothelial denudation and the presence of endothelium on the samples.

Clinical end point : The patients will be followed up to assess their quality of life, angina, revascularisation.

Appendix 2: Study final protocol flow chart