A randomised study comparing Vein Integrity and Clinical Outcomes (VICO) in open vein harvesting and two types of endoscopic vein harvesting for coronary artery bypass grafting.

By

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ABSTRACT

Background:

Coronary Artery Bypass Grafting (CABG) surgery is one of the most commonly performed surgical procedures to improve the symptoms of coronary artery disease. The Long Saphenous Vein (LSV) is typically used as a graft to bypass the blocked coronary arteries. The traditional way of harvesting the LSV is to make a long skin incision in the patient's leg. This technique has a high rate of incidence of wound complications and postoperative pain and poorer patient satisfaction. Endoscopic Vein Harvesting (EVH) techniques, introduced more than a decade ago, reduce these complications and improve quality of life. Findings regarding the safety and efficacy of EVH techniques and the quality of the vessel harvested by this technique are contradictory. Adoption of EVH techniques is still inconsistent globally and it is not completely accepted by all cardiac centres. Many studies are available in the literature measuring either histological outcome or clinical outcome in relation to different harvesting techniques. However, there remains no definitive randomised data available directly correlating harvesting-induced vein damage with clinical outcome.

The aim of this Vein Integrity and Clinical Outcome (VICO) randomised trial was designed to assess the direct relationship between the histological damage caused during different methods of vein harvesting and clinical outcome post coronary artery bypass surgery.

Methods:

100 patients were randomised in each group: Group 1 consists of closed tunnel CO_2 endoscopic vein harvesting (EVH) (CT-EVH) and Group 2 consists of open tunnel CO_2 EVH (OT-EVH) with the control Group 3 consists of standard open vein harvesting (OVH) with a total of 300 patients in this study. All the veins were harvested by an experienced practitioner who has performed >2000 OVH and >250 EVH. 1cm x 3 segments from three different parts of the vein were obtained for all patients (n=900). The histological levels of damage (endothelial and muscular layers) of the harvested vein and post clinical outcome for Major Adverse Cardiac Events (MACE) were measured using validated measuring tools. Health economic (cost effectiveness, EQ-5D) and health-related quality of life (SF-36) data were also recorded to assess the impact of these surgical techniques.

Results:

The level of endothelial disruption was greatest in the OT-EVH group in the proximal, distal and random samples (all $p<0.001$). The level of medial layer disruption was greatest in CT-EVH, with the least disruption observed in OVH for proximal, distal and random samples (all $p<0.001$). Internal muscle migration was greatest in OT-EVH compared to the other groups for proximal, distal and random samples (all $p<0.001$). Smooth muscle circular layer detachment was observed on a much greater scale in the endoscopic groups compared to OVH in proximal ($p=0.008$), distal ($p<0.001$) and random ($p=0.001$). Smooth muscle longitudinal layer detachment was consistent between groups in proximal ($p=0.113$) and distal ($p=0.380$) samples but was greater in endoscopic groups compared to OVH ($p=0.012$).

Secondary clinical outcomes demonstrated no significant differences in composite MACE scores at 3, 6, 12, 18 and 24 months. The quality adjusted life in years (QALYs) gain per patient was: 0.11 ($p<0.001$) for closed tunnel CO_2 EVH and 0.07 ($p=0.003$) for open tunnel CO_2 EVH compared with open vein harvesting. The likelihood of being cost-effective, at a pre-defined threshold of £20,000 per QALYs gained was: 75% for closed tunnel EVH, 19% for open tunnel EVH and 6% for open vein harvesting.

Conclusion:

In this study, open vein harvesting was associated with better preservation of vein layers in non-distended proximal samples than endoscopic vein harvesting. Both EVH groups displayed some degree of histological damage; OT-EVH was associated with more endothelial disruption. Clinical outcomes suggest that histological findings do not directly contribute to MACE outcomes. Gains in health status were observed and cost-effectiveness was better with CT-EVH compared with the other two surgical techniques. These results suggest that EVH can be utilised safely, but with careful selection of patients.

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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Parents: P. Krishnamoorthy and K. Banumathy

Sisters: Mahesh, Rajee, Jagan

Brother: KasiRaman

Husband: Raj

Children: Harine Raaj and Sam Raaj

Neighbour: Peter Wray

For their tremendous support throughout this study.

LIST OF ABBREVIATIONS

ACC : American College of Cardiology

ADP : Adenosine Di-Phosphate

AHA : American Heart Association

CABG : Coronary Artery Bypass Grafting

CAD: Coronary Artery Disease

CCS : Canadian Cardiac Classification System

CI : Confidence Interval

CO2 : Carbon dioxide

ECG : Electrocardiography

ECHO : Echocardiography

EDRF : Endothelial Derived Relaxing Factors

EVH : Endoscopic Vein Harvesting

HCO3 : 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

PaCO2 : 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

LIST OF ORAL PRESENTATIONS

1. **Validation of the endothelial staining markers CD31 and CD34 in immunohistochemistry of the long saphenous vein.**

   B Krishnamoorthy Mphil [1], WR Critchley MSc [2], JB Barnard [1], PD Waterworth MD FRCS [1], AC Caress PhD [3], J Fildes PhD [2] , N Yonan MD, FRCS[1].

   Presented in World Society of Cardiothoracic surgery (WSCTS) on August 2015 at Royal College of Surgeons, Edinburgh, GB.

2. **Interim results of a randomised study comparing Vein Integrity and Clinical Outcomes (VICO) between different vein harvesting techniques for bypass surgery.**

   B Krishnamoorthy Mphil [1], WR Critchley MSc [2], AC Caress PhD [3], J Fildes PhD [2] , N Yonan MD, FRCS[1].

   Presented in Society of Cardiothoracic surgery (SCTS) on March 2016 at International Convention Centre, Birmingham, GB.

3. **Randomised control trial comparing the effect of carbon-dioxide insufflation on vessel integrity using different types of vein harvesting for coronary artery bypass surgery- Interim results.**

   Bhuvaneswari Krishnamoorthy[1], Faisal Hashmi[1], Isaac Kadir[1], Paul Waterworth[1], Ann Caress[2], James Fildes[2], NizarYonan[1] , Wythenshawe hospital (GB).

   Presented in Scandinavian cardiothoracic surgery conference (SATS) on September 2016 at Harpa Centre, Iceland.

4. **Randomised control trial comparing the effect of carbon-dioxide insufflation and histological damage on different types of vein harvesting for coronary artery bypass surgery.**

   Bhuvaneswari Krishnamoorthy[1], Nehru Devan[1], John Carey[1], Ann Caress[2], James Fildes[2], NizarYonan[1] , Wythenshawe hospital (GB).

   Presented in European Cardiothoracic conference (EACTS) on October 2016 at International conference centre, Barcelona, Spain.

5. **Randomised control trial comparing endoscopic and open vein harvesting for coronary artery bypass grafting: Histological damage on distended and non-distended long saphenous vein.**

   Bhuvaneswari Krishnamoorthy[1], Nehru Devan[1], Janesh Nair[1], Andreas Paschalis[1], Innois Dimarkis[1], Ann Caress[2], James Fildes[2], Nizar Yonan[1] , Wythenshawe hospital (GB).

   Presented in European Cardiothoracic conference (EACTS) on October 2016 at International conference centre, Barcelona, Spain.

6. **A randomised study comparing the cost-effectiveness of two types of endoscopic versus traditional open vein harvesting for coronary artery bypass surgery.**

   Bhuvaneswari Krishnamoorthy[1], Alexander Thompson[2], Katherine Payne[2], Ann Caress[3], James Fildes[4], Nizar Yonan[1] , Wythenshawe hospital (GB).

   Accepted and will be presented in Society of Thoracic Surgeons conference (STS) on January 2017 at Houston, Texas.

LIST OF PUBLICATIONS

1. An overview of minimally invasive vein harvesting. Accepted for publication and minor corrections done with Editor on Betham Open access, Saudi Arabia publications (2016). **B Krishnamoorthy,** Dimarakis I, Critchley WR, Caress A, Fildes JE, Yonan N. **(IN PRESS)**

2. A validation of endothelial staining markers comparing CD34 vs CD31 for coronary artery bypass surgery. (2016). Madridge journal of cardiology. **B Krishnamoorthy**, W Critchley, R V Venkateswaran, A Caress, J Fildes, N Yonan. October 2016.

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1. Randomised controlled trial comparing the effect of carbon-dioxide insufflation on vessel integrity using two types of endoscopic and open vein harvesting for coronary artery bypass surgery. **B Krishnamoorthy**, W R Critchley, J Nair, I Malagon, J Carey, J Barnard, P Waterworth, J E Fildes, A Caress, N Yonan.

2. Randomised control trial comparing endoscopic and open vein harvesting for coronary artery bypass grafting: Histological assessment on distended and non-distended long saphenous vein. **B Krishnamoorthy**, W R Critchley, M Mohammed, N Devan, I Kadir, R Venkateswaran, A Caress, J E Fildes, N Yonan.

3. A randomised study comparing Vein Integrity and Clinical Outcomes (VICO) in open vein harvesting and two types of endoscopic vein harvesting for coronary artery bypass grafting – The VICO trial. **B Krishnamoorthy**, W R Critchley, A J Thompson, K Payne, J Morris, A Caress, J E Fildes, N Yonan.

CHAPTER 1

Introduction

1. INTRODUCTION

1.0 : CORONARY ARTERY DISEASE

The rising incidence of diabetes and obesity in the Western world has increased the risk of cardiovascular diseases, which is likely to become the main cause of death globally in the next 15 - 25 years (Murray and Lopez, 1997). Cardiovascular diseases are the predominant factor in almost 38% of all deaths in North America and Europe, with coronary artery disease (CAD) being the most common cause of death in both men and women above 65 years (Hansson, 2005). CAD is caused by the gradual deposition of atherosclerotic plaques and intraluminal thrombosis, which leads to pathogenesis of unstable angina, acute myocardial infarction and sudden death (Figure 1) (Epstein et al., 1992).

Myocardial infarction (MI) occurs once the atherosclerotic plaque inhibits blood flow through the coronary arteries to the heart muscles. It was previously thought that narrowing of the arterial lumen due to continuous growth of plaque smooth-muscle cells was responsible for myocardial infarction. However, many angiographic studies have revealed that activation of the plaque, coronary spasm and thrombus formation over the plaque are responsible for infarction and ischemia of the myocardium (Figure 2) (Hansson, 2005; Davies, 1996).

1.1.1: TREATMENT

The first line of treatment for CAD is medical management such as nitroglycerin, aspirin, beta-adrenergic blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors and drug-eluting stents (Scott et al., 1994; Passamani et al., 1985; Zubiate et al., 1977). Interestingly, a lot of work has been performed to assess the use of aspirin and its effects on reducing the risk of a first MI by 44% (Hennekens, 1989) and the inhibition of prostaglandin synthesis (Vane, 1987). It is also proven that the formation of thromboxane A_2, which aggregates the effects of platelets and vasoconstrictive prostaglandin, is inhibited when aspirin acetylates cyclooxygenase (Ridker et al., 1997; Vane, 1987). Hence, it remains controversial whether low doses of aspirin will prevent coronary disease events by inhibiting the inflammatory process.

The medical management of CAD remains challenging, despite the introduction of newer techniques such as Percutaneous Trans-Coronary Angioplasty (PTCA), balloon angioplasty, bare metal stents and drug eluting stents. However, surgical management via Coronary Artery Bypass Grafting (CABG) yields significantly lower mortality rates than medical management (Yusuf et al., 1994; Windecker et al., 2014).

1.1.2: CORONARY ARTERY BYPASS GRAFTING

Since its introduction in 1960, CABG has become the most common surgical procedure in cardiac surgery. CABG relieves angina pectoris, all symptoms of CAD and improves quality of life (Favaloro, 1968; Dee, 2003; Eifert et al., 2010). CABG is carried out to bypass the blocked coronary arteries by using autologous arterial and venous conduits obtained from the patient (Figure 3). The number of bypasses depends upon the number of blocked coronary arteries (Figure 4). The surgery is performed by splitting open the breast bone in the midline and exposing the heart by cutting the outer covering of the pericardium (Mullany, 2003). The main conduits are arterial grafts (internal mammary arteries and radial arteries) and venous conduits (long saphenous and short saphenous veins) (Legare et al., 2004).

1.1.3: ARTERIAL CONDUITS

Arterial conduits play a vital role in CABG surgery due to their physical and functional properties. The internal mammary artery and radial artery are the most common arterial conduits used for bypass surgery.

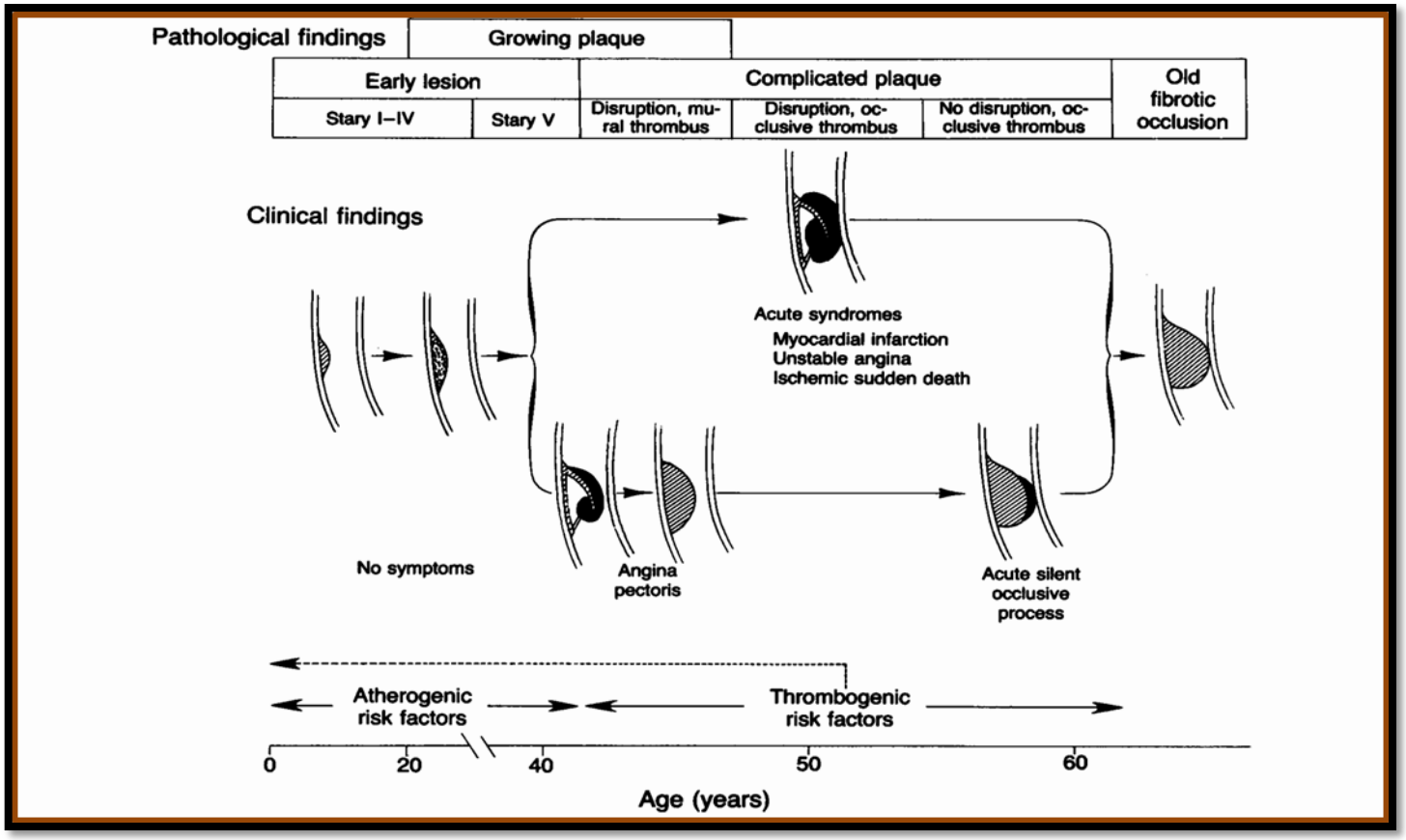

Figure 1: The progression of coronary atherosclerosis with clinical and pathological findings.

This figure represents the different stages of plague growth as well as pathological and clinical findings. This also illustrates the age related risk factors of stages of coronary atherosclerosis (Epstein et al., 1992).

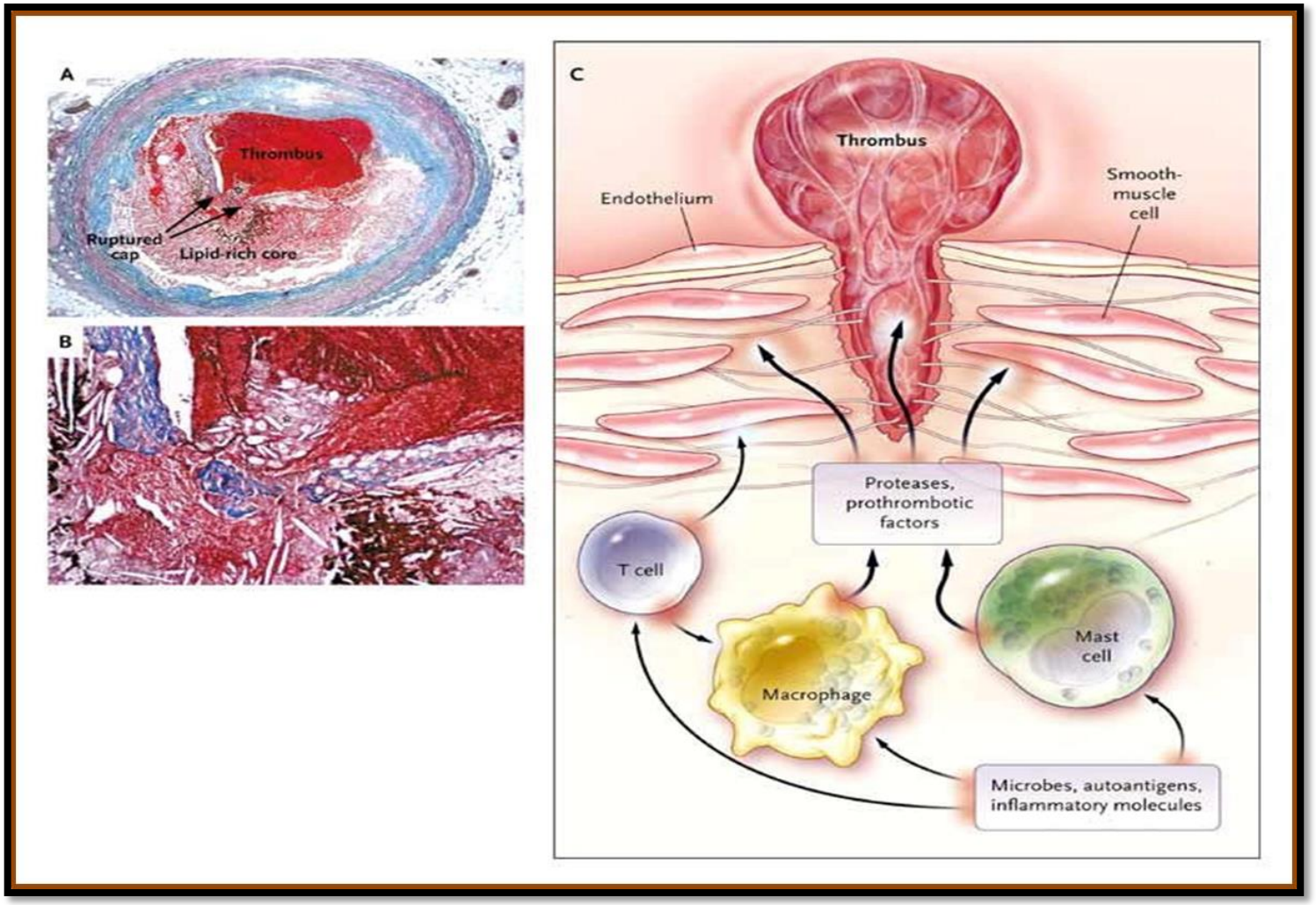

Figure 2: This figure illustrates the process of atherosclerotic lesion formation on the human arteries.

This figure represents the detailed stages of atherosclerotic lesion formation on the arteries and also the interactions between the inflammatory molecules and the arterial tissues (Epstein et al., 1992).

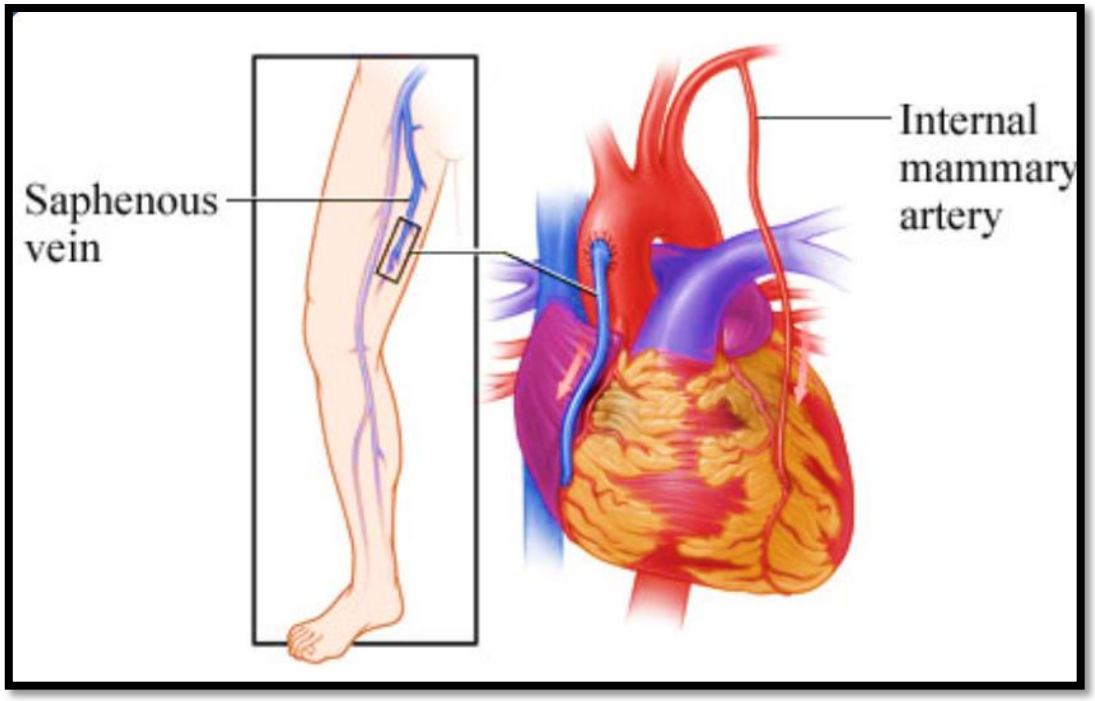

Figure 3: Diagrammatic representation of the coronary artery bypass surgery.

This figure illustrates the bypass surgery of an internal mammary artery disconnected from one end of the chest wall and connected to the left anterior descending artery after the block. Similarly, the long saphenous vein has been harvested from the patient leg and attached to the right coronary artery and aorta. This figure is obtained from WebMed, healthwise incorporation medical reference last accessed on 07/08/14.

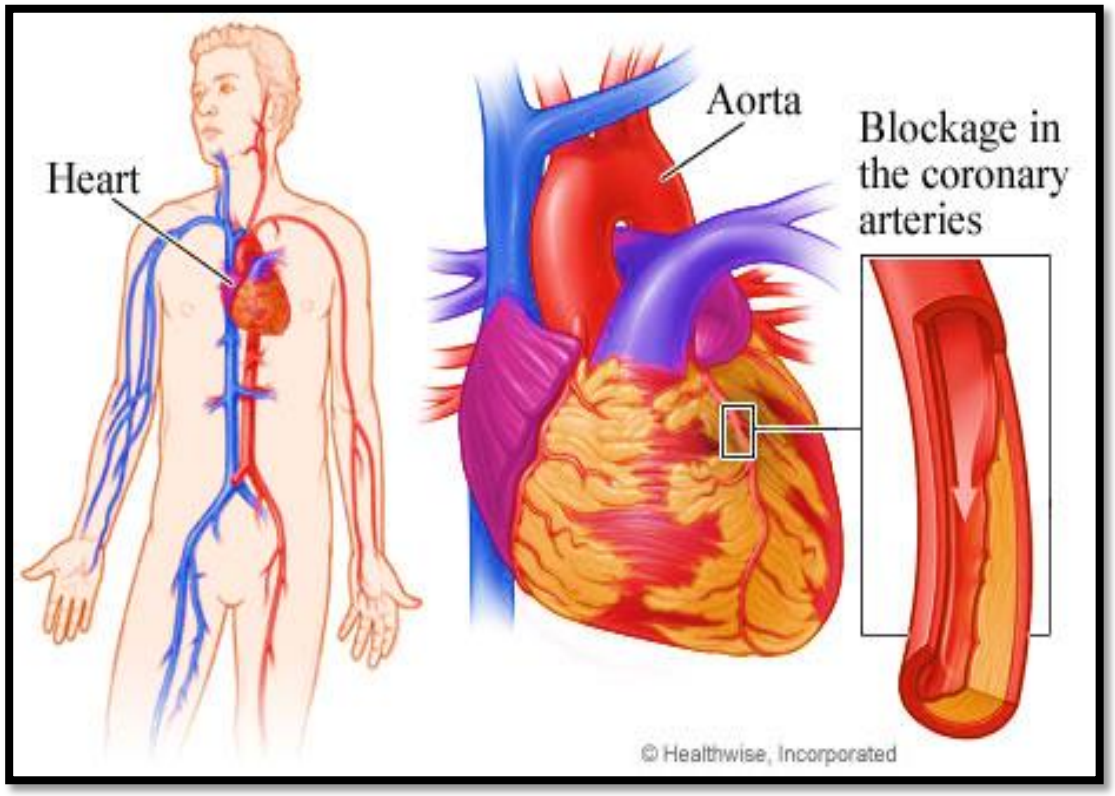

Figure 4: Diagrammatic representation of blocked coronary artery and the human body.

This figure represents the situation of the heart inside the human body and also the blocked left anterior descending coronary artery. The vertical section of the coronary artery illustrates the gradual deposition of the plague inside the artery which narrows the lumen and reduces the normal blood flow beyond this point. This figure is obtained from WebMed, healthwise incorporation medical reference last accessed on 07/08/14.

1.1.4: INTERNAL MAMMARY ARTERY

There are two internal mammary arteries (IMA) in the human body: the right IMA and the left IMA. The arteries are located just behind the breastbone or sternum on either side. The left IMA is the most commonly used conduit due to its easy accessibility to the left anterior descending coronary artery (Figure 5). It was first introduced in 1964 (Mehta and Khan, 2002) and used as a pedicle graft for left anterior descending coronary anastomosis in 1970 (Green et al., 1970). It is associated with a 90% graft patency over a 10 year period (Cheng et al., 2010). It is considered to be a gold standard conduit for bypass surgery due to its high patency rate and its long term survival rate (Cameron et al., 1996). However, it is very difficult to use the limited length of IMA as a pedicle graft for posterior coronary vessels such as the posterior descending artery, posterior left ventricular branch and obtuse marginal (Tabata et al., 2009). As such, the posterior coronary vessels are always grafted using a free long length radial artery and venous conduits.

1.1.5: RADIAL ARTERY

The radial artery was first used by Carpentier and colleagues in 1971 (Carpentier et al., 1973) but it was abandoned because of graft failure within a 2 year period (Geha et al., 1975). Mostly, the failure of the radial artery conduit was a consequence of graft spasm and severe intimal hyperplasia caused by mechanical traction and dilation (Curtis et al., 1975; Fisk et al., 1976). Interestingly, the discovery of patent radial artery grafts 15 years after bypass surgery increased the popularity of its use (Acar et al., 1992). To reduce spasm, there are a few modifications carried out during harvesting of the radial artery, including pedicle graft technique (harvested with surrounding fat tissues) and non-touch technique (minimal handling of the direct radial artery tissues) (Fremes et al., 1995). Many centres utilise the arterial conduits of the IMA and radial artery for single or double bypass surgery, although the long saphenous vein is still the preferred conduit for multi-vessel bypass surgery due to its long length.

1.1.6: Anatomical comparison of arterial and venous conduits

Anatomically, the internal mammary artery has a discontinuous internal elastic laminal layer, a thin medial layer with multiple elastic lamina and an increased production of nitric oxide. The absence of a thick muscular layer explains the reduced tendency for vascular spasm and development of atherosclerosis (Taggart, 2013). However, the radial artery has a thin continuous intima of endothelial cells, an internal elastic lamina and thick medial smooth muscle cells which are prone to immediate vascular spasm, occlusion and thrombosis (Ruengsakulrach et al., 1999). In contrast, the long saphenous vein has a thinner, less elastic and more muscular medial layer, which more commonly leads to thrombosis and re-occlusion compared to the internal mammary artery with 50% of grafts failing within 5 to 10 year (Fitzgibbon et al., 1996).

1.1.7: Venous Conduits

The long and short saphenous veins are the alternative conduits whenever arterial conduits are not possible or not long enough for bypass surgery. The use of the short saphenous vein is very limited due to its short length (10-15cm), small luminal diameter (varying from 2.8mm to 4.2mm) and the anatomical difficulties involved during harvesting (Ibrahim and Refaat, 2007). The long saphenous vein (Figure 6) is most commonly used due to its long length (60-90cm) and its lack of invasiveness (Favaloro, 1969), and was first used in 1967 (Favaloro et al., 1971; Mehta and Khan, 2002). There are different methods of harvesting the long saphenous vein, including open vein harvesting, bridging and endoscopic vein harvesting.

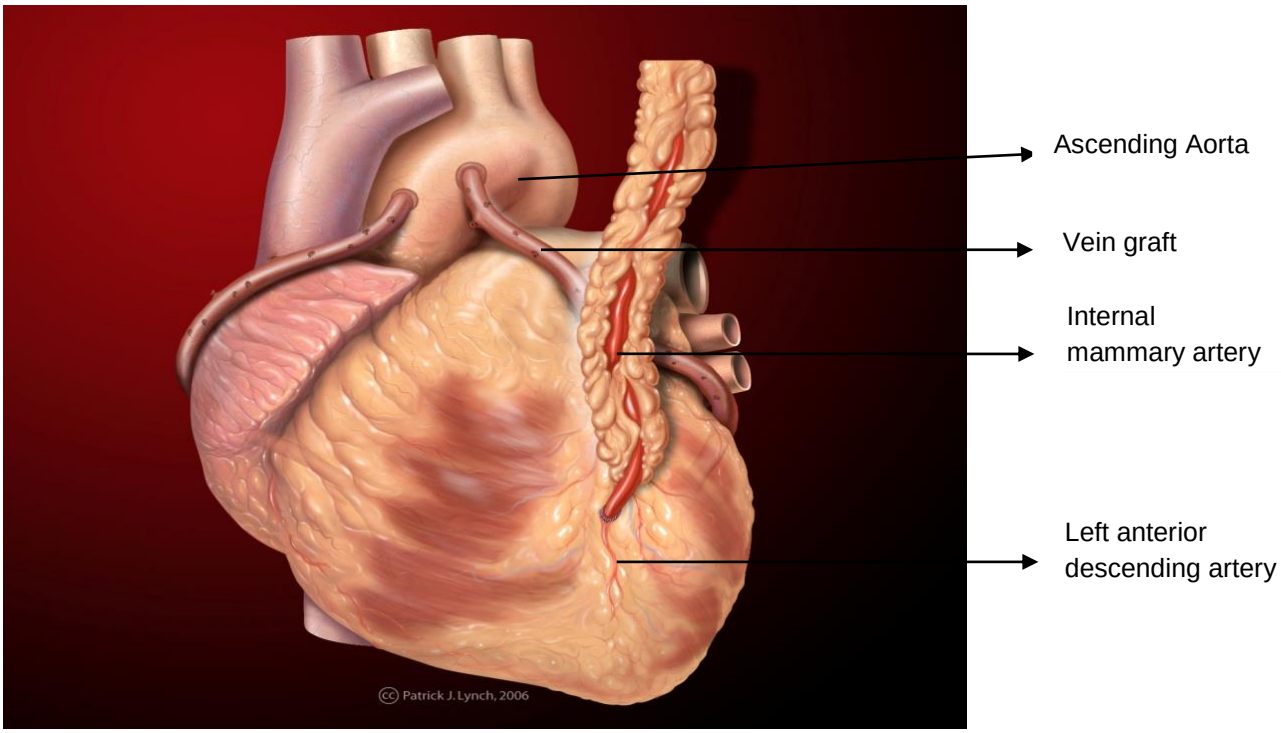

Figure 5: Diagrammatic representation of the heart with coronary artery bypass grafts.

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. This figure is obtained from WebMed, healthwise incorporation medical reference last accessed on 07/08/14.

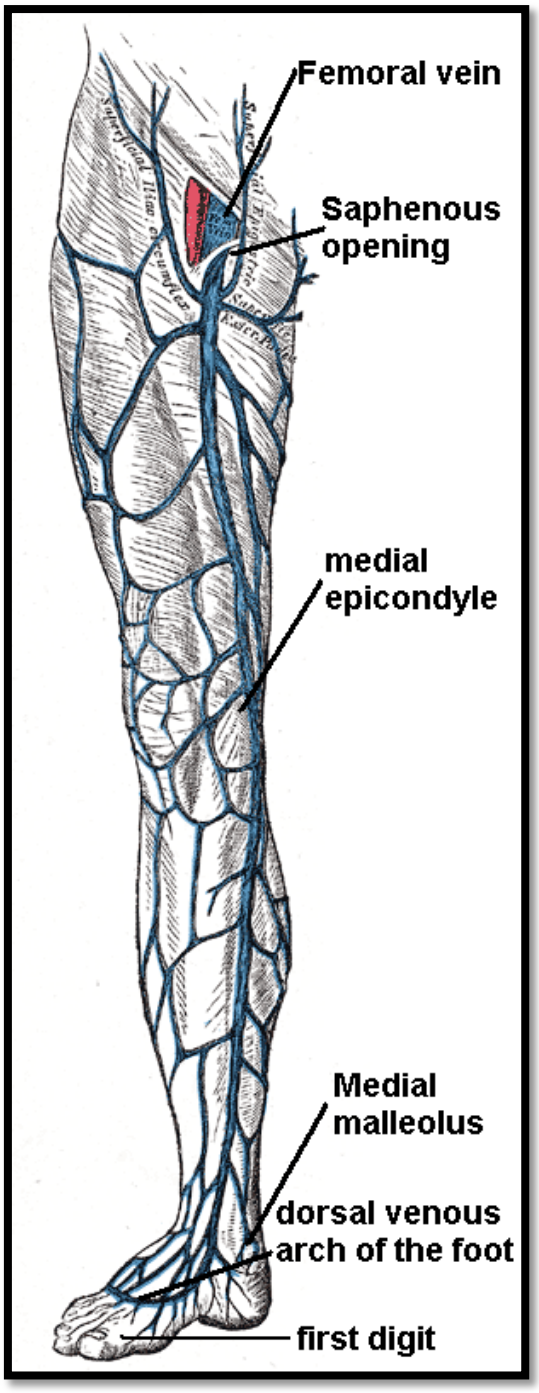

Figure 6: Diagrammatic representation of the long saphenous vein on the human body.

This figure illustrates the detailed anatomical course of the long saphenous vein. Following its origin, it passes anterior to the medial malleolus in the foot, and then runs along the medial aspect of the leg, posterior to the medial epicondyle of the femur before passing anteriorly to connect with the femoral vein in the groin. This figure is obtained from Medscape medical images last accessed on 07/08/14.

There are clear advantages and disadvantages for the use of each different vein harvesting method. However, the use of EVH as a routine surgical procedure remains controversial due to questions regarding long term graft patency, morbidity, vein graft quality and rates of repeat angina and mortality. In the literature review (Chapter 03) all the types of EVH systems in cardiac and other specialities from the initial introduction of endoscopic systems will be considered.

CHAPTER 2

Literature Synthesis

2.0 An overview of literature search and strategy

2.1: Introduction

A literature search was conducted on a number of databases including: Medline, CINHAL, PubMed, Cochrane, and Google Scholar. The aim of the literature review was to assess the findings of histological and clinical studies in order to answer the following research questions:

1. What studies have been conducted comparing different vein harvesting techniques for coronary artery bypass surgery?
2. What histological research studies are available providing data on vein trauma during conduit harvesting?
3. Is there a relationship between the extent of vein trauma during surgery and long term clinical outcomes?
4. What are the cost implications of different harvesting techniques and how do these affect patients' quality of life after coronary artery bypass surgery?

2.1.2: Search approach

This literature search (Appendix 1 and Appendix 2) was conducted during January 2014 to identify studies related to the research questions, and included all relevant studies since 1950. Notably, there was observed to be a paucity of randomised trials and quality research exploring vein harvesting techniques.

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist (Liberati et al., 2009) was used for the literature search. The primary aim of the PRISMA checklist is to help ensure the clarity and transparency of reporting of systematic reviews (Moher et al., 2007; Liberati et al., 2009). Figure 7 illustrates the four-phase PRISMA flow diagram of this literature search.

2:1.3: Search strategy

The literature search was performed in a structured manner using five components, "PICOS": patient population/or the disease (P), the interventions/exposure (I), comparator group (C), the outcome or endpoint (O) and finally study design (S). The PICOS terms used for this study are highlighted in Table 1.

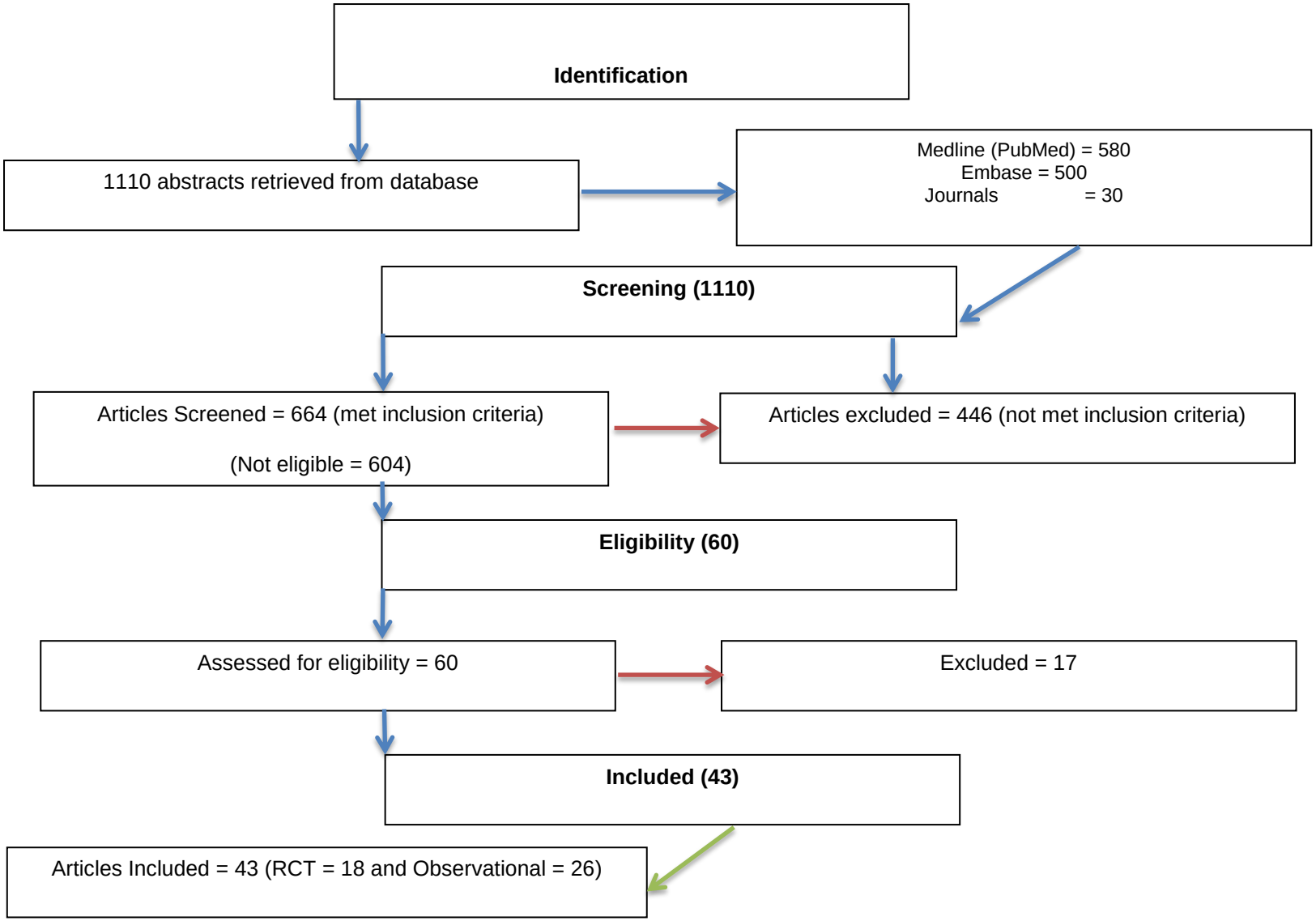

Figure 7: PRISMA flow chart includes a detailed database search history, screening and studies included for this literature synthesis.

Table 1: This table illustrates the "PICOS" search strategy.

Patient /disease	Interventions	Outcomes	Study design
Coronary artery bypass surgery	Endoscopic vein harvesting OR	Clinical outcome OR	Vein trauma OR
Cardiac surgery	Open vein harvesting AND	Major cardiac adverse events OR	During harvesting OR
Endothelial damage	Different vein harvesting methods AND	Level of damage AND	Non-touch technique, pedicle graft. AND
Cost analysis	Evaluation OR	Benefit OR	Health care, cost minimisation, cost effectiveness. OR
Quality of life	Post cardiac surgery AND	Physical and emotional wellbeing of an individual. AND	Health related, general. AND

Table 2: Inclusion of database for literature search.

Database	Origin
Cochrane	Central register for controlled trials. Database for systematic reviews and meta-analysis.
Medline	US National Library of Medicine (1946 – 2014)
PubMed central.	US data base for all scientific literatures (1996 – 2014)
CINAHL	Cumulative Index to Nursing and Allied Health Literature (1937-2014).

Only articles between 1950 and 2014 were included in the search, as this corresponds to the time in which CABG surgery has been performed (Table 2). Only full articles written in English were retrieved from the database due to the lack of a translator.

2:1.4: SEARCH KEY TERMS USED FOR THIS LITERATURE REVIEW

The search term for this project was identified through Medical Subject Headings (MeSH), keywords, online synonyms finder and thesaurus.

MeSH terms were carefully used to identify studies and to exclude studies which utilised short saphenous vein and femoral vein conduits. As such, the “long saphenous vein” or “greater saphenous vein” terms were used to ensure the transparency and uniformity of meaning. Table 3 illustrates the MeSH terms utilised for the literature review.

Table 3: MeSH terms and abbreviations.

Heading	Detailed definitions	MeSH and search terms
Saphenous Vein	A superficial vein running from the feet to the groin.	Vein (long saphenous vein, greater saphenous vein)
Coronary artery bypass surgery	Bypass surgery to restore the blood supply to the heart after blockage.	Conduits, bypass, coronaries.
Wound infection	A clean wound gets infected due to bacterial or fungal invasion.	Leg wound infection, surgical site infection, donor leg wound infection.
Health related quality of life	Patients' quality of life after surgery.	Health care, general quality of life, cardiac related quality of life, age related quality of life.
Health economics	The cost analysis which is used in relation to a medical or surgical intervention.	Cost evaluation, NHS procedural cost and cost-benefit analysis.
Outcomes	Clinical outcomes post-surgery.	Health related clinical outcomes, positive and negative outcomes.
Vein histological outcomes.	Structural and morphological alterations in the vein conduit following retrieval and surgery.	Endothelium, muscle layer, adventitia, secretions.

2:1.5: RESULTS OF DATABASE SEARCH

Table 4 demonstrates the number of hits per database within each section of the search. The results take into account the following restrictions: only animal, human and English language studies with publications until July 2014 are included.

This search brought a total of 1110 articles abstract for review. All abstracts were carefully read and only the relevant ones were chosen for further review to answer the research question. A total of 664 abstracts and papers were screened, 446 excluded because they did not meet the inclusion criteria. There were a total of 60 articles left after eligibility checking from 664, although only 43 (18 RCTs and 28 Observational) were determined to be relevant for answering the research questions and were thus included for this review. A total of 17 studies were excluded because these articles compared endoscopic harvesting with other minimally invasive techniques rather than open vein harvesting. Some of the articles were also only available in languages other than English.

2:1.6: INCLUSION AND EXCLUSION CRITERIA FOR SEARCH STRATEGY

To ensure a comprehensive search was performed, the inclusion criteria were kept as wide as possible within the cardiac surgical speciality. However, the histological inclusion criteria were widened to include studies from outside the cardiac surgery speciality. This allowed the retrieval of data describing the properties of healthy and diseased human veins and research of saphenous veins that might be relevant. The inclusion and exclusion criteria are illustrated in Table 5.

Table 4: Number of articles collected across each database.

Database and journals	Search terms	ST	ST	Total
Medline(PubMed)	12869	12763	28780	54412
Embase	15446	27153	1215610 (cost)	1258209
Journals Annals of thoracic surgery etc.	46169	83592	1429	131190
Total	74484	123508	1245819	1443811

Table 5: Inclusion and exclusion criteria for studies to be assessed in this review

Lists	Inclusion criteria	Exclusion criteria
Study design	- Randomised controlled trials, - Cohort trials, - Observational studies, - Non-randomised studies, - Case reports, - Letter to editors, - Editorial comments, - Retrospective studies. - In vitro studies. - Histological studies.	None
Interventions	- Coronary artery bypass surgery - Minimally invasive vein harvestings techniques such as open vein, standard vein harvesting, traditional vein harvesting, endoscopic vein harvesting, bridging vein harvesting techniques. - Laparoscopic surgeries. - Effect of carbon-di-oxide on the tissues. - Effect of carbon di-oxide air embolism on the blood.	- Vein stripping, - Mayo vein stripping techniques.
Outcomes	- Short term and long term clinical outcomes, - Health economic evaluation. - Cost related outcomes, - Patient benefit outcomes, - Patient satisfaction, - Health Quality of life (HRQoL) outcomes, - Histological outcomes. - Effect of diabetes and vascular diseases on the saphenous vein.	None.

2:1.7: REASON FOR NOT CONDUCTING A SYSTEMATIC REVIEW

A systematic review of the clinical and histological studies on this subject would be ideal for this research. However, as there was a recent systematic review published in 2013 (267525 patients) providing up-to date evidence from 1998 until 2013, this was not repeated.

2:1.8: QUALITY OF TRIAL REPORT ISSUES

There are three main methods to assess the quality of clinical trials such as individual markers, checklists and scales (Jadad et al., 1996). Importantly, scales have theoretical benefit over other methods, because they provide quantitative approximations of quality that can be duplicated easily and amalgamated formally into the peer review process (Moher et al., 1996). Assessing the quality of a trial is very important because it gives the health care professional the opportunity to estimate the probability that the study results are valid and truthful (Moher et al., 1995).

There have been many scales and checklists developed to date. The first scale was published in 1981 (Chalmers et al., 1981) and checklists were first published in 1961 (Badgley, 1961). Currently, almost 33 scales and 53 checklists (Sanderson et al., 2007) to assess the quality of Randomised Control Trial (RCTs) have been published.

There are a few limitations of the current scales and checklists, such as lack of sub-scales, which provides a profile of the strengths and weakness of each methodological concern and their exclusion of any consideration of external validity (Downs and Black, 1998). After, a detailed search on Medline and Cochrane regarding which checklist is suitable to use for RCTs and non-RCTs, it was accepted that the Downs and Black checklist (Appendix 3) was the most suitable for this literature review.

2:1.9: QUALITY ASSESSMENT

All 43 abstracts were read carefully and relevant full PDF papers were downloaded and printed for quality assessment. The full text was read carefully and the following were noted: year of publication, year of submission, date accepted for publication, authorship, sponsor details, study design, sample size, risk factors, operative time, harvesting time, number of grafts, number of conduits obtained from the patient, primary outcomes, secondary outcomes, power calculation, statistical analysis and results of the study.

In this assessment, the Jadad scale (Appendix 4) was used for all RCT studies, which uses seven items to provide a possible score of 0-poor and 5-excellent. All the observational studies were assessed using the Downs and Black 27 items checklist, with a possible score from 0-27.

A total of 18 RCTs, 9 prospective non-RCTs and 17 retrospective studies were included in this thesis. The tables 06 - 17 illustrate the explanation of the studies in detail with their scoring marks.

Table 6: Summary of randomised studies comparing open and endoscopic harvesting (1).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Jadad (0-5) and Downs & Black score (0-27).
Allen 1998 Annals of thoracic surgery.	- RCT -n=109 (51:EVH & 58: OVH).	- Ethicon Endopath.	- Post op Pain. - Length of stay. - Myocardial Infarction. - Mortality.	- 6 weeks	- Small sample size to look at the difference of clinical outcomes. - No short term benefits such as wound infection compared between groups.	- 02/18.
Folliguet 1999 Arch Mal Coeur Vaiss. (Archives des maladies du Coeur et des vaisseaux) French but requested Library for English version. Got help from French surgeon.	- RCT - n=120 (EVH:60 & OVH: 60)	- Ethicon Endopath and Vasoview Guidant.	- Wound infection. -Incidence of Haematoma. - Length of stay. - Myocardial Infarction. - Mortality.	- 7 days.	- Very short term follow up. - Most wound infections happen within a 2 week period. - Used two types of EVH system but did not separate them.	- 01/15
Hayward 1999 Annals of thoracic surgery.	- RCT - n=100 (EVH: 50 & OVH: 50).	- Ethicon Endopath.	- Wound infection. - Incidence of Haematoma. - Length of stay.	- 6 Weeks	- Poorly designed RCT. - Not adequately powered.	- 01/16

Table 7: Summary of randomised studies comparing open and endoscopic vein harvesting (2).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Jadad (0-5) and Downs & Black score (0-27).
- Isgro 1999 - European Journal of Cardiothoracic Surgery.	- RCT. - n=208 (EVH: 103 & OVH: 105)	- Vasoview Guidant.	- Wound infection. - Incidence of Haematoma.	- In-hospital admission only.	- Inappropriate design. - Just prospective data collection without any validated scoring system for wound infection.	- 0/13.
- Puskas 1999 - Annals of thoracic surgery.	- RCT - n=97 (EVH: 47 & OVH: 50).	- Ethicon Endopath.	- Wound infection. - Incidence of Haematoma. - Post op pain. - Length of stay.	- 1 month	- Design poor for an RCT. - Only reported medians for pain score and length of hospital stay not mean score. - Validated scoring tool used for the assessment. - 4 week follow up period.	- 3/19.
- Carpino 2000 - Journal of Thoracic Cardiovascular surgery.	- RCT - n=132(EVH:66& OVH:66).	- Vasoview Guidant.	- Wound infection. - Length of stay.	- 2 weeks	- Poor design. - Short term follow up in regards to wound infection.	- 01/20

Table 8: Summary of randomised studies comparing open and endoscopic vein harvesting (3).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Jadad (0-5) and Downs & Black score (0-27).
- Cisowski 2000. - Medical Science Monitor International Basic research journal.	- RCT - n=45 (EVH:30 & OVH:15).	- Ethicon Endopath.	- Wound infection. - Incidence of Haematoma.	- 7 days.	- Not adequately designed RCT. - Short term follow up. - Concealment – not reported.	- 01/16.
- Fabricius 2000 - Annals of thoracic surgery.	- RCT. - n=61 (EVH:31 & OVH:30)	- Ethicon Endopath.	- Wound infection. - Incidence of Haematoma.	- 6 days.	- Poor RCT design. - No concealment. - Short term follow up.	- 00/17.
- Kiaii 2002 - Journal of Thoracic Cardiovascular surgery.	- RCT - n=144 (EVH: 72 & OVH: 72).	- Ethicon Endopath.	- Wound infection. - Length of stay. - Myocardial Infarction. - Mortality.	- 6 weeks.	- Poor design for RCT& No concealment. - No validated scoring system and 5 patients converted & 10 patients lost to follow-up.	- 01/21.
- Schurr 2002. - Journal of Thoracic Cardiovascular surgery.	- RCT. - n=140 (EVH: 80 & OVH: 60).	- Vasoview Guidant	- Wound infection. - Incidence of Haematoma - Length of stay.	- 3 months.	- Validated scoring tool & Good follow up period including readmissions in relation to the wound. - External validity not reliable due to poor reporting of the results.	- 03/21

Table 9: Summary of randomised studies comparing open and endoscopic vein harvesting (4).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Jadad (0-5) and Downs & Black score (0-27).
- Bonde 2004 - Annals of thoracic surgery.	- RCT - n=108 (EVH: 52 & OVH: 56).	- Ethicon ClearGlide.	- Wound Infection. - Post op Pain. - Myocardial Infarction. - Mortality.	- 2 and 3 year follow up.	- Design appropriate. - Random adequate. - Drop outs included.	- 03 / 21.
- Perrault 2004. - Journal of Thoracic Cardiovascular surgery	- RCT - n= 32 (EVH: 17 & OVH:15)	- Vasoview Guidant	- Wound Infection. - Length of stay. - Graft failure & Mortality - Myocardial Infarction.	- 10 months.	- Design appropriate. - Very small sample size. - Not adequate powered.	- 03 / 19.
- Yun 2005 - Journal of Thoracic Cardiovascular surgery.	- RCT - n= 197 (EVH:97 & OVH:100)	- Vasoview Guidant	- Wound infection. - Incidence of Haematoma. - Graft failure. - Mortality.	- 6 months.	- Poor design. - Not adequate concealment. - No description of dropouts. - Poor internal validity.	- 01 / 22

Table 10: Summary of randomised studies comparing open and endoscopic vein harvesting (5).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Jadad (0-5) and Downs & Black score (0-27).
- **Schultz 2006** - Journal of Cardiothoracic surgery.	- RCT - n=200 (EVH: 100 & OVH/MIVH: 100)	- Ethicon Clearglide	- Wound infection. - Incidence of Haematoma.	- 12 weeks	- Too many patients converted to OVH in the EVH. - Not appropriately designed & Drop outs not included.	- 03 / 20
- Andreasen 2008 - European Journal of Cardiothoracic Surgery.	- RCT - n= 129 (EVH: 66 & OVH: 63).	- Vasoview 5 & 6.	- Wound infection. - Incidence of Haematoma. -Post op Pain and Myocardial Infarct. - Length of stay & Mortality.	- 1 month.	- Inappropriate randomisation. - Not reported mean for length of hospital stay; only median reported.	- 03 / 25
- Au 2008 - Journal of Cardiac Surgery.	- RCT - n= 114 (EVH: 54 & OVH: 60)	-Terumo VirtuoSaph.	- Wound Infection. -Post op Pain. - Mortality.	- 21 days	- Very short term mortality assessment. So, internal validity is questionable. - Validated scoring used for pain and wound assessment.	- 03 / 23
- Wang 2011. - Heart Surgery Forum.	- RCT - n= 40 (EVH: 20 & OVH: 20)	- Vasoview.	- Myocardial Infarction. - Mortality.	- I month.	- Inappropriate design. - Designed for histological studies and followed up the patients. - Not reliable results.	- 00 / 17

Table 11: Summary of prospective observational studies comparing open and endoscopic vein harvesting (1).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Downs & Black score (0-27).
- Pagni 1998 - Annals of Thoracic Surgery.	- Prospective non-randomised. - n= 90 (EVH: 50 & OVH: 40)	- Ethicon EVH Kit.	- Wound Infection. -Post op Pain. - Mortality. - Myocardial Infarction.	- 2, 4 and 6 weeks	- Small sample size. - Used validated scoring for pain only. - Drop out mentioned. - Telephone follow-up. - Learning curve – 25 patients mentioned.	- 18.
- Crouch 1999 - Annals of Thoracic Surgery.	- Prospective non-randomised. - n= 568 (EVH: 180 & OVH: 388).	- Vasoview.	- Wound Infection. - Length of stay.	- 6 weeks	- No mention of validated tool. - Conversion methods have been discusses. - No assessors blinded. - No follow up mentioned.	- 19.

- Marty 2000. **-** Heart Surgery Forum.	- Prospective non-randomised. - n= 40 (EVH: 22 & OVH: 18).	- Karl Storz Endoscope.	- Wound infection. - Incidence of Haematoma.	- In hospital admission.	- Small sample size. - Cases selected on surgeon preference. - No validated tools. - Follow up period not clearly mentioned.	- 16.
- Rodrigus 2001 - Heart Surgery Forum.	- Prospective non-randomised. - n = 158 (EVH: 131 & OVH:27).	- Vasoview.	- Wound infection. - Incidence of Haematoma.	- 6 weeks	- Not mentioned how they followed up patients. - Not reliable data due to one group having greater sample size compared to other group, which had only 10% of the number. - Learning curve mentioned. - Not powered, no prior sample calculation assessed. - No validated scoring system.	- 15.

Table 12: Summary of prospective observational studies comparing open and endoscopic vein harvesting (2).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Downs & Black score (0-27).
- Bitondo 2002 - Annals of Thoracic Surgery.	- Prospective non-randomised. - n=225 (EVH: 154 & OVH: 106).	- Vasoview.	- Wound infection. - Incidence of Haematoma.	- 6 and 8 weeks.	- Poorly designed study. - Operator preferred method. - No validated tools. - Conversion group mentioned. - Only analysed the data of infected patients not all patients.	- 17
- Vaidyanathan 2008. - Asian cardiovascular and thoracic annals.	- Prospective non-randomised. - n= 161 (EVH: 81 & OVH: 80).	- Vasoview.	- Wound infection. - Incidence of Haematoma. - Myocardial Infarction. - Mortality.	- In hospital admission.	- Learning curve mentioned. - No validated tools. - No follow up period. - Concealment not appropriate. - Results not reliable because the choice of the vein grafting method depends upon the surgeon.	- 17.

- Simek 2008 - The Journal of Cardiovascular surgery (Torino)	- Prospective non-randomised. - n= 300 (EVH: 180 & OVH: 120).	- Vasoview.	- Wound infection. - Incidence of Haematoma. - Length of stay.	- 7 days, 3months and 1year.	- No validated scoring mentioned. - No follow up period.	- 19.

Table 13: Summary of prospective observational studies comparing open and endoscopic vein harvesting (3).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Downs & Black score (0-27).
- Chou 2009. - Journal of Zhejiang University SCIENCE B.	- Prospective non-randomised. - n= 348 (EVH: 270 & OVH: 78).	- Vasoview.	- Wound infection. - Incidence of Haematoma. - Post op Pain. - Length of stay. - Myocardial Infarction. - Mortality.	- 12 months	- Well-designed study. - Validated scoring used. - Fisher exact and student t test used for statistical analysis.	- 21.
- Zenati 2011 - Journal of Thoracic Cardiovascular surgery	- Prospective non-randomised. - n= 894 (EVH: 341 & OVH: 553).	- No data available. Surgeons have been informed by the researcher to use whatever available EVH system for this study.	- Graft Failure. - Myocardial Infraction. - Mortality.	- 1 year.	- Well-designed study. - Flow diagram. - Appropriate statistical analysis done. - Angiographic done for all patients at one year.	- 20.

Table 14: Summary of retrospective studies comparing open and endoscopic vein harvesting (1).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Downs & Black score (0-27).
- Morris 1998 - Annals of Thoracic Surgery.	- Retrospective. - n= 51 (EVH: 27 & OVH: 24).	- Vasoview	- Wound infection. - Incidence of Haematoma. - Post op Pain.	- In hospital admission.	- Validated scoring system for pain assessment. - Non-parametric statistical analysis for smaller sample size. - Learning curve minimum 25 cases to obtain accuracy.	- 14.
- Davis 1998 - Journal of Thoracic Cardiovascular surgery	- Retrospective. - n= 138 (EVH: 110 & OVH:28 + 99).	- Ethicon EVH Kit.	- Wound infection. - Post op Pain. - Length of stay. - Mortality.	- 6 weeks.	- No validated scoring on wound assessment. - Non parametric test and multiple linear regressions. - Learning curve related problems explained.	- 15.
- Coppoolse 1999. - - European Journal of Cardiothoracic Surgery	- Retrospective. - n=600 (EVH:300 & OVH:300)	- Karl Storz EVH system	- Wound Infection.	- 3 weeks.	- Wound healing, graft quality and pain assessment carried out without any validated scoring system. - Learning curve observed minimum of 100 cases.	- 12.

- Kan 1999 - Journal of Cardiac Surgery.	- Retrospective. - n=119 (EVH:60 & OVH:59)	- Ethicon Endopath.	- Wound infection. - Incidence of Haematoma.	- In hospital admission.	- No validated tools. - No extended follow up period.	- 17.
- Allen 2000. - Heart Surgery Forum.	- Retrospective. - n=919 (EVH: 276 & OVH: 643).	- Ethicon EVH Kit.	- Wound infection. - Incidence of Haematoma.	- 6 weeks.	- No assessors blinded. - No validated tools.	- 18

Table 15: Summary of retrospective studies comparing open and endoscopic vein harvesting (2).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Downs & Black score (0-27).
- Galbraith 2000 - Journal of endovascular therapy.	- Retrospective. - n= 212 (EVH:77 & OVH: 135.	- Endosaph Tyco system.	- Wound infection. - Incidence of Haematoma. - Length of stay. - Mortality.	- 30 days.	- No validated tools for wound assessment. - No assessors blinded with the techniques. - Well written paper.	- 19.
- Felisky 2002. - The American Journal of Surgery.	- Retrospective. - n=720 (EVH:380 & OVH: 340.	- Vasoview.	- Wound infection. - Incidence of Haematoma. - Myocardial Infarction. - Mortality.	- In hospital admission.	- Learning curve assessed. - validated scoring for wound assessment. - very short term clinical outcome assessed.	- 18
- Lai 2006. - Texas Heart Institute Journal.	- Retrospective. - n=1573 (EVH:588 & OVH: 985).	- ClearGlide, Ethicon.	- Wound Infection. - Length of stay.	- 30 days	- Learning curve mentioned around 15 to 35 cases. - No closure time of the donor leg.	- 22
- Lopes 2009 - New England Journal of Medicine.	- Retrospective. - n=3000 (EVH:1753 & OVH: 1247).	- Not mentioned (later disclosed in letter to editor: 90% Vasoview and 10% Ethicon).	- Graft failure. - Mortality.	- 3 years	- Not designed to compare the vein harvesting techniques. - Secondary to CTMIP.	- 17.

Table 16: Summary of retrospective studies comparing open and endoscopic vein harvesting (3).

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Downs & Black score (0-27).
- Kirmani 2010 - Journal of Cardiothoracic surgery.	- Retrospective. - n= 176 (EVH: 71 & OVH: 105).	- Vasoview	- Wound Infection. - Post op. Pain. - Length of stay. - Mortality.	17 & 37 months.	- Major adverse cardiac events follow up. - - Well written paper with methodology. - Validated scoring.	- 23
- Ouzounian 2010. - Annals of Thoracic Surgery.	- Retrospective. - n= 5825 (EVH: 2004 & OVH: 3821)	- Vasoview.	- Wound Infection. - Myocardial Infarction. - Mortality.	- 6 months	- Good sample size to assess the clinical outcomes. -Validated scoring system.	- 25
- Dacey 2011 - Circulation.	- Retrospective. - n= 8542 (EVH: 4480 & OVH:4062).	- Not mentioned.	- Wound Infection. - Myocardial Infarction. - Mortality.	- 4 years.	- The endoscopic system not mentioned. - Collected retrospective data from database.	- 23
- Ad 2011. - Journal of Cardiovascular surgery.	- Retrospective. - n= 1988 (EVH: 1734 & OVH: 254)	- Vasoview.	- Wound infection. - Myocardial Infarction. - Mortality.	- 39 months.	- Validated scoring system for wound assessment. - Database analysis.	- 20
- Grant 2012. - Heart	- Retrospective. - n= 4709 (EVH: 586 & OVH: 4123).	- Vasoview	- Myocardial Infarction. - Mortality.	- 22 months	- Multicentre data collection. - Database analysis.	- 16

Table 17: Summary of recent studies comparing open and endoscopic vein harvesting.

Author, year & Journal	Research design and total sample size	EVH Device used	Outcomes	Follow up period	Comments	Quality assessment Jadad (0-5) and Downs & Black score (0-27).
- Williams 2012 - JAMA	- Retrospective. - n= 235,394 (EVH: 122, 899 & OVH: 112,495)	- No data available.	-Wound complications. - Myocardial Infarction - Mortality.	- 3 years	- Observational National database study. - Mainly secondary outcome measures analysed.	- 24
- Andreas 2013. -Interactive cardiovascular and Thoracic surgery.	- Retrospective. - n= 885 (EVH: 262 & OVH :623)	- Vasoview.	- Wound complications. - Mortality.	- 30 days, 1 and 2 years.	- Validated scoring for wound assessment. - Observational data analysis.	- 18.
- Brat 2013 - Biomed Pap Med Fac Univ Palacky Olomouc Czech.	- RCT. - n= 100 (EVH:50 & OVH:50)	- VirtuoSaph Terumo.	- Leg morbidity. - Incidence of Haemotoma. - Endothelial damage.	- 7 days and 1 month.	- Poor design. - No appropriate methodology. - No validated scoring system.	2/17.

2.1.10: DISCUSSION

There were no relevant studies identified which directly compared the three vein harvesting surgical techniques, which thus necessitated our carrying out the pre-trial work.

The Medical Research Council's (2008) complex intervention guidance states that "Best practice is to develop interventions systematically, using the best available evidence and appropriate theory, then to test them using a carefully phased approach, starting with a series of pilot studies targeted at each of the key uncertainties in the design, and moving on to an exploratory and then a definitive evaluation. The results should be disseminated as widely and persuasively as possible, with further research to assist and monitor the process of implementation" (Craig et al., 2008).

We therefore carried out a non-randomised pre-trial study during the training period with n=140 samples which has demonstrated 70% endothelial denudation in the closed tunnel CO_2 EVH system compared to 20% denudation in the open tunnel CO_2 EVH technique. However, there was no statistically significant difference in clinical outcomes between the groups at 4 year clinical follow up.

2.1.11: LIMITATIONS OF THIS LITERATURE REVIEW

The main limitation for this review is the exclusion of studies from foreign languages due to translation issues. Many of the studies found in the literature used only open-tunnel or closed tunnel endoscopic vein harvesting compared with standard open vein harvesting. There are no studies directly related to all three vein harvesting techniques for bypass surgery. However, the pre-trial work from our study has given some insight, yet there is a lack of control group which raises the question of the validity and reliability of the study.

2.1.12: LITERATURE REVIEW SUMMARY

This chapter has identified that there is a gap in the knowledge regarding the scientific and clinical outcomes of venous conduit after coronary artery bypass surgery. The pre-trial findings from our study also demonstrate that there is an urgent need for a randomised controlled trial on this subject. Our pre-trial work highlights the trauma to the vein conduit; however, it remains unclear whether this directly relates to long term clinical outcomes which were not present in the current literatures.

These findings need further exploration to answer the main aims of this study. The main research questions that still need to be answered are:

1. Is the endothelial damage demonstrated in the pre-trial work due to endoscopic technique itself or occurred due to some other reasons?
2. Is there is any difference on the vessel damage of the vein between the three groups?
3. Is there is any correlation between endothelial damage and clinical outcomes?

2.1.13: RECENT EVIDENCE FROM 2014 TO 2016.

The main literature search and review was conducted in 2014 and this thesis was written on 2016. The second literature review was conducted to identify any new evidences added into this research area. A study published in Annals of Surgery (van Diepen et al., 2014) which analysed data retrospectively from the PREVENT-IV trial (Lopes et al., 2009), in order to compare the two EVH techniques (open tunnel (n=390) and closed tunnel (n=1159)). The authors compared the incidence of vein graft failure (p=0.724) and composite clinical outcome (p=0.221), and concluded that there are no statistically significant differences between the two EVH surgical techniques. The other clinical studies (Hess et al., 2014; Yoshimoto et al., 2014; Amouzeshi et al., 2016), meta-analysis (Sastry et al., 2013; Markar et al., 2010), reviews (Krishnamoorthy et al., 2016; Bisleri et al., 2013), learning curve (Eifert et al., 2010; Arora et al., 2015), EVH cost analysis (Luckraz et al., 2016) and histological studies (Hashmi et al., 2015; Nezafati et al., 2014) yielded data favourable to EVH but all concluded that there was still need a randomised trial.

However, there is no data available regarding a direct comparison of histological and clinical with the open vein harvesting. So, this raises questions with regard to the effects of EVH, such as practitioner training related problems, immediate vein graft failure due to surgical trauma to the conduit and whether patient risk factors are directly related to the poor outcomes observed in the Prevent IV trial. This clearly highlights the need for a randomised study comparing the histological and clinical outcome between these three surgical methods.

2.20: SUMMARY OF CHAPTERS

This thesis was written in the format of a series of journal article chapters because it has various experimental methods that link together to investigate the effect of vein harvesting techniques on the vein integrity and clinical outcomes. Each chapter is self-contained and can be read without reference to the rest of the thesis. Subsequently, the contents from the introduction may be repeated in the introduction of each chapter to address our primary research question. The quality of the vein and clinical outcome post CABG surgery can be affected by many factors. Some of the important factors arising from the literature synthesis/review were explored in detail and set out as individual chapters.

In **chapter 3**, the aim of the literature review was to explore the advantages and disadvantages of the different vein harvesting systems/techniques. This included investigating whether each vein harvesting technique had any impact on quality of the vein and clinical outcomes. If there was an indication of damage, the current evidence was assessed on how each technique affects the different layers of the vein. At the end of the review, the physical or mechanical factors which may affect the quality of the vein were explored.

In **chapter 4**, the study protocol was written with all elements of complete histological, clinical, and health economics methods, power calculation, statistical analysis, scoring methods and consort diagram.

In **chapter 5**, the aim of this chapter was to bring together all the histological, clinical and health economic methods used in Chapters 6 – 9, to make it easier for the reader to understand the work undertaken and provide greater detail regarding methods than is possible within the confines of a publication.

In **chapter 6**, the main focus was on validating the endothelial staining markers. The most commonly used endothelial markers in human tissue research studies are CD31 and CD34. The main aim of this chapter was to explore which endothelial marker would work best for the vein immunohistochemistry analysis. The main reason being that slides are scored by new imaging techniques rather than old style microscopic scoring. The new scoring technique provided lots of benefits such as convenient scoring at any time, no need for the microscope and image clarification.

In **chapter 7**, the literature review highlighted that the duration of vein exposure to the pressurised carbon dioxide tunnel during EVH may affect the quality of the vein. Vein quality and carbon dioxide absorption were assessed in non-distended, non-touch proximal vein samples obtained using all three vein harvesting techniques. For this analysis, only H1 (proximal) vein samples were used for histological level damage analysis. We believed that the use of proximal samples would give the true effect of damage caused by the carbon dioxide insufflation because the distal and random vein samples had undergone various surgical handling and preparation that can affect the credibility of the study.

In **chapter 8**, the chapter aimed to determine whether the distended, minimally distended and non-distended vein samples harvested by three different types of vein harvesting had any direct impact on muscular and endothelial layers. Endothelial stretching and detachment were assessed using a validated scoring system, 900 vein samples were stained using Haematoxylin & Eosin staining. Longitudinal and circular muscle detachment, internal & external muscle migration, and hypertrophy were assessed in 900 vein samples which were stained by Picrosirius red muscular stain.

In **chapter 9**, the main aim of the research was addressed, by exploring whether differences in vein integrity between the three vein harvesting techniques were associated with differences in clinical outcomes post CABG surgery, at different follow-up points (3, 6, 9, 12, 18, 24, 36 and 48 months). As a secondary outcome, the relationship between composite and individual Major Adverse Cardiac Events (MACE) scores and health related quality of life (baseline, 3 months and 1 year) was explored. Cost effectiveness was compared between all three surgical techniques (costed from the day of surgery and post-surgery). Validated scoring systems (EQ-5D and modified cardiac specific SF-36) were used to assess Quality Adjusted Life in years (QALYs) and unit/incremental costs.

In **chapter 10**, presents the overall discussion and conclusions from the studies undertaken and considers their strength and weaknesses. In addition, this chapter highlights clinical and research recommendations.

CHAPTER 3

Literature review

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An overview of a minimally invasive vein harvesting methods: Literature Review

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3.0 An overview of a minimally invasive vein harvesting methods

3.1: Introduction

Although the internal thoracic artery exhibits an excellent patency rate of 90% (Cheng et al., 2010) and other arterial conduits have been established as preferred conduits for use in Coronary Artery Bypass Grafting (CABG) surgery, the long saphenous vein (LSV) remains most widely used. This is due to the LSV being readily available, relatively easy to harvest, versatile, resistant to spasm and is of proven efficacy (Favaloro, 1969). There are different harvesting techniques used to retrieve the vein, such as open, bridging and endoscopic vein harvesting (Kayacioglu et al., 2007). It is estimated that over 800,000 CABG surgeries are performed worldwide, and of these, between 20,000 and 28,000 CABG surgeries are performed annually in the United Kingdom. Research into the optimal technique to use for retrieval may have significant clinical and financial implications. There remains a requirement for a review of evidence based practice to assist the surgeon and patient in selecting the appropriate vein harvesting method.

The aim of this review is to highlight the advantages and disadvantages of the aforementioned vein harvesting methods for CABG in relation to the current literature.

3.1.2: Parameters for assessing efficacy and safety

Aside from mortality, an endpoint for many clinical studies, a significant proportion of cardiothoracic trials utilise the Major Adverse Cardiac Events (MACE) scoring system, which provides criteria for evaluating post-operative outcome. Criteria include cardiac death (acute myocardial infarction, sudden cardiac death or congestive heart failure), nonfatal acute myocardial infarction or target vessel revascularisation. Studies evaluating vein harvesting methods in particular also consider graft patency (failure and occlusion), recovery time (time until discharge), wound complications (inflammation, infection, seroma and haematoma etc.), scarring (cosmesis), patient satisfaction, cost and operating time (DeLaria et al., 1981; Brandt et al., 2003; Lavee et al., 1989; L'Ecuyer et al., 1996). However, the mechanisms contributing to vein graft occlusion are multifarious and it has been demonstrated that approximately 30% of vein grafts occlude within the 1st year after surgery, increasing to more than 50% after 10 years (Wan et al., 2012; Izzat et al., 1994; Angelini et al., 1989a). Another

parameter which is often overlooked despite its importance is the endothelial integrity of the harvested vessel.

3.1.3: ADVENTITIAL LAYER AND ITS IMPORTANCE

The adventitial layer consists of a complex of nourishing micro vessels; the vasa vasorum, lymphatic vessels and autonomic nerves (Dashwood et al., 2009). This layer also contains a resident group of macrophages, T-cells, B-cells, mast cells and dendritic cells which carry out important immune surveillance functions and co-ordinate downstream responses (Majesky et al., 2011; Galkina et al., 2006; Swedenborg et al., 2011). Therefore, the adventitial layer has a complex structure of interacting cell types, and displays a number of molecular mechanisms responsible for homeostasis as well as the repair of the vessel wall following injury (Majesky et al., 2011). This highlights the importance of preservation of the adventitial layer during vein harvesting. The vasa vasorum provides oxygen and nutrients to the outer layer of the vessel wall (Wolinsky and Glagov, 1967; Heistad et al., 1981; Heistad and Marcus, 1979; Williams and Heistad, 1996). It also acts as a principal route for leukocyte transfer into atherosclerotic lesions, and modulating the vasa vasorum microvasculature affects plaque growth (Majesky et al., 2011). Research studies carried out on the arterial surrounding indicates neointimal lesion formation (Majesky et al., 2011; Dashwood et al., 2009) as a result of stripping of the vasa vasorum. As such, failing to preserve this layer appropriately during harvesting can affect the quality of the conduit and ultimately alter vein graft patency.

3.1.4: MEDIAL SMOOTH MUSCLE LAYER

The medial layer consists of an inconspicuous inner layer of longitudinal muscle fibres and a more prominent outer layer of circular muscle layers. The inner longitudinal muscular layer becomes thicker at the site of the valves (Milroy et al., 1989; Barboriak et al., 1974). Any trauma to the intimal layer of the vein causes smooth muscle proliferation which leads to hyperplasia and neointimal lesion formation (Lehmann et al., 1989).

3.1.5: ENDOTHELIUM

The mechanical and functional importance of the endothelium is often understated in relation to other layers of the vein. The vascular endothelium (a monolayer of cells lining blood vessels) is an active paracrine, endocrine and autocrine cell layer that is essential for the regulation of vascular tone and maintenance of vascular homeostasis. Not only does it serve as a physiologic barrier between blood constituents and the sub endothelium, but it is also responsible for the regulation of leukocyte and platelet adhesion, regulation of thrombosis, fibrinolysis and mediation of inflammation (Thatte and Khuri, 2001; Davies and Hagen, 1995).

Endothelial dysfunction may be responsible for several risk factors associated with coronary disease (Anastasiou et al., 1997). Mechanical insults, i.e. vasoactive substances and mediators released by white blood cells and platelets are detected by the endothelium (Angelini et al., 1989b). The endothelium responds by synthesising and releasing biological mediators that maintain vascular homeostasis (Loscalzo and Welch, 1995). The most important endogenous vasodilator is nitric oxide (NO). The vasodilatory effect of NO is not its sole benefit as it also exhibits significant vasoprotective properties. Reduced bioavailability of NO, due to either enhanced degradation or reduced formation, leads to endothelial dysfunction and consequent vascular pathology such as atherosclerosis (Michel and Feron, 1997; Dimmeler and Zeiher, 2000).

Conversely, platelet aggregation and coagulation can be induced via damage to the endothelium. Vasospasm, occlusive intimal hyperplasia and accelerated atherosclerosis can also occur as a result. It is therefore imperative to maintain endothelial homeostasis and prevent vessel injury during vein harvesting.

Endothelial integrity can be compromised due to the effects of temperature, pH (Hussaini et al., 2011), surgical distension (Angelini et al., 1989b) and composition of storage solution (Cavallari et al., 1997).

3.1.6: VEIN PREPARATION

The preparation of the vein after harvesting also plays a major role in graft occlusion. Current evidence suggests that surgical manipulation during harvesting and preparation causes more evident morphological disruption and functional impairment to the vein (Angelini et al., 1989b; Harskamp et al., 2014; Mills and Everson, 1995). Graft preparation with excessive vein distension and manual handling can increase the risk of vasospasm, thrombogenesis, occlusive intimal hyperplasia and stenosis (Thatte and Khuri, 2001; Catinella et al., 1982; Angelini et al., 1985; Barboriak et al., 1974; Hussaini et al., 2011). Therefore, it is vital to preserve the vein with minimal distension, in an appropriate storage solution and also to reduce surgical manipulation as much as possible (Wilbring et al., 2011; Harskamp et al., 2014).

The vein conduits used in CABG often go into vascular spasm, and this is overcome by saline distension, thus damaging the endothelium. However, the effects of different harvesting techniques on the endothelial layer are yet to be sufficiently evaluated. Vein segments harvested using the "traditional method" are also associated with increased luminal endothelial/adventitial injury and down regulation of eNOS at these regions compared with veins harvested using a 'no touch' technique (Rueda et al., 2008; Tsui et al., 2002; Tsui et al., 2001).

3.1.7: OPEN VEIN HARVESTING

Autologous vein harvesting for CABG was first described using the traditional Open Vein Harvesting (OVH) (Figure 8) method in 1967(Mehta and Khan, 2002; Favaloro et al., 1971). The LSV is harvested under direct vision which normally entails a longitudinal incision from the ankle up to the groin, although the length of the skin incision depends on the number of vein conduits required for surgery (Waqar-Uddin et al., 2009). 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 (Brandt et al., 2003). Common complications include; postoperative pain, leg oedema, cellulitis, serous drainage, subcutaneous fat tissue necrosis and delayed healing (DeLaria et al., 1981; Horvath et al., 1998; Khan et al., 2010). These complications can delay post-operative recovery and increase the length of

hospital stay. Significantly reduced patient satisfaction has been documented (Khan et al., 2010) and as a result, these problems stimulated a demand for minimally invasive vein harvesting methods (MIVH) which provide improved cosmetic outcomes (Khan et al., 2010).

In light of surgical advancements, MIVH techniques (Bridging technique, Mayo stripper and endoscopic vein harvesting) have rapidly developed to supersede the traditional OVH technique.

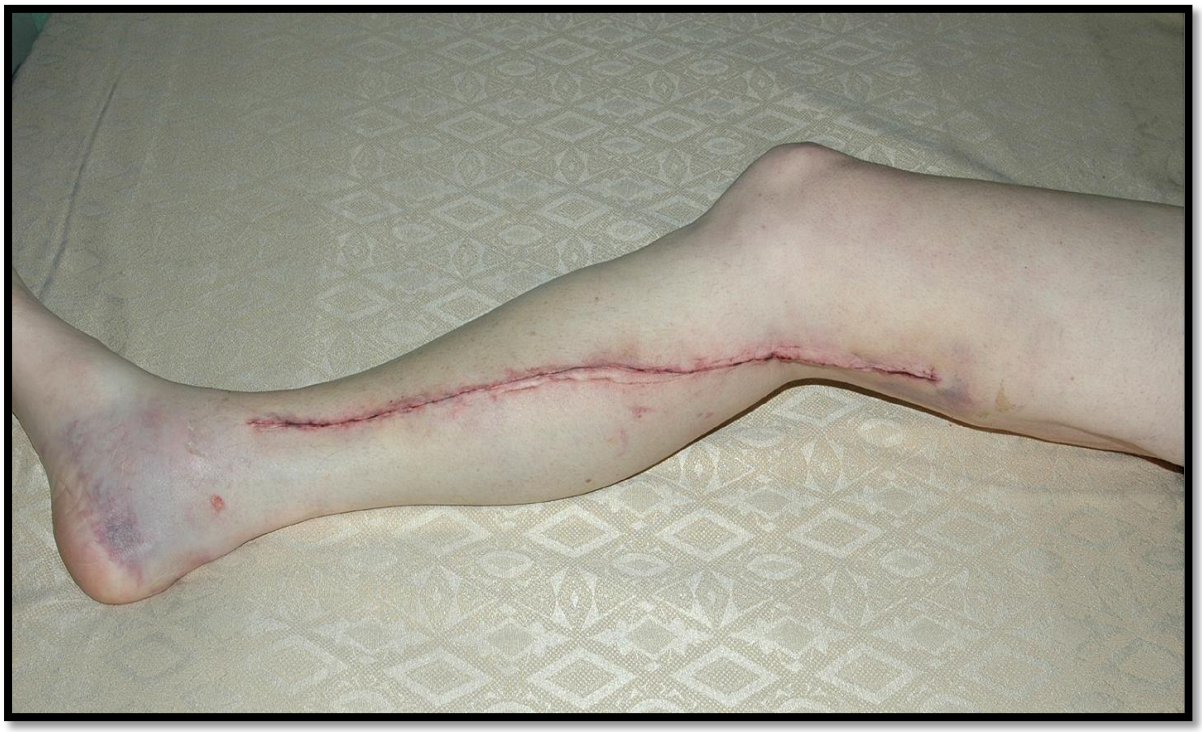

Figure 8: Open vein harvesting

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

3.1.8: BRIDGING TECHNIQUE

The standard bridging technique (SBT) (Figure 9) is an alternative to the traditional open method. This technique involves a number of 2-3cm incisions with 5-6cm gaps from ankle to groin. The number of incisions required is again dependent upon the length of conduit required for surgery (Khan et al., 2010). 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 multiple incisions (Reed, 2008). The bridging method can be carried out using a traditional west retractor, langenbeck retractor, laryngoscope retractor and the SaphLite retractor system. Current evidence comparing these retractors is provided in tables 18, 19 and 20.

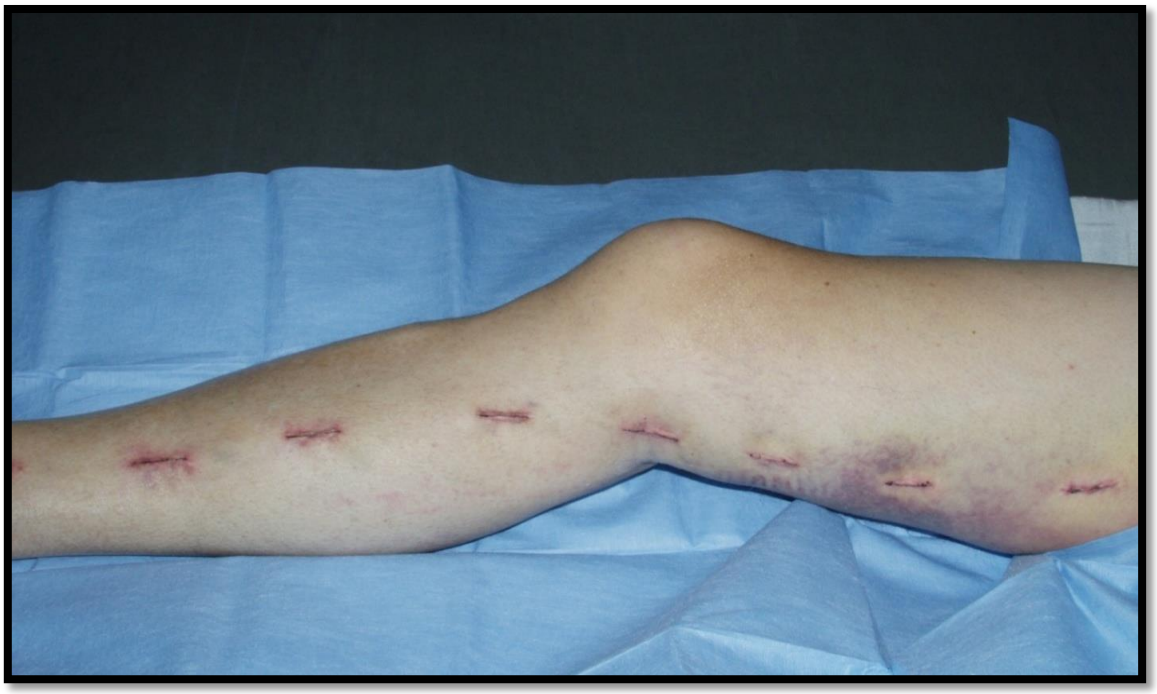

Figure 9: Bridging technique.

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

Table 18: Studies comparing different types of bridging technique with other MIVH or OVH.

Study	Device	Study design	Sample size	Findings	p-value
Horvath et al (Horvath et al., 1998)	- Richardson retractor (SBT). - Ethicon endoscopic (EVH).	Non-RCT	SBT (n=29) EVH (n=31)	- Patient demographics. - Length of vein graft. - Total vein operative time. - Total wound complications.	- p=0.0038. - p=0.05. - p=0.0001. - p=0.0048.
Khan et al (Khan et al., 2010)	- West and Langenbeck (SBT). - Traditional Open Technique (TOT).	RCT	SBT (n=50) TOT (n=50)	- Pain assessment. - Patient satisfaction. - Wound assessment. - Saphenous neuropathy	- p<0.001 - p<0.001 - p<0.001 - p<0.001.
Krishnamoorthy et al (Krishnamoorthy et al., 2012b)	- West and Langenbeck (SBT). - Open vein harvesting (OVH). - Maquet Vasoview Hemopro Endoscopic vein (EVH).	RCT	SBT (n=50) OVH (n=50) EVH (n=50)	- Post-operative pain (6weeks). - Haematoma formation. - Patient satisfaction. - Cosmetic Scarring. - Hospital stay.	- Reduced pain on movement, SBT and EVH – p=0.005 /p<0.001. - P<0.001 on EVH vs other two methods. - p<0.001 better EVH. - p<0.001 in MIVH group. - p=0.002 (EVH vs OVH) - p=0.128 (EVH vs SBT).

Table 19: Studies comparing different types of bridging technique (SaphLite) with other harvesting methods.

Study	Device	Study design	Sample size	Findings	p-value
Greenfield et al (Greenfield et al., 2001)	SaphLITE retractor (Genzyme Biosurgery, Cambridge, MA).	Non-RCT, with no comparison group.	n=305	- Length of vein. - Harvest time. - Injury to vein. - Side branch tear. - Length of stay. - Wound infection. - Pain assessment. -	- 46.0±15.2 cm - 43.4±17.6min - 0.7% - 21.0% - 6.8±4.8 - 2.3% - 41%.
Feyrer et al (Feyrer et al., 2006)	-Group 1 – OVH. - Group 2 – CBT. -Group 3 - SaphLITE	RCT.	n=110	- Conduit quality - Postoperative pain.	- No significance. - No significance.
Black et al (Greenfield et al., 2001)	- Group 1 OVH. - Group 2 SaphLITE.	- RCT - Isometric tension studies, smooth muscle contractile function.	n=40	- Harvest time - Vein length - No. of repairs - Acetylcholine - Bradykinin - Sodium nitroprusside -	- 0.94 - 0.13 - <0.001 - 0.32 - 0.52 - 0.39
Cook et al (Cook et al., 2004)	- Group 1 SBT -Group 2 OVH	Isometric experiments.	1- n=20 2- n=8	- high potassium depolarising - Phenylephrine contractions. - Endothelial cell function.	- 0.70 - 0.41 - 0.007 (with acetylcholine OVH more relaxed than SBT).

Table 20: Studies comparing different types of bridging technique (MayoStripper) with other harvesting methods.

Study	Device	Study design	Sample size	Findings	p-value
O'Regan et al (O'Regan et al., 1997)	- Mayo stripper. - Open vein harvesting.	Experimental.	n=12 on same patients.	- Vascular reactivity. - Light microscopy. - One year follow up.	- p>0.05. - No significance. - Estimation of 92% vein patency on Magnetic resonance angiography.
Nowicki et al (Nowicki et al., 2004)	- Mayo stripper (G1) - OVH (G2).	RCT -Immunohistochemistry CD31 and NOS.	G1 (n=100). G2 (n=100).	- Luminal CD31. - Lack of CD31 in vasa vasorum. - Luminal NOS - Lack of NOS in vasavasorum.	- p=0.05. - p=0.02. - p=0.05. - p=0.02.
Mahmood et al (Mahmood et al., 2006)	- OVH (A) - Mayo stripper(B)	RCT. - Vasomotor studies (calcium ionophore, sodium nitroprusside and apocynin)	Group A (n=38). Group B (n=39).	- Length of wound - Vein harvest time - Pain score. - Calcium Ionophore. - Apocynin. - Sodium Nitroprusside.	- p<0.001. - p=0.002 - p=0.43 - p=0.04. - p=0.7. - p=0.6.

As a result of these short term clinical findings, we may conclude that the bridging method of vein harvesting is a safe and effective technique. However, there is still a need for more randomised controlled trials to assess the long term clinical outcome in association with histological findings.

3.1.9: ENDOSCOPIC VEIN HARVESTING

Since its introduction almost 15 years ago, EVH (Figure 10) has been increasingly used for CABG due to a number of factors. The surgical techniques and devices used in EVH continue to evolve as studies evaluate their safety and efficacy. This technique involves a thin endoscope 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 (Crouch et al., 1999). Previous studies have reported that EVH significantly lowers the wound infection rate to 4-6.3% compared to 14.8-28.3% in the OVH group (Allen et al., 1998; Kiaii et al., 2002).

Despite the significant short term benefits, there remains concern with EVH regarding long-term outcome such as graft patency and MACE criteria. Although EVH is initially time consuming, the duration of the surgical procedure has been demonstrated to shorten considerably in association with the learning curve. Estimations of the extent of the learning curve for EVH vary from 30 (Ramakrishnan and Nainar, 2013) to 100 (Kiani et al., 2012) cases. The quality of the vein during the learning curve period remains questionable due to the nature of the procedure, which requires good hand eye co-ordination and endoscopic skills.

There are different types of endoscopic vein harvesting systems available in the market and they vary significantly with regard to the methods utilised for vein harvesting. The devices are classified according to the use of CO_2 in an open or closed tunnel system.

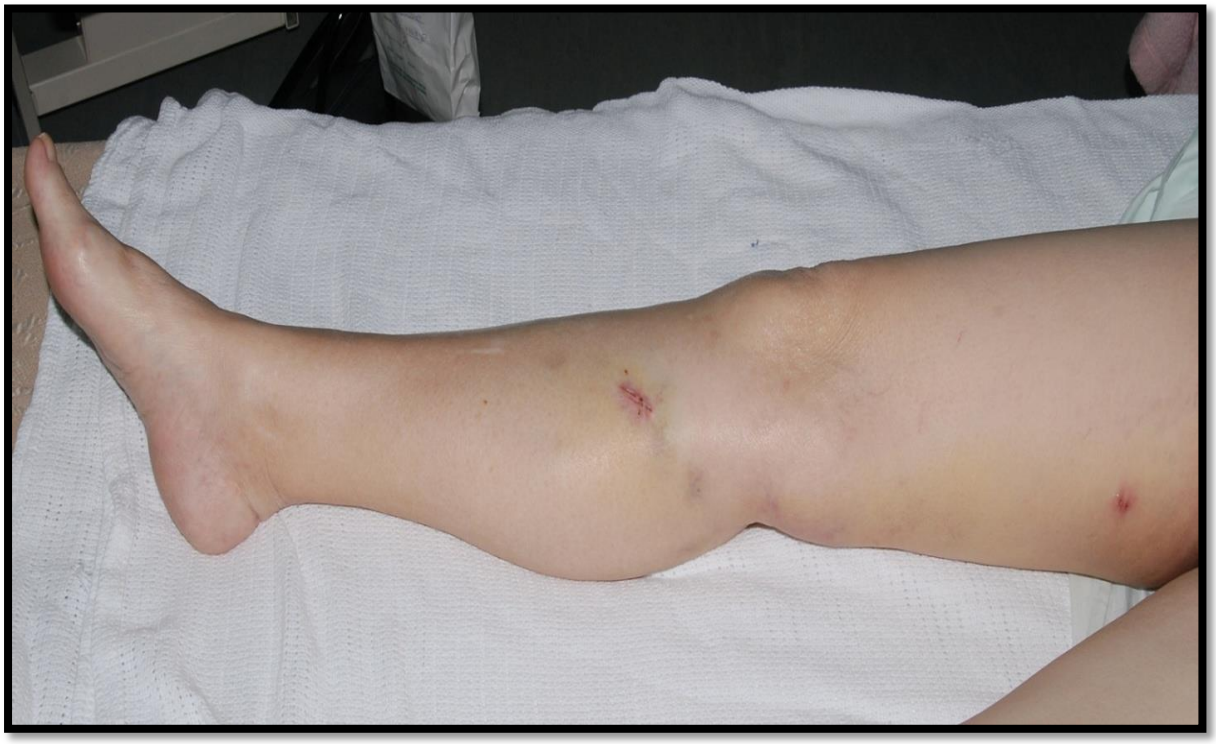

Figure 10: Endoscopic vein harvesting.

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 taken from a Society of Cardiothoracic Surgery conference abstract.

The differences between open and closed tunnel CO_2 are widely considered to be important in determining the overall effects of endoscopic vein harvesting (Bisleri et al., 2013)**.** However, van Diepen et al (van Diepen et al., 2013) demonstrated that there is no difference in patient clinical outcomes between these two EVH techniques.

3.1.10: CLOSED TUNNEL CO_2 SYSTEM

This procedure (Figure 11) involves making a 2cm longitudinal/oblique/vertical skin incision just above or 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), and 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 or saline depending upon local surgical practice.

The systems available in the market include the latest 8^{th} generation of Vasoview Hemopro2® (Maquet Cardio vascular, LLC, Wayne, NJ) and Virtuosaph® (Terumo Olympus® Medical cardio vascular group).

3.1.11: VASOVIEW® NEW TECHNOLOGY

The Vasoview Hemopro2® (Figure 12) has many advantages in that it represents an all in one package and is easy for operators to handle. The technological advances of the latest iteration also reduce thermal spread. The latest training in this system involves reducing port balloon incision site vein damage by not inflating the balloon and by making the incision as small as possible. Additionally, the vein is dissected along with the vasa vasorum and a little of the surrounding tissues, and reduces the use of the C-ring to avoid trauma to the vein. Finally, the reduced flow of CO_2 (10-12mmHg) avoids creating high pressure within the tunnel.

3.1.12: VIRTUOSAPH®

The Virtuosaph (Figure 13) has an atraumatic conical tip with centred ring and CO_2 delivered at the tip. In addition, this product utilises an open system distal insufflation with non-occlusive trocar to reduce the risk of CO_2 embolism. In order to improve visibility during harvesting, the Virtuosaph has developed a unique wiper to clean the endoscope lens. This system uses a bipolar diathermy for cauterising the side vein branches.

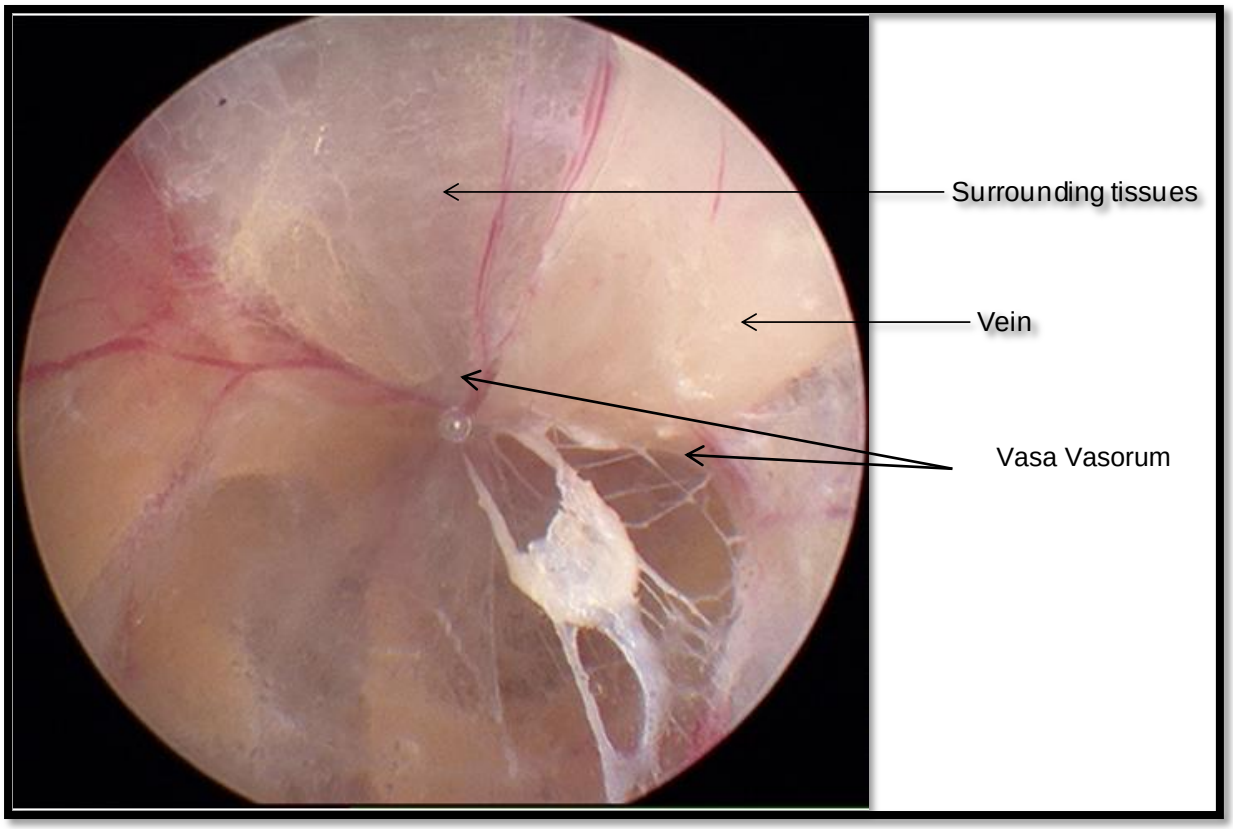

Figure 11: Closed tunnel CO2 EVH system.

This picture demonstrates the location of the vein and the conical tip within the tunnel on the leg. The vasa vasorum and surrounding tissues are pushed away by the carbon dioxide insufflation.

Figure 12: Vasoview® Hemopro2 EVH system.

This picture illustrates the Maquet endoscopic vein harvesting insertion handle with Hemopro2 insertion. This image was obtained from Maquet company representative from their product catalogue.

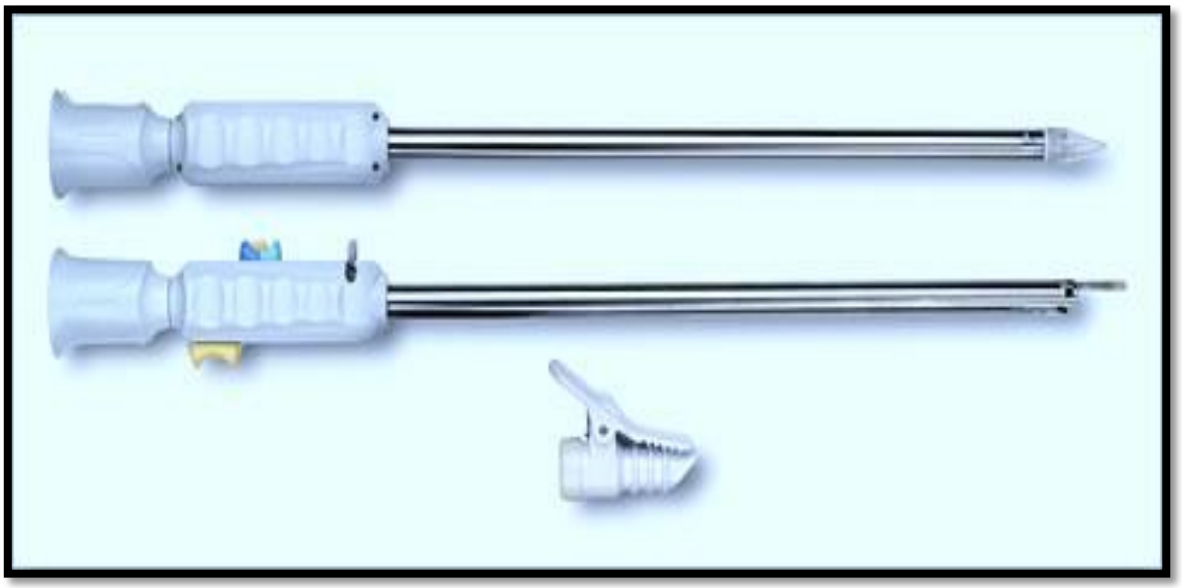

Figure 13: Virtuosaph™EVH system.

This picture illustrates the Terumo company endoscopic vein harvesting kit with its adaptor. This image was obtained from Terumo company representative from their product catalogue.

3.1.13: OPEN TUNNEL CO_2 EVH SYSTEM

In a similar manner to the closed tunnel CO_2 system an incision is made just above or below the knee, however, this procedure (Figure 14 & 14a) differs significantly 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 the 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 or saline depending upon local surgical practice.

3.1.14: SORIN VASUCLEAR®

The Sorin group launched their new modification with the latest flexible technology (Sorin Vasuclear®) (Figure 16) in 2011. This open tunnel CO_2 product has evolved from clearglide to VC15 –VC23 to prevent any vein trauma and optimise better easy use for the operators.

The Karl Storz® Freiburg (Figure 15) model EVH system also utilises an open tunnel CO_2 system but is reusable to improve cost effectiveness. It has special features of ergonomic retractor handle and integrated insufflation channel for smoke evacuation.

There are clear advantages and disadvantages for the use of each of these systems. However, the use of EVH as a whole remains controversial due to questions regarding long term graft patency, morbidity, vein graft quality and rates of repeat angina and mortality. In this review, we will be considering all the types of EVH systems in cardiac and other specialities from their initial introduction. An overview of the basic experimental research studies performed to date is included in Table 21 and Table 22.

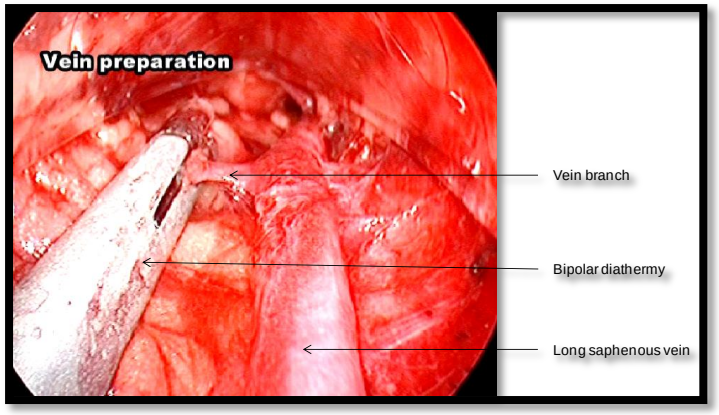

Figure 14: Open tunnel CO2 EVH system (Vasuclear)

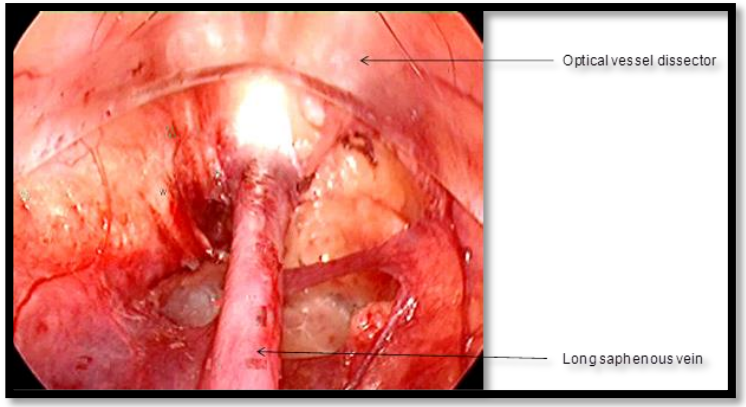

Fig 14a: Open tunnel CO2 EVH system (Vasuclear)

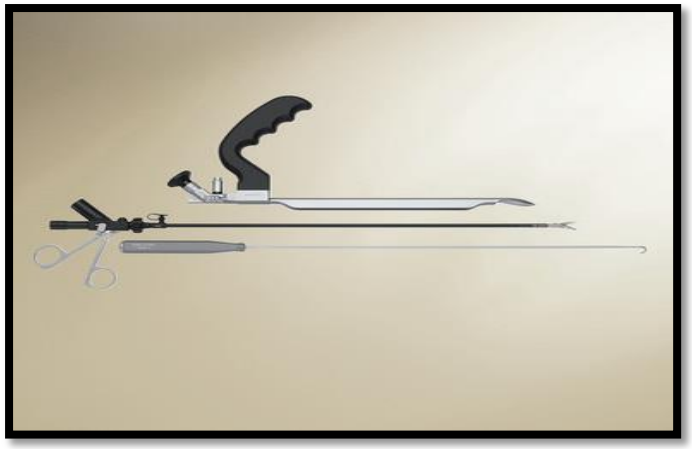

Figure 15: Karl Storz® FREIBURG model (Google image last accessed 07/08/14)

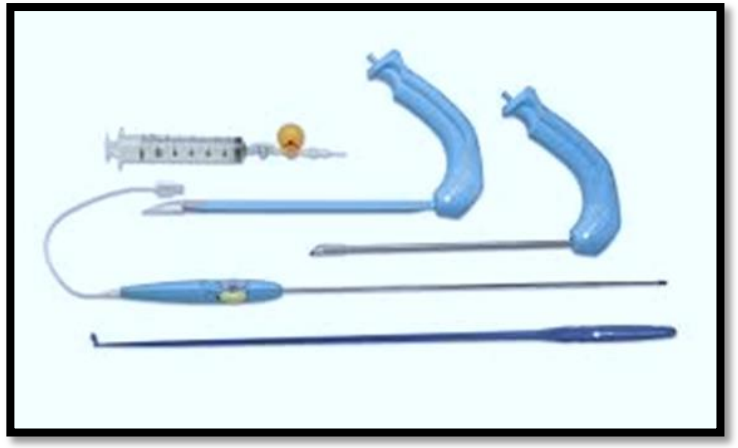

Figure 16: Vasuclear® Sorin EVH system (Google image last accessed 07/08/14).

Table 21: Comparison of endoscopic vein harvesting with other methods (Histological and Immunochemistry studies).

Author	Device	Design (experimental)	Sample size	Study findings	p-value
Rousou et al (Rousou et al., 2009)	- Vasoview EVH system. - Open vein harvesting.	Immunofluorescence (caveloin, endothelial nitric oxide synthase, von Willebrand factor and cadherin) and Western blot techniques.	n=5 (EVH) n=5 (OVH)	Esterase activity (cell viability) was significantly higher in OVH group.	p<0.0001
Cable et al (Cable et al., 1998)	Procine vein model. - Ethicon (Cincinnati,OH). - Open vein harvesting.	Light and electron microscopy. Verhoeff-van Gieson stain and hematoxylin-eosin stain	n=5 (EVH) n=5 (OVH)	No significant loss of endothelial cell or connective tissue in both groups.	p=0.68
Meyer et al (Meyer et al., 2000)	- Vasoview Balloon dissection system. - Open vein harvesting.	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.	n=9 (EVH) n=5 (OVH)	No difference in the intima, media and adventitia layer between both groups.	No statistical analysis was performed in this study.
Griffith et al(Griffith et al., 2000)	- Ethicon (Cincinnati,OH). -Open vein harvesting.	Hematoxylin-eosin (endothelial), Mason's trichrome (smooth muscle) and elastin (elastic lamina) staining	n=88 (EVH) n=82 (OVH)	Minor histological alterations but more significantly, no differences between both groups.	p=0.88
Crouch et al (Crouch et al., 1999)	- Vasoview EVH system. -open vein harvesting.	Immunoperoxidase stains (vimentin, Factor VIII and CD31).	n=4 (EVH) n=4 (OVH)	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 (Fabricius et al., 2000)	- No EVH device information - Mini Harvest system. - Open vein harvesting.	- RCT Electron microscopy, Hematoxylin-eosin and Giemsa stains.	a. n=31 (EVH) b. n=31 (light coupled retractor) c. n=30 (OVH)	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 (Rinia-Feenstra et al., 2000)	- Vasoview Balloon dissection system. - mediastinoscopy (no device information. - Open vein harvesting.	8ml organ bath filled with oxygenated Krebs-Henseleit solution of 37°C (pH 7.4).	n=6 (OVH) n=4 (mediastinoscopy) n=5 (EVH)	No significant differences in the vascular integrity between these groups.	p=0.46

Table 22: Comparison of endoscopic vein harvesting with other methods (Histological and Immunochemistry studies).

Author	Device	Design(experimental)	Sample size	Study findings	p-value.
Nezafati et al(Nezafati et al., 2014)	- Vasoview Hemopro2 - Open vein harvesting	-Immunohistochemistry evaluation of vWf, e-cadherin, Caveolin and eNOS.	EVH – n=30. OVH – n=17.	No significant difference between the EVH and OVH in relation to IHC.	p>0.05
Alrawi et al(Alrawi et al., 2001a)	- Ethicon (Cincinnati,OH). - "No-touch" open vein harvesting.	-Light, scanning and transmission electron microscopy to evaluate endothelial cell separation (EC), detachment, basement membrane exposure(BM) and collagen exposure(CE) and EC oedema.	n=90 samples from 45 patients.	No significant difference of endothelial damage between the techniques.	EC detach – p=0.378 BM - p=0.624 CE - p=1.0 EC oedema – p=0.368.
Hussaini et al(Hussaini et al., 2011)	- Terumo Virtosaph EVH - "No-touch" open vein harvesting.	- Structural and functional assays, immunofluorescence, multiphoton microscopy.	n=19 (thigh region – EVH) (Lower leg – OVH)	No statistically significant on any assays expect calcium mobilisation and NO production after bradykinin stimulation.	Esterase activity p<0.2478

Histological studies have identified both positive and negative findings of the endoscopic technique compared to open vein harvesting. However, there is a need for a definitive and well-designed randomised controlled histological study. Many smaller randomised trials, non-randomised trials, meta-analyses and systematic reviews have been conducted to compare clinical outcome post-surgery between EVH and OVH. Endoscopic vein harvesting was questioned in a high profile study for its safety and efficacy by Lopes et al in 2009, using results from their PREVENT IV database (Lopes et al., 2009). However, further studies dispute these findings are included in table 23. Importantly, the Lopes study wasn't designed for the purposes of a comparison between EVH and OVH. Table 23 indicates the most recent meta-analyses performed to evaluate differences between OVH and EVH. Table 24 demonstrates some of the most recent findings outside of cardiac surgery comparing OVH with EVH.

Table 23: Current meta-analyses evaluating EVH vs OVH.

Author	Studies included	Devices used	Study findings	p-value.	Comments
Deppe et al(Deppe et al., 2013b) systematic review with meta-analysis	43 studies (16 RCT, 27 OT) with 27,789 patients	- Ethicon endopath. -Vasoview Guidant. - Karl Storz Endoskope. - VirtuoSaph Terumo. - Clearglide Ethicon. - SaphLITE system. - Open vein harvesting.	-Wound Infection - Postoperative pain - Vein graft failure - MI - Mortality	- p<0.0001 CI-95% OR=0.27 (0.22 to 0.32) - p=0.0026 CI-95% (-2.07 to -0.44). - p<0.0001 CI-95% OR=1.38 (1.01 to 1.88) - p=0.8465 CI-95% OR=0.89 (0.69 to 1.16) - p=0.3452 CI-95% OR=0.90 (0.77 to 1.06)	- Significant difference between EVH and OVH. - Increase in EVH compared to OVH. - No significant difference. - No significant difference.
Sastry et al (Sastry et al., 2013) Meta-analysis	44 studies (19 RCT, 25 Non-RCT) with 267,525 patients.	- Ethicon endosurgery. - Karl Storz, Germany. - Guidant Origin. -SaphLITE system. -VirtuoSaphTerumo -Vasoview, Maquet. - Open vein harvesting.	- Wound infection (31 studies) - Postoperative pain (12 studies) -MI within 30days post- surgery (12 studies) - Vein graft occlusion (4 studies). - Repeat angina (4 studies, median of 2.6years) - Repeat Vascularisation (7 studies, median of 2.3 years).	p<0.0001 CI-95% SRR=0.31 (0.23-0.42) - p=0.001, CI-95% SMD= -1.48 (-2.38 to -0.59) - p=0.26 , CI-95% SSR=0.87 (0.68 -1.11) - p=0.004, CI-95% SSR=1.39 (1.11-1.75) - p=0.81, CI-95% SSR=1.06 (0.49-2.25). - p=0.06, CI-95% SSR=1.16 (0.99-1.36)	Significant difference. EVH had better outcome than OVH. - Less pain on EVH. - No significant difference. - Inconclusive - OVH had greater patency compared to EVH. - No significant difference. - No significant difference.

Table 24: Comparison of vascular studies on EVH and OVH.

Author	Studies included	Devices used	Study findings	p-value.	Comments
Jauhari et al (Jauhari et al., 2014) A systematic review in lower extremity arterial bypass.	-18 studies (cohort and case series) with 2.343 patients. -Mostly Long saphenous veins and SSV (1.9% on 197 patients) harvested. -Open vein harvesting and Endoscopic vein harvesting.	- Vasoview Guidant - Ethicon, Endopath. - Endosaph, Coviden. - Vasoview Hemopro. - Open vein harvesting.	Graft patency 5 studies 4 studies 6 studies 2 studies	- no significant difference. - Inferior patency rate in EVH at 12, 36 and 60 month follow up. - p=0.28 CI-95% HR-1.294 (1.03-1.63) - p<0.001	- The author concluded that the available data in lower extremity bypass are heterogeneous and of poor quality. So, the conclusion can't be made with this data. - Diabetic patients in EVH group had worse patency rate than OVH.
Erdoes et al(Erdoes and Milner, 2005) Infrainguinal bypass	- Prospective study. - No comparison group. n= 197 – EVH group. Long saphenous veins.	- Vasoview, Guidant.	Wound complications Readmission Graft failure	- 7.5% (197) - 2.5% (197) - 10/197.	- Very poorly designed study.
Gazoni et al (Gazoni et al., 2006) Knee arterial bypass surgery	- Prospective study. - 27 month follow up. - EVH (n=29) - OVH (n=59). Long saphenous veins.	- Vasoview 6, Guidant. - Open vein harvesting.	- Pain at rest. - Length of stay (days) - Death - Wound complications - interventions, patency - <30days - >30days - 21 months	- p=1.00 - p=0.87 - p=1.00 - p=1.00 - p=0.29 - p=0.03 - p=0.12	EVH had improved patency rate and decreased wound complications.
Eid et al (Eid et al., 2014) lower extremity bypass.	- Retrospective study. - 22 months follow up. - EVH (n=39). - OVH (n=49).	- Vasoview 7, Maquet. - Open vein harvesting.	- Wound infection - Length of hospital stay. - Graft patency rate. - Vascular re- interventions.	- p<0.001 - p=0.26. -p=0.007 at 3 years. - p<0.001	- EVH superior. - No significance. - Better in OVH group. - Better in OVH group.

Therefore, recent studies suggest that EVH has superior short term benefits of improved wound healing, reduced scarring, reduced length of hospital stay and greater patient satisfaction. The graft patency, quality of conduit harvested, traction and manipulation during the learning curve period is still questionable. The patency of the graft and the incidence of graft failure after bypass surgery are multifarious processes and other factors need to be considered while evaluating the safety of the minimally invasive harvesting methods.

3.1.15: INDEPENDENT FACTORS AFFECT CLINICAL OUTCOMES

Factors independent of vein harvesting which influence postoperative clinical outcomes must also be considered, as these not only impact upon the interpretation of aforementioned studies, but also may influence decisions on which harvesting technique to use according to the patients baseline characteristics.

The most consistent predictors of post-CABG mortality (as identified by the recent report of the ACC/AHA Task Force on practice guidelines) are priority of surgery, age, prior heart surgery, female gender, left ventricular ejection fraction, percentage of left main coronary artery stenosis and number of major coronary arteries with significant stenosis. The ACC/AHA guidelines identify the urgency of operation, advanced age, and one or more prior coronary artery bypass surgeries as being correlated with the greatest risk. Other mortality-related variables include coronary angioplasty during index admission, recent myocardial infarction, a history of angina, ventricular arrhythmias, chronic heart failure and mitral regurgitation. Importantly, co-morbidities such as diabetes, cerebrovascular diseases, peripheral vascular disease, chronic obstructive pulmonary disease and renal dysfunction play a vital role in the post-operative clinical outcomes.

3.1.16: Conclusion:

All minimally invasive techniques have their own unique advantages and disadvantages. Practitioners should not relinquish procedures to others because of the learning curve involved. It is not a failure if endoscopic vein harvesting is not performed in all cases or has to be converted to other techniques during surgery due to clinical reasons. Not all patients are suitable for endoscopic vein harvesting and in those circumstances other minimally invasive techniques should be considered to reduce the wound complications, postoperative pain, length of hospital stay and increase patient satisfaction. Finally, endoscopic vein harvesting itself has not been proven to be significantly worse than open techniques in any study that had an appropriate design. However, with recent advancements in endoscopic equipment, there is the potential that higher quality vein conduits can be obtained, although this relies on appropriate training, patient selection, understanding the importance of preserving the vein structures and harvesting the vein with all complete layers without any major trauma. Importantly, as Ramakrishnan and colleagues (Ramakrishnan and Nainar, 2013) have said, there is a need for physician assistants to have appropriate training with laparoscopic or thoracoscopic skills, and this will improve the standard of their endoscopic vein harvesting. We also strongly believe that there is an urgent need for a structured training programme which will combine all of these components.

The major question arising from this review is why endoscopic training for cardiac surgery falls short compared to other surgical specialities (dentistry, general surgery etc.). These specialities have structured training programmes for novice practitioners, yet this is not translated into vein harvesting. It is important to consider that the vein obtained by any minimally invasive harvesting or traditional harvesting technique will need to re-perfuse the heart for many years and so the quality matters. Interestingly, the new slogan of Health Medical Education England (HEE) reads “Better training, Better Care in future”. Incorporating improved training in minimally invasive vein harvesting may lead to significantly improved clinical outcomes.

CHAPTER 4

STUDY PROTOCOL

A RANDOMISED STUDY COMPARING VEIN INTEGRITY AND CLINICAL OUTCOMES (VICO) IN OPEN VEIN HARVESTING AND TWO TYPES OF ENDOSCOPIC VEIN HARVESTING FOR CORONARY ARTERY BYPASS GRAFTING – THE VICO STUDY PROTOCOL.

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Key words: Coronary artery bypass grafting, cardiac surgery, endoscopic vein harvesting, vein integrity.

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4.0 ABSTRACT

Background:

The Vein Integrity and Clinical Outcome (VICO) randomised trial is designed to assess the direct relationship between the histological damage caused during different methods of vein harvesting and clinical outcome post-surgery. Many studies are available in the literature measuring either histological outcome or clinical outcome in relation to different harvesting techniques. However, there remains no definitive randomised data available directly correlating harvesting-induced vein damage with clinical outcome.

Methods and design:

We aimed to randomise 100 patients in each group: Group 1 consists of closed tunnel CO_2 endoscopic vein harvesting (EVH) (CT-EVH), Group 2 consists of open tunnel CO_2 EVH (OT-EVH) and Group 3 forms a control group consisting of standard open vein harvesting (OVH). This provided a total of 300 patients in this study. All the veins will be harvested by an experienced practitioner for this study. We have planned to analyse the histological level of damage in three different parts of the harvested vein with the post clinical outcome using validated measuring tools. This study will also explore the health economical cost (EQ-5D), quality of life (SF-36) impact on these surgical methods.

Discussion:

We believe that this study will generate scientific and clinical data which may provide a definite answer of whether the vein damage caused during harvesting is operator, procedure or patient dependent. This will also be the ground work for comparing if the histological level damage during harvesting will have any effect on long term vein graft patency.

4.1: BACKGROUND

Coronary Artery Bypass Grafting (CABG) is one of the most frequently performed cardiac surgical procedures. Vein harvesting can be performed using an open (OVH) or endoscopic (EVH) technique. There are two methods of EVH – closed tunnel (CT-EVH) and open-tunnel (OT-EVH), which differ on the basis of CO_2 pressurisation. Importantly, it remains unclear whether there is any difference with regard to vein integrity and clinical outcome between these two methods.

4.1.2: VEIN INTEGRITY

Maintaining the structural integrity of harvested conduits is essential to a successful graft (DeLaria et al., 1981; Thatte and Khuri, 2001; LoGerfo et al., 1983; Rousou et al., 2009). Injury to the endothelium may cause denudation, which promotes platelet aggregation, intimal proliferation and hyperplasia; all of these significantly increase the risk of graft failure (Mills and Everson, 1995). Endoscopic harvesting requires more manipulation and handling of the vein, compared with the traditional non-touch OVH method (Bonchek, 1980). The clinical consequences of this are the subject of fierce debate.

An influential New England Journal of Medicine paper by Lopes and colleagues reported that EVH was associated with >75% graft occlusion, repeat vascularisation, myocardial infarction and sudden death (Lopes et al., 2009). Several centres have closed EVH programmes in response to the Lopes findings. However, subsequent studies demonstrate no major difference between OVH and EVH in mortality and morbidity outcomes (Dacey et al., 2011; Yun et al., 2005). More importantly, a cohort study comparing 8542 patients over four years reported that patients undergoing EVH had a lower mortality than those undergoing OVH (11.3% for EVH versus 13.8% for OVH; $p<0.001$) (Dacey et al., 2011).

Recently, a systematic review with meta-analysis of 27,789 patients concluded that EVH reduced leg wound infections without increasing mid-term risk for vein graft failure and mortality (Deppe et al., 2013a). However, there remains a paucity of high quality studies that have explored the potential risk of endothelial damage in direct relation to clinical outcome.

4.1.3: Wound Complications

Vein harvesting is traditionally performed as an open procedure, but this is associated with a number of postoperative wound complications, the rates of which range from 5% to 44% (DeLaria et al., 1981). Moreover, several studies have found that EVH significantly lowers wound infection rates - OVH 15-28% vs. EVH 4-6% (Deppe et al., 2013a; Desai et al., 2011; Kiani and Poston, 2011). A recent cost analysis study reported that the cost of readmissions for wound complications at 30 days was considerably higher in patients who have undergone OVH compared with EVH (£10,905 vs. £5,074) (Athanasiou et al., 2003; Carpino et al., 2000).

4.1.4: Other Gaps in Knowledge

Patient satisfaction and reduced economic burden are key priorities in the modern surgical world. The recent systematic review (Deppe et al., 2013a) highlighted the lack of high quality data regarding the cost difference between OVH and EVH. Little is known about patient satisfaction and its comparison with different approaches to vein harvesting. Although studies have compared OVH and EVH, comparisons have either been made against only one form of EVH (open and closed tunnel). No study has yet directly compared all three types of vein harvesting technique, nor a head to head comparison between the two EVH systems. This is an important omission, since these two forms of EVH may impact vein integrity differently.

4.1.5: Recent evidence in 2015

A study published in Annals of Surgery by Diepen et al (Sean van Diepen et al., 2013) retrospectively analysed data from the PREVENT-IV trial (Lopes et al., 2009), in order to compare the two EVH devices (open tunnel (n=390) and closed tunnel (n=1159)). The authors compared the incidence of vein graft failure (p=0.724) and composite clinical outcome (p=0.221), and concluded that there is no statistical differences between the two EVH surgical techniques. The other clinical studies (Hess et al., 2014; Mizumoto et al., 2015; Yoshimoto et al., 2014), meta-analysis (Sastry et al., 2013; Deppe et al., 2013b; Markar et al., 2010; Hess et al., 2014), reviews (Bisleri and Muneretto, 2015a; Raja and Sarang, 2013; Bisleri et al., 2013), learning curve (Arora et al., 2015; Krishnamoorthy et al., 2015; Kim do et al., 2015) and histological studies (Hashmi et al., 2015; Nezafati et al., 2014) concluded that EVH is favourable but still there is a need of a randomised trial.

However, there is no data available on direct comparison of scientific and clinical with the open vein harvesting control. So, this raises many questions with regard to the effects of EVH, such as practitioner training related problems, immediate vein graft failure due to surgical trauma to the conduit and whether patient risk factors are directly related to the poor outcomes observed in the Prevent IV trial. This clearly highlights the need for a randomised study comparing the scientific and clinical outcome between these three surgical methods.

4.1.6: NEED FOR A TRIAL

There is a paucity of randomised studies comparing EVH with OVH, and no data available comparing closed tunnel CO_2 with open CO_2 tunnel dissection. The current lack of definitive evidence and the resulting polarisation of opinion regarding vein harvesting technique are resulting in variation in clinical practice. In 2005, the International Society for Minimally Invasive Cardiac Surgery (ISMICS) held a consensus conference which recommended that EVH should now be considered a standard technique for vein harvesting. By contrast, the National Institute for Health and Clinical Excellence (NICE) currently recommends that EVH only be used as part of research or audit programmes, until its clinical effectiveness has been proven. The need for further high quality research to guide practice in this area has been recognised, including by NICE, which in 2010 recommended that an appropriate comparative assessment of OVH and EVH should be undertaken, which should include clinical outcomes, health economics and patient satisfaction.

4.1.7: RESEARCH QUESTIONS

1. Is there any difference in conduit integrity following retrieval with the OVH, CT-EVH and OT-EVH techniques?
2. Are there any differences in clinical outcomes (ie mortality, graft failure, myocardial infection) between OVH, CT-EVH and OT-EVH?
3. Is there any association between vein integrity and clinical outcomes?
4. Are there any differences in patient reported outcomes (ie health-related quality of life and satisfaction) between OVH, CT-EVH and OT-EVH?
5. Are there any differences in cost between these techniques?

4.1.8: PRIMARY AIMS

1. To assess the integrity of conduits harvested using the OVH, CT-EVH and OT-EVH techniques.
2. To assess whether there is any association between histological changes and clinical outcomes.
3. Comparison of the effect of carbon di-oxide on the tissue level on proximal samples. Full biochemistry data will be obtained and will be reported as separate outcomes.
4. Comparison of distended and non-distended vein samples will also be analysed and reported as a separate study outcomes.

4.1.9: SECONDARY AIMS

1. To determine the incidence of adverse clinical outcomes (ie mortality, graft failure, myocardial infection) and compare between the OVH, CT-EVH and OT-EVH groups.
2. To compare patient reported outcomes (ie health-related quality of life and satisfaction) between OVH, CT-EVH and OT-EVH.
3. To perform a health economic cost analysis associated with the three vein harvesting techniques.

4.1.10: METHODS

The study will be conducted as a single centre 3-armed randomised clinical trial based at the cardiothoracic department and transplant research laboratory, University Hospital of South Manchester NHS Foundation Trust, Manchester. The practitioner involved in this study has carried out more than 250 endoscopic vein harvesting and more than 2000 open vein harvesting surgical procedures.

4.1.11: RECRUITMENT

Patients will be screened using a two stage assessment process of inclusion and exclusion criteria.

4.1.12: INCLUSION CRITERIA

1. Patients aged over 18 years of age undergoing CABG surgery providing written informed consent will be recruited into this study.
2. All elective and urgent in patients will be included.
3. Patients who need at least one length of long saphenous vein.
4. Patients who are undergoing on-pump CABG surgery.
5. Patients are having single LIMA and vein grafts will be included.

4.1.13: EXCLUSION CRITERIA

1. Any patient refusing or withdrawing consent will be excluded from the study.
2. Patients undergoing emergency surgery.
3. Contra-indication to a surgical technique, which includes varicosities of the long saphenous vein, small or thin legs (<7.5cm diameter at the lower calf) or superficial LSV (less than ½ cm deep from the skin), determined using ultrasound scans will also be excluded.
4. Enrolled in other clinical trials.
5. Patients undergoing off-pump CABG surgery.

4.1.14: RANDOMISATION

We aim to randomise 100 patients per group assuming a feasible recruitment of 300 patients from a total of 960 CABG procedures performed at UHSM. EVH is currently performed as a routine procedure in UHSM. All the patients who provide written consent to take part in the study will be recruited and included in the randomisation. The patients will be randomised into three groups using block randomisation which will be provided by an independent statistician. The independent research assistant will conceal the allocation of each patient in a sealed envelope which will be provided to the practitioner on a daily basis in order to determine the group. The concealed envelope will only be opened once the patient has been anaesthetised for surgery. Our pilot results concluded that diabetes

patients are at higher risk of post-surgery repeat angina due to the progressive nature of the disease. As such, we will be using stratified block randomisation to ensure that age, sex are evenly spread between each group.

4.1.15: METHODS OF RECRUITMENT AND ALLOCATION

Patients will be allocated to one of the three groups (OVH, CT-EVH and OT-EVH). All the information regarding the procedure, study code and allocation of treatment will be kept confidential from the research team. The detailed recruitment and group allocation is attached in CONSORT diagram in page 113.

4.1.16: CLINICAL

All clinical data will be collected by two research team members, as a part of their involvement in this study. Research data collection is part of their normal work. Researchers will be blinded to the procedure allocation, thus reducing any potential bias during data collection.

4.1.17: HISTOLOGICAL

The samples will be collected by the principal investigator after the procedure and stored with relevant study code at -80°C in a secure laboratory.

4.1.18: BLINDING OF TISSUE SAMPLES

1. All the samples will be coded in the operating theatre prior to being sent to the laboratory. The codes will be kept confidential by the principal investigator.
2. Once the samples have been processed and stained for immunohistochemistry, they will be labelled from 1-300 to avoid any additional bias during scoring of the slides.
3. All the slides will be digitally scanned and the images will be scored by 5 independent assessors. The consultant histopathologist will score the slides rather than the images.

4.1.19: Sample size, power calculation

4.1.19a: Primary outcome: endothelial integrity

In the non-randomised pilot study, less than 20% of open tunnel CO_2 and greater than 50% of closed tunnel CO_2 patients had zero endothelial integrity.

With just 100 patients per group (assuming a feasible recruitment of 300 patients over 40 months), the study would have 80% power to detect differences in the percentage of patients with zero endothelial integrity of 15% or more, eg 20% vs 35%.

4.1.19b: Secondary outcome: composite end point MACE at 12 months

In the non-randomised pilot study, 19% of closed tunnel CO_2 patients had MACE compared to 13% of open tunnel CO_2 patients (ie only a 6% difference in incidence). In order to have 80% power to detect this magnitude of difference, over 600 patients in each group (1200 in all) would be required.

With just 100 patients per group (assuming a feasible recruitment of 300 patients over 40 months), the study would have 80% power to detect differences in the percentage of patients with MACE of 13% or more, eg: 13% vs 26% (using a simple chi-square test with the conventional 5% significance level).

(MACE defined as having one of the following outcomes: death, repeat angina, re-intervention, myocardial infarction / ischaemia, stroke, atrial fibrillation or graft blockage).

4.1.20: Data Analysis

The percentage of patients with zero endothelial integrity will be compared between the two randomised groups using firstly a simple chi-square test, followed by logistic regression analysis to incorporate any potential confounding factors. The percentage of patients with MACE in each group will be assessed using similar statistical methodology. Thus, no allowance is made for testing differences between the three groups in pairs, using three pair-wise comparisons.

4.1.21: METHODS FOR MINIMISING POTENTIAL STUDY BIAS

1. This study is single centred, owing to the nature of the research. We aim to determine the causation of any underlying histological vein damage. Observations from our pilot study and previous endoscopic procedures demonstrate histological vein damage can be caused by practitioner inexperience when performing endoscopic surgery.
2. To reduce bias caused by different operators carrying out different techniques, one experienced practitioner, who has carried out more than 250 endoscopic vein harvesting and more than 2000 open vein harvesting will be harvesting all the veins for this study. Importantly, EVH has been associated with a long learning curve, which varies from 30 (Desai et al., 2011) to 100 (Cadwallader et al., 2009) cases. The use of a single experienced practitioner will allow us to control for this.
3. The principal reason for using a sole operator for this study is to minimise the incidence of practitioner skill error. Varied practitioner skill would markedly impair the validity of any findings between endoscopic vein harvesting methods. In addition, evidence provided by our recent pilot study suggests experienced practitioners optimise vein quality through improved hand eye coordination.
4. Computerised randomisation will be provided by an independent statistician. The concealed envelope will be kept by an independent person to reduce study bias.
5. The manual immunohistological staining method has the potential for slight bias. The histological protocol is well developed and has been used in many endothelial studies. However, experience developed during our pilot work has allowed the team to improve the protocol by staining slides in batches of 12. Nevertheless, the potential exists for batch staining variation. Therefore, we will utilise automated, computerised immunohistological staining at the UHSM histology department. This system can perform staining with 120 slides, which reduces human error and bias.

4.1.22: Surgical intervention

4.1.22a: Open vein harvesting - Control group

In normal practice, a long incision will be made from ankle to thigh depending upon the length of vein required for surgery. For the purpose of this study, if the patient requires two lengths of vein, it will be harvested from just below the knee (approximately 9cm). If the patient requires three lengths of vein, it will be harvested from 4cm above the medial malleolus bone. The vein side branches will be ligated with 4-0 vicryl ties and titanium clips on both sides. The leg wound will be closed in layers and a dressing and pressure bandage will be applied (Krishnamoorthy et al., 2012b).

4.1.22b: Closed tunnel CO2 - Group 1

We will be using a Maquet Vasoview Hemopro2® vein harvesting system which involves a pressurised CO_2 tunnel for vein dissection. A 2-3cm incision will be made just above or below the knee (approximately 9cm) depending upon the length of vein (1 or 2) required for surgery. The long saphenous vein will be exposed and dissected using a West retractor and a Langenbeck retractor. A 30mm, 0° endoscope with a sharp, clear dissecting cone on the tip will be inserted through the skin incision. After 3cm of anterior dissection, the balloon will be inflated to seal the incision port. The vein will be dissected from the surrounding tissues anteriorly and posteriorly until reaching the femoral junction in the groin. The vein side branches will be ligated with 4-0 vicryl ties and titanium clips on both sides. The small leg wound will be closed in layers and a dressing and pressure bandage will be applied (Krishnamoorthy et al., 2012b).

4.1.22c: Standardisation

- The CO_2 tunnel pressure will be set to 10 - 12mmHg and a flow rate of 3 litres per minute will be applied for all cases. A minimal amount (10ml) of trocar cuff air inflation will be used to reduce the trauma to the vein.

- The vein branches will be cut from the insertion port towards the thigh or ankle to minimise the trauma to vein branches. The major stress on the base of the branch during

harvesting causes intimal injury which leads to platelet adherence, release of mitogenic proteins, smooth muscle cell proliferation and intimal hyperplasia (Davis et al., 1998a; Brown et al., 2007a).

4.1.22D: HEPARIN

- All the patients in this EVH group will be administered intravenous heparin just 5 minutes before sealing the skin insertion port, which reduces the intraluminal clot strand formation inside the vein during CO_2 insufflation (Brown et al., 2007a).
- Our pre-trial study demonstrated that patients who received anticoagulant therapy until the day of surgery experienced increased bleeding in the tunnel. As a result, only 2500 units of intravenous heparin will be administered for these patients.
- 5000 units of intravenous heparin will be administered for all other patients in this group.

4.1.22E: ENDOSCOPIC VEIN HARVESTING METHOD 2

We will be using the Sorin Clearglide® vein harvesting system. A 2-3cm incision will be made just above or below the knee (approximately 9cm) depending upon the number of vein lengths (1 or 2) required for surgery. Initially, the long saphenous vein will be exposed and dissected using a West retractor and a Langenbeck retractor. A 30mm, 0° telescope with a Clearglide dissecting retractor will be introduced through the skin incision. The CO_2 insufflator will be set up at a continuous flow rate of 3 litres per minute and 0mmHg pressure. The vein will be dissected from the surrounding tissue anteriorly and posteriorly until reaching the femoral junction in the groin. The vein side branches will be ligated with 4-0 vicryl ties and titanium clips on both sides. The small leg wound will be closed in layers and a dressing and pressure bandage will be applied.

4.1.23: STANDARDISATION FOR ALL THREE GROUP TECHNIQUES

1. The vein will be harvested with fat and adventitial layers. The conduit will be harvested 2 to 3 mm away from the main vein.
2. All the branches will be cut with at least 1cm length wherever possible.
3. The vein will be inflated with heparinised arterial blood with minimal inflation pressure.
4. The cardioplegia vein perfusion pressure will be standardised to 70mmHg for all cases.
5. All patients requiring three lengths of vein will have the conduits harvested from the ankle to the thigh. For patients who require one or two lengths, these will be harvested from just below or above the thigh.
6. The measurement of partial pressure of arterial carbon-dioxide ($PaCO_2$), $EtCO_2$ and also any changes to the ventilator settings during the vein harvesting procedure will be monitored and recorded for this study.
7. All endoscopic vein harvesting patients will have a leg drain on the wound (Krishnamoorthy et al., 2012a) which will be opened 10 minutes after protamine sulphate is given. However, in the open vein harvesting group, only patients who received antiplatelet medication until the day of surgery will have the leg wound drain inserted.

4.1.24: STUDY OUTCOME AND MEASUREMENTS

The primary outcome of this study will be whether histological changes occurring in the long saphenous vein correlate with clinical outcome post-surgery on CABG patients.

4.1.25: LABORATORY BASED ASSESSMENT OF THE ENDOTHELIUM IN COLLECTED SAMPLES

Endothelial integrity will be determined using standard streptavidin/peroxidase techniques. Briefly, samples will be dehydrated using xylene/alcohol before embedding in paraffin and sectioning to 4um using a microstat. Sections will be placed on poly-l-lysine coated histology slides, rehydrated, and endogenous peroxidase activity inhibited using hydrogen peroxide. Sections will then be incubated

with endothelial specific antibodies, including CD31 and CD34, which will be localised and visualised on a section of vessel. CD31 or PECAM-1 is a 130 kDa member of the immunoglobulin superfamily 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, which 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 colorimetric reaction. Following this, samples will be counter-stained using haematoxylin and eosin, and endothelial integrity will be visualised using microscopy.

In addition to endothelial marker (CD31 or CD34), Picrosirius Red muscular stain will be used to assess the circular and longitudinal muscle morphology and Haematoxylin and Eosin will be used for basic vein structural assessments. All samples will be initially assessed by the Principal Histopathologist at UHSM, and then graded by five independent assessors using a previously reported scale system (0-100%) which will be grouped into four categories, where 0 represents no endothelium and 4 represents continuous endothelial layer (Fischlein et al., 1994). A validated scoring system will be used to grade muscular damages in the vein muscle layers on a scale of 0-3 (normal, mild, moderate and severe). The endothelial damage on Haematoxylin and Eosin stained slides will be assessed on a scale of 0-3 (normal, mild, moderate and severe).

4.1.26: COLLECTION OF CLINICAL DATA

General demographic baseline data including pre-operative risk factors will be collected. Intra-operative data includes pre-surgical coronary vessel analysis, number of grafts, type of conduits and cardioplegia choice. In-hospital mortality and community mortality will be obtained from validated registry data and post-mortem reports. A validated disinfect wound scoring system will be used within the first 30 days to evaluate incidence of wound infection. A modified Likert scale will be utilised to determine patient satisfaction. The major clinical outcome will be assessed in terms of Major Cardiac Adverse Event (MACE) incidence, collected at 3 month intervals within the first year, and then at 1,3 and 5 years. Health related quality of life will be assessed every three months via telephone interview using the SF-36 and EQ-5D questionnaires. Use of telephone follow-up, rather than post, provides enhanced data quality/completeness and minimises respondent burden, taking account of patient age (many will be elderly) and the question volume. Our pilot work and also supporting literature (Curtis

and Redmond, 2009) suggests that the use of postal (and email) questionnaires for follow-up, yields low response rates.

4.1.27: PLANNED STATISTICAL ANALYSES

Simple descriptive summary statistics (percentages, means, medians, range and standard deviation) will be calculated. The distribution of data will be assessed by analysing skewness, kurtosis and histogram plots.

4.1.28: HISTOLOGICAL AND CLINICAL OUTCOME ANALYSIS

The percentage of patients with zero endothelial integrity will be compared between the three randomised groups using firstly a simple chi-square test, followed by multiple logistic regression analysis to incorporate adjustments for potential confounding factors such as age, sex and diabetes.
The percentage of the MACE total score in each group at the end of follow-up will be assessed using similar statistical methodology as for endothelial integrity. Baseline and finalisation follow-up SF-36 and EQ-5D scores will be summarised and compared between the three groups using analyses of covariance. Repeated 3-monthly scores will be assessed using longitudinal regression modelling. Data will be analysed using SPSS v20. Statistical significance will be taken as $p \leq 0.05$.

4.1.29: FREQUENCY OF DATA ANALYSES

Data will be analysed every quarter of the data collection timeframe, with mid-term analyses to ensure no serious adverse events accrue for the participants.

4.1.30: HEALTH ECONOMIC ANALYSIS

The primary aim of the economic analysis is to compare the cost and clinical outcome of the three vein harvesting approaches. Unit cost data will be attached to the resource use data and collected during surgery, along with in-patient admission, 3 months, 12 months to 5 year follow up. Descriptive statistics will be used to summarise the mean costs and their variations. The mean cost per patient, and total cost for each approach will be calculated then analysed alongside the data on health status. It will be collected using the EQ-5D, to help understand the relative costs and outcomes of the three vein harvesting approaches. Appropriate statistical methods will be used to compare the cost and health status data, taking into account the skewed nature of the data (for example, boot strapping methods used to analyse cost data).

4.1.31: LIMITATION OF THIS STUDY

This study will not provide post-surgery angiographic evidence for all patients. However, patients who are symptomatic will undergo cardiac MRI scans, angiograms and any other relevant investigations which will be addressed in this study.

4.1.32: DISCUSSION

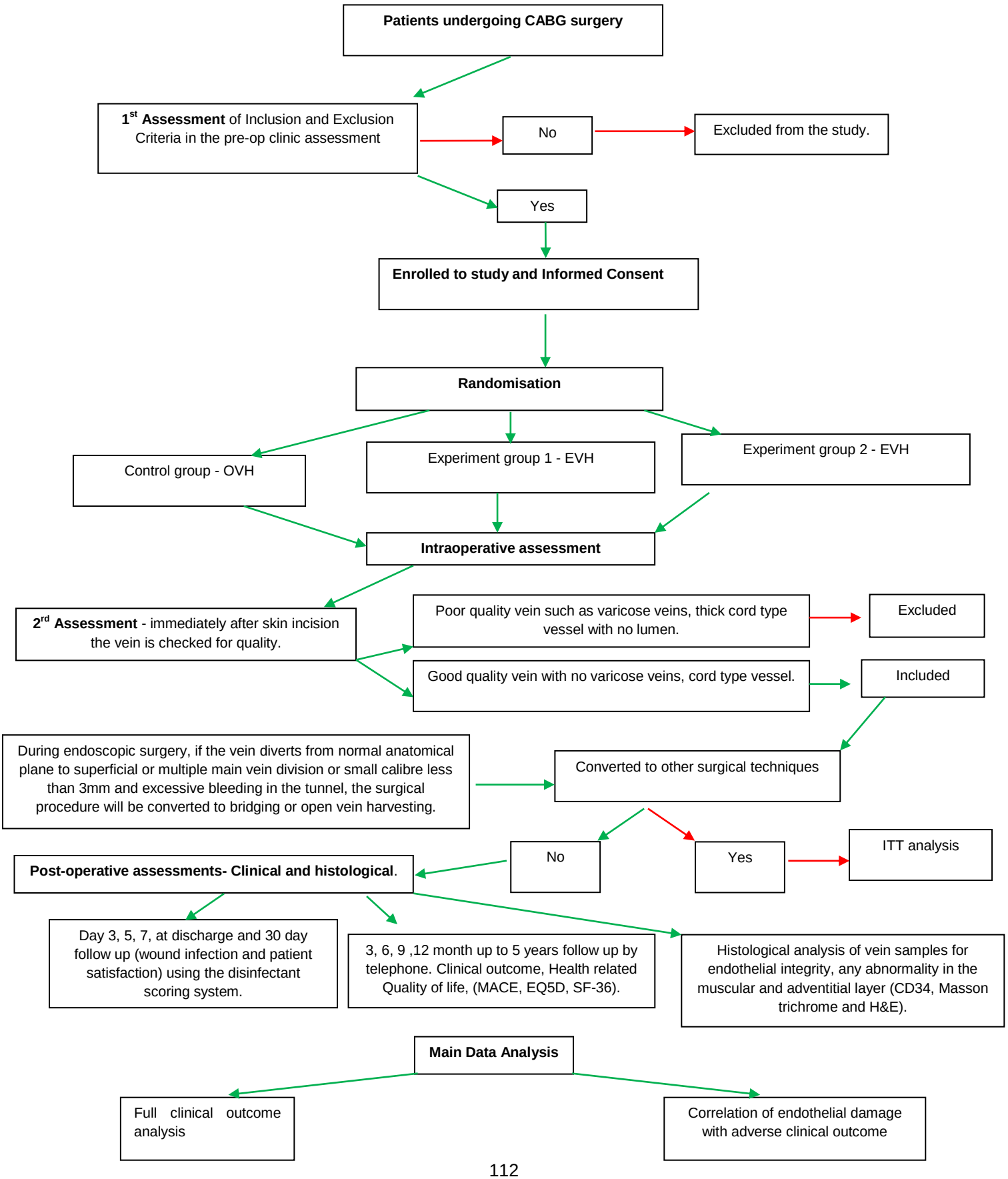
Vein harvesting techniques can potentially cause structural damage to the vessel wall leading to graft failure as shown in angiographic and ultra-structural studies revealing mural thinning and endothelial cell damage (Kennedy and Tedgui, 1995; Catinella et al., 1982). Some vein studies concentrating on the biological effects of endothelial layer impairment demonstrated that myointimal proliferation affects short and long term graft performance (Kennedy and Tedgui, 1995; Dhein et al., 1991; Furchgott and Zawadzki, 1980).

Impairment of the endothelial layer in OVH samples was demonstrated during pre or post-surgical preparation while distending (Angelini et al., 1989b; Gundry et al., 1980) or stretching the vein(Bonchek, 1980; Hasse et al., 1981; Bush et al., 1986). Manderson et al (Manderson and Campbell, 1986) suggest that histological studies of veins harvested using different minimally invasive techniques should be performed periodically on different timings to assess endothelial integrity, since endothelial denudation leads to intimal and medial layer repair with neointimal thickening.

Meticulous preservation of the layers of the saphenous vein during harvesting is an important factor in determining graft patency rate (Alrawi et al., 2001b). There continues to be concern that excessive manipulation of the vein via EVH may cause trauma to the vessel leading to early graft failure and stenosis (DeLaria et al., 1981; Cable and Dearani, 1997; Slaughter et al., 1998; Tevaearai et al., 1997; Wipke-Tevis et al., 1996). We believe that the use of CO_2 during EVH can affect the endothelium of the LSV. It is crucial to delineate the effects of CO_2 pressure on vessel integrity and clinical outcome following CABG. This trial will provide insight into the effects of pressurised CO_2 on the vessel, and will be compared to both non-pressured CO_2 EVH and OVH.

We believe that this trial will provide important clinical data that is currently lacking in the literature, and can provide an answer to the concerns and controversies around the vein harvesting techniques for coronary artery bypass surgery.

4.33: CONSORT STUDY FLOW DIAGRAM



4.34 COCHRANE RISK OF BIAS TOOL

Selection bias (allocation)

- Computerised random allocation by the independent statistician.

- Concealed by opaque envelope carried out by the non-research team member and main allocation sheet held in their campus.
- Opened after the patients were anaesthetised for the surgery.

Detection bias (blinding outcome assessors)

- All clinical data collected by the research team with numeric coded about the procedure.
- All histological vein samples were numeric coded and completely blinded to the groups.
- All scores were done by the independent assessors and controlled by the team without involving the operating practitioner.
- All vein samples were auto stained (CD34, Picrosirius Red, H&E and to avoid any manual error).

Performance bias (blinding participants)

- All patients were blinded to the study (only came to know after surgery about procedure didn't have any idea about what group they were allocated).

- All patients were recruited and consented by the surgical team not by the operating practitioner.

Attrition bias

- No missing outcome data expect mortality.
- Patients who moved house telephone numbers obtained from the GP surgery.
- Patients whose veins were not used after the surgical procedure will be included in the main analysis as Intent to treat analysis and protocol adherent analysis.

Reporting bias

- All outcome measures will be reported.
- Histological, clinical, health economics, wound infection, effects of carbon-di-oxide.

CHAPTER 5

General Methods

5.0: General Methods

The aim of this chapter is pull together the histological, clinical and health economics methods for chapters 6, 7, 8, 9 into one chapter, providing greater detail than is possible within the confines of a publication. All the immunohistochemistry standard protocols were provided by The University of Manchester (Archibald Vivian (AV) Hill Histology Laboratory) and University Hospital of South Manchester (UHSM) NHS Foundation Trust Histology Laboratory.

The H&E, CD34 and Picrosirius red staining were performed by fully automated machines in a batch of 64 slides at a time to avoid any manual staining errors. However, the student did manually generate some of the sample slides as a practice run to understand the full staining protocol and to gain experience.

5.1: Immunohistochemistry methods

The VICO study composed of a few histological methods such as

- Validation of endothelial staining comparing CD34 and CD31.
- Effect of carbon dioxide absorption – basic H&E staining to assess the endothelial stretching and endothelial detachment of the vein in three types of vein harvesting techniques.
- Effect of distension and non-distended vein damages – basic H&E stain, Picrosirius red muscular staining to assess the endothelial disruption, circular and longitudinal muscle migration, hypertrophy, detachment.
- If vein damage has any direct impact on clinical outcomes – basic H&E stain, CD34 endothelial stain, Picrosirius red muscular staining to assess the endothelial continuity, muscular damages.

5.1.2: Vein sample collection

Immediately after the long saphenous vein (LSV) by open or closed tunnel or open tunnel endoscopic vein harvesting, 3cms (1 x 1cm) was taken. It was coded as proximal sample (H1) which was undistended, distal sample (H3) which was distended with 10mmHg heparinised blood flush to check for leakages. Finally, random sample (H2) which was fully distended and also undergone all surgical preparation/handling and cardioplegia solution (70mmHg). A total of 2700 vein samples underwent

the histology method process. The detailed number of the specimen is mentioned in the chapter manuscript method sections.

5.1.3: VEIN SAMPLE PRESERVATION

The 1cm x 3 vein samples were carefully cut by using number 11 sharp blade and immediately placed directly into three 4% formalin solution pots (the constitution of formalin was 1:10 ratio of formalin and distilled water pH 7.4). Formalin storage of vein samples prevents the alternation of the vein tissue structures through decomposition by chemical cross-linking of proteins and the removal of water from the tissues (Krishnamoorthy, 2014).

5.1.4: DEHYDRATION AND PARAFFIN EMBEDDING OF THE VEIN SAMPLES

To dehydrate the vein samples, the samples were washed through a series of alcohol concentrations (ethanol) which ranged from 30%, 50%, 70%, 80%, 90%, 95% and 100% for 70 minutes (10mins in each solution). The vein samples were then incubated in Ethanol and Xylene solution for another hour in the following sequence:

- 2:1 ethanol: xylene for 10-15mins.
- 1:1 ethanol: xylene for 10-15mins.
- 1:2 ethanol: xylene for 10-15mins.

Finally, all the vein samples were placed in a 100% ethanol solution for three different time points (10-15mins) to ensure complete water removal from the tissues. After completing the dehydration process, the samples were again washed in xylene three times to clean the tissues before immersing in paraffin wax. The samples were transferred to 50:50 solutions of xylene and paraffin and finally, the samples were immersed in 100% paraffin at 56° C for three hours to allow accurate infiltration of the vein samples. The vein samples were placed vertically (to obtain cross section of the vein segments) into an embedding mould (small plastic/metal cassette). The melted paraffin was poured from the semi-automated paraffin machine over the cassette to form a block. These blocks were allowed to cool on an ice machine before sectioning.

5.1.5: SECTIONING OF THE VEIN SAMPLES

A Leica (2255™) rotary microtome was used to section the paraffin embedded vein blocks. The orientation incidence angle of 3-4° was kept with a use of an allen key and a fresh sharp microtome blade was used, starting from right hand side of the sample and gradually moved to left as it blunts. Before starting the sectioning, excess wax on each paraffin block was carefully chipped away using a solid 23 handled scalpel to avoid any damage to the tissues and blade. The blocks were placed on the ice 10 minutes before sectioning to allow softening of the paraffin cassette. The paraffin block cassette was positioned by keeping the label towards the right of the machine and lowered to just above the blade. Few times the wax was trimmed off from the block until the vein tissue appear on the top of the cassette. Coarse and fine brushes were used to clean the microtome and pick up the sections from the water bath.

The microtome was turned on a section mode from safety mode; the hand wheel was gently rotated in a continuous manner to create a ribbon of straight 5µm thin cross sections. The sections were immediately moved by using a fine brush into a warm water bath (45 to 50° C). The fine brush was used to separate each section and poly-l-lysine special histology slides were dipped into the water bath to scoop the section onto the middle of the slides. These specialised histology slides were used instead of standard histology slides to avoid the loss of tissues during microwave/pressure cooker preparation for antigen retrieval (Krishnamoorthy, 2014). The sections were drained carefully and dried on a slide rack on a hot plate. Finally, the slides were left to dry overnight in an incubator at 37° C and stored in a racked storage box at room temperature.

5.1.6: HAEMATOXYLIN AND EOSIN (H&E) STAINING

To avoid manual staining error, the automated carousel stainer Shandon Varistain™ 24-4 was used. It stains 64 slides per basket, theoretically (64 x 24) per run with 10 slide carriers. A total of 900 vein sample slides were stained using this machine. The procedure is a standard protocol (Appendix 5), obtained from Mr. Peter Walker (Histology Research Technician), in the AV Hill building histology laboratory at The University of Manchester. The slides were arranged and placed on top of the slide carrier vertically into the allocated slots. The machine was reset and program 1 was selected for H&E staining. The stainer canopy was elevated by pressing the load button on the control panel. Immediately, the slide carrier was pushed into the support hanger at the bottom of the canopy. The

agitation button was pressed which moved the slides gently up and down in the reagent. The canopy was closed by pressing the automatic mode on the panel to start the H & E staining process (detailed staining process is written on Appendix 5). Once the sequence completed, the slide carrier is removed and dried before inserting the slides into the coverslip automated machine (Thermo Scientific ClearVue™). This coverslip machine provides high quality pressure and vacuum control system and does 400 slides in an hour. It automatically identifies and delivers the correct amount of mountant without any micro bubbles, which is a major problem for manual cover slipping. The mountant is a mixture of distyrene, plasticiser and xylene (DPX), a colourless synthetic resin mounting medium.

5.1.7: PICROSIRIUS RED STAINING PROTOCOL

To avoid manual staining error, the Shandon Varistain™ 24-4 which is an automated carousel type stainer, was used. It stains 64 slides per basket, theoretically (64 x 24) per run with 10 slide carriers. A total of 900 vein sample slides were stained using this machine. The procedure is a standard protocol (Appendix 6) obtained from Mr. Peter Walker (Histology Research Technician) in the AV hill histology laboratory at The University of Manchester.

We used pre-prepared Sirius red F3B (C.I.35782) which is 0.5g in 500ml of saturated aqueous solution of picric acid. In order to ensure good saturation, little solid picric acid was added. The pre-prepared stain keeps for at least 3 years and can be used many times. The staining kit (Sigma-Aldrich (Direct Red 80) 24901-250, polyscience, Inc) was used for this study. In order to prepare the acidified water, we used 5ml acetic acid (glacial) to one litre of distilled water.

The sections were dewaxed by heating at 50°C for 30 minutes and dipped in Xylene solution three times for 5 minutes. Then, sections were then dehydrated by washing for 2 minutes in various grades of ethanol (100%, 100%, 100%, 95%, 95%, and 70%) and finally washed in water. The slides were stained for nuclei with Weigert's haematoxylin for 8 minutes and then washed for 10 minutes in running tap water. To give near-equilibrium staining, the slides were left in Picrosirius red stain for one hour. Immediately, after the hour, the slides were washed in two changes of acidified water (0.5% acetic acid) then a Varistain™ agitator removed any water from the slides.

The slides were dehydrated over increasing grades of ethanol (70%, 100% and 100%) and cleared in xylene. The dehydration method was carried out very quickly by the machine because ethanol washes reduce the contrast between Picrosirius red staining and picric acid. Finally, the slides were mounted using a permanent mounting medium (DPX) by a coverslip automated machine (Thermo Scientific ClearVue™).

5.1.8: CD 34 STAINING

To avoid any manual staining error, we used a fully automated Bond Aspirating Probe Cleaning System for Immunohistochemistry™ which delivers speed, efficiency and quality slide staining. It stains 140 to 170 slides per day. A total of 900 vein sample slides were stained using this machine. The procedure is a standard Immunohistochemistry (IHC) protocol and it was obtained from University Hospital of South Manchester NHS Foundation Trust histology laboratory from Mrs. Catherine Wilmot (Histology scientist).

The slides were soaked and de-waxed two times in 100% xylene for 10 minutes, then rehydrated in 100% ethanol solution for six minutes and 95%, 70%, 50% solutions for three minutes individually. To remove any excess alcohol from the tissues, the slides were dipped and rinsed under running tap water for five minutes. 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.

The antigen retrieval was performed by placing the slides on a metal rack in a pre-warmed 0.01M citrate buffer (pH6.0) that was 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 safeguard the blocking agent within the drawn 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.

The excess horse serum was removed by flicking the slides, then the CD34 antibody (LEICA™) was diluted in a ratio of 1:30, using the DAKO™ antibody diluent and vortexed to methodically mix the solutions. The primary antibody was added to the samples and incubated for 1 hour and 10 minutes. All the slides were washed in 0.05M TRIS buffer (pH 7.6) solution and sited on a mechanical shaker

for 15 minutes to remove excess primary antibodies. The secondary antibody incubation was carried out by adding one to three drops of ImmPRESS™ universal anti-mouse/rabbit IgG reagent (polymerised reporter enzyme) to the samples and incubated for 30minutes in a moist box. Finally, the slides were then washed in TRIS buffer for a further 15 minutes (Krishnamoorthy, 2014).

Once the sequence was completed in the machine, the slide carrier was removed and dried before inserting the slides into the coverslip automated machine (Thermo Scientific ClearVue™).

5.1.9: SCORING OF THE HISTOLOGY SLIDES

Each slide was allocated a random number before any assessors assigned a score. The slides were imaged using Pannoramic 250™ slide scanner at The University of Manchester. This machine has a special high-NA Carl Zeiss™ optic lens to achieve maximum resolution of up to 0.16 µm per pixel image. Samples were scored by five blinded, independent and fully trained assessors using Pannoramic Viewer™ software for efficient image viewing, annotation and archiving purposes. All the scores were verified by an independent Consultant Histopathologist at UHSM. None of these assessors were involved at any stage of this research project. The slides were assessed for endothelial integrity (variability was >15%). A validated scoring system (Fischlein et al., 1994) 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).

The Picrosirius red scoring, which was obtained from The University of Manchester histology lab, was based on the following criteria:

Area of damage	**Scores**	**Detailed scores**
Circular and longitudinal muscle hypertrophy	On a scale of 0 – 3.	0 – normal. 1 – mild. 2 – moderate. 3 – severe.
Medial muscle detachment	0 % to 100%	0% - no detachment. <10% 11 – 25% 26 – 50% 51 – 75% 76 – 100% - complete detachment.
Circular and longitudinal muscle migration (internally and externally)	On a scale of 0 – 3.	0 – normal. 1 – mild. 2 – moderate. 3 – severe.

The H& E scoring, which was obtained from The University of Manchester histology lab was based on the following criteria:

Area of damage	**Scores**	**Detailed scores**
Endothelial damage	Normal endothelial layer	Grade 0.
	Stretched layer	1.1- mild. 1.2- moderate. 1.3- severe.
	Detached layer	2.1- mild. 2.2- moderate. 2.3- severe.
	Partial endothelial loss	Grade 3
	Complete loss of endothelial layer	Grade 4.

5.2: HEALTH ECONOMICS

To evaluate the health economics perspective, complete cost data, EQ- 5D-3L and SF36 was collected at baseline, 3 months and 12 months interval period. We calculated full surgical, medical costs based on resource utilisation and clinical events during the surgical procedure, hospitalisation and prospective postoperative follow up. We counted number of surgical items used in both groups, sutures, disposable kits, medications, wound infection costs, antibiotics usage in hospital and community, any adverse events, length of hospital stay, readmission costs, reintervention costs (angiogram, ECG, Chest x-ray, MRI scan, CT heart scan, stenting the coronary arteries), theatre costs, surgeon and allied health professionals costs, cardiologists, GP, district nurse costs as well as any applied cost weights in UK pounds to calculate costs of the surgical procedure. All community costs post-surgical procedures were obtained from GP surgeries, cardiology departments and outpatient departments from other neighbouring hospitals.

The detailed EQ-5D-3L was also collected for this study, which is a generic instrument involving of two sections: a 5-dimension single summary health status index and a self-rated visual analogue scale which ranges from 0 (best imaginable health state) to 100 (worst health state) (EuroQol, 1990). The cost-effectiveness analysis was carried out by the total costs assessed against the effects in terms of Quality Adjusted Life in Years (QALY) based on the EQ-5D-3L. In addition, the estimated incremental cost per QALY from the hospital service was compared with the willingness to pay threshold of £20,000 to £30,000 per extra QALY which is currently used by the National Institute of Health and Care Excellence (NICE) (Excellence., 2013) . The total costs were derived by intervention plus or minus any subsequent differences in the NHS costs.

5.3: CLINICAL METHODOLOGY

All the clinical data were collected prospectively into a relational database. General demographics including age, sex, race, body mass index, hospital admission, pre catheterisation basic information's and history of angina were collected. Other preoperative risk factors such as hypertension, smoking, family history of coronary artery disease, diabetes, peripheral vascular disease, hypercholesterolemia, previous myocardial infarction/ myocardial ischemia, previous Percutaneous Transluminal Coronary Angioplasty (PTCA), Parsonnet score which is a simplified Canadian risk scoring system to estimate the cardiac surgical mortality risk and finally European system for Cardiac Operative Risk Evaluation (Euroscore) were documented.

All intraoperative data including number of coronary vessels grafted, number of grafts planned, types of conduits harvested, surgical timings, details of the members of staff who completed the surgery and cardioplegia details was recorded. In hospital mortality, community mortality outcomes were collected from validated registry and post-mortem reports from the Coroner's Court. Long-term Major Adverse Cardiac Events (MACE) outcomes were measured for this study at different time points (3, 6, 9, 12, 24, 36, 48 months) post-surgery. The MACE was defined as post CABG recurrent angina, MI, target vessel revascularisation, coronary artery/vein graft stenting, stroke and death (Krishnamoorthy, 2014).

Repeat angina was classified using the Canadian Cardiovascular Society grading system (CCS) which is a validated scoring system for standardisation of angina grade ranging from I-IV. Class I specifies angina with sustained, strenuous exertion, Class II characterises slight limitation with angina upon vigorous action, Class III represents moderate limitation with symptoms during everyday activity and Class IV indicates severe limitation and inability to perform any activity with angina even at rest (Campeau, 1976). Breathlessness was assessed using the New York Heart Association (NYHA) scoring system which ranges from I-IV (Raphael et al., 2007). 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. Electro cardiogram (ECG), nuclear test for ischemia, dobutamine stress test, 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) (Sianos et al., 2005; Krishnamoorthy, 2014). All the patients were followed up by the telephone interview from day of surgery, 3, 6, 9, 12, 24, 36, 48 months using a validated MACE questionnaire. In addition, the symptomatic and non-symptomatic patients' notes were obtained from the outpatient clinics, other community hospitals, GP surgeries, the cardiology database; district nurses files and consultant's secretaries' online notes.

CHAPTER 6

Validation of endothelial stain markers

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6: VALIDATION OF THE ENDOTHELIAL MARKERS CD31 AND CD34 IN IMMUNOHISTOCHEMISTRY OF THE LONG SAPHENOUS VEIN FOR CORONARY ARTERY BYPASS SURGERY

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Key words: Coronary artery bypass surgery, endothelium, immunohistochemistry, blood vessels.

6.1: Abstract

Objectives:

Endothelial injury during a surgical intervention can significantly affect the functional status of the vein. The endothelial layer plays a vital role in the long saphenous vein for ensuring smooth blood flow and the prevention of vasoconstriction and thrombi formation within the blood vessels. There are few histological studies that compare the different vein harvesting techniques that have studied endothelial layer integrity using CD31 and CD34 on human long saphenous veins.

Methods:

Non-distended vein samples measuring 1cm were obtained from ten consecutive traditional open vein harvesting patients and were automatically processed and stained using immunohistochemistry for CD31 and CD34. The colour, intensity and distribution of the staining on the tissues were scored blindly by five independent scientists and an expert histopathologist for this study.

Results:

The CD34 antibody demonstrated greater colour staining ($p<0.007$), intensity ($p<0.019$) and distribution ($p<0.007$) compared to CD31.

Conclusion:

Our study indicates that CD34 provides a more reliable endothelial marker in the long saphenous vein than CD31. The results of this study can be translated into other immunohistochemistry studies looking at the quality of the endothelium on the vein in cardiac and vascular surgical studies.

6.2: INTRODUCTION

Coronary Artery Bypass Grafting (CABG) Surgery is the most commonly performed procedure in cardiac surgery. This method involves bypassing blocked coronary arteries using arterial and venous conduits. Despite the use of arterial conduits providing improved long term graft patency, venous conduits such as the long saphenous vein are still widely used in multiple bypass surgery due to its long length and easy availability. However, donor leg wound complications are among the most common post-surgical problems, which may occur in 5% to 44% of cases (DeLaria et al., 1981). Minimally invasive vein harvesting methods have been developed to reduce the risk of wound complications and post-operative morbidity. However, these techniques have been associated with greater risk of damaging the vein layers, particularly of disrupting the endothelium, during surgery. If this occurs, platelets become aggregated and induce endothelial denudation, promoting intimal proliferation and hyperplasia, leading to graft occlusion, which may subsequently result in poor long term graft patency (Catinella et al., 1982).

Previous studies have focused on endothelial damage occurring during harvesting of the long saphenous vein, predominantly via assessment of CD31 expression. CD31 is a 130-kDa transmembrane glycoprotein, which demonstrates strong homogeneous expression in all human pulmonary endothelial cells but is also expressed to a lesser extent on platelets and some leukocyte subsets (Muller et al., 2002). CD34 has also been used as a marker of endothelial cells. However, a methodical comparison of the quality of these markers would be beneficial, especially considering that the molecular and functional characteristics of endothelial cells can vary on the vascular tree between the different vessels around the body (Pusztaszeri et al., 2006). The 110-kDa transmembrane glycoprotein CD34 demonstrates a more heterogeneous expression and is particularly found on endothelial cells of: capillaries, arteries, veins, arterioles and venules (Kawanami et al., 2000). Additional markers expressed on endothelial cells, such as von Willebrand Factor (vWF) and Fli1, have been utilised previously for the identification and detection of these cells. The glycoprotein vWF has important roles in platelet adhesion following injury, and is expressed on endothelial cells in a range of settings. However, vWF has been previously demonstrated to have weak expression on capillary endothelium and its use may be complicated sub endothelial expression in certain tissues. Fli1 is consistently expressed by endothelial cells in a range of tissues; however it is also present

within the nucleus of haematopoietic cells, especially lymphocytes and is a useful marker for diagnostic evaluation and detection of vascular tumours. This study evaluated only the use of CD31 and CD34 for assessment of endothelial integrity on the long saphenous vein due to the nature of their expression patterns.

Immunohistochemistry (IHC) remains the gold standard for studying the morphological status of the vein. Although previous studies have used IHC to score endothelial integrity, none have directly compared the quality of CD31 or CD34 staining following vein harvesting for CABG surgery. This is the first study to compare the difference in colour, intensity and distribution of CD31 and CD34 expression on long saphenous vein sections with the purpose of identifying a standard marker for future IHC for use in assessing endothelial integrity.

6.3: METHODS

6.3.1: INFORMED CONSENT AND ETHICAL APPROVAL

An overview of the experimental design for this study is included in Figure 17. Ethical approval was provided by the Greater Manchester North East - National Research Ethics Committee (NREC) as part of the vein integrity and clinical outcomes (VICO) randomised controlled trial. All the patients provided written informed consent for the trial. The VICO trial is designed to assess the direct relationship between endothelial damage and the clinical outcomes. This validation of staining study was designed to elite the correct endothelial marker for the VICO trial full sample analysis. Samples of proximal long saphenous veins were collected from the lower leg using the open vein harvesting technique from ten consecutive patients. Vein conduits retrieved by minimally invasive vein harvesting techniques were not included in this study to ensure a reliable sample was retrieved with intact endothelium. Vein samples that were not surgically distended were utilised in this study to provide a reliable result indicative of viable endothelium in the long saphenous vein.

6.3.2: SAMPLE PROCESS AND STAINING

We used a fully automated staining protocol from UHSM to avoid any bias of manual handling/error. The following staining procedures were performed by the automated machine as per standard Immunohistochemistry (IHC) protocol.

Samples were cut into approximately 1cm sections and placed into a solution of 4% formalin in distilled water (1:10 ratio of formalin and distilled water pH 7.4). Samples were processed using the standard operating procedures of the Histopathology Laboratory at UHSM. The samples stored in formalin preclude modification of the tissue structures through decomposition by chemical cross-linking of proteins and the removal of water from the tissues (Krishnamoorthy, 2014).

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. At the end, the samples were placed in a 100% ethanol solution to ensure complete water removal.

After the dehydration procedure was finished, the samples were washed in xylene to clean the vein tissues in preparation for soaking in paraffin wax. At first, the samples were soaked in a 50:50 solution of absolute ethanol and xylene for three hours. The samples were transported 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 moved to an embedding mould (small plastic cassette) and melted paraffin was poured over the mould from an automated machine to form a block. These paraffin wax blocks were allowed to cool before commencing sectioning of the vein samples.

The paraffin embedded samples were then cut into 4 µm-thin sections and placed onto poly-l-lysine histology slides, dewaxed and rehydrated in graded alcohols ranging 100% ethanol solution for 6 minutes and 95%, 70%, 50% solutions for 3 minutes individually. At the end, the samples were placed and rinsed under running tap water for 5 minutes to remove any residual alcohol from the vein tissues. Endogenous peroxidase activity was quenched by incubation in a 0.3% hydrogen peroxide aqueous solution for 15 minutes at room temperature. The heat-induced epitope retrieval method by means of a pressure cooker was used for antigen retrieval of vein cross sections. The samples were

incubated in 2.5% ready to use normal horse serum (VectorLabs ImmPRESS) for antigen retrieval at room temperature for 30 minutes.

The efficacy of anti-CD31 and anti-CD34 antibodies (Dako, Cambridgeshire, UK) to stain the saphenous vein endothelium was compared following automated tissue immunohistochemistry using both antibodies at a 1:30 dilution in DAKO™ antibody diluent (Dako, Cambridgeshire, UK) according to manufacturer's protocol. The primary antibody was added to the vein 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 antibody. Detection was performed using one to three drops of ImmPRESS™ HRP universal anti-mouse/rabbit IgG reagent antibody polymer detection kit (Vector Laboratories, UK) and incubated for 30 minutes in a moist box. The slides were then washed in TRIS buffer for a further 15 minutes.

The slides were incubated in an ImmPACT™ DAB (3, 3'- diaminobenzidine) peroxidase (HRP) substrate (Vector Laboratories, UK, Cat # SK - 4100) to bind the secondary antibody. This DAB substrate solution was prepared by linking 1ml chromogen substrate and one drop of peroxidase (enzyme). This solution was vortexed to ensure proper mixing. The slides were then incubated for 5 minutes to accomplish adequate sample staining intensity. Haematoxylin and eosin staining was performed for the evaluation of the saphenous vein, as a method for counterstaining (Figure 18) for one minute and immediately washed under running tap water.

The slides were allocated a random number before assessors started scoring them. The slides were imaged using Pannoramic 250™ slide scanner at the University of Manchester. This machine has a special high-NA Carl Zeiss™ optic lens to achieve maximum resolution of up to 0.16 µm per pixel image. Samples were scored by five blinded, independent and fully trained assessors by using Pannoramic Viewer™ software for efficient image viewing, annotation and archiving purposes. All scores were verified by a UHSM Consultant Histopathologist. None of these assessors were involved at any stage of this research project. Slides were scored based on the colour, intensity and staining distribution of CD31 and CD34 using the following validated scoring system: "1" neg-none, "2/+" mild, "3/++" moderate and "4/+++" intense (Nezafati et al., 2014; Pusztaszeri et al., 2006).

6.3.3: STATISTICAL ANALYSIS

All data was expressed as mean score±standard deviation, with differences between the two sets of results determined using the Chi-square test for categorical variables. A p-value of <0.05 was considered statistically significant. SPSS 19.0 software was used for all calculations.

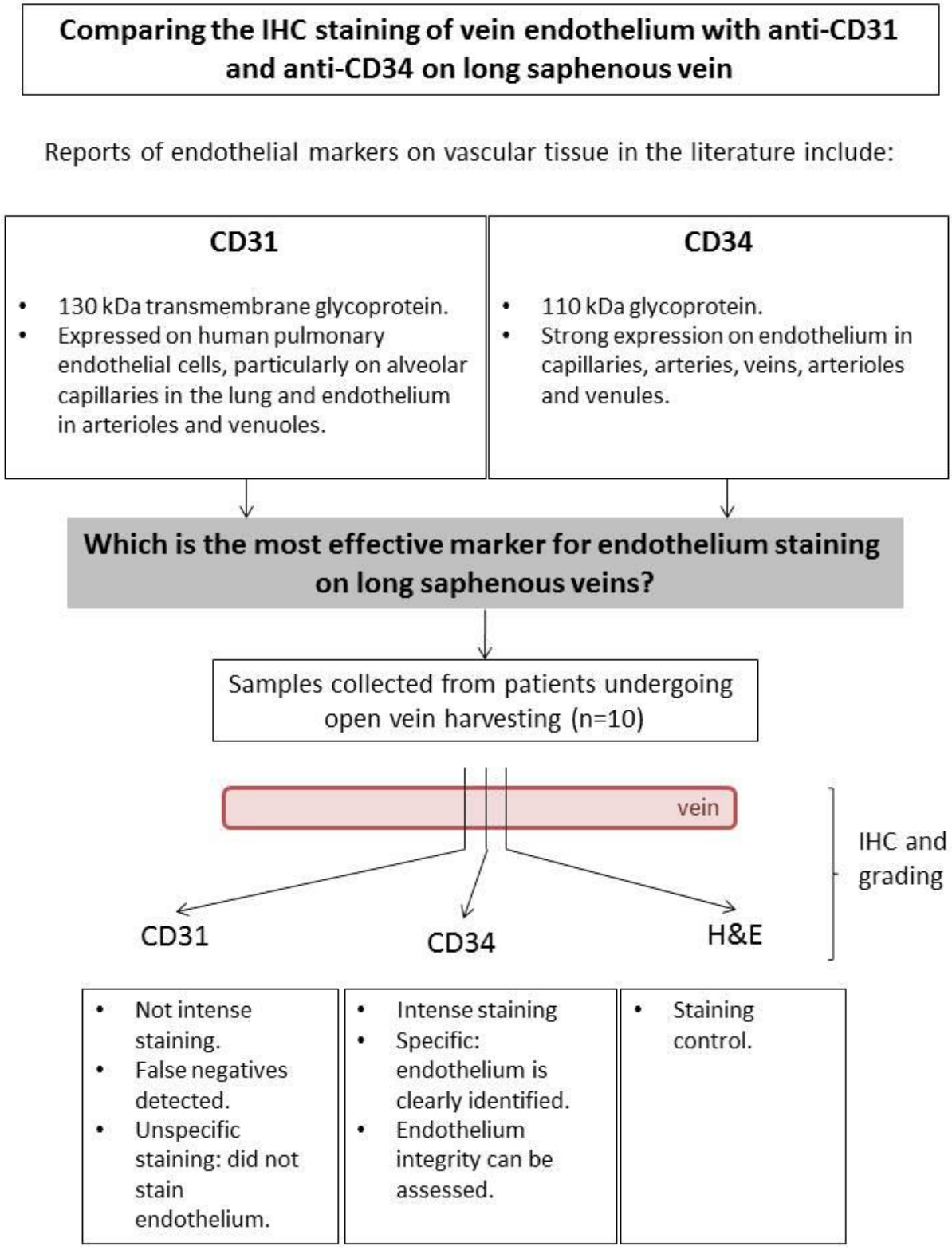

Figure 17: Experimental design to compare endothelial markers CD31 and CD34.

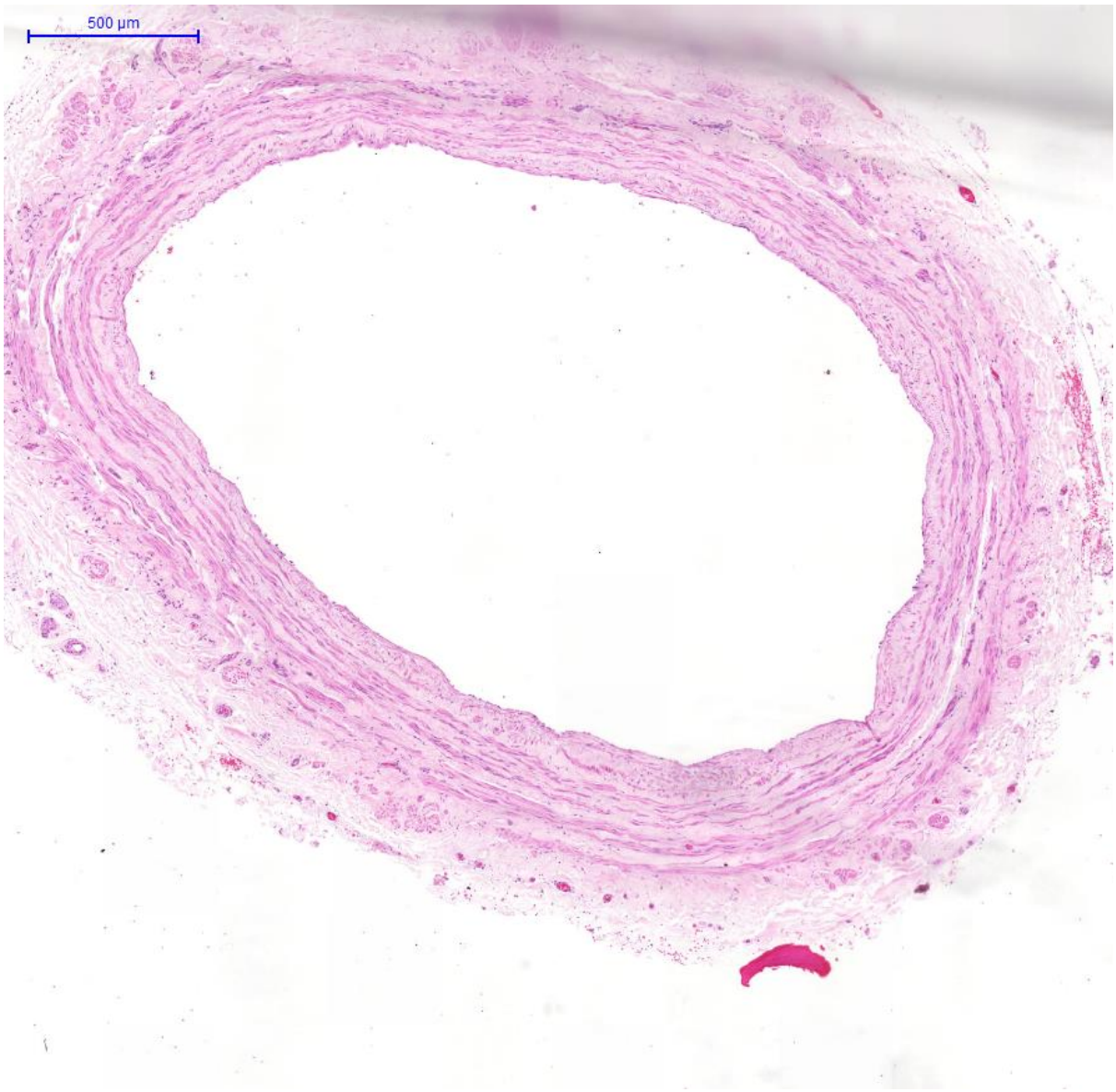

Figure 18: A cross section of the vein stained by Haematoxylin and Eosin stain.

This figure demonstrates that the Haematoxylin and Eosin stained long saphenous vein as a basic control stain before staining with the endothelial CD34 and CD31 stain.

6.4: RESULTS

Consecutive saphenous vein sections were stained using anti-CD31 and anti-CD34 antibodies. A significantly different pattern of expression was found in terms of colour, intensity and distribution as follows:

6.4.1: COLOUR

The relative colour of CD34 expression on the veins was found to be more distinct than that of CD31 (1.00±0.00 vs. 3.60±0.55, $p<0.007$, see Figure 19).

6.4.2: INTENSITY

Endothelial cell staining was found to be significantly more intense with the use of anti-CD34 antibody, compared to the mild staining of CD31 (1.80±0.45 vs. 3.40±0.55, $p=0.019$, see figures 3c and 3d).

6.4.3: DISTRIBUTION

CD34 staining was more widely distributed across the tissue compared to CD31, with improved coverage of endothelial cells (1.00±0.00 vs. 3.60±0.55, $p=0.007$). The CD34 stain was uniformly distributed along the endothelial layer of the saphenous vein. In addition, small capillary vessels on the adventitial layer were also effectively stained. In contrast, CD31 staining using anti-CD31 was found to be irregular along the endothelial layer.

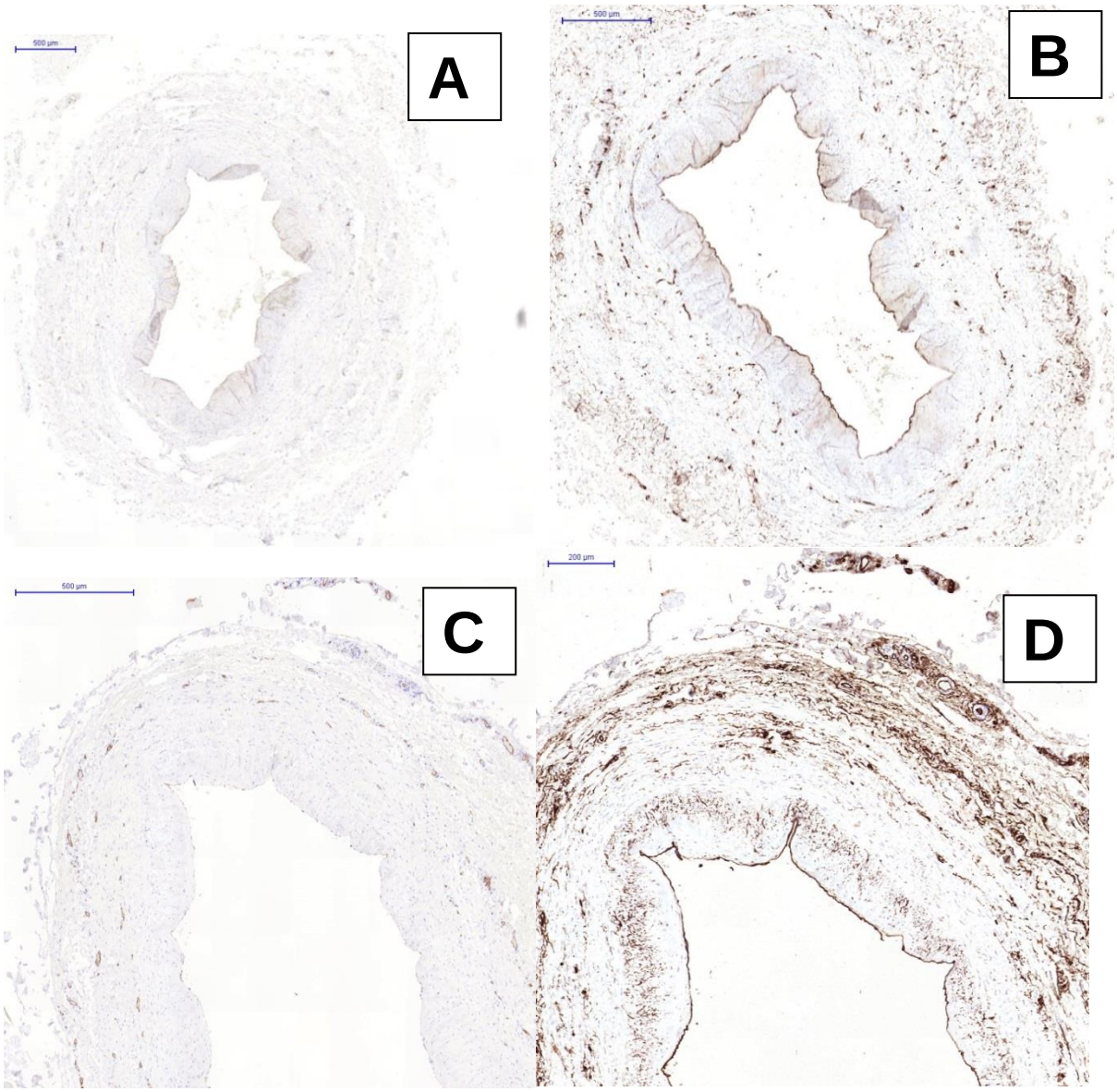

Figure 19: A cross section of a vein stained with CD31 and CD34.

This figure: (A) CD31 negative colour expression on the cross section of the long saphenous vein. (B) CD33 positive colour expression on the cross section on the long saphenous vein. (C) CD31 mild intensity on the cross sectioned vein. (D) CD34 severe intensity on the cross sectioned vein. Pictures A-C magnified: 500µm and D: 200µm.

6.5: DISCUSSION

This study aimed to compare the use of CD31 and CD34 as markers of endothelium on human long saphenous vein. Our findings indicate that CD34 stains in a more intense and regular manner, including the endothelium of small arterioles and venules located in the tunica intima, compared to CD31. Modern bio imaging techniques represent a fundamental area for evaluation of tissue samples at a cellular level, yet highly optimised staining is required for reliable scoring. This is particularly true for large scale studies when significant numbers of samples need to be assessed as high throughput automated methods can be utilised where bright and distinct staining is present.

The majority of studies assessing endothelial integrity have employed CD31 as the key marker, which is expressed on ~90% of endothelial tumours (De Young et al., 1998), ~90% of vascular tumours and sinusoids of the spleen (Ben-Izhak et al., 2001). CD31 is also strongly expressed on the surface of circulating platelets, monocytes, neutrophils and intracellular junctions, making interpretation difficult (Muller et al., 2002). Its frequent use in analogous studies, without evidence of systematic comparison with other markers, led to its acceptance as the best single marker for this purpose. In contrast, CD34 is assumed to play a major role in the formation of endothelial adherence junctions, which are the key components of angiogenesis (Tanigawa et al., 1997; Young et al., 1995). It is also present on lympho-haematopoietic stem and progenitor cells, leukemic cells and embryonic fibroblasts. In routine clinical practice, this marker is used for leukaemia diagnosis using immunohistochemistry and for the purification of immunological stem cells for clinical transplantation (Kirkpatrick et al., 1985; Sauter et al., 1998). Most of these studies focused on comparing these markers on a macro rather than a micro vascular level, which could pose important biological and physiological differences. In addition, there is limited evidence comparing CD31 and CD34 in human long saphenous vein.

Further knowledge regarding the expression pattern of specific endothelial phenotypes on the vascular tree is important to evaluate the effectiveness of these markers. Although previous studies did not perform a comparison between CD31 and CD34 in human long saphenous vein, the results of our study suggest that CD34 is a superior marker to CD31 in determining the presence of endothelial cells on the vessel luminal wall.

In conclusion, the use of CD34 provides a stronger and more distinct staining pattern for endothelium in human long saphenous vein samples when compared to CD31. This study provides novel evidence regarding the use of these markers which could have important clinical utility, such as an indicator of endothelial denudation following harvesting for coronary artery bypass surgery.

CHAPTER 7

Understanding the impact of carbon dioxide absorption on the harvested veins and systemic physiological alterations on these patients during surgery.

Submitted for publication

7. RANDOMISED CONTROLLED TRIAL COMPARING THE EFFECT OF CARBON-DIOXIDE INSUFFLATION ON VESSEL INTEGRITY USING TWO TYPES OF ENDOSCOPIC AND OPEN VEIN HARVESTING FOR CORONARY ARTERY BYPASS SURGERY.

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Keywords: Coronary artery bypass surgery, Open vein harvesting, closed tunnel endoscopic vein harvesting, open tunnel endoscopic vein harvesting, endothelial integrity.

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7.0 ABSTRACT

Objective:

To assess whether the use of carbon dioxide (CO_2) insufflation has any impact on integrity of long saphenous vein comparing two types of endoscopic vein harvesting (EVH) and traditional open vein harvesting.

Methods:

A total of 301 patients were prospectively randomised into three groups. Group 1 control arm of open vein harvesting (OVH) (n=101), Group 2 closed tunnel (CO_2) EVH (CT-EVH) (n=100) and Group 3 open tunnel (CO_2) EVH (OT-EVH) (n=100). Each group was assessed to determine the systemic level of Partial arterial CO_2 ($PaCO_2$), end tidal CO_2 ($EtCO_2$) and pH. Three blood samples were obtained at baseline, 10 minutes after start of EVH and 10 minutes after the vein was retrieved. Vein samples were taken immediately after vein harvesting without further surgical handling to measure the histological level of endothelial damage. A modified validated endothelial scoring system was used to compare the extent of endothelial stretching and detachment.

Results:

The level of end tidal CO_2 was maintained in the OT-EVH and OVH groups but increased significantly in the CT-EVH group ($p=0.451$, $p=0.385$ and $p<0.001$). Interestingly, partial arterial CO_2 also did not differ over time in the OT-EVH group ($p=0.241$) whereas $PaCO_2$ reduced significantly over time in the OVH group ($p=0.001$). A profound increase in $PaCO_2$ was observed in the CT-EVH group ($p<0.001$). Consistent with these patterns, only the CT-EVH group demonstrated a sudden drop in pH over time ($p<0.001$) whereas pH remained stable for both OT-EVH and OVH groups ($p=0.105$ and $p=0.869$ respectively). Endothelial integrity was better preserved in the OVH group compared to OT-EVH or CT-EVH groups ($p=0.012$) and was not affected by changes in CO_2 or low pH. Significantly greater stretching of the endothelium was observed in the open tunnel endoscopic OT-EVH group compared to the other groups ($p=0.003$).

Conclusion:

This study demonstrated that different vein harvesting techniques have an impact on endothelial integrity; however this does not appear to be related to the increase in systemic absorption of carbon dioxide or to the pressurised endoscopic tunnel. The open tunnel endoscopic harvesting technique vein had more endothelial stretching compared to the closed tunnel endoscopic technique; this may be due to manual dissection of the vein. Further research is required to evaluate the long term clinical outcome of these vein grafts.

7.1: INTRODUCTION

Open vein harvesting is the traditional long saphenous vein retrieval method for Coronary Artery Bypass Grafting (CABG) and is associated with significant morbidities (Andreasen et al., 2008; Krishnamoorthy et al., 2012b) post-surgery. Endoscopic Vein Harvesting (EVH) has been adopted for CABG as a result of reduced postoperative pain, reduced incidence of wound complications and improved patient satisfaction (Krishnamoorthy et al., 2012b). Whilst EVH is currently used routinely in our hospital, many centres in the United Kingdom have not adopted this technique or have ceased its use due to issues related to the quality of the vein (Krishnamoorthy et al., 2015), carbon dioxide insufflation (Neuberger et al., 1996; Vitali et al., 2000) and long term patency (Lopes et al., 2009).

Carbon dioxide insufflation is a method used in EVH to create a subcutaneous tunnel in the leg, thereby opening up the tissue space for dissection and clear visualisation (Maslow et al., 2006). Previous studies have highlighted that the systemic absorption of CO_2 (Vitali et al., 2000) and rarely gas embolism (Lin et al., 2003) can lead to life threatening events, which has led to questions about the safety of EVH (Lin et al., 2003). Careful attention is paid to EVH cases with safeguards in place, such as trans-oesophageal echocardiography (TOE) and end-tidal CO_2 monitoring, although there is a paucity of information regarding vein tissue level absorption of the gas.

No previous studies have directly investigated the effect of CO_2 in relation to histological level vein tissue trauma. The aim of this study was to explore the effects of CO_2 insufflation and histological evidence of vein tissue damage in three different types of vein harvesting.

7.2: METHODS

The study was approved by the NRES committee North West-Greater Manchester East and written informed consent was obtained from all study participants in accordance with institutional research ethics review board guidelines. Between 2011 to 2015, 301 patients who underwent CABG were recruited (see CONSORT diagram page no: 159). Simple block randomisation was performed by an independent statistician and patients were allocated into one of 3 groups:

Group 1 - (Control arm): 101 patients receiving traditional open vein harvesting (OVH).

Group 2 - (Intervention 1): 100 patients receiving closed tunnel CO_2 EVH (CT-EVH).

Group 3 - (Intervention 2): 100 patients receiving open tunnel CO_2 EVH (OT-EVH).

The allocation was performed using sequentially numbered opaque, sealed envelopes. A designated and independent research assistant had responsibility for the list. The practitioner opened the envelope once the patient had been anaesthetised in the operating room to avoid any cancellation of surgery.

Vein sample tissue storage and handling was covered by the Human Tissue Act licence held by the Institution Research and Development office. All veins were harvested by an experienced surgical practitioner who had performed at least 250 cases in each EVH technique and more than 2000 open vein harvests.

Patients were excluded from the study if they required emergency CABG surgery, if they did not want to participate, if they had a previous history of varicose veins or had thin superficial veins (Davis et al., 1998b; Krishnamoorthy et al., 2016).

7.2.1: SAMPLE STORAGE AND PROCESSING

An un-distended 1cm vein sample was obtained from the proximal region of the vessel from each patient and processed immediately to assess the direct effects of CO_2 absorption without any potential confounding effects from surgical handling and distension. Samples were cut and placed into a solution of 4% formalin in distilled water (pH 7.4). The samples were immersed before inserting the

vessel cannula into the vein for checking leakages and as such underwent no distension. There were a total of 301 vein samples obtained from these patients, which were then numerically coded to allow blinding of the laboratory histologist.

7.2.2: HISTOLOGY AND STAINING

All embedded vein samples were sectioned at 5µm by a Leica 2255 fully automatic microtome. Haematoxylin and Eosin staining was performed by a Shandon Varistain™ 24-4 automatic slide stainer to evaluate endothelial preservation. Endothelial integrity was classified based upon endothelial preservation and severity of abnormality: Grade 0 (normal endothelium), 1.1 (mild stretching), 1.2 (moderate stretching), 1.3 (severe stretching), 2.1 (mild detachment), 2.2 (moderate detachment), 2.3 (severe detachment).

Samples were blindly scored by five independent experienced assessors and a consultant histopathologist.

7.2.3: SURGICAL TECHNIQUES

In cases where 1-2 lengths of vein were required, these were harvested from mid-calf to thigh. When 3 lengths of vein were required, these were harvested from ankle to thigh. The traditional open vein harvesting control group were started either from the mid-calf or from the medial malleolus by a longitudinal leg skin incision (Krishnamoorthy et al., 2012b). The vein was taken with some perivascular fat and vein side branches were ligated with 4/0 vicyrl ties and metal ligaclips. For intervention Group 1, we used the Maquet Vasoview™ Hemopro 1&2 closed tunnel endoscopic vein harvesting system. To create a closed tunnel dissection, the CO_2 insufflator was set to flow rate 3 litres/minute with a constant pressure of 10mmHg. Most studies that have reported CO_2 embolism have used the company recommendation of 12–15mmHg pressurised tunnel in the leg (Chavanon et al., 1999; Chen et al., 2006). In our centre, we use 10mmHg pressure for the CT- EVH group in order to avoid any systemic complications (Tamim et al., 2008). For intervention Group 2, we used the Sorin ClearGlide™ open tunnel CO_2 system, and the CO_2 insufflator was set to flow rate of 3 litres/ min with 0mmHg pressure. From our previous experience, an open EVH system allows normal venous blood flow in the vein during the vein harvesting due to the lack of a pressurised tunnel.

7.2.4: STANDARDISATION

A significant increase in $PaCO_2$ is associated with a decrease in arterial pH. In order to account for this, the anaesthetist normally adjusts the ventilator by increasing the patient's minute ventilation (Neuberger et al., 1996). In this study, artificial ventilation settings were not changed during the study period. All the patients were fully heparinised and went on bypass once the vein harvesting was completed. However, CT-EVH group patients received 5000 units of heparin before starting the retrieval in order to avoid intraluminal clot formation inside the vessel (Brown et al., 2007b). Patients who were on anticoagulant until the day prior to surgery were administered only 2500 units instead of 5000 units to avoid major bleeding inside the tunnel.

7.2.5: SYSTEMIC CO_2 MEASUREMENTS

In addition to basic demographics, all patients had trans-oesophageal echo (TOE) performed for close monitoring of CO_2 bubbles. We collected three consecutive blood samples for CO_2 analysis at baseline after induction, 10 minutes after vein harvesting started and 10 minutes after vein harvesting was completed. The levels of partial CO_2 (Pa CO_2), end-tidal CO_2 (Et CO_2), pH, respiratory rate, Fraction of Inspired Oxygen (FiO_2) and tidal volume were also recorded to determine any pattern of acidity and hypercarbia.

Veins were considered to be exposed to CO_2 during the entire harvesting process from start of insufflation to removal of the vein from the leg.

7.2.6: POWER CALCULATION AND ANALYSIS

The primary outcome measure was to determine the extent of histological tissue damage by three vein harvesting techniques in relation to systemic CO_2 levels. The sample size required to address the primary end point was calculated on the basis of our previous pilot histological work due to unavailability of any previous studies in this area. With 91 patients in each of the three groups (CT-EVH, OT-EVH, OVH), i.e. 273 in total, the study would have 80% power to detect difference in the percentage with zero vein 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% significance level. In total, 301 patients were recruited in order to allow for a 10% drop out rate.

All categorical data was assessed using the chi square test and expressed as number (percentage). The distribution of all continuous data was formally assessed via the Shapiro-Wilk test. Continuous data is expressed as mean±standard deviation or median [interquartile range] for parametric and non-parametric data respectively. Comparisons between the three groups were performed by ANOVA or Independent samples Kruskal-Wallis test for parametric and non-parametric data respectively.

7.3: RESULTS

7.3.1: DEMOGRAPHICS

A full description of pre-operative demographics is detailed in Table 25. No conversions to traditional open harvesting from endoscopic groups were performed. A slightly higher body mass index (BMI) more left main stem disease and more current smokers were observed in the CT-EVH group.

7.3.2: INTRAOPERATIVE DETAILS

All surgical timings were recorded to establish the duration for which the vein conduit was exposed to CO_2 during retrieval and to determine the overall surgical duration required to obtain the veins. Our study demonstrates a greater vein harvesting time, and thus exposure to CO_2, in the CT-EVH group compared to the other groups ($p=0.028$), with the fastest retrieval achieved in the OT-EVH group. This translated into an extended overall surgical time for the leg in the CT-EVH group ($p<0.001$) compared to the other groups, although the fastest time to completion of the leg surgery was in the OVH group. A full overview of vein graft harvest timings is provided in Table 26. The number of vein grafts required for the surgery did not differ significantly between the groups ($p=0.138$).

No differences in any recorded ventilator variables were observed. An overview of intraoperative data is provided in Table 26.

Table 25: Demographic data including pre-operative co-morbidities, risk factors and cardiac history. Categorical variables are expressed as number (percentage). Continuous variables are expressed as either mean±standard deviation (parametric data) or median [interquartile range] (non-parametric data). PTCA=Percutaneous Transluminal coronary angioplasty.

Demographic variables		**Group**		
		OT-EVH (n=100)	**OVH (n=100)**	**CT-EVH (n=100)**
Age (years)		66.92±10.08	65.96±9.34	64.06±10.20
Sex (M/F)		82/18 (82.0%/18.0%)	79/21 (79.0%/21.0%)	79/21 (79.0%/21.0%)
Body Mass Index (BMI)		27.77 [6.41]	27.93 [5.45]	28.78 [6.54]
Urgency	*Elective*	46 (46.0%)	49 (49.0%)	41 (41.0%)
	Urgent	54 (54.0%)	51 (51.0%)	59 (59.0%)
Diabetes	*Diet controlled*	8 (8.0%)	6 (6.0%)	4 (4.0%)
	Tablet controlled	21 (21.0%)	27 (27.0%)	22 (22.0%)
	Insulin controlled	8 (8.0%)	11 (11.0%)	4 (4.0%)
Canadian Cardiovascular Society	*I*	17 (17.0%)	17 (17.0%)	12 (12.0%)
	II	25 (25.0%)	29 (29.0%)	33 (33.0%)
	III	45 (45.0%)	45 (45.0%)	46 (46.0%)
	IV	13 (13.0%)	9 (9.0%)	9 (9.0%)
New York Heart Association	*I*	27 (27.0%)	32 (32.0%)	40 (40.0%)
	II	45 (45.0%)	35 (35.0%)	26 (26.0%)
	III	26 (26.0%)	25 (25.0%)	29 (29.0%)
	IV	2 (2.0%)	8 (8.0%)	5 (5.0%)
STEMI		18 (18.0%)	19 (19.0%)	29 (29.0%)
NSTEMI		42 (42.0%)	48 (48.0%)	44 (44.0%)
Previous PTCA		16 (16.0%)	12 (12.0%)	20 (20.0%)
Previous MI		52 (52.0%)	43 (43.0%)	54 (54.0%)
Multivessel disease		82 (82.0%)	81 (81.0%)	86 (86.0%)
Left main stem		25 (25.0%)	25 (25.0%)	40 (40.0%)
Hypertension		87 (87.0%)	83 (83.0%)	88 (88.0%)
Smoking	*Never smoked*	32 (32.0%)	33 (33.0%)	23 (23.0%)
	Previous smoker	52 (52.0%)	54 (54.0%)	47 (47.0%)
	Current smoker	16 (16.0%)	13 (13.0%)	30 (30.0%)
Hypercholesterolemia		96 (96.0%)	90 (90.0%)	92 (92.0%)
Peripheral vascular disease		19 (19.0%)	20 (20.0%)	21 (21.0%)
Left ventricular ejection fraction	*>50%*	74 (74.0%)	74 (74.0%)	72 (72.0%)
	30-50%	21 (21.0%)	18 (18.0%)	22 (22.0%)
	<30%	5 (5.0%)	8 (8.0%)	6 (6.0%)

Table 26: Surgical data showing the full breakdown of surgical timings and the number of vein grafts harvested. Continuous data is expressed as median [interquartile range] and analysed by the Independent samples Kruskal-Wallis test. Categorical variables are expressed as number (percentage) and assessed by the χ^2 test.

Variable	Group			p-value
	OT-EVH	OVH	CT-EVH	
Harvesting time (mins)	19.86 [11.64]	22.26 [17.65]	23.40 [12.48]	**0.031**
Full leg surgery time (mins)	42.93 [20.46]	42.73 [25.43]	53.50 [22.50]	**<0.001**
Total surgery time (mins)	226.77 [56.99]	222.65 [58.34]	228.46 [67.72]	0.806
Bypass time (mins)	93.00 [49.00]	90.00 [43.00]	92.00 [35.75]	0.698
Cross-clamp time (mins)	54.00 [37.00]	58.00 [34.75]	57.00 [23.00]	0.841
Number of vein grafts				
1	26 (26.0%)	26 (26.0%)	13 (13.0%)	0.130
2	54 (54.0%)	51 (51.0%)	57 (57.0%)	
3	20 (20.0%)	22 (22.0%)	30 (30.0%)	
4	0 (0.0%)	1 (1.0%)	0 (0.0%)	
Length of vein obtained (cm – mean±SD)	34.86±12.90	35.60±13.71	39.23±12.09	**0.039**
FiO2	0.50 [0.17]	0.50 [0.10]	0.50 [0.11]	0.270
Respiratory Rate (bpm)	12.00 [2.00]	12.00 [2.00]	12.00 [2.00]	0.601
Tidal Volume (ml)	500.00 [67.50]	500.00 [100.00]	500.00 [50.00]	0.287

7.3.3: SYSTEMIC CO_2 AND PH MEASUREMENTS

Baseline $EtCO_2$ levels were consistent across the 3 groups (median [interquartile range]: 4.40 [0.60] vs. 4.30 [0.65] vs. 4.50 [0.70] for OT-EVH vs. OVH vs. CT-EVH respectively, p=0.137). However, baseline $PaCO_2$ levels were significantly lower in the CT-EVH group compared to the other 2 groups (5.25 [0.9] vs. 5.40 [0.80] vs. 4.90 [0.90] for OT-EVH vs. OVH vs. CT-EVH respectively, p<0.001). Baseline pH was also similar between groups (mean±standard deviation: 7.40±0.05 vs. 7.40±0.04 vs. 7.40±0.05 for OT-EVH vs. OVH vs. CT-EVH respectively, p=0.666).

$EtCO_2$ did not alter over time during harvesting in either OT-EVH or OVH group (p=0.451 and p=0.385 respectively); however, $EtCO_2$ increased significantly over time in the CT-EVH group (p<0.001, Figure 20). Interestingly, $PaCO_2$ also did not differ over time in the OT-EVH group (p=0.241) whereas $PaCO_2$ reduced significantly over time in the OVH group (p=0.001). A profound increase in $PaCO_2$ was observed in the CT-EVH group (p<0.001, Figure 20). Consistent with these patterns, only the CT-EVH group demonstrated a decrease in pH over time (p<0.001) whereas pH remained stable for both OT-EVH and OVH groups (p=0.105 and p=0.869 respectively, Figure 21).

7.3.4: ENDOTHELIAL INTEGRITY

Conduit endothelial integrity was assessed in terms of intimal stretching and detachment and compared between groups on proximal undistended vein samples. The number of samples with normal preserved endothelium (defined as absence of stretching or detachment) (Figure 22) varied between groups, with greatest preservation in the OVH group (54.0%), compared to either endoscopic group (39.0% and 34.0% for CT-EVH and OT-EVH respectively, p=0.012). Samples with intimal stretching (Figure 23) were further evaluated and severity varied significantly between groups, with more stretching graded as severe in the OT-EVH group (13 (13.0%)), compared to OVH (0 (0.0%)) and CT-EVH (5 (5%)) groups (p=0.003, Table 27). Samples with intimal detachment were further graded on severity, although no significant differences were observed (p=0.245, Table 27).

Table 27: Histological data demonstrating the level of intimal stretching and intimal detachment in each group. Data is expressed as number (percentage) and was analysed using the χ^2 test.

Group	**Intimal Stretching**				**p-value**
	Normal	**Mild**	**Moderate**	**Severe**	
OT-EVH	34 (34.0%)	38 (38.0%)	13 (13.0%)	13 (13.0%)	0.003
OVH	54 (54.0%)	35 (35.0%)	8 (8.0%)	0 (0.0%)	
CT-EVH	39 (39.0%)	34 (34.0%)	20 (20.0%)	5 (5.0%)	
Group	**Intimal Detachment**				**p-value**
	No detachment	**Mild**	**Moderate**	**Severe**	
OT-EVH	98 (98.0%)	1 (1.0%)	1 (1.0%)	0 (0.0%)	0.245
OVH	97 (97.0%)	0 (0.0%)	2 (2.0%)	1 (1.0%)	
CT-EVH	98 (98.0%)	2 (2.0%)	0 (0.0%)	0 (0.0%)	

7.4: DISCUSSION

It has been well documented that the use of CO_2 insufflation causes hypercarbia and tissue acidosis, yet despite this, EVH procedures still utilise this gas because of its non-flammable properties, low toxicity and low cost (Neuberger et al., 1996). Other noble gases such as helium and argon have been suggested in laparoscopic surgeries but still there is no clear evidence for their benefit. Importantly, in this study we did not experience any CO_2 embolism or significant hypercarbia because of the low pressure setting of 10mmHg rather than company recommendation of 12mmHg to 15mmHg. We suggest that the use of a reduced pressure tunnel in the CT-EVH system may minimise any systemic complications. Our study also demonstrates significantly increased $PaCO_2$ level and significant decreases in arterial pH levels in the CT-EVH group. Despite this, tissue integrity remains similar or slightly better maintained compared to that observed in the OT-EVH group.

Our study data demonstrates that the method of vein harvesting utilised does impact on endothelial integrity. The OVH control group illustrated the greatest endothelial preservation compared to endoscopic techniques. However, veins obtained using the CT-EVH method demonstrated greater preservation of normal, continuous endothelium than veins retrieved by the OT-EVH technique. This enabled us to further our understanding about the effect of prolonged vein exposure to an acidic environment and pressurised CO_2 tunnel. The current literature suggests that the optimal pH for endothelial cell viability ranges between pH 7.3-7.4, below which the acidic environment can damage vessel viability (Rousou et al., 2009; Krishnamoorthy et al., 2016). Our findings demonstrate that despite the drop in pH in the CT-EVH, conduit integrity is not adversely affected.

In this study, we observed longer harvesting time for CT-EVH compared to the other groups, which can increase the length of vein exposure to CO_2. Yet, the CT-EVH group more often required the retrieval of 3 lengths of vein compared to the other groups, although this did not demonstrate statistical significance. Longer harvesting time was not associated with reduced endothelial integrity.

Usually, the tunnel created by CO_2 insufflation allows for easy dissection and visibility (Potapov et al., 2007; Banks et al., 2002). This promotes absorption of CO_2 by the adjacent tissues, including, to some extent, the vein walls. Greater vessel compression is expected with CT-EVH because of the

pressure in the tunnel produced by insufflation. Again, in our study, this did not impact upon the incidence of endothelial layer detachment or endothelial stretching.

The severe endothelial stretching observed in the OT-EVH group may be due to the increased manual handling of the vein due to lack of tunnel and the design of the EVH equipment. Open tunnel EVH requires manual dissection and thus traction stresses on the vein. Additionally, it is required that the practitioner works very close to the vein, which is not the case in the closed tunnel technique due to the greater access created by the CO_2 tunnel. This helps the practitioner to work away from the vein and obtain the vein with surrounding tissues. The current evidence stresses that the veins harvested with surrounding tissues as a pedicle has a higher patency rate compared to skeletonised veins (Samano et al., 2015).

7.5: LIMITATIONS

Patients who underwent CT-EVH for vein harvesting received heparin, which was not provided to those in the other two groups according to local standard endoscopic guidelines. We do not fully understand the role of heparin on the vascular structures and vessel wall, which may have complicated our findings. We did not perform any optical coherence tomography (OCT) to assess whether there was any intraluminal clot formation in the OT-EVH system. Our study focused only on the structural integrity of the vein but functional viability such as nitric oxide production, a potent endothelium-dependent vasorelaxant synthesised from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS) (Krishnamoorthy et al., 2016) are also very important. The endothelial denudation affects the functional capacity of the vein and leads to graft failure which weren't explored in this study.

Our study also confirms that the veins obtained by the traditional open harvesting approach demonstrate a better preservation of the endothelium compared to the endoscopic groups. However, we cannot discount the fact that the practitioner had greater experience in OVH (>2000 cases at the start of the study) than endoscopic harvesting (>250 cases of each technique). This may have contributed to the differences observed.

7.6: Conclusion

Our study provides clear evidence to suggest that conduit integrity is not adversely affected by a small drop in pH induced by CO_2 insufflation. Furthermore, we highlight that the use of a less pressurised tunnel (10mmHg rather than 12-15mmHg) can maintain pH at levels suitable for endothelial integrity for the duration required for vein harvesting.

7.7: Clinical impact

The use of a low pressure CO_2 tunnel does not impact upon the quality of the harvested vein on a histological level. So, EVH can be safely undertaken without concern about CO_2 exposure, acidic environment or risk of embolism.

7.8: Acknowledgement

We would like to acknowledge the histopathology scientist Mr. Peter Walker from the University of Manchester, Dr. Julie Morris, Statistician and Reader from University of Manchester and all cardiothoracic surgeons and anaesthetists for their support on this study.

7.9: Conflict of Interest

There was no conflict of interest.

7.10: Funding and support

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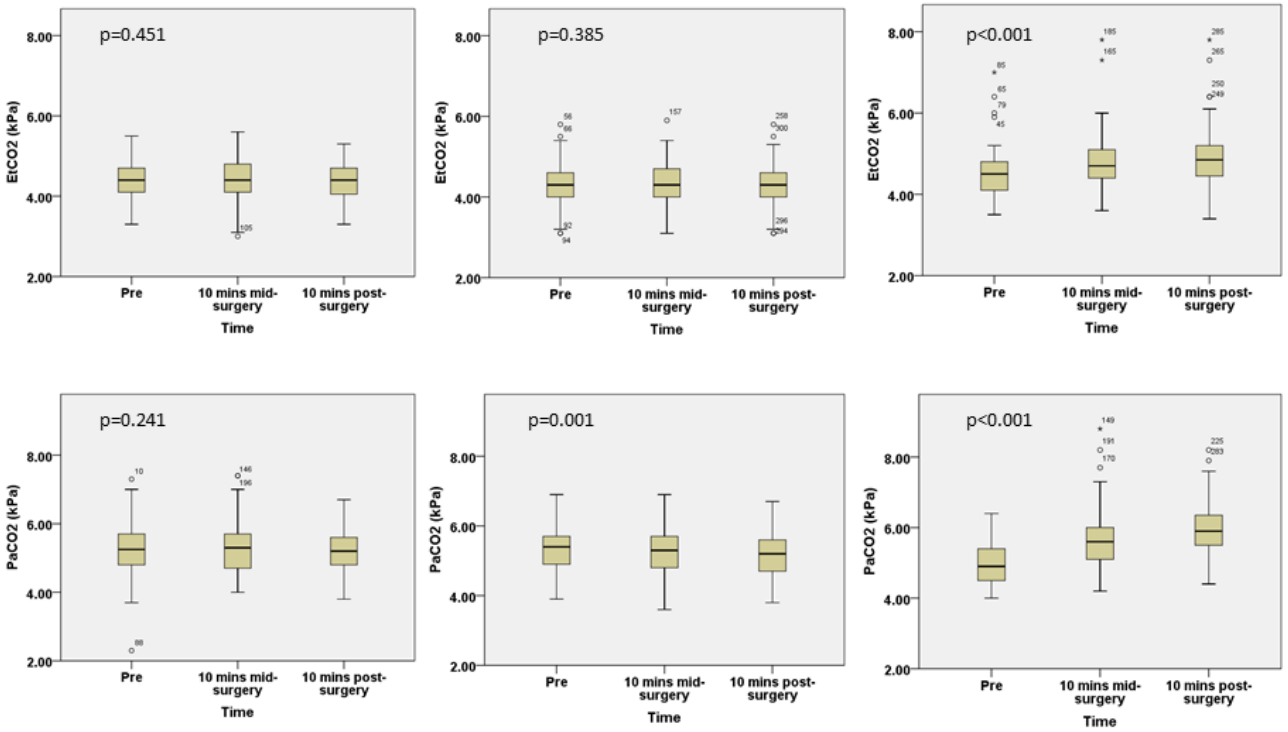
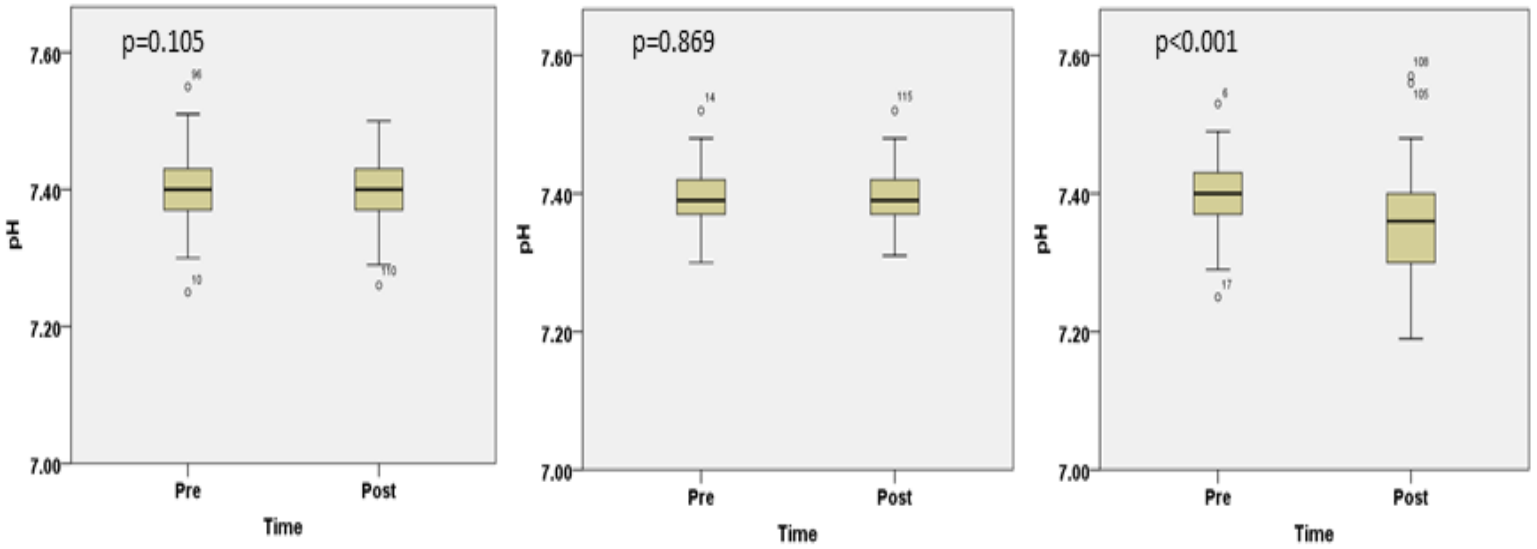
Box Plots



a) OT-EVH b) OVH c) CT-EVH

Figure 20: Graphs indicating the change in $PaCO_2$ and $EtCO_2$ levels over time in each harvesting group.$PaCO_2$ and $EtCO_2$ were consistent across all time points in the OT-EVH group (a). $PaCO_2$ was significantly reduced over time in the OVH group (b), although $EtCO_2$ remained constant. Significant increases in both $EtCO_2$ and $PaCO_2$ were observed in the CT-EVH group (c).



a) OT-EVH b) OVH c) CT-EVH

Figure 21: Graphs indicating the change in pH over time during vein harvesting. A significant drop in pH was observed in the CT-EVH group (c), whereas both OT-EVH (a) and OVH (b) groups maintained consistent pH throughout.

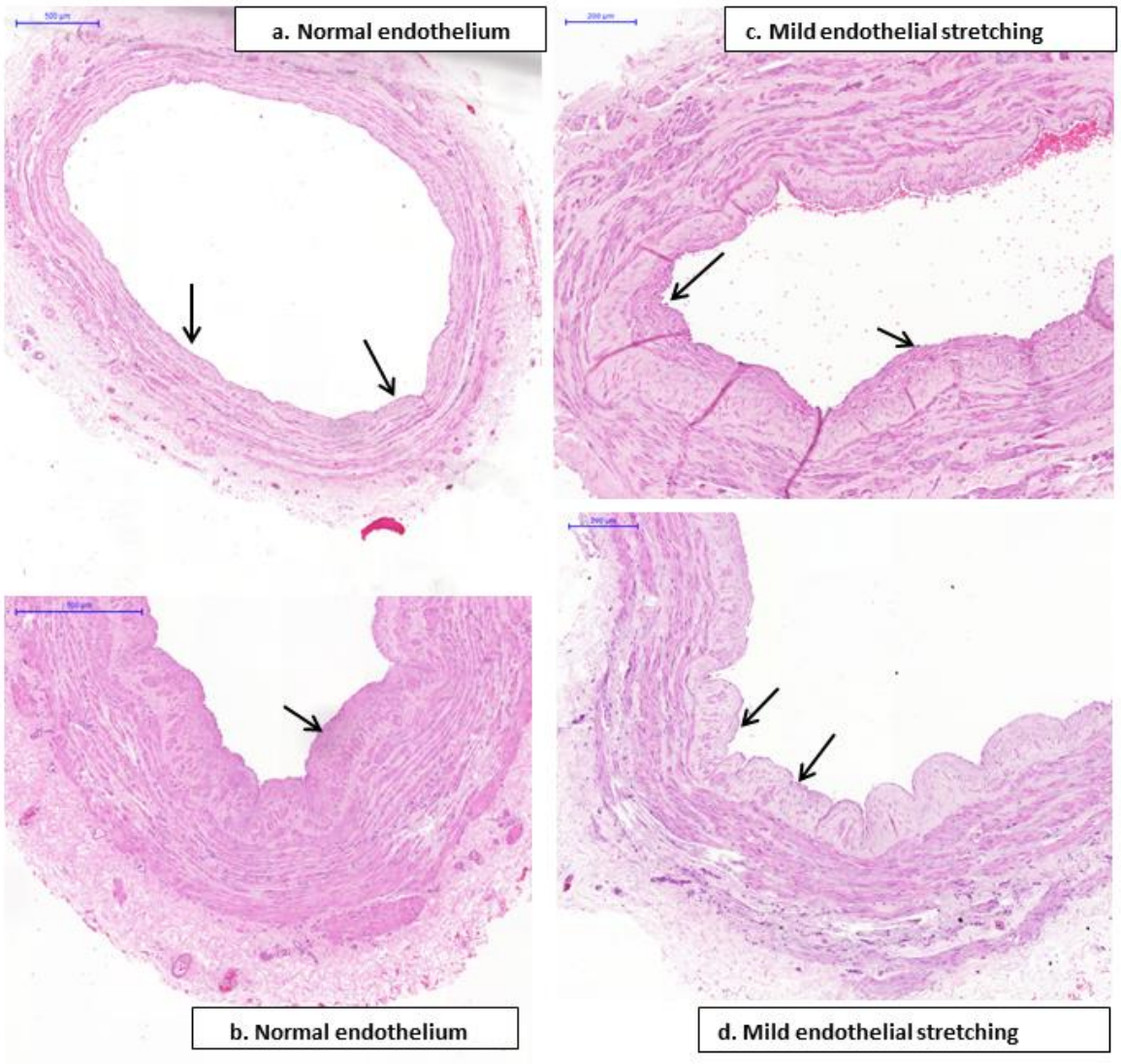


Figure 22: Haematoxylin & Eosin staining showing normal endothelium (a and b) and mild endothelial stretching (c and d).

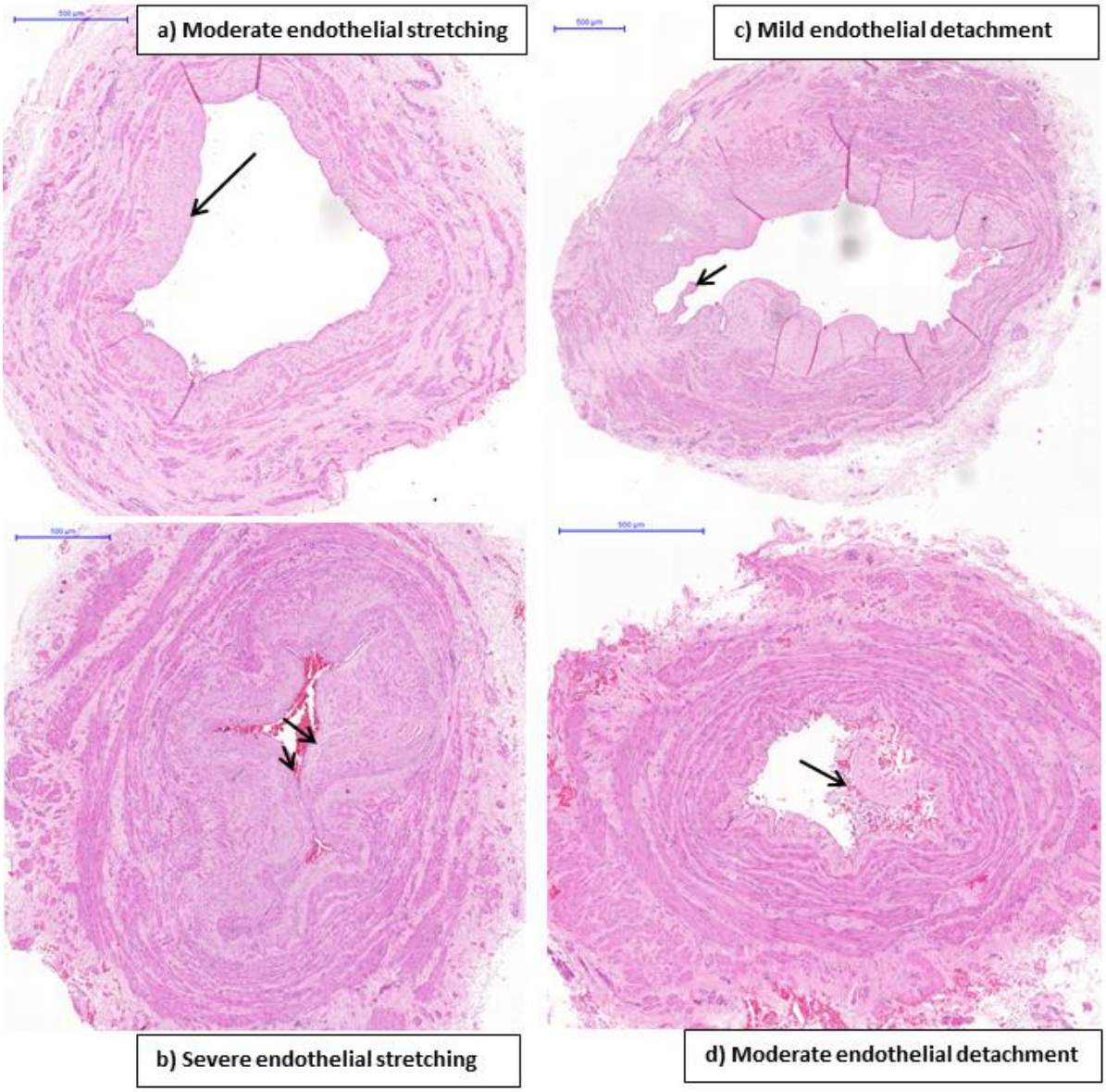
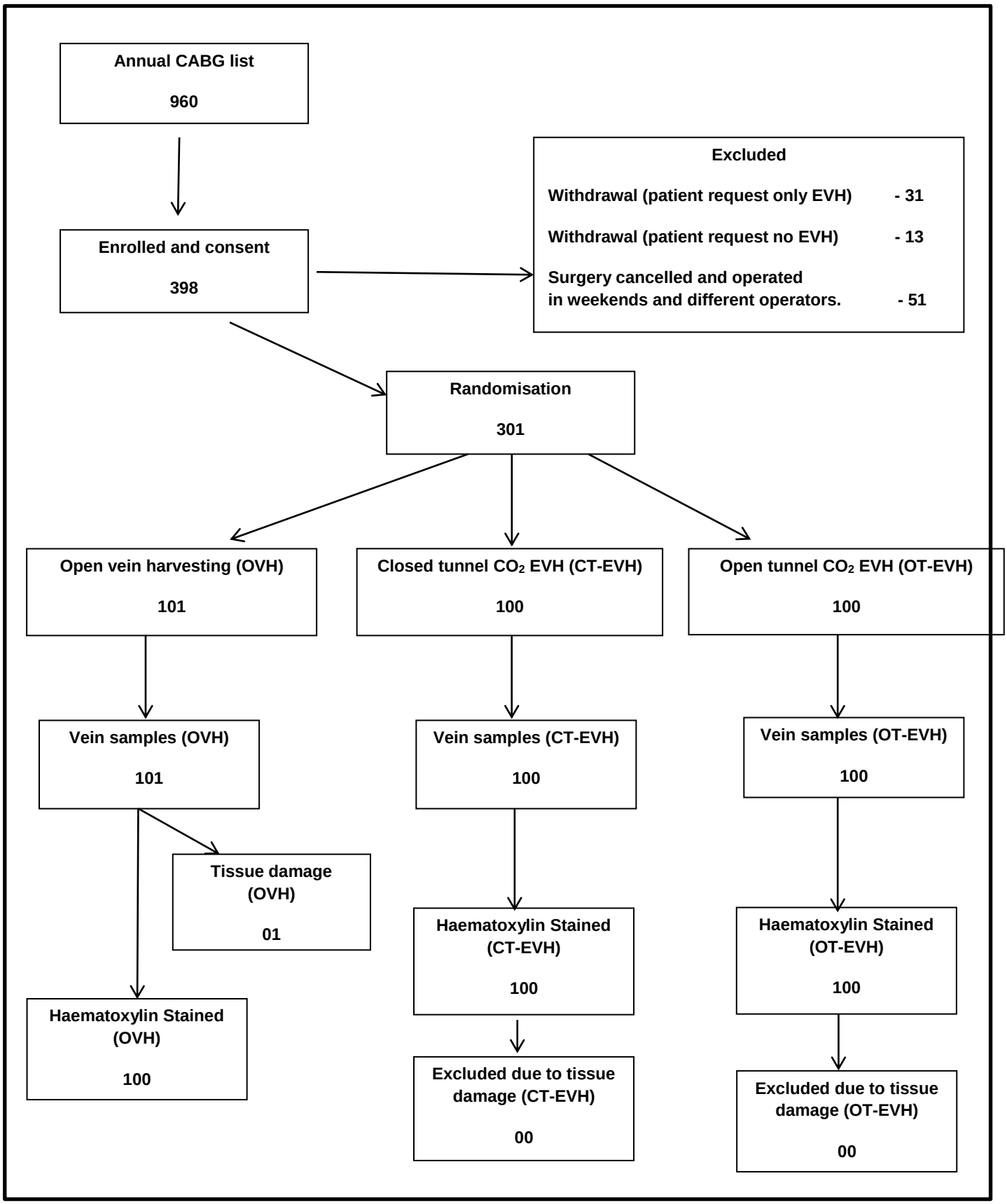


Figure 23: Haematoxylin-eosin staining illustrating moderate & severe endothelial stretching (a and b) and mild and moderate endothelial detachment (c and d).

CONSORT diagram



CHAPTER 8

Understanding the effect of pressure distension the long saphenous veins after different vein harvesting techniques causes histological damage.

Submitted for publication

8.0 RANDOMISED CONTROL TRIAL COMPARING ENDOSCOPIC AND OPEN VEIN HARVESTING FOR CORONARY ARTERY BYPASS GRAFTING: HISTOLOGICAL ASSESSMENT ON DISTENDED AND NON-DISTENDED LONG SAPHENOUS VEIN.

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Keywords: Coronary artery bypass surgery, open vein harvesting, endoscopic vein harvesting, muscle migration, intimal stretching.

ISRCTN: 91485426

8.01 ABSTRACT

Background:

Vein graft damage can induce intimal hyperplasia leading to narrowing of the lumen and thrombus formation. Surgical trauma to the vein incurred during harvesting, as well as with the distension and handling of the vein, leads to smooth muscle migration, which causes intimal thickening and proliferation. However, the extent of damage caused by minimally invasive and traditional open vein harvesting techniques remains unknown. The aim of this study was to compare structural differences and damage caused by the distension of the saphenous vein harvested by three different surgical techniques.

Methods:

We randomly assigned n=301 patients to open vein harvesting (OVH), closed tunnel endoscopic vein harvesting (CT-EVH) and open tunnel endoscopic vein harvesting (OT-EVH). A 1cm vein sample was collected from the proximal region (undistended, n=300), the distal region (distended with 20mmHg pressure, n=300) and a random sample (underwent surgical trauma, 70mmHg pressure cardioplegia and distension, n=300). Picrosirius red (n=900) was used to assess viability of the longitudinal muscle layer. Samples were blindly scored by five independent researchers and a histopathologist.

Results:

The level of intimal disruption was greatest in the OT-EVH group in proximal, distal and random samples (all $p<0.001$). The level of medial layer disruption was greatest in CT-EVH, with least disruption observed in OVH for proximal, distal and random samples (all $p<0.001$). Internal muscle migration was greatest in OT-EVH compared to the other groups for proximal, distal and random samples (all $p<0.001$). Smooth muscle circular layer detachment was much greater in endoscopic groups compared to OVH in proximal ($p=0.008$), distal ($p<0.001$) and random ($p=0.001$). Smooth muscle longitudinal layer detachment was consistent between groups in proximal ($p=0.113$) and distal ($p=0.380$) samples but was greater in endoscopic groups compared to OVH ($p=0.012$).

Conclusion:

In this study, open vein harvesting was associated with better preservation of vein layers in non-distended proximal samples than endoscopic vein harvesting. Although both EVH groups displayed histological damage, OT-EVH was associated with more intimal disruption. Data obtained from this study suggests that the EVH technique can be utilised as a routine procedure in coronary bypass surgery but careful patient selection and type of EVH equipment need to be taken into consideration.

8.1: INTRODUCTION

Cardiac surgery is moving towards the use of total arterial grafts for Coronary Artery Bypass Grafting (CABG) due to their high patency rate (Tranbaugh et al., 2015). However, the long saphenous vein (LSV) is still commonly used for multiple bypass graft surgery because of its ease of access and long length (Hwang et al., 2012). Minimally invasive vein harvesting techniques have been adopted in some centres in order to avoid the complications and morbidity associated with traditional open vein harvesting (Teixeira et al., 2015). However, the use of endoscopic vein harvesting remains controversial amid questions about the quality of the retrieved vein. Previous studies have demonstrated the potential benefits of a number of approaches that improve the quality and long term patency of the LSV, including the adoption of the no-touch technique (Souza et al., 2009), minimal manipulation and the use of an external support venous stent (Krejca et al., 2002).

Vein graft failure is one of the major concerning factors post CABG surgery, and is induced by intimal hyperplasia of the vein. This leads to narrowing of the vessel lumen and thrombosis formation (Sayers et al., 1991; Hess et al., 2014). The current literature suggests that trauma to the vein incurred during harvesting (Tsui and Dashwood, 2002), as well as with distension and handling of the vein (Dashwood and Loesch, 2014) leads to migration of the smooth muscles causing intimal thickening and proliferation. However, other studies have also demonstrated that the loss of the endothelial layer impairs the function of the vein via reduced release of prostacyclin and endothelium-derived relaxing factors, which could play an important role in the development of graft failure (Sayers et al., 1991; Angelini et al., 1989b).

There remains a significant gap in the literature with regards to how different vein harvesting techniques impact upon the structural layer of the conduit. Furthermore, no study has been performed that clearly demonstrates the extent to which surgical preparation, manual handling and distension of the vein between harvesting and grafting induces changes to venous structure, therefore affecting conduit quality. The aim of this study was to assess structural damage to the vein layers on a histological level and compare between surgically distended and non-distended LSV harvested by three different vein harvesting techniques.

8.2: METHODS

8.2.1: STUDY DESIGN

Ethical approval was gained from the NRES committee North West Greater Manchester East and all study participants provided written informed consent. In total, 301 patients who underwent CABG surgery between 2011 and 2015 were randomised into 3 groups. All vein conduits were harvested by a surgical practitioner with experience of at least 250 cases using each retrieval method. A total of 900 vein samples were obtained from the study participants (see in the consort diagram on page174). Vein samples were coded as H1 (non-distended proximal vein sample), H3 (distended distal vein sample after 20mmHg flush to check for leakages) and finally H2 (vein sample taken following all surgical trauma, 70mmHg cardioplegia flush pressure and distension).

Group 1 - (Control arm): 101 patients undergoing traditional open vein harvesting (OVH).

Group 2 - (Intervention 1): 100 patients undergoing closed tunnel endoscopic vein harvesting (CT-EVH).

Group 3 - (Intervention 2): 100 patients undergoing open tunnel endoscopic vein harvesting (OT-EVH).

All samples were blindly scored by five independent assessors and a consultant histopathologist.

8.2.2: SURGICAL TECHNIQUES

The site for long saphenous vein harvesting was dependent upon the number of conduits required. When 1-2 conduits were required, the vein was retrieved from mid-calf to thigh, whereas when 3 lengths were required, harvesting was performed from ankle to thigh. All OVH samples were retrieved either from the mid-calf or from the medial malleolus following a longitudinal leg skin incision. All veins were retrieved with attached perivascular fat and side branches were ligated with 4/0 vicyrl ties and metal liga clips. Closed tunnel EVH was performed using the Maquet Vasoview™ Hemopro2 system. The CO_2 insufflator was set to a continuous flow rate of 3 litres/min at 10mmHg pressure to provide a closed tunnel to facilitate dissection. Open tunnel EVH was performed using the Sorin ClearGlide™ harvesting system with the CO_2 insufflator set to a flow rate of 3 litres/min with 0mmHg pressure.

8.2.3: SAMPLE STORAGE AND PROCESSING

Three samples measuring approximately 1cm (1 each of H1, H2 and H3) were obtained from every patient. Samples were fixed using 4% formalin in distilled water (pH 7.4). The H1 proximal samples vein were obtained and immersed prior to cannulation for flushing to detect leakages. All veins were distended to a pressure of 20mmHg and then the H3 distal samples were subsequently obtained. Finally, the H2 random samples were obtained at the end of surgery following all manipulation and distension. All numerically coded vein samples were transported in a formalin container to the research lab.

8.2.4: HISTOLOGY ASSESSMENT

8.2.4A: PICROSIRIUS RED: ASSESSMENT OF STRUCTURAL VIABILITY OF MUSCULAR AND INTIMA LAYER.

A standard protocol was followed for all staining with Picrosirius red. Vein sections were deparaffinised and hydrated in distilled water, and nuclei were subsequently stained for 8 minutes with Weigert's haematoxylin A and B solutions. All slides were washed with running tap water for 10 minutes before incubation for 1 hour at room temperature in 0.1% (w/v) Direct Red 80/Picrosirius red (Sigma-Aldrich Ltd, Dorset, UK) with saturated aqueous solution of picric acid (Sigma-Aldrich Ltd, Dorset, UK). Slides were then washed in two changes of acidified water, drained and blotted dry with two filter papers. Finally, the slides were rapidly dehydrated using 2-3 changes of 100% ethanol, xylene, and then coverslip attached using a resinous medium.

Longitudinal and circular muscle layer viability was scored using a validated scoring system with minor modifications. Muscular hypertrophy, detachment and muscle migration (external and internal) were graded on a scale from 0-3 (representing normal, mild, moderate or severe; representative images displayed in Figure 24). The percentage of disruption to the intima and medial connective tissue was graded on a continuous scale from 0-100 (no disruption to complete disruption).

8.2.4B: IMAGE SCORING AND ANALYSIS

Picrosirius red and Haematoxylin & Eosin stained slides were digitally scanned and imaged by the Pannoramic viewer 250™ slide scanning system with high NA Carl Zeiss™ optics, allowing a maximum image resolution of up to 0.16µm per pixel. All digital slide images were viewed, annotated and archived using Pannoramic viewer 250 and all scorers utilised images within this software for the purpose of scoring. All scores were transferred to IBM SPSS version 22 (IBM, Armonk, NY) for analysis.

8.2.5: STATISTICAL ANALYSES

All statistical analyses were performed using SPSS version 22 (IBM, Armonk, NY). Data distribution was formally assessed using the Shapiro-Wilk test. Parametric data are expressed as mean±standard deviation and non-parametric data are expressed as median [interquartile range]. Categorical variables were analysed using the chi square test. Scale variables were analysed using either ANOVA or the Kruskal-Wallis test depending upon data distribution.

8.3: RESULTS

8.3.1: DEMOGRAPHICS

Demographic variables were recorded for patients in each of the three vein harvesting groups. A full description of pre-operative demographics is detailed in Table 28. No conversions to traditional open harvesting from endoscopic groups were performed. The CT-EVH group contained more current smokers, a higher incidence of left main stem disease and had a higher median BMI than the other groups.

8.3.2: SMOOTH MUSCLE DETACHMENT

Circular layer detachment differed significantly between groups in the H1 samples, with least detachment in the OVH group (1.0%), compared with OT-EVH (18.6%) or CT-EVH (19.0%) groups ($p=0.008$). In H2 samples, the OVH group again displayed least detachment (2.0%) compared with OT-EVH (43.9%) or CT-EVH (36.4%) groups ($p<0.001$). Finally, circular layer detachment was significantly least prevalent in H3 samples from the OVH group (4.0%) compared with OT-EVH (17.1%) and CT-EVH (17.3%) groups ($p=0.001$) (Table 29).

Detachment of the longitudinal layer did not differ significantly between groups in H1 samples (8.2% vs. 3.0% vs. 4.0% for OT-EVH, OVH and CT-EVH respectively, $p=0.113$). This was also true for H3 samples (8.3% vs. 4.0% vs. 7.1% for OT-EVH, OVH and CT-EVH respectively, $p=0.380$). However, a significantly greater proportion of H2 samples from veins harvested endoscopically displayed longitudinal detachment (5.1% and 10.1% for OT-EVH and CT-EVH respectively) compared with samples obtained by OVH (0.0%), where no detachment was observed ($p=0.012$) (Table 29).

8.3.4: SMOOTH MUSCLE MIGRATION

External muscle migration did not significantly differ between groups in H1 ($p=0.121$), H2 ($p=0.230$) or H3 ($p=0.084$) samples. However, internal muscle migration was observed in significantly more H1 samples from the OT-EVH group (86.6%) compared with OVH (13.1%) or CT-EVH (48.0%) groups ($p<0.001$). A similar pattern was observed in H2 samples, with greatest migration in OT-EVH (43.9%) compared with OVH (11.0%) and CT-EVH (29.3%) groups ($p<0.001$). Again, for H3 samples, greatest internal muscle migration was observed in OT-EVH (70.1%) compared with OVH (7.0%) and CT-EVH (47.5%) groups ($p<0.001$).

8.3.5: DISRUPTION (INTIMA AND MEDIAL LAYER)

Intimal disruption was observed at significantly greater levels in H1 samples from the OT-EVH group followed by CT-EVH and OVH groups ($p<0.001$). This was also true in H2 samples, where OT-EVH displayed the greatest disruption, followed by CT-EVH and OVH ($p<0.001$). Intimal disruption was also highest in H3 samples from the OT-EVH group, followed by CT-EVH and OVH groups ($p<0.001$) (Figure 25).

Medial disruption was observed at significantly greater levels samples from the CT-EVH group followed by OT-EVH and then the OVH group. This was true for H1, H2 and H3 samples (all $p<0.001$) (Figure 25).

Table 28: This table provides an overview of the demographics of each group.

Demographic variables		**Group**		
		OT-EVH (n=100)	**OVH (n=100)**	**CT-EVH (n=100)**
Age (years)		66.92±10.08	65.96±9.34	64.06±10.20
Sex (M/F)		82/18 (82.0%/18.0%)	79/21 (79.0%/21.0%)	79/21 (79.0%/21.0%)
Body Mass Index (BMI)		27.77 [6.41]	27.93 [5.45]	28.78 [6.54]
Urgency	*Elective*	46 (46.0%)	49 (49.0%)	41 (41.0%)
	Urgent	54 (54.0%)	51 (51.0%)	59 (59.0%)
Diabetes	*Diet controlled*	8 (8.0%)	6 (6.0%)	4 (4.0%)
	Tablet controlled	21 (21.0%)	27 (27.0%)	22 (22.0%)
	Insulin controlled	8 (8.0%)	11 (11.0%)	4 (4.0%)
Canadian Cardiovascular Society	*I*	17 (17.0%)	17 (17.0%)	12 (12.0%)
	II	25 (25.0%)	29 (29.0%)	33 (33.0%)
	III	45 (45.0%)	45 (45.0%)	46 (46.0%)
	IV	13 (13.0%)	9 (9.0%)	9 (9.0%)
New York Heart Association	*I*	27 (27.0%)	32 (32.0%)	40 (40.0%)
	II	45 (45.0%)	35 (35.0%)	26 (26.0%)
	III	26 (26.0%)	25 (25.0%)	29 (29.0%)
	IV	2 (2.0%)	8 (8.0%)	5 (5.0%)
STEMI*		18 (18.0%)	19 (19.0%)	29 (29.0%)
NSTEMI		42 (42.0%)	48 (48.0%)	44 (44.0%)
Previous PTCA*		16 (16.0%)	12 (12.0%)	20 (20.0%)
Previous MI		52 (52.0%)	43 (43.0%)	54 (54.0%)
Multivessel disease		82 (82.0%)	81 (81.0%)	86 (86.0%)
Left main stem		25 (25.0%)	25 (25.0%)	40 (40.0%)
Hypertension		87 (87.0%)	83 (83.0%)	88 (88.0%)
Smoking	*Never smoked*	32 (32.0%)	33 (33.0%)	23 (23.0%)
	Previous smoker	52 (52.0%)	54 (54.0%)	47 (47.0%)
	Current smoker	16 (16.0%)	13 (13.0%)	30 (30.0%)
Hypercholesterolemia		96 (96.0%)	90 (90.0%)	92 (92.0%)
Peripheral vascular disease		19 (19.0%)	20 (20.0%)	21 (21.0%)
Left ventricular ejection fraction	*>50%*	74 (74.0%)	74 (74.0%)	72 (72.0%)
	30-50%	21 (21.0%)	18 (18.0%)	22 (22.0%)
	<30%	5 (5.0%)	8 (8.0%)	6 (6.0%)

* PTCA=Percutaneous Transluminal Coronary Angioplasty, STEMI=ST Elevated Myocardial Infarction.

Table 29: This table illustrates the extent of muscular layer detachment in proximal, distal and random samples of the long saphenous vein.

Sample	Group	Normal	Mild	Moderate	Severe	p-value
Circular layer detachment						
H1 (proximal)	OT-EVH	79 (81.4%)	12 (12.4%)	4 (4.1%)	2 (2.1%)	0.008
	OVH	97 (99.0%)	0 (0.0%)	1 (1.0%)	0 (0.0%)	
	CT-EVH	81 (81.0%)	13 (13.0%)	3 (3.0%)	3 (3.0%)	
H2 (random)	OT-EVH	55 (56.1%)	15 (15.3%)	20 (20.4%)	8 (8.2%)	<0.001
	OVH	97 (98.0%)	2 (2.0%)	0 (0.0%)	0 (0.0%)	
	CT-EVH	63 (63.6%)	22 (22.2%)	11 (11.1%)	3 (3.0%)	
H3 (distal)	OT-EVH	70 (72.9%)	17 (17.7%)	7 (7.3%)	2 (2.1%)	0.001
	OVH	95 (96.0%)	4 (4.0%)	0 (0.0%)	0 (0.0%)	
	CT-EVH	71 (72.7%)	15 (15.2%)	11 (11.1%)	1 (1.0%)	
Longitudinal layer detachment						
H1 (proximal)	OT-EVH	89 (91.8%)	1 (1.0%)	5 (5.2%)	2 (2.1%)	0.113
	OVH	95 (96.9%)	3 (3.1%)	0 (0.0%)	0 (0.0%)	
	CT-EVH	96 (96.0%)	1 (1.0%)	1 (1.0%)	2 (2.0%)	
H2 (random)	OT-EVH	93 (94.9%)	0 (0.0%)	4 (4.1%)	1 (1.0%)	0.380
	OVH	99 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	
	CT-EVH	89 (89.9%)	5 (5.1%)	3 (3.0%)	2 (2.0%)	
H3 (distal)	OT-EVH	88 (91.7%)	2 (2.1%)	1 (1.0%)	5 (5.2%)	0.012
	OVH	95 (96.0%)	3 (3.0%)	1 (1.0%)	0 (0.0%)	
	CT-EVH	91 (92.9%)	1 (1.0%)	2 (2.0%)	4 (4.1%)	

8.4: DISCUSSION

Vein grafts are commonly used in multiple bypass surgery due to the requirement for several long conduits. However, venous grafts are more prone to occlusion (Konerding et al., 1996) and failure (Zhang et al., 2002) than arterial grafts (Kanellaki-Kyparissi et al., 2005), due to exposure to the high pressure arterial system (Kanellaki-Kyparissi et al., 2005). The increased pressure in the arterial circulation can cause increased expression of adhesion molecules, production of growth factors and release of extracellular proteins which leads to thickening and proliferation of the intima (Westerband et al., 2001; Cheanvechai et al., 1975). Consequently, it is very important to investigate whether the vein harvesting technique can accelerate graft occlusion. This is the first study which has examined the different parts of the harvested vein (proximal, random and distal) samples to establish the effect of distended (grafts used for bypass surgery), minimally distended and non-distended veins. From the results of this study, we can demonstrate that there are changes to the vein at a histological level, which is related to the type of harvesting method used for coronary artery bypass surgery.

In relation to intimal changes induced by different vein harvesting methods, the proximal samples more frequently had mild disruption when harvested by OT-EVH compared to the other techniques. Severe disruption of the intimal layer and elongation of the intimal surface leads to constriction of the vessel lumen and finally graft occlusion (Wali and Eid, 2002; Kanellaki-Kyparissi et al., 2005). However, only a small proportion of the vein samples had severe intimal disruption that may be due to the manual dissection occurring in closer proximity to the vein in OT-EVH group.

There are a number of factors that induce endothelial disruption and it is very important to understand the histological level of damage occurring at each stage of harvesting and surgical preparation. Our study demonstrates that despite the different harvesting techniques used, the random distended vein samples displayed similar intimal integrity between the groups, suggesting that distension during surgical preparation itself induces structural changes to the vein. Cardioplegia solutions, surgical preparation and distension of the vein used during surgery can promote acute endothelial injury which leads to cell deficiency and damage to the cytoskeleton (Davies et al., 1999; Macchiarelli et al., 1994; Kanellaki-Kyparissi et al., 2005).

Furthermore, we have assessed the extent of smooth muscle hypertrophy, migration and detachment which is commonly associated with surgical distension and manipulation of the harvested vein. Our study results demonstrate that smooth muscle damage was caused more in the circular layer than the longitudinal layer of the vein and was greatest in the endoscopic groups rather than OVH. Similar results were also seen in a canine model (LoGerfo et al., 1983) which demonstrates that vein grafts prepared by a minimal mechanical manipulation reduces endothelial and medial smooth muscle injury (Quist and LoGerfo, 1992).

Current evidence illustrates that the pressure imposed on the harvested vein such as shear stress, radial and longitudinal deformation leads to medial layer stretching and damage to the contractile elements of the vein (Hocking et al., 2011). In addition to pressure, manual manipulation of the vein causes smooth muscle hypertrophy and migration of the muscle towards the vessel lumen (Meyer et al., 2000; Fabricius et al., 2000; Krishnamoorthy et al., 2016). Despite taking all the precautions of reducing the mechanical trauma and minimal pressure distension of the vein between 20-70mmHg to avoid intimal hyperplasia, the histological level damage seen in OT-EVH may be due to the design of the equipment which involves working very close to the vein.

8.5: Clinical significance

This is the only study to directly compare the different vein harvesting techniques in terms of their impact on the structural integrity of the conduit. We demonstrate that the integrity of the vein can be altered significantly by the technique utilised for harvesting, with least effect induced by OVH. Our findings suggest that further improvements to the training programme for endoscopic technique could minimise the impact caused during harvesting.

8.6: Conclusion

The level of histological damage induced directly by open vein harvesting was minimal compared to that induced by endoscopic harvesting, particularly OT-EVH. However, differences to the intimal layer are no longer observed between groups following distended surgical preparation, suggesting that the harvesting technique has relatively low impact on intimal integrity. Other layers are affected more significantly by harvesting, although improving practitioner technique in order to stay away from the vein may reduce additional mechanical trauma during harvesting. From this study, we demonstrate

that the EVH technique can be carried out safely, although patient selection and type of EVH equipment needs to be carefully considered.

8.7: LIMITATIONS

To understand the full impact of harvesting on the veins, it is always important to assess the vasomotor functional status of the vein and monitor gene expression changes, although these were beyond the remit of this study. We have also not examined the functional viability of the vein using transmission and scanning electron microscopy which may add some additional data. The improved conduit integrity observed in the OVH group may be accounted for by the greater experience of the practitioner in this technique (>2000 cases) compared with endoscopic harvesting (>250 cases in each technique). Advanced training may minimise the effect caused by EVH.

8.8: ACKNOWLEDGEMENT

We would like to acknowledge the histopathology scientist Mr. Peter Walker from the University of Manchester, all cardiothoracic surgeons and anaesthetists for their support on this study.

8.9: CONFLICT OF INTEREST

There was no conflict of interest.

8.10: FUNDING AND SUPPORT

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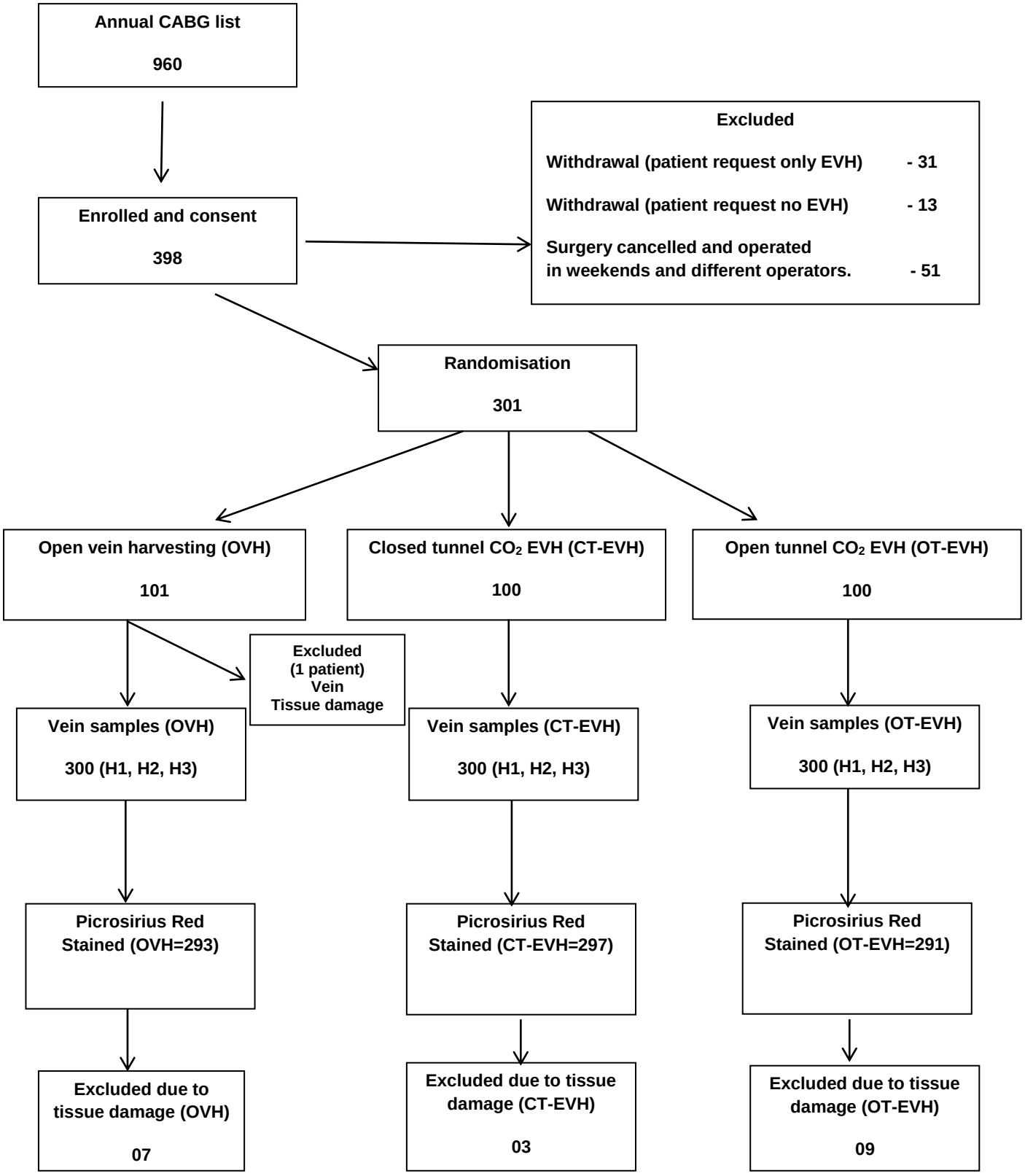
Consort diagram



Consort diagram illustrates the detailed enrolment of the patient, histological vein samples and tissue damage.

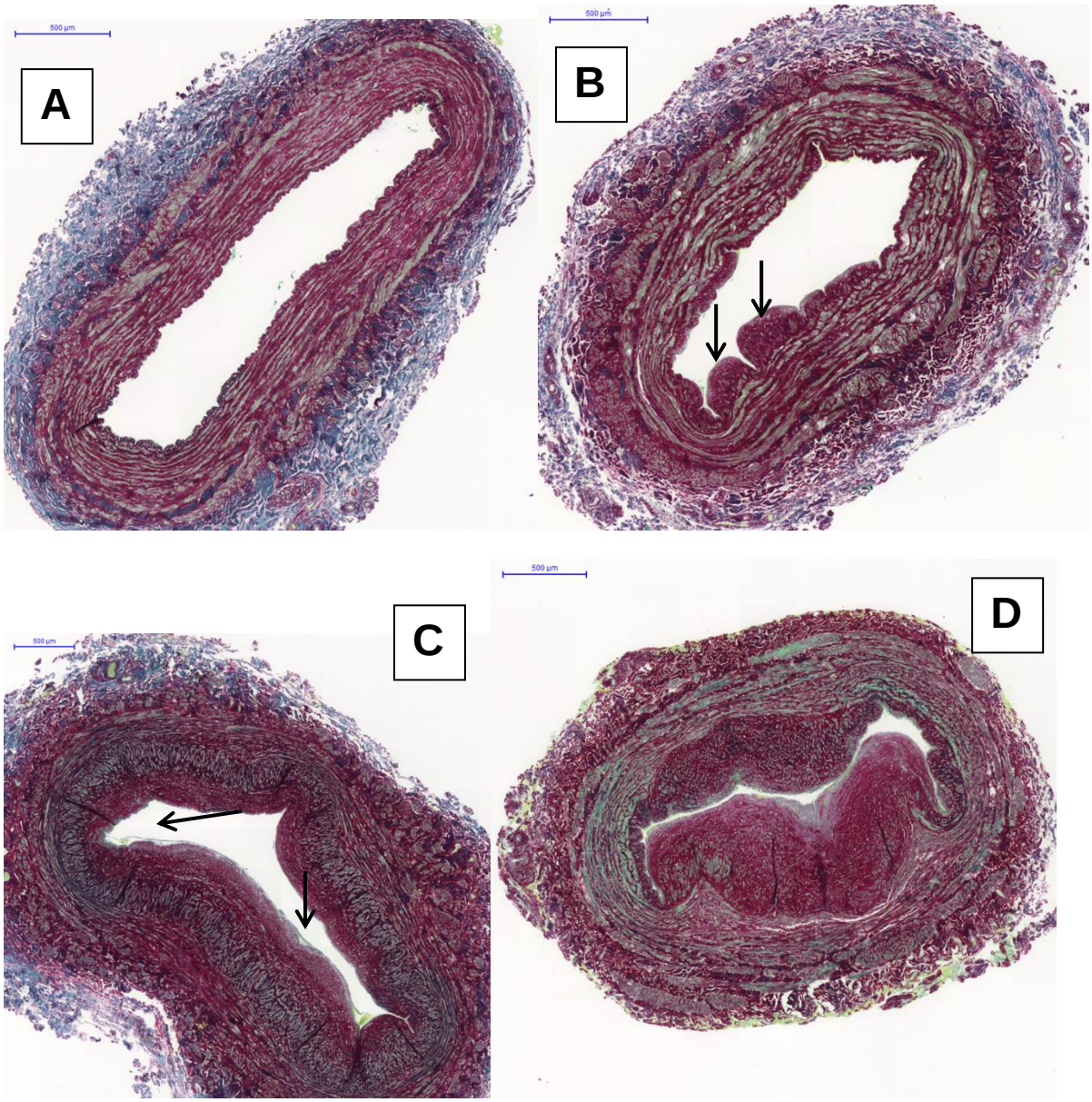


Figure 24: These pictures illustrate the cross sectional long saphenous vein are stained by Picrosirius red and magnified to 500µm. A) Normal appearance of the vein B) black arrow points the mild hypertrophy of the circular muscle and muscle migration C) black arrow illustrates the intimal detachment and intimal stretching D) Severe circular hypertrophy which almost occluded the vein.

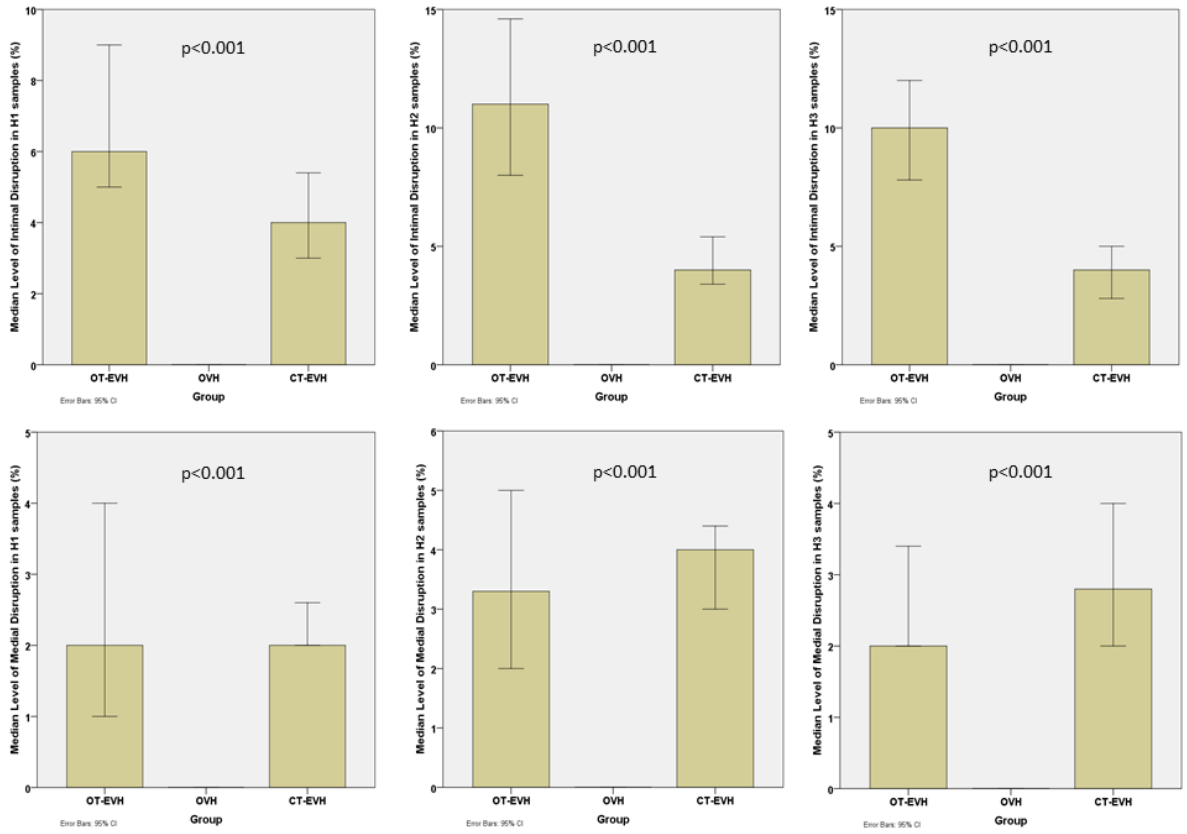


Figure 25: This figure illustrating the median level of intimal and medial disruption in proximal, distal and random vein samples.

CHAPTER 9

Comparison of vein integrity and clinical outcomes (VICO) between three types of vein harvesting surgical techniques for coronary artery bypass grafting. The VICO trial

Submitted for publication

9.0 A RANDOMISED STUDY COMPARING VEIN INTEGRITY AND CLINICAL OUTCOMES (VICO) IN OPEN VEIN HARVESTING AND TWO TYPES OF ENDOSCOPIC VEIN HARVESTING FOR CORONARY ARTERY BYPASS GRAFTING – THE VICO TRIAL.

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Keywords: Coronary artery bypass surgery, Open vein harvesting, Closed tunnel endoscopic vein harvesting, Open tunnel endoscopic vein harvesting, endothelial integrity.

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9.01 ABSTRACT

Current consensus statements maintain that endoscopic vein harvesting (EVH) should be standard care in Coronary Artery Bypass Grafting (CABG) but vein quality and clinical outcomes have been questioned with EVH. The Vein Integrity and Clinical Outcome (VICO) trial was designed to assess the impact of different vein harvesting methods on vessel damage and if this contributes to clinical outcomes following CABG. We randomly allocated n=300 patients into: Closed tunnel CO_2 EVH (n=100), Open tunnel CO_2 EVH (n=100) and traditional Open vein harvesting (n=100) groups. The primary end-point was endothelial integrity of the harvested vein. Secondary end-points included clinical outcomes (major adverse cardiac events, MACE), use of healthcare resources and impact on health status (quality-adjusted life years, QALYs).

The OVH group demonstrated marginally better endothelial integrity in random samples (85% vs. 88% vs. 93% for CT-EVH, OT-EVH and OVH, p<0.001). CT-EVH displayed the lowest longitudinal hypertrophy (1% vs. 13.5% vs. 3%, p=0.001). However, no differences in endothelial stretching were observed between groups (37% vs. 37% vs. 31%, p=0.62).

Secondary clinical outcomes demonstrated no significant differences in composite MACE scores at 3, 6, 12, 18 and 24 months. The QALY gain per patient was: 0.11 (p<0.001) for closed tunnel CO_2 EVH and 0.07 (p=0.003) for open tunnel CO_2 EVH compared with open vein harvesting. The likelihood of being cost-effective, at a pre-defined threshold of £20,000 per QALY gained was: 75% for closed tunnel, 19% for open tunnel and 6% for open vein harvesting.

Our study demonstrates that harvesting techniques do impact upon integrity of different vein layers, albeit with only a small effect. Secondary outcomes suggest that histological findings do not directly contribute to MACE outcomes. Gains in health status were observed and cost-effectiveness was better with CT-EVH.

9.1.1: INTRODUCTION

The long saphenous vein (LSV) remains the preferred conduit for multiple Coronary Artery Bypass Grafting (CABG) surgery due to its long length, and endoscopic vein harvesting (EVH) has demonstrated reduced postoperative morbidity and improved patient satisfaction (Allen et al., 2005; Krishnamoorthy et al., 2012b). Two EVH techniques exist: closed tunnel EVH (CT-EVH) and open tunnel EVH (OT-EVH), which differ on the basis of CO_2 pressurisation and instrumentation.

There is major debate regarding vein quality and long term clinical outcomes following EVH, largely due to the findings of a major study (Lopes et al., 2009), which revealed poorer outcomes with EVH. However, this raised questions about the use of different systems (CT-EVH was used for the majority of EVH cases in that study), case selection, operator experience (Bisleri and Muneretto, 2015b) and other comorbidities (Sabik et al., 2006). Previous studies (Chernyavskiy et al., 2015; van Diepen et al., 2014; Hess et al., 2014) and systematic reviews (Sastry et al., 2013; Deppe et al., 2013b) have highlighted the need for an appropriately designed clinical trial to establish the effect of harvesting on vein integrity, downstream costs and clinical outcomes (Markar et al., 2010). This was reinforced by the International Society of Minimally Invasive Cardiac Surgery (Allen et al., 2005) (ISMICS) and the National Institute for Health and Care Excellence (Kip et al., 2008; Barnard et al., 2011) (NICE).

We designed a prospective single centre 3-armed randomised study comparing vein damage and clinical outcomes between two types of EVH (closed and open tunnel) and traditional open vein harvesting (OVH). A trial-based cost-effectiveness analysis was prospectively integrated within the study design to generate evidence on the cost-effectiveness of the vein harvesting techniques.

9.2: METHODS

9.2.1: STUDY DESIGN

The study was approved by the NRES Committee and conducted following the principles of the Declaration of Helsinki and Good Clinical Practice. This study was undertaken at the University Hospital of South Manchester NHS Foundation Trust and was overseen by an external steering committee, clinical trial unit, public patient involvement and safety monitoring board. The trial was registered on the NIHR and the IRAS trial registry prior to commencing patient recruitment. We also registered the trial on the International Standard Randomised Controlled Trial Registry (ISCTRN: 91485426) in line with EU regulation 536/2014 (the trial was submitted on 30th April 2014 and EU regulation 536/2014 was released on 27th May 2014. The trial was fully registered on 18th September 2014).

Consented patients were prospectively recruited between November 2011 and May 2015 from the cardiac waiting list (please see the Consort diagram on page number: 206). Patients who received single internal mammary artery and individual vein grafts by on-pump bypass were included (full study protocol describing recruitment, clinical and health economics data collection, method of histological scoring and standard techniques included in chapter 4). Exclusion criteria included: emergency CABG, superficial LSV (less than ½ cm below the skin) or varicose LSV and/or small or thin legs (<7.5cm diameter at the lower calf), determined via by an ultrasound Sonasite™ scans (Krishnamoorthy et al., 2012b).

Patients were randomised to one of three groups with a 1:1:1 allocation ratio. Computerised simple block randomisation using random block sizes was performed by an independent statistician. Patient allocation was revealed to the practitioner once the patient was anaesthetised. Data gathering researchers, the statistician, health economist and histologist were completely blinded to the study group assignments.

9.2.2: SURGICAL TECHNIQUES

OVH and EVH were performed as previously described (Krishnamoorthy et al., 2012b; Krishnamoorthy et al., 2015). All veins were harvested by an experienced surgical practitioner (>250 cases for each EVH technique and >2000 open harvesting cases). Harvesting was started either from the mid-calf (1-2 vein lengths) or from the medial malleolus (3 lengths) (Krishnamoorthy et al., 2012b). For group 1, a Maquet Vasoview™ Hemopro2 closed tunnel endoscopic vein harvesting (CT-EVH) system was used. For group 2, a Sorin ClearGlide™ open tunnel (OT-EVH) system was used. The CO_2 insufflator was set to 3 litres/ min with 0mmHg pressure. Following completion of harvesting, patients received full heparinisation followed by cardio-pulmonary bypass. CT-EVH patients received 5000 units of heparin before EVH to avoid intraluminal clot formation (Brown et al., 2007b).

9.2.3: HISTOLOGICAL ASSESSMENT

2700 vein samples were numerically coded to ensure laboratory blinding. Surgically undistended vein samples (n=900) were obtained proximally at the port of entry and coded H1. Distal vein samples (n=900) obtained after 20mmHg heparinised blood flush to check for leakages were coded H3. Following vein grafting, a random sample was obtained from the remaining conduit, and coded H2 (n=900).

Therefore H2 samples underwent all distension and manipulation as required for surgical preparation. As such, these samples provide the best possible representation of the entire vein at different stages following harvesting that could be achieved given the logistics of the operation. A computerised immunohistochemistry protocol was used to stain CD34 (a validated endothelial marker) (Hashmi et al., 2015) of each vein sample from batch 1 (n=900; H1, H2, H3).

A validated scoring system was used to grade endothelial integrity (Hwang et al., 2012) (0-100% intact (positive staining), Figure 26). The second batch of 900 vein samples was stained with Picrosirius red muscular and collagen stain (80-picrosirius red; Sigma-Aldrich Ltd, Dorset, UK) to assess structural damage in the muscular layers. We refined/ modified the existing scoring system for simplicity, which was used to grade muscular hypertrophy, detachment, muscle migration on a scale of 0-3 (normal, mild, moderate, severe, see Figure 27).

The final batch of 900 vein samples was stained with Haematoxylin & Eosin (H&E) to assess endothelial stretching and detachment. Endothelial damage was graded on a scale of 0-3 (normal, mild, moderate and severe) (Hashmi et al., 2015). All slides were scanned using a Pannoramic 250™ slide scanning system. All histology images were scored by 5 independent assessors and validated by a consultant histopathologist.

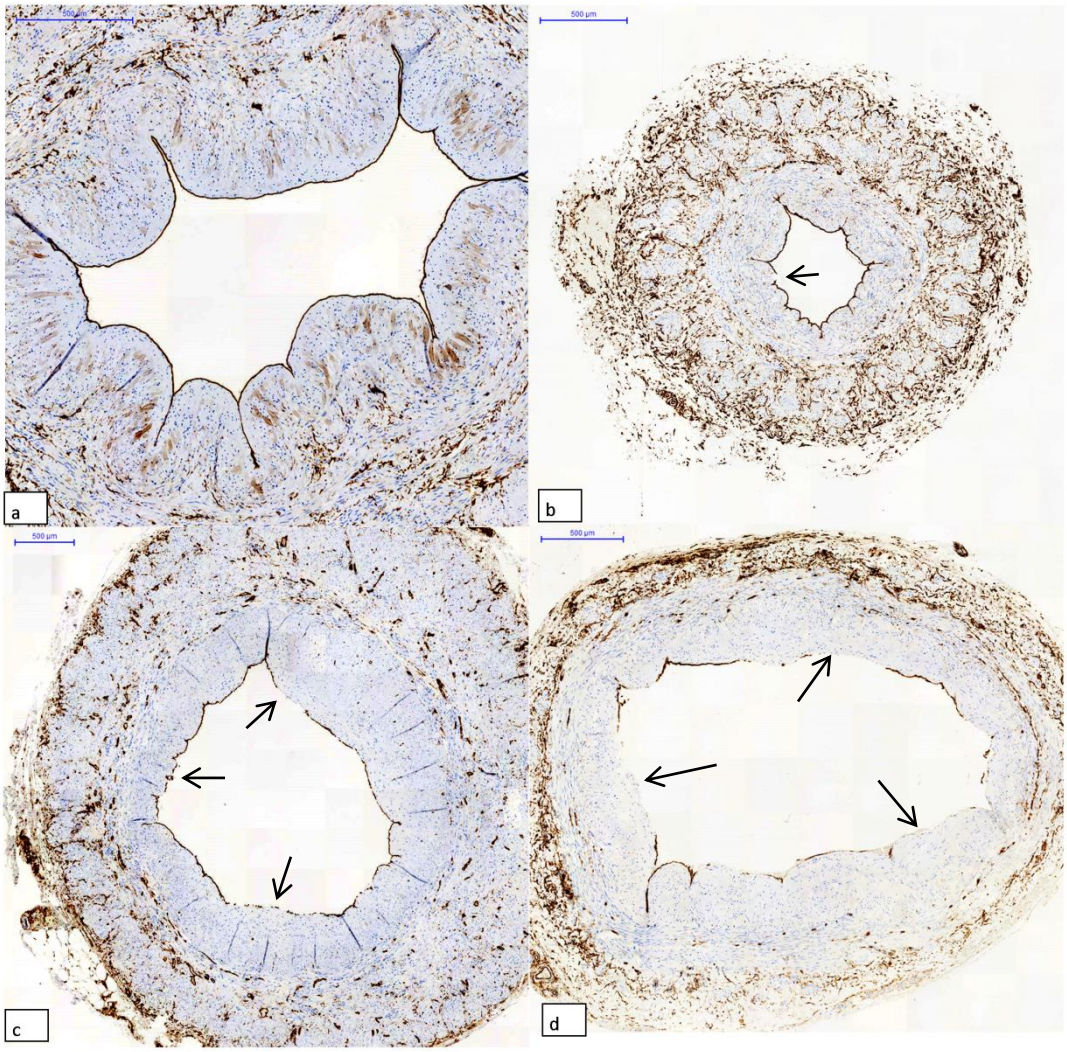


Figure 26: CD34 endothelial staining of long saphenous vein samples demonstrating (a) normal continuous endothelium, (b) mild endothelial disruption, (c) moderate endothelial disruption and (d) severe endothelial disruption. ↑ indicates site disruption.

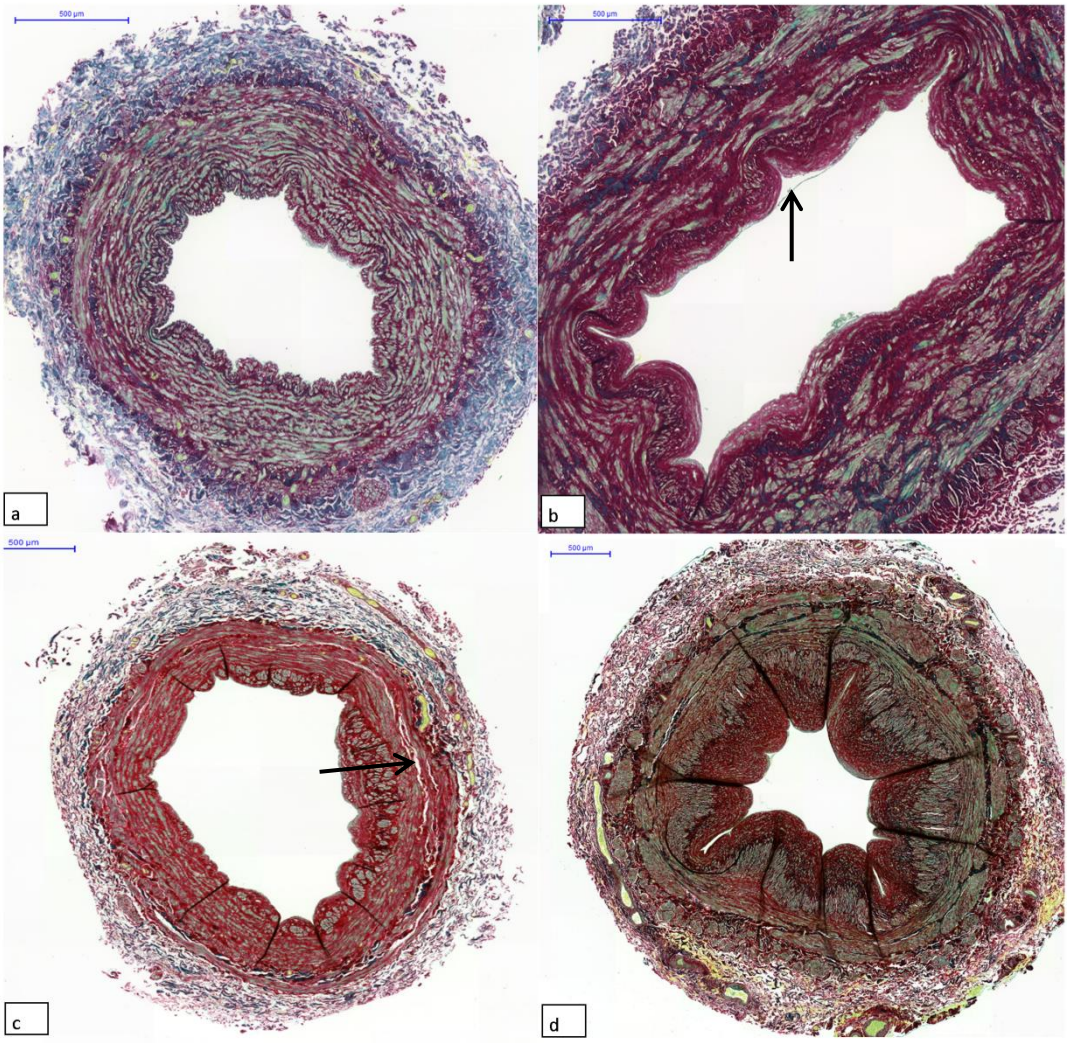


Figure 27: Picrosirius red staining of long saphenous vein samples demonstrating (a) normal vein structures, (b) mild intimal detachment, (c) detachment within the longitudinal muscle layer and (d) moderate circular hypertrophy. ↑ indicates site of defined injury.

9.2.4: STUDY OUTCOME MEASURES

The primary outcome measure was severity of histological damage to the vein conduits. The association between histological damage and pre-defined clinical outcomes was then assessed. Complete demographics, intraoperative details, incidence of wound infection and General Practitioner/district nurse visits were recorded.

The secondary end-points included incidence of Major Adverse Cardiac Events (MACE), use of healthcare resources and impact on health status. MACE was defined as repeat angina, breathlessness, myocardial ischemia/infarction, re-intervention, stroke and death. MACE were determined by telephone interviews, clinic letters, general practitioner and coroner reports at 3 month intervals until 12 months and then at 18, 24, 36 and 48 months. Only symptomatic MACE patients underwent cardiac MRI scans and angiograms were reviewed by an independent cardiologist and a cardiac surgeon.

An NHS and social services perspective was used for the scope of the collection of healthcare resources. All healthcare resources associated with treatment and follow-up care was recorded prospectively. For a full list of healthcare utilisation data collected (Table 30) and unit costs which were sourced from the procurement and finance department at the hospital and national databases where relevant for follow-up care (Hwang et al., 2012; 2015). The vein harvesting procedure was micro-costed, with the fixed cost of the vein harvesting equipment fully absorbed in each arm of the trial. The length of time within theatre required for vein harvesting was recorded and costed. The impact on health status was assessed using the EQ-5D-3L, a generic preference-based measure at 3 and 12 months and converted into Quality Adjusted Life Years (QALYs) using a published national tariff (Dolan, 1995). A one year time horizon was chosen and so no discounting was applied to the cost or QALY data.

Table 30: This table illustrates the full resource use and unit costs included in this cost analysis.

Resource use and unit costs in the analysis							
Resource-use item	**Cost type**	**Fixed cost total**	**Unit cost***	**Source Year**	**Source**	**Years need inflating**	**Unit cost**
Vein Extraction							
West retractor (one off payment)	Fixed	£ 78.80	£ 2.75	2014	Finance department	1	£ 2.78
Sterilisation 1	Variable		£ 2.00	2014	Finance	1	£ 2.02
Langenbeck retractor small(one off payment)	Fixed	£ 46.52	£ 3.32	2014	Finance	1	£ 3.36
Sterilisation 2	Variable		£ 2.00	2014	Finance	1	£ 2.02
Vein harvesting set (cut down)one off payment	Fixed	£ 293.70	£ 6.59	2014	Finance	1	£ 6.66
Sterilisation 3	Variable		£ 12.00	2014	Finance	1	£ 12.13
Disposables (in theatres and ward, community)			-				
Leg vacuum drain size 10	Variable		£ 7.52	2013	Procurement	2	£ 7.67
Dressings large each	Variable		£ 1.15	2013	Procurement	2	£ 1.17
Dressings small each	Variable		£ 0.66	2013	Procurement	2	£ 0.67
Bandages 6" each	Variable		£ 0.84	2013	Procurement	2	£ 0.86
Sutures							
2/0vicryl each	Variable		£ 3.45	2013	Procurement	2	£ 3.52
3/0monocryl	Variable		£ 3.50	2013	Procurement	2	£ 3.57
Vicryl ties4/0 each	Variable		£ 4.22	2013	Procurement	2	£ 4.31
Drain stitch each	Variable		£ 1.57	2013	Procurement	2	£ 1.60
Swabs(5 pieces per pack)	Variable		£ 1.12	2013	Procurement	2	£ 1.14
Red ligaclips pack 4	Variable		£ 27.60	2013	Procurement	2	£ 28.16
Blue ligaclips pack 4	Variable		£ 29.20	2013	Procurement	2	£ 29.79
Theatre time	Variable		£ 15.12	2013	Procurement	2	£ 15.43
Total leg operation surgery timings							
EVH- disposable kit	Variable		£ 550.00	2013	Procurement	2	£ 561.10
Camera drapes	Variable		£ 190.58	2013	Procurement	2	£ 194.43
Lens cleaner	Variable		£ 79.59	2013	Procurement	2	£ 81.20
CO2 tubing	Variable		£ 173.13	2013	Procurement	2	£ 176.63
Light lead one off payment	Fixed	£ 295.00	£ 0.06	2013	Procurement	2	£ 0.06
Telescope one off payment	Fixed	£ 2,571.00	£ 0.47	2013	Procurement	2	£ 0.48
Hemopro2 cable one off payment	Fixed	£ 220.00	£ 0.04	2013	Procurement	2	£ 0.04

TV, camera monitor stack machine one off payment for 10 years	Fixed	£ 35,725.00	£ 19.27	2013	Procurement	2	£ 19.66
Power supply Hemopro2 one off payment	Fixed	£ 4,025.00	£ 2.17	2013	Procurement	2	£ 2.22
Follow-up care							
ECG per visit	Variable		£ 62.00	2013	Finance	2	£ 63.25
ECHO per visit	Variable		£ 111.00	2013	Finance	2	£ 113.24
Cardiac MRI scan per visit	Variable		£ 534.00	2013	Finance	2	£ 544.78
Angiogram visit urgent	Variable		£ 3,213.87	2013	Finance	2	£ 3,278.75
Angiogram day case	Variable		£ 1,367.36	2013	Finance	2	£ 1,394.96
PTCA elective	Variable		£ 3,045.28	2013	Finance	2	£ 3,106.76
PTCA day case	Variable		£ 2,978.67	2013	Finance	2	£ 3,038.80
GP visit	Variable		£ 53.00	2015	PSSRU	0	£ 53.00
District nurse home visits	Variable		£ 24.00	2015	PSSRU	0	£ 24.00
Antibiotic	Variable		£ 7.20	2015	Pharmacy	0	£ 7.20
Cardiology follow-up	Variable		£ 97.78	2013	Finance	2	£ 99.75
Cardiac surgeon follow-up	Variable		£ 189.69	2013	Finance	2	£ 193.52
Pacemaker stay and cost of the device etc)	Variable		£ 1,495.00	2013	Finance	2	£ 1,525.18
wound infection full package(includes readmission, itu, ward, retheatre procedure, vac therapy)	Variable		£ 7,250.00	2013	Finance	2	£ 7,396.36
Hospital stay	Variable		£ 250.00	2013	Finance	2	£ 255.05
Medications	Variable		£ 1,000.00	2015	Pharmacy	0	£ 1,000.00
Surgical intervention	Variable		£ 6,000.00	2015	Finance	0	£ 6,000.00

*****For fixed costs, the unit cost is fully absorbed and was applied on a per-operation basis

9.2.5: POWER CALCULATION AND STATISTICAL ANALYSIS

To generate an accurate power calculation we undertook a non-randomised pilot study comparing the impact of the different vein harvesting techniques on endothelial integrity using 140 patients. Based on this pilot data we calculated that 91 patients in each of the three groups (OVH, CT-EVH and OT-EVH), i.e. 273 in total, would provide 80% power to detect differences in the percentage with zero endothelial integrity of 20% or more (for example 20% vs. 40%) in this study. This calculation was based on a comparison of two groups using a simple chi-square test, with continuity correction at the 5% significance level. A recruitment strategy requiring a total of 300 patients with a 10% drop out rate was used.

Clinical outcomes in our pilot study demonstrated that 19% of closed tunnel CO_2 patients experienced MACE compared to 13% of open tunnel CO_2 patients (ie. only a 6% difference in incidence). Demographics were summarised as frequencies/percentages for categorical variables and means/median with standard deviation/interquartile range as appropriate for continuous variables. Endothelial integrity as determined by CD34 expression was presented as median percentage integrity and other histological outcomes were presented as median scores and analysed using the Kruskal-Wallis test. Composite and individual MACE events were analysed at each time point using the chi-square test. All tests were performed as two-tailed analyses and p-values <0.05 were considered significant.

The area under the curve method was used to generate Quality Adjusted Life Years (QALYs) for each patient. Incremental costs, incremental QALYs and incremental net benefit at a decision-maker's threshold of £20,000 per QALY were calculated using ordinary least squares regression controlling for baseline disease severity using EQ-5D and the Canadian Cardiovascular Society grading score. Statistical uncertainty was considered by using a non-parametric bootstrap method (Briggs et al., 1997) accommodating for the correlation between costs and QALYs.

9.2.6: PRE-TRIAL WORK

A pre-trial study was designed to determine study sample size for the primary end-point and demonstrated that OT-EVH (n=70) better preserved conduit endothelium compared to CT-EVH (n=70) (median 65.0% vs. 11.4%, p<0.001, Figure 28). However, no significant differences were observed between groups for MACE events including repeat angina (p=0.62), breathlessness (p=0.80), re-intervention (p=1.00), myocardial infarction/ischaemia (p=1.00) or mortality (p=0.44) up to 4 years post-surgery (Table 31).

9.3: RESULTS

9.3.1: DEMOGRAPHICS

398 patients were enrolled but 24.6% were excluded from the study based on predefined exclusion criteria. 300 patients underwent randomisation and there were no clinically relevant differences between groups (Table 32). A higher BMI, more left main stem and current smokers were observed in the CT-EVH group. Intraoperative variables were recorded, including surgical timings and number of veins required (Table 33).

9.3.2: HISTOLOGICAL OUTCOMES

9.3.21: ENDOTHELIAL INTEGRITY: CD34

Endothelial integrity was better preserved in the OVH group in proximal samples compared to endoscopic techniques (median percentage integrity [IQR]: 91.50 [12.50] vs. 91.63 [10.56] vs. 95.75 [6.69] for CT-EVH vs. OT-EVH vs. OVH respectively, p<0.001, Figure 29). Random samples from the OVH group displayed greatest endothelial integrity compared to the other groups (85.25 [21.13] vs. 87.50 [21.00] vs. 92.71 [13.13] for CT-EVH vs. OT-EVH vs. OVH respectively, p<0.001, Figure 29). However, no statistical difference was observed in distal samples (92.25 [10.88] vs. 91.75 [13.81] vs. 95.38 [9.25] for CT-EVH vs. OT-EVH vs. OVH respectively, p=0.07, Figure 29).

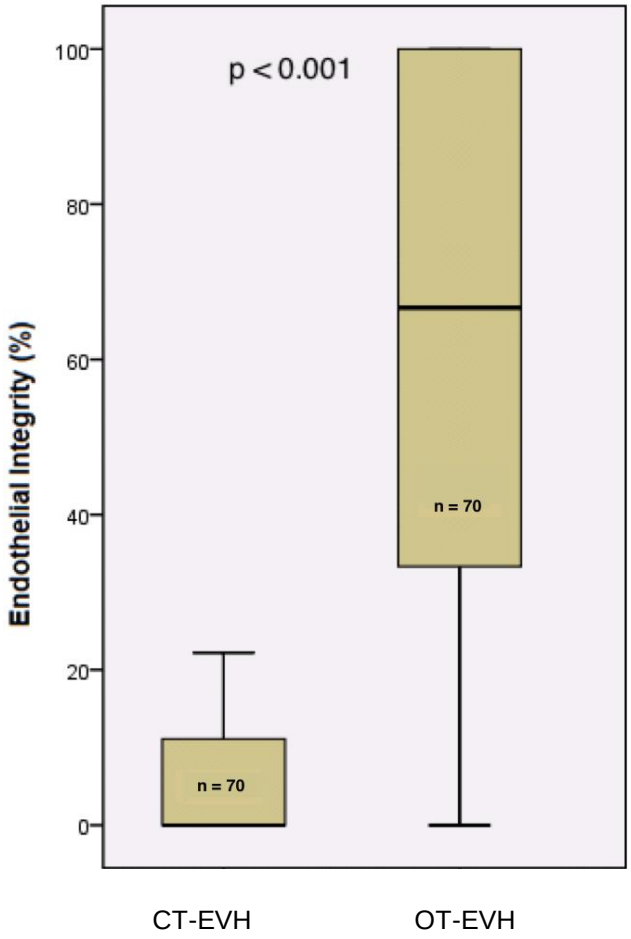


Figure 28: This box plot represents a comparison of the endothelial integrity of veins obtained via closed tunnel CO2 and open tunnel CO2 EVH systems. Veins obtained using the open CO_2 method (OT-EVH) exhibited significantly greater endothelial integrity compared to those obtained using the closed tunnel CO_2 technique (CT-EVH).

Table 31: Pilot work four years clinical outcome MACE data.

Variable	CT-EVH (n=70)	OT-EVH (n=70)	p-value
	Number (percentage)		
Repeat angina			
3 months	4 (5.8)	1 (1.4)	0.209
6 months	7 (10.1)	2 (2.9)	0.097
9 months	5 (7.4)	2 (2.9)	0.441
12 months	7 (10.3)	4 (5.9)	0.531
18 months	8 (11.8)	4 (5.9)	0.365
24 months	7 (10.3)	3 (4.5)	0.325
48 months	2 (3.1)	1 (1.5)	0.619
Repeat breathlessness			
3 months	9 (13.0)	12 (17.4)	0.636
6 months	10 (14.5)	13 (19.1)	0.501
9 months	9 (13.2)	7 (10.3)	0.791
12 months	9 (13.2)	13 (19.1)	0.486
18 months	9 (13.2)	16 (23.5)	0.183
24 months	9 (13.2)	9 (13.4)	1.000
48 months	10 (15.4)	8 (12.3)	0.800
Repeat interventions			
3 months	3 (4.3)	1 (1.4)	0.619
6 months	3 (4.3)	3 (4.4)	1.000
9 months	2 (2.9)	6 (8.8)	0.274
12 months	6 (8.8)	5 (7.4)	1.000
18 months	6 (8.8)	1 (1.5)	0.115
24 months	3 (4.4)	3 (4.5)	1.000
48 months	2 (3.1)	1 (1.5)	1.000
Myocardial Infarction/Ischaemia			
3 months	4 (5.8)	0 (0.0)	0.120
6 months	4 (5.8)	1 (1.5)	0.366
9 months	3 (4.4)	1 (1.5)	0.619
12 months	5 (7.4)	2 (2.9)	0.441
18 months	4 (5.9)	3 (4.4)	1.000
24 months	4 (5.9)	1 (1.5)	0.366
48 months	2 (3.1)	1 (1.5)	1.000
Mortality			
3 months	1 (1.4)	1 (1.4)	1.000
6 months	1 (1.4)	2 (2.9)	1.000
9 months	2 (2.9)	2 (2.9)	1.000
12 months	2 (2.9)	2 (2.9)	1.000
18 months	2 (2.9)	2 (2.9)	1.000
24 months	2 (2.9)	3 (4.3)	1.000
48 months	6 (8.8)	4 (5.8)	0.532
Post-operative PTCA	4 (5.7)	1 (1.6)	0.366
Vein graft patency			
No flow limitation	8 (61.5)	5 (100)	0.264
Flow limited	2 (15.4)	0 (0.0)	
Completely blocked	3 (23.1)	0 (0.0)	
ACC/AHA coronary artery score			
Discrete (<10mm) lesion	1 (7.1)	0 (0.0)	0.343
Tubular (10-20mm) lesion	3 (21.4)	0 (0.0)	
Diffuse (>2cm) lesion	10 (71.4)	6 (100.0)	

Table 32: Demographic data including pre-operative co-morbidities, risk factors and cardiac history.

Demographic variables		**Group**		
		OT-EVH (n=100)	**OVH (n=100)**	**CT-EVH (n=100)**
Age (years)		66.92±10.08	65.96±9.34	64.06±10.20
Sex (M/F)		82/18 (82.0%/18.0%)	79/21 (79.0%/21.0%)	79/21 (79.0%/21.0%)
Body Mass Index (BMI)		27.77 [6.41]	27.93 [5.45]	28.78 [6.54]
Urgency	*Elective*	46 (46.0%)	49 (49.0%)	41 (41.0%)
	Urgent	54 (54.0%)	51 (51.0%)	59 (59.0%)
Diabetes	*Diet controlled*	8 (8.0%)	6 (6.0%)	4 (4.0%)
	Tablet controlled	21 (21.0%)	27 (27.0%)	22 (22.0%)
	Insulin controlled	8 (8.0%)	11 (11.0%)	4 (4.0%)
Canadian Cardiovascular Society	*I*	17 (17.0%)	17 (17.0%)	12 (12.0%)
	II	25 (25.0%)	29 (29.0%)	33 (33.0%)
	III	45 (45.0%)	45 (45.0%)	46 (46.0%)
	IV	13 (13.0%)	9 (9.0%)	9 (9.0%)
New York Heart Association	*I*	27 (27.0%)	32 (32.0%)	40 (40.0%)
	II	45 (45.0%)	35 (35.0%)	26 (26.0%)
	III	26 (26.0%)	25 (25.0%)	29 (29.0%)
	IV	2 (2.0%)	8 (8.0%)	5 (5.0%)
STEMI		18 (18.0%)	19 (19.0%)	29 (29.0%)
NSTEMI		42 (42.0%)	48 (48.0%)	44 (44.0%)
Previous PTCA		16 (16.0%)	12 (12.0%)	20 (20.0%)
Previous MI		52 (52.0%)	43 (43.0%)	54 (54.0%)
Multivessel disease		82 (82.0%)	81 (81.0%)	86 (86.0%)
Left main stem		25 (25.0%)	25 (25.0%)	40 (40.0%)
Hypertension		87 (87.0%)	83 (83.0%)	88 (88.0%)
Smoking	*Never smoked*	32 (32.0%)	33 (33.0%)	23 (23.0%)
	Previous smoker	52 (52.0%)	54 (54.0%)	47 (47.0%)
	Current smoker	16 (16.0%)	13 (13.0%)	30 (30.0%)
Hypercholesterolemia		96 (96.0%)	90 (90.0%)	92 (92.0%)
Peripheral vascular disease		19 (19.0%)	20 (20.0%)	21 (21.0%)
Left ventricular ejection fraction	*>50%*	74 (74.0%)	74 (74.0%)	72 (72.0%)
	30-50%	21 (21.0%)	18 (18.0%)	22 (22.0%)
	<30%	5 (5.0%)	8 (8.0%)	6 (6.0%)

Categorical variables are expressed as number (percentage) and assessed by the χ^2 test. Continuous variables are expressed as either mean±standard deviation (parametric data) or median [interquartile range] (non-parametric data) and analysed by ANOVA or Independent samples Kruskal-Wallis test respectively. * STEMI- ST elevated myocardial infarction, NSTEMI – Non ST elevated myocardial infarction, PTCA – Percutaneous coronary angioplasty.

Table 33: Intraoperative variables were recorded for each surgery.

Variable	Group			p-value
	OT-EVH	OVH	CT-EVH	
Harvesting time (mins)	19.86 [11.64]	22.26 [17.65]	23.40 [12.48]	**0.031**
Full leg surgery time (mins)	42.93 [20.46]	42.73 [25.43]	53.50 [22.50]	**<0.001**
Total surgery time (mins)	226.77 [56.99]	222.65 [58.34]	228.46 [67.72]	0.806
Bypass time (mins)	93.00 [49.00]	90.00 [43.00]	92.00 [35.75]	0.698
Cross-clamp time (mins)	54.00 [37.00]	58.00 [34.75]	57.00 [23.00]	0.841
Number of vein grafts				
1	26 (26.0%)	26 (26.0%)	13 (13.0%)	0.130
2	54 (54.0%)	51 (51.0%)	57 (57.0%)	
3	20 (20.0%)	22 (22.0%)	30 (30.0%)	
4	0 (0.0%)	1 (1.0%)	0 (0.0%)	
Length of vein obtained (cm – mean±SD)	34.86±12.90	35.60±13.71	39.23±12.09	**0.039**

Intraoperative variables were recorded for each surgery. Longer harvesting times and overall leg surgery time was required for CT-EVH and OVH. The number of vein grafts required was not significantly different between groups.

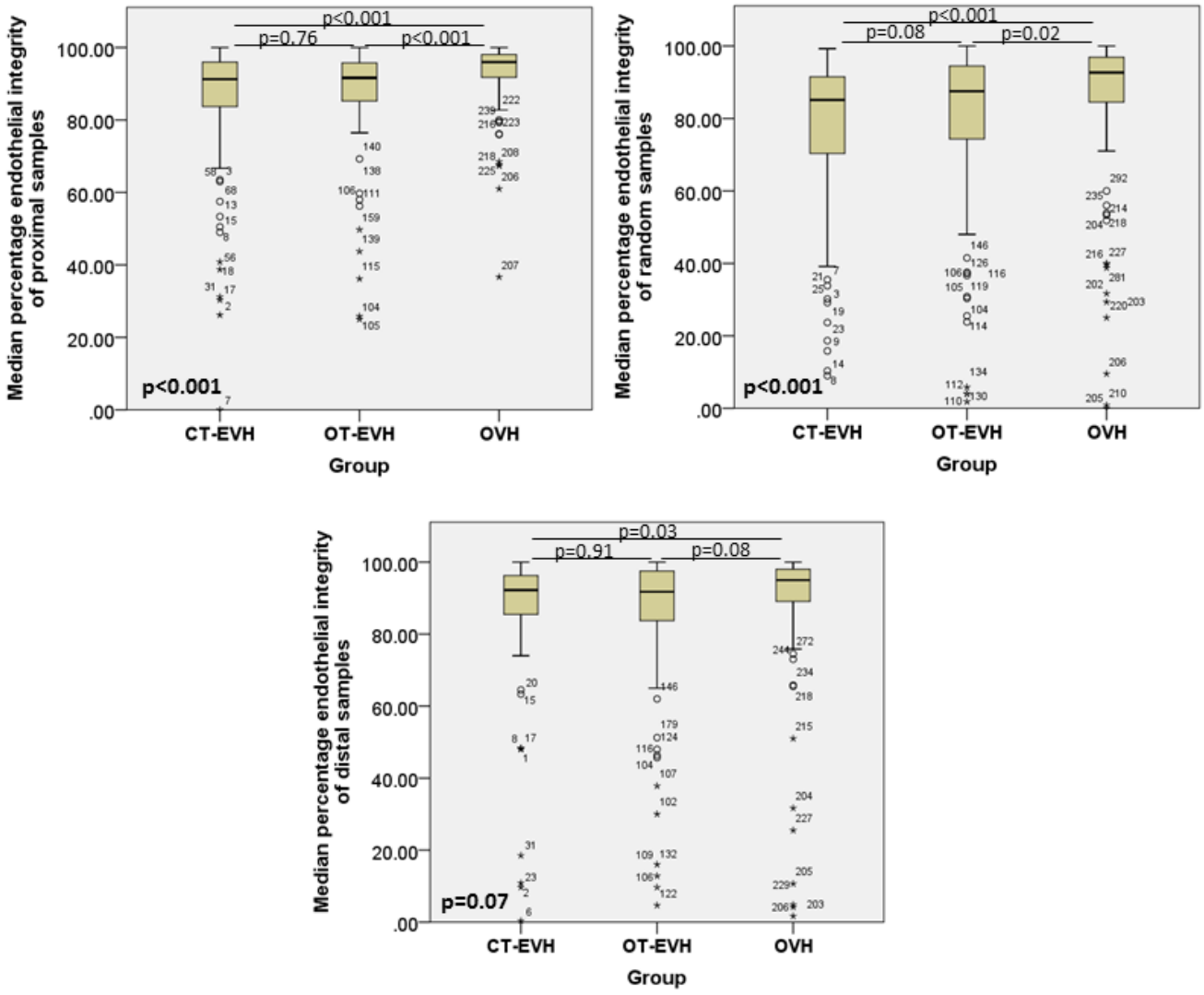


Figure 29: This figure illustrates the median percentage endothelial integrity on proximal (H1), random (H2) and distal (H3) vein samples between CT-EVH, OT-EVH and OVH groups.

9.3.22: MUSCULAR MORPHOLOGY: PICROSIRIUS RED AND H&E

Endothelial stretching of proximal vein samples was greatest in OT-EVH group (66.0%), followed by CT-EVH (61.0%), with least stretching in OVH (46.0%, p=0.01). No differences in endothelial stretching were observed between groups in distal (53.5% vs. 51.5% vs. 41.0% for OT-EVH, OVH and CT-EVH respectively, p=0.16) or random (37.4% vs. 31.3% vs. 36.7% for OT-EVH, OVH and CT-EVH respectively, p=0.62) samples. The level of endothelial detachment was consistent between groups in proximal (2% vs. 3% vs. 2% for OT-EVH, OVH and CT-EVH, p=0.25), distal (4% vs. 1% vs. 6% for OT-EVH, OVH and CT-EVH, p=0.63) and random (5% vs. 2% vs. 5% for OT-EVH, OVH and CT-EVH, p=0.47) samples.

The circular muscle layer displayed greatest hypertrophy in proximal samples from the OT-EVH group (65.6%) followed by CT-EVH (45.0%) and OVH (14.3%, p<0.001). A similar pattern was observed in distal (46.3% vs. 24.2% vs. 38.8% for OT-EVH, OVH and CT-EVH, p<0.001) and random (35.4% vs. 14.1% vs. 31.3% for OT-EVH, OVH and CT-EVH, p=0.01) samples. The longitudinal muscle layer displayed greatest hypertrophy in proximal samples from the OT-EVH group (56.2%) compared to OVH (5.1%) and CT-EVH groups (23.0%, p<0.001). Greatest longitudinal hypertrophy was observed in distal samples from the OT-EVH group (26.3%), followed by CT-EVH (8.2%) and OVH (1.0%, p<0.001). Moreover, OT-EVH displayed greatest longitudinal hypertrophy in random samples (13.5%), compared to OVH (3.0%) and CT-EVH (1.0%, p=0.001).

9.3.23: SECONDARY OUTCOMES – CLINICAL EVENTS

Composite and individual MACE scores were analysed in this study to avoid any varying definitions of composite outcomes. Kip et al (Kip et al., 2008) suggested that authors should focus separately on safety and effectiveness outcomes.

9.3.24: COMPOSITE MACE SCORES

The incidence of composite MACE events were analysed at 3, 6, 12, 18 and 24 months. No significant differences were observed between groups at any point (Table 34). With n=300 up to 24 months, MACE events occurred in 13% of CT-EVH, 14% of OT-EVH and 12% of OVH patients. Endothelial integrity did not differ between patients with or without MACE at 24 months in proximal samples (median percentage integrity [IQR]: 93.58 [11.42] vs. 92.33 [7.54] for MACE-free and MACE-

affected respectively, p=0.48), distal samples (93.08 [11.81] vs. 96.25 [11.50], p=0.26) or random samples (88.75 [18.56] vs. 87.25 [23.92], p=0.64).

9.3.25: INDIVIDUAL MACE EVENTS

The secondary outcomes demonstrated that no significant difference in MACE events was observed between groups, other than slightly higher mortality at 3 and 6 months (p=0.05 and p=0.03 respectively), in the OT-EVH group (Table 35) although these deaths were not MACE related mortalities. Atrial fibrillation occurred in 9 patients and vein graft blockage was noted in 6 patients, although incidence was not influenced by group (p=0.69 and p=0.42 respectively, Table 36). No statistically significant difference in MACE outcomes at 3, 6, 12, 18 and 24 months was observed between operators (p=0.76, p=0.78, p=0.26, p=0.23 and p=0.21 respectively).

9.3.26: COST EFFECTIVENESS ANALYSES

Both EVH techniques led to an increase in the initial vein harvesting costs versus OVH. The use of CT-EVH increased costs by £1180 (p<0.001) whilst OT-EVH increased costs by £981 (p<0.001) per patient over OVH. The major increase in cost of the two EVH approaches versus OVH was a consequence of the fixed resources required for EVH (such as visual equipment) and variable costs required for each operation (such as tubing and camera drapes). In total, the additional equipment and suture cost for CT-EVH versus OT-EVH was £1015 (p<0.001) and £1020 (p<0.001) respectively but there was no statistical difference between the two EVH approaches. However, CT-EVH required longer in surgery than OT-EVH leading to a higher theatre time harvesting cost (£159; p<0.001). For follow-up care, CT-EVH led to a reduction in downstream costs for of £814 (p=0.002) whilst OT-EVH led to a reduction of £598 (p=0.03) versus OVH. Overall, both EVH methods led to net cost increases over OVH, by £274 (p=0.34) for CT-EVH and £436 (p=0.16) for OT-EVH per patient.

CT-EVH had an increase in QALYs of 0.11 per patient (p=0.001) whilst OT-EVH had an increase in QALYs of 0.07 per patient (p=0.003) versus OVH. CT-EVH had an incremental net-benefit per patient of £1927 (p<0.001) versus OVH and a 75% likelihood of being cost-effective for a decision-maker's threshold of £20,000 whilst OT-EVH had an incremental net-benefit per patient of £950 (p=0.12) versus OVH and a 19% likelihood of being cost-effective. OVH had a low likelihood (6%) of being cost-effective (Figure 30 and Figure 31).

Table 34: This table illustrates the MACE events composite outcomes at 3, 6, 12, 18 and 24 months.

MACE events Composite Outcomes	Groups				p-value
	CT-EVH	OT-EVH	OVH	Total	
3 months	05/100 (5.0%)	07/100 (7.0%)	05/100 (5.0%)	17/300 (5.7%)	0.78
6 months	07/100 (7.0%)	10/100 (10.0%)	05/100 (5.0%)	22/300 (7.3%)	0.39
12 months	09/100 (9.0%)	13/100 (13.0%)	11/100 (11.0%)	33/300 (11.0%)	0.67
18 months	13/100 (13.0%)	14/100 (14.0%)	12/100 (12.0%)	39/300 (13.0%)	0.92
24 months	11/85 (12.8%)	14/86 (17.9%)	11/85 (11.5%)	36/256 (14.1%)	0.77

The Pearson chi square test was used and expressed in numbers and percentages. The composite outcomes include repeat angina, re-intervention, mortality, breathlessness, vein graft failure, stroke and myocardial infarction/ischaemia.

Table 35: This table illustrates the detailed breakdown of major adverse cardiac events occurred to the study population from 3 months to 48 months. All categorical data is expressed as number (percentage).

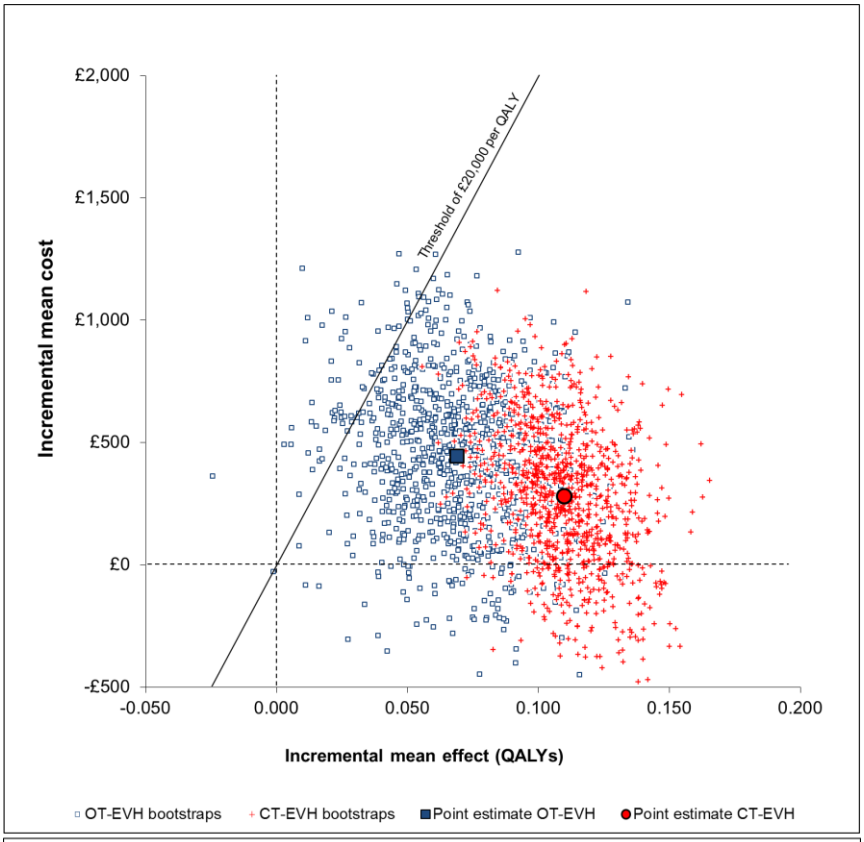
Time	Number of participants	MACE event	CT-EVH	OT-EVH	OVH	p-value
3 months	n=297	Repeat angina	0 (0.0%)	2 (2.1%)	3 (3.0%)	0.24
		Breathlessness	5 (5.0%)	2 (2.1%)	5 (5.0%)	0.48
		Reintervention	0 (0.0%)	2 (2.1%)	2 (2.0%)	0.36
		Myocardial infarction/ischaemia	0 (0.0%)	2 (2.1%)	2 (2.0%)	0.36
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=300	Survival	100 (100.0%)	97 (97.0%)	100 (100.0%)	0.05
6 months	n=294	Repeat angina	1 (1.0%)	0 (0.0%)	1 (1.0%)	0.62
		Breathlessness	7 (7.0%)	2 (2.1%)	3 (3.0%)	0.18
		Reintervention	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
		Myocardial infarction/ischaemia	1 (1.0%)	0 (0.0%)	0 (0.0%)	0.38
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=300	Survival	100 (100.0%)	95 (95.0%)	99 (99.0%)	0.03
9 months	n=293	Repeat angina	1 (1.0%)	0 (0.0%)	3 (3.0%)	0.18
		Breathlessness	6 (6.1%)	1 (1.1%)	3 (3.0%)	0.15
		Reintervention	0 (0.0%)	0 (0.0%)	2 (2.0%)	0.14
		Myocardial infarction/ischaemia	1 (1.0%)	0 (0.0%)	3 (3.0%)	0.18
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=300	Survival	99 (99.0%)	95 (95.0%)	99 (99.0%)	0.10
12 months	n=291	Repeat angina	2 (2.0%)	2 (2.1%)	3 (3.1%)	0.86
		Breathlessness	7 (7.1%)	2 (2.1%)	3 (3.1%)	0.18
		Reintervention	0 (0.0%)	2 (2.1%)	0 (0.0%)	0.13
		Myocardial infarction/ischaemia	2 (2.0%)	1 (1.1%)	2 (2.1%)	0.83
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=300	Survival	99 (99.0%)	95 (95.0%)	97 (97.0%)	0.25
18 months	n=289	Repeat angina	3 (3.1%)	3 (3.2%)	3 (3.1%)	1.00
		Breathlessness	8 (8.2%)	3 (3.2%)	2 (2.1%)	0.09
		Reintervention	2 (2.1%)	1 (1.1%)	2 (2.1%)	0.83
		Myocardial infarction/ischaemia	2 (2.1%)	1 (1.1%)	2 (2.1%)	0.83
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=300	Survival	97 (97.0%)	95 (95.0%)	97 (97.0%)	0.69

24 months	n=289	Repeat angina	3 (3.7%)	1 (1.2%)	1 (1.2%)	0.45
		Breathlessness	5 (6.1%)	1 (1.2%)	0 (0.0%)	0.03
		Reintervention	1 (1.2%)	1 (1.2%)	0 (0.0%)	0.60
		Myocardial infarction/ischaemia	2 (2.4%)	0 (0.0%)	0 (0.0%)	0.14
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=300	Survival	97 (97.0%)	95 (95.0%)	97 (97.0%)	0.69
36 months	n=125	Repeat angina	1 (2.3%)	1 (2.6%)	0 (0.0%)	0.57
		Breathlessness	4 (9.3%)	1 (2.6%)	0 (0.0%)	0.08
		Reintervention	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
		Myocardial infarction/ischaemia	1 (2.3%)	0 (0.0%)	0 (0.0%)	0.38
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=133	Survival	43 (95.6%)	38 (88.4%)	44 (97.8%)	0.15
48 months	n=79	Repeat angina	1 (3.8%)	1 (4.2%)	0 (0.0%)	0.55
		Breathlessness	0 (0.0%)	1 (4.2%)	0 (0.0%)	0.31
		Reintervention	0 (0.0%)	1 (4.2%)	0 (0.0%)	0.31
		Myocardial infarction/ischaemia	1 (3.8%)	1 (4.2%)	0 (0.0%)	0.55
		Stroke	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
	n=85	Survival	26 (89.7%)	24 (88.9%)	29 (100.0%)	0.19

Table 36: This table illustrates the incidence of post-operative complications and investigations carried out for the participants post CABG surgery during the follow up period from the day of surgery until 24 months

Variable	**CT-EVH**	**OT-EVH**	**OVH**	**p-value**
Chest wound numbness	57 (57.0%)	39 (40.2%)	52 (52.0%)	**0.05**
Chest wound tenderness	49 (49.0%)	34 (35.1%)	42 (42.4%)	0.14
Leg wound numbness	3 (3.0%)	10 (10.3%)	52 (52.5%)	**<0.001**
Leg wound tenderness	3 (3.0%)	7 (7.2%)	36 (36.4%)	**<0.001**
Arrhythmias	2 (2.0%)	3 (3.0%)	2 (2.1%)	0.87
Atrial fibrillation	2 (2.0%)	3 (3.0%)	4 (4.1%)	0.69
Ventricular fibrillation/tachycardia	0 (0.0%)	0 (0.0%)	0 (0.0%)	---
Pacemaker fitted	2 (2.0%)	2 (2.0%)	1 (1.0%)	0.83
MRI performed	2 (2.0%)	6 (6.0%)	3 (3.1%)	0.30
Angiography performed	2 (2.0%)	5 (5.2%)	5 (5.2%)	0.43
Total Angina until 24 months	4 (4.0%)	7 (7.0%)	6 (6.0%)	
Cause of MACE events until 24 months.				
Vein not used	2*	1	0	---
Native artery disease	1	1*	2	
Previous stent blocked	2*	1	1	
LIMA blocked	1*	2*	2	
Vein graft insertional stenosis	0	2*	2	
Vein graft blockage	0	2*	0	

In addition to the incidences, the detailed causes of MACE events have been listed. *****represents that the same patient had multiple MACE causes.



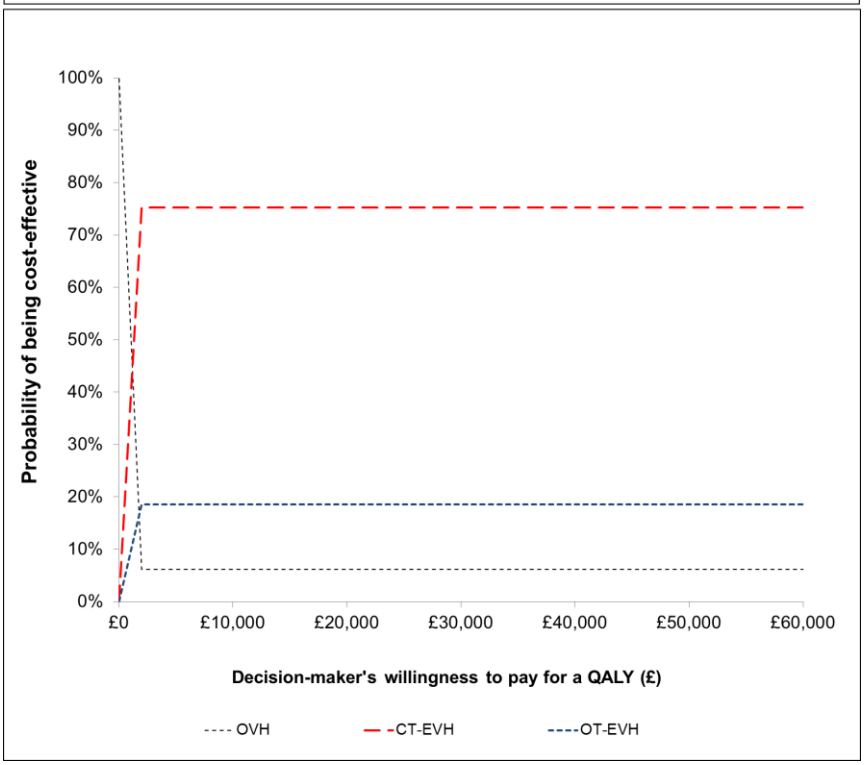


Figure 30: (Above) Cost-effectiveness plane showing incremental costs and QALYs of CT-EVH and OT-EVH versus OVH. Bootstrap replicates show the uncertainty with the larger points showing the point estimates. A cost-effectiveness threshold of £20,000 per QALY is presented.

Figure 31: (Below) Cost-effectiveness acceptability curve for OVH, CT-EVH and OT-EVH.

9.4: SAFETY AND CLINICAL RELEVANCE

At 24 months, composite MACE events were observed in 39 patients; (OVH (12/39), CT-EVH (13/39) and OT-EVH (14/39)). 289 patients survived with non-cardiovascular mortality in 11 patients due to ischemic bowel, pneumonia, liver failure and cancer. No mortality associated with cardiovascular events was observed. Until 24 months, MACE repeat angina events (Table 34) were observed in 17 patients. Follow-up MRI and angiogram evaluation in symptomatic patients concluded that angina was caused by vein grafts not being used due to calcified/small coronaries (3/17), native artery disease progression (4/17), vein graft insertional site stenosis (4/17), vein graft blocked (2/17), previous patent stent blocked (4/17) and left internal mammary artery insertional site stenosis (5/17). Multiple causes were observed in 5 patients.

9.5: DISCUSSION

This is the first study with direct head to head comparison of two EVH techniques with traditional OVH in relation to histological vein integrity and clinical outcomes. We report there was some vein injury in EVH compared to OVH (with loss of endothelial integrity, increased endothelial stretching, and muscle hypertrophy most severe in OT-EVH compared to CT-EVH and OVH). Severe stretching and muscle migration has been associated with graft occlusion (Kanellaki-Kyparissi et al., 2005; Wali and Eid, 2002), yet only a small proportion of vein samples had severe intimal stretching in the OT-EVH group, and our sub-analysis suggested that this was not associated with MACE events.

In 2009, a major non-randomised study concluded that EVH had higher rates of vein graft failure and mortality within 12-18 months post-surgery (Lopes et al., 2009). However, secondary outcomes from our randomised study demonstrate no statistically significant difference in composite or individual MACE scores with EVH from 3 to 24 months. Furthermore, MACE scores did not correlate with vessel injury. This corroborates findings from previous studies describing positive clinical outcomes (Allen et al., 2005; Markar et al., 2010; Sastry et al., 2013) with both EVH and OVH and provides insight into the risk factors for MACE.

Repeat angina and re-intervention in patients in this study were mainly due to grafting technique and technical error (Shah et al., 1995; Krishnamoorthy et al., 2016), poor target artery quality(Hess et al., 2014), progression of native coronary artery diseases (Sabik et al., 2006) and previous stent blockage

post CABG surgery. Whilst the importance of grafting technique is highlighted by our findings, we did not observe significant intra-operator effects on MACE outcomes.

According to the ISMICS consensus statement (Allen et al., 2005), studies comparing OVH versus EVH have focused only on the cost of wound complications(Brandt et al., 2003), readmissions and hospital stay (Puskas et al., 1999) but no analysis of incremental cost-effectiveness has been conducted. Our study highlights that both EVH techniques led to modest net increases in cost versus OVH during surgery. However, both EVH techniques substantially reduced post-surgery costs and improved patients' health-related quality of life leading to relatively large gains in QALYs when compared with other technologies. The use of CT-EVH was associated with lower costs and better outcomes when compared with OT-EVH but higher costs and better outcomes than OVH. Therefore CT-EVH may represent the optimal cost-effective technique for vein harvesting.

9.6: LIMITATIONS

This study was designed to utilise a single experienced practitioner from one centre to determine the impact of harvesting techniques. Different operators will inevitably introduce variability in surgical skills which could confound a true comparison. The practitioner had experience of >2000 OVH cases but only 250 EVH cases and this may have implications on surgical timings, quality of the OVH vein conduit and post-operative complications, which need to be taken into consideration when interpreting the data. Also, not all study participants underwent routine angiogram or cardiac MRI scans due to ethical, financial restrictions within the NHS and risks involved due to patients' age. The current study is underpowered to detect small differences in clinical outcomes as >1000 patients would be required in each arm, which would not be possible for a single centre study. However, this study was designed with graft histology as the primary outcome as this has been understudied to date and is an important area. For these purposes, a single centre, single practitioner model was most appropriate. Also, we performed comparisons of MACE incidence at multiple time points, which could increase the likelihood of type 1 error and obtaining statistically significant results by chance. However, we did not detect statistical differences in individual MACE events at any time point, and so type 1 error did not contribute to our conclusions.

9.7: Conclusion

Our study demonstrated that endoscopic vein harvesting does have minimal damage to the layers of the vein and did not increase the incidence of MACE. Endoscopic vein harvesting also provides better health-related quality of life, QALYs and is more cost effective than open vein harvesting post CABG surgery. Therefore endoscopic vein harvesting can be utilised for vein harvesting safely with appropriate patient selection, equipment and better structured training in future practitioners. This study provides a base for future multicentre studies and also clarifies that histological damage is minimal when practitioners are experienced.

9.8: Acknowledgement

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9.9: Conflict of Interest

There was no conflict of interest.

9.10: Funding and support

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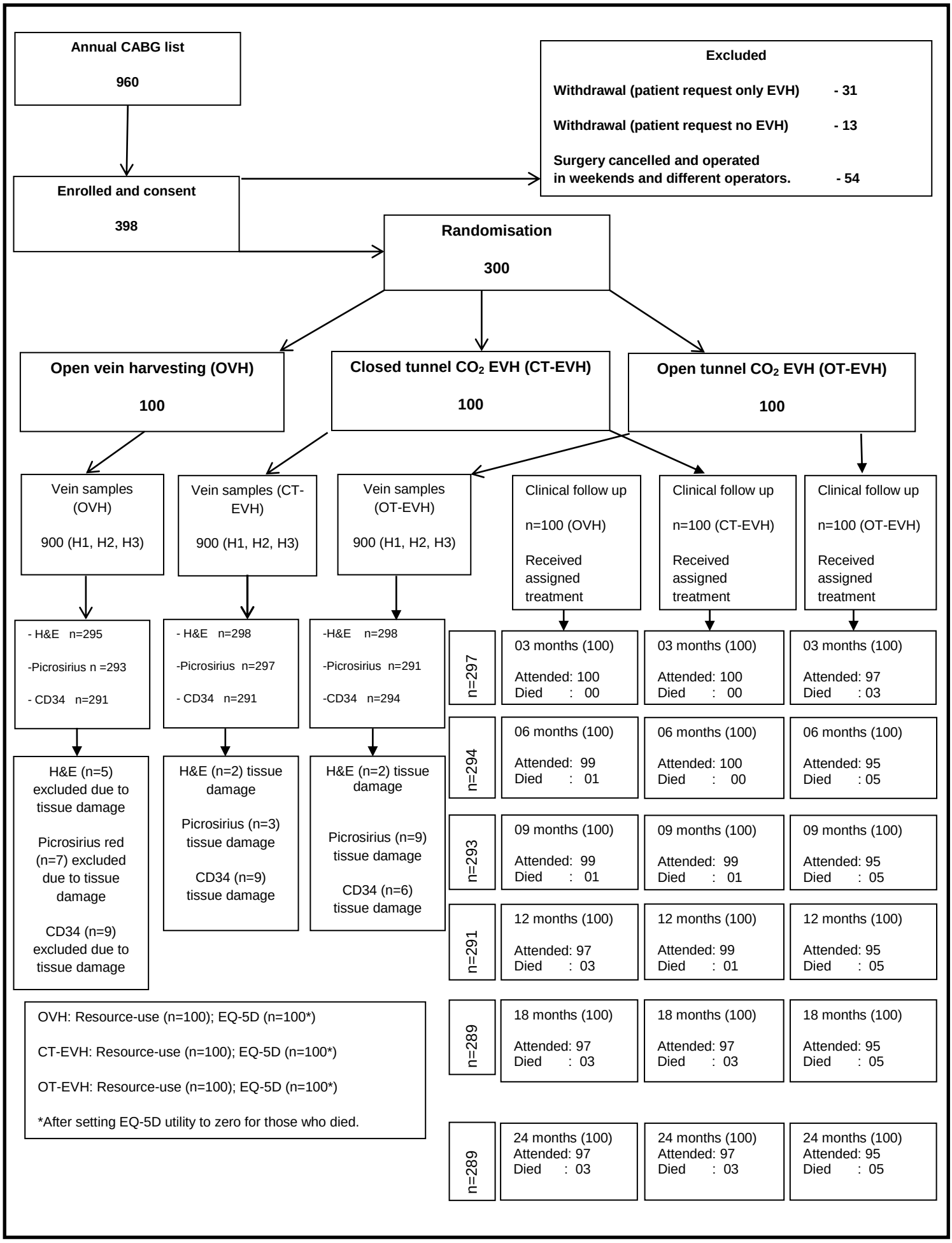
Consort diagram demonstrates the detailed enrolment, treatment and follow up of the VICO trial patients.



CHAPTER 10

Overall discussion and conclusion of the VICO trial studies in this thesis

10: Discussion and Conclusions

This VICO trial addresses several questions in the literature (Lin et al., 2003; Kanellaki-Kyparissi et al., 2005; Dashwood and Loesch, 2014; Cook et al., 2004; Angelini et al., 1989b) including a major non-randomised study (Lopes et al., 2009) published by the New England Journal of Medicine in 2009. Although, recent research articles have published evidence suggesting there are no significant differences between vein harvesting methods, there is still a paucity of well-powered, randomised controlled trials to determine the safety and efficacy of EVH.

This thesis brings together the impact of carbon dioxide absorption on the vein during vein harvesting, histological evidence of pressure related distension on the veins during surgical preparation, whether vein integrity on different layers has any direct correlation with clinical outcomes and finally cost effectiveness and health related quality of life in patients who have undergone different vein harvesting surgical techniques. By writing the PhD in alternative thesis, the chapters incorporated in this study have been exposed to an external peer review process.

This final chapter provides a transitory summary of the overall results and the strengths and limitations of each study. In addition, the chapter will also have any clinical recommendations from this research and also for future research.

10.01: DISCUSSION OF PRINCIPAL OUTCOMES

10.1.1: VALIDATION OF THE ENDOTHELIAL MARKERS

10.1.1: STRENGTHS OF THIS STUDY

This study (Chapter 6) was conducted to validate the available endothelial markers and choose the most appropriate marker vein sample assessment. It provides evidence that CD34 is a more reliable endothelial marker for LSV than CD31. Predominately, previous studies that have focused on endothelial damage on the LSV used CD31. CD31 is a 130-kDa transmembrane glycoprotein with homogenous expression especially on human pulmonary endothelial cells (Muller et al., 2002). In contrast, CD34 is a 110-kDa transmembrane glycoprotein with heterogeneous expression especially on endothelial cells of capillaries, veins, arteries, arterioles and venules (Kawanami et al., 2000). These studies clearly indicate that the CD31 is better for the major vessels for their homogenous expression. However, CD34 is specifically expressed in small vascular structures. Overall this validation experiment demonstrated that CD34 had a significant pattern of expression in terms of more distinct colour, intense staining and was widely distributed across the long saphenous vein tissue compared to CD31. This study provided novel evidence regarding the selection of appropriate endothelial markers. The findings have substantial relevance for future IHC vein assessment.

10.1.2: LIMITATIONS OF THIS STUDY

This study was a single centre study with only 10 vein samples, which can raise the generalisability of the findings. To further validate the findings of this study we would need multiple experiments with different antibodies and positive control. Some antibodies require samples to be conserved in a precise manner. The concentration, incubation time and antigen retrieval is also being different between antibodies. We used normal horse serum for antigen retrieval but there are other serums (i.e.: goat, donkey and sheep) that can have different effects.

The other limitation of this study is different IHC makers such as ERG1 antibody which is very good and provides clear nuclear staining than other current markers. Current literature claims that ERG1, ETS family transcription factor is very good in expressing the endothelial cells than CD31 and CD34 (Miettinen et al., 2011). In presence of necrosis and haemorrhage on the tissues, the CD31 can cause diffuse immunoreactivity which can be misinterpreted.

10.2: Effect of carbon dioxide on harvested veins

10.2.1: Strengths of the study

This study (Chapter 7) was established to assess if carbon dioxide (CO_2) insufflation causes histological damage to vein tissues or alters systemic parameters. The study demonstrated that minor histological damage on the vein was not caused by low pH or pressurised (10mmHg) tunnel on the leg. We also observed that the closed tunnel CO_2 EVH group had high partial pressure oxygen and partial pressure CO_2 levels but this did not correlate with tissue damage to the vein. Endothelial stretching was high in the open tunnel CO_2 EVH group which may be due to manual dissection/handling without a pressurised tunnel. This is first study directly comparing tissue damage in a pressurised tunnel and non-pressurised tunnel.

10.2.2: Limitations of the study

Studies have highlighted that heparin rapidly crosses the arterial wall and can cause damage to the endothelium (Lovich and Edelman, 1995). Patients who underwent CT-EVH received heparin (5000 units) or a heparin flush, which was not given to those in the other two groups according to local standard endoscopic guidelines. Other studies also demonstrated that heparin injections can cause a moderate level of endothelial damage (Kinhult et al., 2003). Interestingly, the patients will be fully heparinised (vary from 20000 to 25000units) prior to cardiopulmonary bypass and it will be circulating in the vascular system for 2 to 3 hours. So, the effect of administering 5000units heparin for EVH will affect the endothelium of the vein is questionable. This area need to be explored by a well-designed trial to understand the role of heparin on the veins.

Our study focused only on the structural integrity of the vein but functional viability is also an important factor for nitric oxide production, a potent endothelium-dependent vasorelaxant synthesised from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS) (Krishnamoorthy et al., 2016).

10.3: HISTOLOGICAL EVIDENCE OF DISTENDED AND NON-DISTENDED LONG SAPHENOUS VEIN

10.3.1: STRENGTHS OF THE STUDY

This study (Chapter 8) explored the structural differences and damage caused by the distension and non-distension of the saphenous vein harvested by three different surgical techniques. There remains a significant paucity in the literature with regards to how different vein harvesting techniques impact the structural layer of the conduit. Moreover, no study has been performed that clearly demonstrates the extent to which surgical preparation, manual handling and distension of the vein between harvesting and grafting induces changes to venous structure, therefore affecting conduit quality. The open vein harvesting technique was associated with better preservation of the endothelium on proximal samples. The distal and random samples had no significant differences between the groups. Both EVH surgical techniques demonstrated some degree of structural damage to the vessel wall. However, OT-EVH displayed more endothelial disruption than CT-EVH and open vein harvesting. From this study, EVH can be adopted safely but with careful patient selection and EVH equipment.

10.3.2: LIMITATIONS OF THE STUDY

To determine the full impact of structural damage, it is important to assess the vasomotor function and changes of gene expression but these were outside the remit of this study. In addition, we did not look at the functional viability of the vein by electron microscopy and fluorescence; which may provide some additional data.

10.4: HISTOLOGICAL DAMAGE HAS NO DIRECT IMPACT ON CLINICAL OUTCOMES AND COST EFFECTIVENESS

10.4.1: STRENGTH OF THE STUDY

This study (Chapter 9) assessed whether histological level damage has any direct correlation on clinical outcomes post coronary artery bypass surgery. Open vein harvesting produced better vessel integrity in all layers of the vein compared to both endoscopic vein harvesting techniques. However, the differences in damage between the groups were minimal. The secondary clinical outcomes of our study demonstrated no significant differences between all three groups at different time points. The individual and composite MACE scores were also similar between groups. The closed tunnel endoscopic group had better health related quality of life scores and was also more cost-effective than the other two techniques.

10.4.2: LIMITATIONS OF THE STUDY

To understand the full impact of these surgical techniques, an angiogram on all patients post-surgery would be the best method to assess patency of the bypassed vein. However, it is very psychologically distressing for the patients to know that their grafts are getting blocked even though they do not have any clinical symptoms. Due to the age factor it is not possible for all patients to undergo angiogram because renal function reduces with ageing. To validate the findings of this study, a multicentre trial would be the best option. However, as it was conducted at a single centre and with a single practitioner, this study did enable specific evaluation of the techniques without any operator induced variability.

10.5: RECOMMENDATION FROM THIS STUDY

As a result of the study findings there are a number of recommendations that have emerged.

10.5.1: IMPLICATIONS FOR CLINICAL PRACTICE

- The use of a less pressurised tunnel with 10 mmHg carbon dioxide insufflation during endoscopic vein harvesting would provide optimum pressure rather than the company recommendation of 12 – 15mmHg. The risk of carbon dioxide embolism, pressure related vein damages and low pH are minimal with a low pressurised tunnel. Therefore, this study could result in changes to current clinical practice and change in company guidelines.
- We have found that there was no direct relationship between histological damage and clinical outcomes on post coronary artery bypass surgery patients. This study also highlighted that the quality of the vein improves by experience of the practitioner. The quality of the EVH training programme remains to be challenging. Attention should be given to improving the training programme and the need for a multicentre trial.

10.5.2: RECOMMENDATIONS FOR FUTURE RESEARCH

A number of recommendations for future research can be made, such as:

- A larger, multicentre study is needed to validate the findings of this single centre study. It was not possible for a PhD student to obtain a larger sample size of more than >1000 in each group to see differences in clinical Major Cardiac Adverse Events (MACE) outcomes. The future study should incorporate histological damage, clinical outcomes, angiographic imaging to detect any different grade graft stenosis, cost analysis and health related quality of life between different types of vein harvesting techniques.
- It is important to understand the current EVH training programme and design a structured training programme to improve the quality of vein conduits obtained by novice practitioners.

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Appendix 1: Search history in Medline for literature review (1).

Number	Place	Keywords	Results
01	MEDLINE	"coronary artery bypass graft".ti,ab;	7620
02	MEDLINE	cabg.ti,ab;	12763
03	MEDLINE	"coronary artery bypass surgery".ti,ab;	6407
04	MEDLINE	*CORONARY ARTERY BYPASS/;	28780
05	MEDLINE	1 OR 2 OR 3 OR 4;	38231
06	MEDLINE	(vein* AND harvesting).ti,ab;	800
07	MEDLINE	*SAPHENOUS VEIN/tr [tr=Transplantation];	3319
08	MEDLINE	*TISSUE AND ORGAN HARVESTING/;	3605
09	MEDLINE	vein*.ti,ab;	166259
10	MEDLINE	8 AND 9;	422
11	MEDLINE	6 OR 7 OR 10;	4093
12	MEDLINE	5 AND 11;	1514
13	MEDLINE	exp "OUTCOME ASSESSMENT (HEALTH CARE)"/ OR exp POSTOPERATIVE COMPLICATIONS/;	1054927
14	MEDLINE	exp TREATMENT OUTCOME/;	654813
15	MEDLINE	(outcom* OR test* OR measur*).ti,ab;	4659219
16	MEDLINE	(leg AND infect*).ti,ab;	3484
17	MEDLINE	SURGICAL WOUND INFECTION/;	28348
18	MEDLINE	13 OR 14 OR 15 OR 16 OR 17;	5319057
19	MEDLINE	12 AND 18;	999
20	MEDLINE	19 [Limit to: Publication Year 1985-2014 and (Clinical Queries Reviews best balance of sensitivity and specificity or Therapy best balance of sensitivity and specificity or Diagnosis best balance of sensitivity and specificity or Prognosis best balance of sensitivity and specificity)]; 405 results.	405

Appendix 2: Search history in Medline for literature review (2).

Number	Place	Keywords	Results
01	MEDLINE	(coronary AND artery AND bypass AND graft).ti,ab;	12869
02	MEDLINE	(coronary AND artery AND bypass AND surgery).ti,ab;	21940
03	MEDLINE	CABG.ti,ab;	12768
04	MEDLINE	*CORONARY ARTERY BYPASS/;	28780
05	MEDLINE	1 OR 2 OR 3 OR 4;	43974
06	MEDLINE	harvest*.ti,ab;	67536
07	MEDLINE	*TISSUE AND ORGAN HARVESTING/;	3605
08	MEDLINE	6 OR 7	69548
09	MEDLINE	5 AND 8;	1333
10	MEDLINE	exp COSTS AND COST ANALYSIS/;	181481
11	MEDLINE	exp COST-BENEFIT ANALYSIS/ OR exp ECONOMICS, MEDICAL/ OR exp HEALTH CARE COSTS/;	109631
12	MEDLINE	(cost* OR economic* OR evaluat* OR NHS OR analysis).ti;	1039881
13	MEDLINE	10 OR 11 OR 12;	1161567
14	MEDLINE	9 AND 13;	85
15	MEDLINE	14 [Limit to: Publication Year 1950-2014	85
16	EMBASE	(coronary AND artery AND bypass AND graft).ti,ab;	15446
17	EMBASE	(coronary AND artery AND bypass AND surgery).ti,ab;	27153
18	EMBASE	CABG.ti,ab;	20109
19	EMBASE	harvest*.ti,ab;	83357
20	EMBASE	(cost* OR economic* OR evaluat* OR NHS OR analysis).ti;	1215610
21	EMBASE	*CORONARY ARTERY BYPASS GRAFT/ OR *CORONARY ARTERY BYPASS SURGERY/;	28340
22	EMBASE	16 OR 17 OR 21;	46169
23	EMBASE	*HARVESTING/;	560
24	EMBASE	19 OR 23;	83592
25	EMBASE	22 AND 24;	1429

26	EMBASE	exp COST/ OR exp COST BENEFIT ANALYSIS/ OR exp COST CONTROL/ OR exp COST EFFECTIVENESS ANALYSIS/ OR exp COST MINIMIZATION ANALYSIS/;	390265
27	EMBASE	exp HEALTH ECONOMICS/;	613452
28	EMBASE	20 OR 26 OR 27;	1747191
29	EMBASE	25 AND 28;	113
30	EMBASE	29 [Limit to: Publication Year 1950-2014];	113
31	MEDLINE &EMBASE	Duplicate filtered: [14 [Limit to: Publication Year 1950-2014]], [29 [Limit to: Publication Year 1950-2014]];	664 & 60

Appendix 3: Downs and Black checklist for non-randomised studies.

Criteria	Description of criteria (with additional explanation as required, determined by consensus of raters)	Possible answers	score
1	Is the hypothesis/aim/objective of the study clearly described? Must be explicit	yes/no	1/0
2	Are the main outcomes to be measured clearly described in the Introduction or Methods section? If the main outcomes are first mentioned in the Results section, the question should be answered no. ALL primary outcomes should be described for YES	yes/no	1/0
3	Are the characteristics of the patients included in the study clearly described? In cohort studies and trials, inclusion and/or exclusion criteria should be given. In case-control studies, a case-definition and the source for controls should be given. Single case studies must state source of patient	yes/no	1/0
4	Are the interventions of interest clearly described? Treatments and placebo (where relevant) that are to be compared should be clearly described.	yes/no	1/0
5	Are the distributions of principal confounders in each group of subjects to be compared clearly described? A list of principal confounders is provided. YES = age, severity	yes/no	1/0
6	Are the main findings of the study clearly described? Simple outcome data (including denominators and numerators) should be reported for all major findings so that the reader can check the major analyses and conclusions.	yes/no	1/0
7	Does the study provide estimates of the random variability in the data for the main outcomes? In non-normally distributed data the inter-quartile range of results should be reported. In normally distributed data the standard error, standard deviation or confidence intervals should be reported	yes/no	1/0
8	Have all important adverse events that may be a consequence of the intervention been reported? This should be answered yes if the study demonstrates that there was a comprehensive attempt to measure adverse events (COMPLICATIONS BUT NOT AN INCREASE IN PAIN).	yes/no	1/0

9	Have the characteristics of patients lost to follow-up been described? If not explicit = NO. RETROSPECTIVE – if not described = UTD; if not explicit re: numbers agreeing to participate = NO. Needs to be >85%	yes/no	1/0
10	Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the main outcomes except where the probability value is less than 0.001?	yes/no	1/0
11	Were the subjects asked to participate in the study representative of the entire population from which they were recruited? The study must identify the source population for patients and describe how the patients were selected.	yes/no/UTD	1/0/0
12	Were those subjects who were prepared to participate representative of the entire population from which they were recruited? The proportion of those asked who agreed should be stated.	yes/no/UTD	1/0/0
13	Were the staff, places, and facilities where the patients were treated, representative of the treatment the majority of patients receive? For the question to be answered yes the study should demonstrate that the intervention was representative of that in use in the source population. Must state type of hospital and country for YES.	yes/no/UTD	1/0/0
14	Was an attempt made to blind study subjects to the intervention they have received? For studies where the patients would have no way of knowing which intervention they received, this should be answered yes. Retrospective, single group = NO; UTD if > 1 group and blinding not explicitly stated	yes/no/UTD	1/0/0
15	Was an attempt made to blind those measuring the main outcomes of the intervention? Must be explicit.	yes/no/UTD	1/0/0
16	If any of the results of the study were based on “data dredging”, was this made clear? Any analyses that had not been planned at the outset of the study should be clearly indicated. Retrospective = NO. Prospective = YES	yes/no/UTD	1/0/0
17	In trials and cohort studies, do the analyses adjust for different lengths of follow-up of patients, or in case control studies, is the time period between the intervention and outcome the same for cases and controls? Where follow-up was the same for all study patients the answer should yes.	yes/no/UTD	1/0/0

	Studies where differences in follow-up are ignored should be answered no. Acceptable range 1 yr follow up = 1 month each way; 2 years follow up = 2 months; 3 years follow up = 3months.....10years follow up = 10 months		
18	Were the statistical tests used to assess the main outcomes appropriate? The statistical techniques used must be appropriate to the data. If no tests done, but would have been appropriate to do = NO	yes/no/UTD	1/0/0
19	Was compliance with the intervention/s reliable? Where there was non-compliance with the allocated treatment or where there was contamination of one group, the question should be answered no. Surgical studies will be YES unless procedure not completed.	yes/no/UTD	1/0/0
20	Were the main outcome measures used accurate (valid and reliable)? Where outcome measures are clearly described, which refer to other work or that demonstrates the outcome measures are accurate = YES. ALL primary outcomes valid and reliable for YES	yes/no/UTD	1/0/0
21	Were the patients in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited from the same population? Patients for all comparison groups should be selected from the same hospital. The question should be answered UTD for cohort and case control studies where there is no information concerning the source of patients	yes/no/UTD	1/0/0
22	Were study subjects in different intervention groups (trials and cohort studies) or were the cases and controls (case-control studies) recruited over the same time? For a study which does not specify the time period over which patients were recruited, the question should be answered as UTD. Surgical studies must be <10 years for YES, if >10 years then NO	yes/no/UTD	1/0/0
23	Were study subjects randomised to intervention groups? Studies which state that subjects were randomised should be answered yes except where method of randomisation would not ensure random allocation.	yes/no/UTD	1/0/0
24	Was the randomised intervention assignment concealed from both patients and health care staff until recruitment was complete and irrevocable? All non-randomised studies should be answered no. If assignment was concealed from patients but not from staff, it should be answered no.	yes/no/UTD	1/0/0
25	Was there adequate adjustment for confounding in the analyses from which the main findings were drawn? In nonrandomised studies if the effect of the main confounders was not investigated or no adjustment was made in the	yes/no/UTD	1/0/0

	final analyses the question should be answered as no. If no significant difference between groups shown then YES		
26	Were losses of patients to follow-up taken into account? If the numbers of patients lost to follow-up are not reported = unable to determine.	yes/no/UTD	1/0/0
27	Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance <5% Sample sizes have been calculated to detect a difference of x% and y%.	1-5	

* UTD: Unable To Determine.

Appendix 4: The Jadad scale.

The first five items are indications of good quality, and each counts as one point towards an overall quality score. The final two items indicate poor quality and a point are subtracted for each if its criteria are met. The range of possible scores is 0 to 5.

1. Was the study described as randomised (this includes words such as randomly, random and randomisation?

Yes = 1, No = 0.

2. Was the method used to generate the sequence of randomisation described and appropriate (table of random numbers, computers-generated, etc)?

Yes = 1, No = 0.

3. Was the study described as double blind?

Yes = 1, No =0.

4. Was the method of double blinding described and appropriate (identical placebo, active placebo, dummy, etc)?

Yes = 1, No =0.

5. Was there a description of withdrawals and dropouts?

Yes = 1, No =0.

6. Deduct one point if the method used to generate the sequence of randomisation was described and it was inappropriate (e.g: patients were allocated alternately, or according to date of birth, hospital number etc).

Described but inappropriate = -1, Described and appropriate = 0.

7. Deduct one point if the study was described as double blind but the method of blinding was inappropriate (e.g. comparison of tablet vs. injection with no double dummy).

Described but inappropriate = -1, described and appropriate = 0.

Appendix 5: A detailed protocol of Varistain 24-4 Haematoxylin and Eosin (H&E) Staining method.

It was obtained from The University of Manchester, Histology laboratory at AV Hill building.

Steps	Solutions	H&E Staining
1	Xylene	5.00 min
2	Xylene	3.00 min
3	Xylene	3.00 min
4	Ethanol 100%	3.00 min
5	Ethanol 100%	2.00 min
6	Industrial Methylated Spirit (IMS) 90%	2.00 min
7	IMS 70%	2.00 min
8	Tap Water	2.00 (Change daily when in use).
9	Haematoxylin Gills	2.00 min (Filter daily when in use).
10	Tap water	1.00 min (Change after each run).
11	Acetic Acid 5%	0.10 (Change weekly).
12	Tap water	1.00 (Change daily when in use).
13	Blueing Reagent	0:30 sec (Change weekly).
14	Tap water	2:00 min
15	IMS 70%	1.00 min
16	IMS 90%	1.00 min
17	Ethanol 95%	1.00 min
18	Eosin alcoholic	1.30 min
19	Ethanol 100%	2.00 min
20	Ethanol 100%	2.00 min
21	Ethanol 100%	2.00 min
22	Xylene	2.00 min
23	Xylene	3.00 min
24	Xylene	5.00 min
END	Distyrene, Plasticiser and Xylene (DPX), colourless resin medium.	coverslip

This fully automated staining programme takes about 50minutes, which includes the changeover times from one solution to another solution.

Appendix 6: A detailed protocol of Varistain 24-4 Picrosirius Red staining programme.

It was obtained from The University of Manchester, Histology laboratory at AV Hill building.

Step	Solutions	Picrosirius Red Staining
1	Xylene	5.00 min
2	Xylene	3.00 min
3	Xylene	3.00 min
4	Ethanol 100%	3.00 min
5	Ethanol 100%	2.00 min
6	Industrial Methylated Spirit (IMS) 90%	2.00 min
7	IMS 70%	2.00 min
8	Tap Water	2.00 min
9		PASS
10		PASS
11		PASS
12		PASS
13		PASS
14		PASS
15		PASS
16		PASS
17		PASS
18	Picrosirius Red	59.00 min
19	1% Acetic Acid	00:05 (change after each run)
20	Ethanol 100%	00:30 (dispose after two runs)
21	Ethanol 100%	00:45 (Move to position “20” then replace with fresh)
22	Xylene	2.00 min
23	Xylene	2.00 min
24	Xylene	2.00 min
Final	Distyrene, Plasticiser and Xylene (DPX), colourless resin medium.	coverslip

Total fully automated programme takes about 1.5 hours, which includes the changeover times from one solution to another solution.