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Research Article

Endoscopic Vein Harvesting is Associated with Increased Endothelial Micro-Particle Secretion - A Randomised *Ex-Vivo* Analysis

Bhuvaneswari Krishnamoorthy[1,2,3]*, Jung Y Tsang[3], William R Critchley[3], Nizar Yonan[2], James Fildes[3] and Rajamiyer V Venkateswaran[2]

[1]Faculty of Health and Social Care, Edge Hill University, Ormskirk, Lancashire, United Kingdom

[2]Department of Cardiothoracic Surgery, University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom

[3]The Manchester Collaborative Centre for Inflammation Research, Faculty of Medical and Human Sciences, University of Manchester, United Kingdom

***Corresponding Author:** Bhuvaneswari Krishnamoorthy, Lead Surgical Care Practitioner, Cardiothoracic surgery, Manchester Foundation Trust, Programme Director/Senior Lecturer at Edgehill University for MSc Surgical Practice, Honorary Research Associate at The University of Manchester, University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom.

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Abstract

Objectives: Endoscopic vein harvesting (EVH) for coronary artery bypass graft surgery has been associated with both positive and negative clinical outcome in a range of studies. In the worst cases, relationships with increased occlusion, myocardial infarction and mortality have been described. EVH could induce endothelial damage leading to loss of graft function. In an attempt to assess endothelial integrity, this study was designed to compare endothelial injury using endothelial micro-particle secretion as a sensitive marker of dysfunction in vessels harvested using endoscopic vs standard techniques.

Methods: A prospective randomised and technician blinded study design was used to compare standard open technique (SOT, n = 10) versus EVH (n = 5). Once the vein had been harvested, 2cm was cut after being filled with heparinised blood and clipped on either end. All the solution in the vein lumen was washed out using PBS and analysed using flow cytometry to quantify EMPs.

Results: There were significantly more Annexin V+ CD31+ EMPs in the EVH group compared to the SOT group (mean number of events = 454.0 vs 86.5, p = 0.001), and also a significant increase in Annexin V+ MPs in the EVH group (mean number of events = 4558.4 vs 1238.3, p = 0.027).

Conclusion: In this *ex-vivo* study, EMP secretion was elevated in vessels harvested using endoscopic compared to standard open techniques, which is indicative of endothelial injury. This could contribute to the negative associations with EVH. However, our three year follow up clinical data demonstrates no difference in the incidence of repeat angina or re-intervention between these groups. Long-term clinical data is clearly warranted to translate these findings with larger sample size.

Keywords: *Coronary Artery Bypass; Saphenous Vein; Endothelial Micro-Particle; Flow Cytometry; Ex-Vivo; Clinical Outcomes; Micro-Particle; Endothelial Damage*

Introduction

In modern health care systems, surgical specialties are increasingly aiming for smaller post-operative scars through the use of minimally invasive surgical techniques. In coronary artery bypass graft surgery (CABG), the introduction of endoscopic vein harvesting (EVH) for saphenous vein retrieval sparked excitement. Initial studies demonstrated a significant improvement in patient outcomes, with reduced pain, lower incidence of leg wound complications and enhanced patient satisfaction using EVH in comparison to standard

open harvesting techniques. As such, the technique promptly gained the recommendation of International Society for Minimally Invasive Cardiothoracic Surgery (ISMICS) as a standard of care.

Further laboratory and clinical based research is continually being developed and other studies have since published conflicting data with positive and negative outcomes of EVH. It is clear that there are inconsistencies surrounding EVH techniques, and hence there is an apparent need for further research into this field.

Endothelial damage has long been postulated as a mechanism involved in vein graft failure and may contribute to the negative results obtained for EVH [1]. As a measure of this, endothelial micro-particles (EMPs), which have gained recent attention, have been shown to be released during the activation and apoptosis of endothelial cells. Different studies have also demonstrated its role as a marker for endothelial damage and even prognosis of certain pathologies. Our study seeks to compare the endothelial damage incurred by different vein harvesting techniques via the measurement of EMPs, along with a further exploration into the role of EMPs as a marker for endothelial integrity.

Materials and Methods

Patient selection

From June 2011 to July 2011, all patients undergoing CABG with or without concomitant valve surgery in the University Hospital of South Manchester were eligible to enrol for the study. The patients were randomised into two groups as group 1: Standard Open vein harvesting Technique (SOT, n = 10) and group 2: Endoscopic vein harvesting (n = 5 open tunnel CO_2 technique and n = 5 closed tunnel CO_2 technique). The computerised randomisation schedule was provided by an Independent Statistician and the sealed envelope was opened just after the patient has been anaesthetised. Patients were excluded if they had vascular injuries, varicose veins, emergency CABG surgery and any medical history of bleeding diatheses. Baseline pre-operative demographics and clinical outcome data (including the incidence of repeat angina, re-intervention, mortality and myocardial infarction) were recorded. Informed written consent was obtained for all patients and this study was approved by the North West Greater Manchester Ethics Committee.

Surgical technique and sample acquisition

For the SOT, a continuous longitudinal incision was made, and surrounding tissues dissected, taking care not to damage the vein by using a non-touch technique. For EVH techniques, both the open and closed tunnel CO_2 systems were utilised. The open tunnel CO_2 system consisted of a small 2 cm horizontal cut below the knee and used the Sorin Clearglide® system. The closed tunnel CO_2 system started with a 2 cm horizontal incision above the knee, and was followed by the administration of 5000 units of heparin before adopting the Maquet VasoView™ Hemopro system [2].

Once the vein had been removed, 2 clips were placed onto the vein, one at the distal end and the other 2 cm above that point, ensuring that the vein was still filled with heparinised blood. The vein was then cut with a scalpel blade just above the position of the second clip, to free the 2 cm vein sample and allow transfer into a sample pot for immediate transportation to the laboratory for analysis.

Sample processing and endothelial microparticle analysis (EMPs)

In the laboratory, the 2 metal clips that were applied to the vein were removed. The lumen of the vein was gently flushed with 500 µl of PBS, taking with it the small amount of heparinised patient serum remaining in the previously clipped vein, and the resulting solution micro-centrifuged at 13,000g for 20 minutes in order to obtain a platelet depleted solution. The supernatant was retained, and 400 µl of the solution was then incubated for 30 minutes at room temperature with 100 µl PBS-calcium (made with 0.05g calcium chloride mixed thoroughly with 20 ml PBS solution and filtered) and 2.5 µl of each of Cy5-conjugated Annexin V (BD Biosciences) and PE-Dyomics590-conjugated CD31 (Abcam) fluorescent monoclonal antibodies. A further 100 µl PBS-calcium was then added to the solution, along with polystyrene micro-beads for size calibration. The sample was then analysed using an LSR II flow cytometer (BD Biosciences) on medium-flow, and the number of events was recorded for 180 seconds.

Statistical methods

The primary end-point of the study was to analyse the absolute number of EMPs released from the endothelium of each of the SOT and EVH groups. This was achieved via the identification of normality via the Kolmogorov-Smirnov test, and then a comparison using the Independent Samples *t* test. The baseline and pre-operative characteristics of each patient, as well as the properties of the sample vein were compared from the two groups using the Independent Samples *t* test for continuous variables and χ^2 test for categorical variables (applying the Yates' Correction for continuity when necessary for analysing 2 by 2 tables). All statistical calculations were completed on SPSS 19.0 software. Data was deemed significant when the p-value was less than 0.05.

Results

The study group was made up of a total of 15 patients, with 10 in the SOT group and 5 in the EVH group, reflecting the frequencies of each type of practice used inside the University Hospital of South Manchester. The mean age of enrolment was 67.3 ± 10.7, with the majority being male patients (73.3%). Five patients samples in the EVH group (n = 4 in Open tunnel CO_2 and n = 1 in Closed tunnel CO_2) were discarded due to sample damage or flow cytometer system breakdown with storage duration exceeding 3 hours.

Demographics

All patient demographics and background variables were comparable between the 2 groups, with factors all exhibiting insignificance (Table 1). Veins were harvested by 3 trained practitioners, one of which was a senior practitioner, with the proportions for each group showing comparability (p = 0.326). Other peri-operative characteristics were also comparable, also shown in table 2. For the EVH group, one patient had their vein harvested via the open tunnel CO_2 system, whereas the other 4 patients had the closed tunnel CO_2 system.

Factor	SOT (n = 10)	EVH (n = 5)	p value
Baseline Characteristics			
Age	64.9 ± 12.2	72.0 ± 4.9	0.437
Female sex	20%	40%	0.930
BMI (kg/m^2)	29.7 ± 4.1	31.6 ± 3.53	0.173
Diabetes	33.3%	20%	0.579
Hypertension	70%	80%	0.923
Hypercholesterolaemia	70%	20%	0.265
Smoking history	60%	80%	0.739
Stable angina	66.7%	80%	0.597
Unstable angina	20%	0%	0.733
Previous MI	40%	60%	0.687
Cardiac family history	40%	40%	0.803
Multivessel disease	70%	100%	0.437
COPD	30%	40%	0.930
Preoperative Medications			
Beta blocker	90%	100%	0.439
Calcium antagonist	10%	20%	0.649
ACE inhibitor	50%	60%	0.872
Long-acting nitrate	30%	40%	0.803
Diuretic	40%	20%	0.739
Lipid lowering therapy	70%	80%	0.923
Antidepressant	10%	20%	0.649
Aspirin	90%	100%	0.439
Aspirin stopped before operation	80%	60%	0.377
Operative characteristics			
No of grafts used for surgery	2.6 ± 0.6	2.4 ± 0.5	0.245
IMA grafted	90%	80.0%	0.887
No. of grafts harvested	2.3 ± 0.5	1.8 ± 0.8	0.156
Vein harvested by senior practitioner	60%	40.0%	0.326

Table 1: *A table showing the baseline and peri-operative characteristics for each of the vein harvest group, either as the mean ± standard deviation or as a percentage.*

Sample properties

In terms of obtaining a standard sample, variations in length, diameter and weight were noted, though they were not significantly different between the 2 groups (Table 2). The average length of all the samples was 1.88 cm (± 0.17) and the average weight was 0.14g (± 0.05). Note that most samples took over 2 hours to analyse from the acquisition of the sample to having finished flow cytometric analysis.

Sample properties	SOT n = 10	EVH n = 5	p value
Length (cm)	1.86 ± 0.16	1.92 ± 0.22	0.745
Diameter (cm)	0.45 ± 0.10	0.47 ± 0.22	0.662
Area (cm^2)	2.64 ± 0.61	2.89 ± 1.64	0.627
Weight (g)	0.13 ± 0.04	0.16 ± 0.05	0.122
Time taken to analyse (min)	138.20 ± 29.44	150.80 ± 37.86	0.404

Table 2: *A table showing the properties of the samples obtained for each group, all as the mean value ± standard deviation.*

Micro-particle analysis

Both the number of Annexin V+ MPs and Annexin V+ CD31+ MPs in each sample were recorded (Figures 1 and 2). There was a significant increase in Annexin V+ CD31+ MPs in the EVH group compared to the SOT group (mean number of events: 454.0 vs 86.5, p = 0.001) and an increase in Annexin V+ MPs in the EVH group which was also significant (mean number of events = 4558.4 vs 1238.3, p = 0.027).

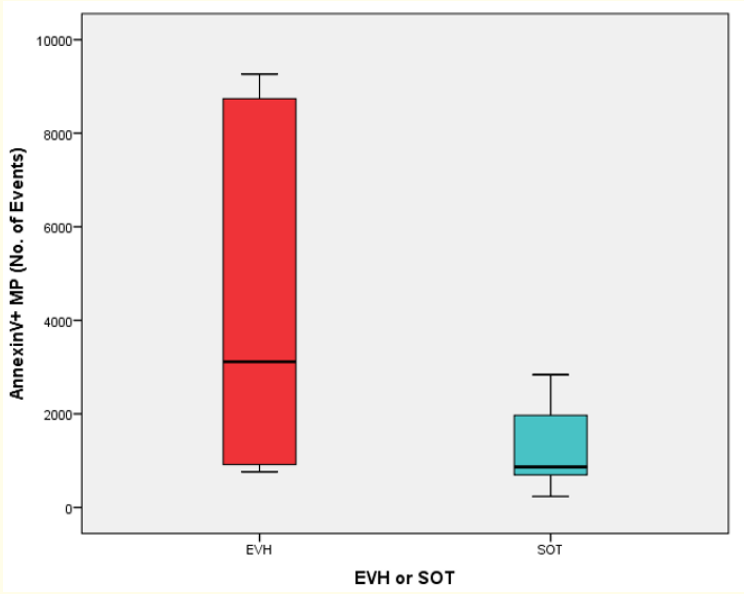


Figure 1: *A box plot showing the distribution of Annexin V+ microparticles in each of the two groups. A significant increase was noted in the EVH group (p = 0.027).*

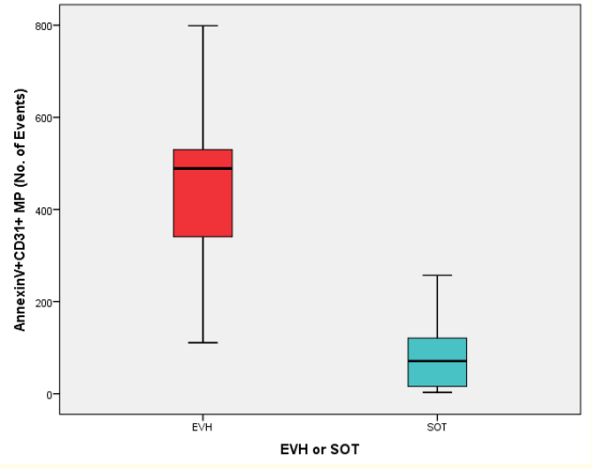
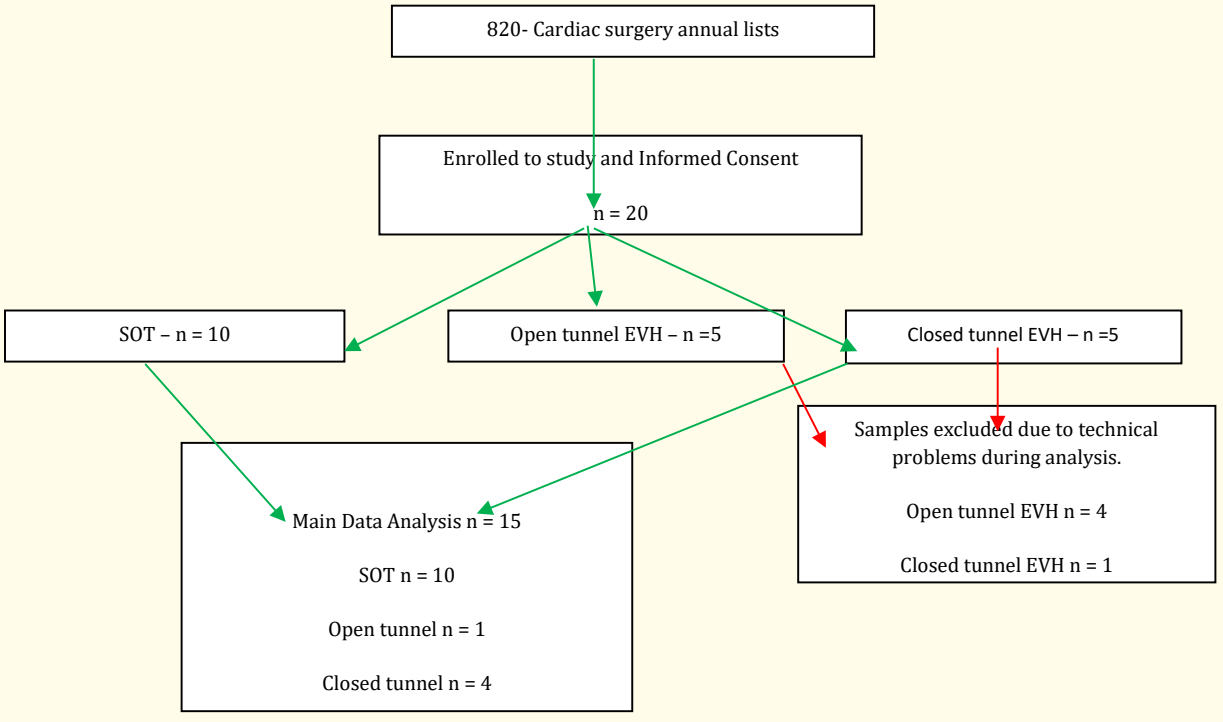


Figure 2: *A box plot showing the distribution of Annexin V+ CD31+ microparticles in each of the two groups. Note the significant increase in Annexin V+ CD31+ microparticles in the EVH group (p = 0.001).*

Clinical outcomes

Over the 3 year follow-up period, no patients in either group experienced repeat angina or myocardial infarction. In addition, all patients across the groups have survived up until 3 years. Two patients had a permanent pacemaker within 2 months of surgery (1 patient in the SOT group and 1 patient in the closed tunnel CO_2 EVH group, p = 1.000). As such, there does not appear to be any clinically apparent differences between the groups with regard to the incidence of major adverse cardiac events, despite the disparities in endothelial micro particle release.



Flow Chart: *Consort study flow diagram.*

Discussion

This study provides an in depth comparison of the number of apoptotic EMPs released into the circulation between traditional open and endoscopic harvesting techniques. Although a significant difference has been noted in the number of Annexin V+ CD31+ EMPs measured in the samples of the EVH group, one must note that other populations of EMPs do exist. The Annexin V+ CD31+ EMPs are mainly regarded as a measure of apoptotic EMPs and are a sensitive indicator of endothelial damage. However, populations of activated EMPs (namely Annexin V+ CD62E+) and even Annexin V- EMPs have also been described, though it remains to be ascertained as to the role that these other populations may contribute to endothelial stress and dysfunction. Importantly, populations of activated EMPs, will have been recorded as part of the Annexin V+ MPs population during our analysis, and will thus have been included in the Annexin V+ MP calculations. In future, it would be interesting to observe whether this individual sub-population is significantly different between the surgical groups.

Our findings demonstrate a significant increase in the population of apoptotic EMPs in the EVH group, which likely corresponds to endothelial damage imparted during EVH. This is consistent with previous findings by Rousou., *et al.* [3], who also found deteriorations in endothelial function after undergoing EVH as compared to SOT. In view of results from multiple studies, there is strong evidence that EVH techniques have detrimental effects on the endothelial layer of grafts. This could be one of the factors contributing to the increased occlusion rates reported by the Lopes paper [4] and also by a recent review by Tennyson., *et al* [5]. Conversely, all these findings are contradictory to earlier studies, which found no significant differences between the SOT and EVH techniques on occlusion rates and in terms of damage to the endothelium [6-8]. However, this may have been because all these studies were restricted to utilising disposable lengths of vein, of which only slices were observed by histology. Inconsistencies could have arisen, since this does not properly reflect the conditions of the actual conduit inside the body and could also miss out points of focal damage to the graft. This is vital since damage caused by EVH most likely results in an isolated point of injury, which can promote thrombosis at the site and hence failure of the conduit even when the rest of the structure appears healthy [8,9].

It is imperative for clinical data to be correlated to our findings in order to determine whether the extent of endothelial micro-particle release is related to downstream patient outcomes. As such, we have followed these patients over 3 years and have demonstrated no significant difference between the groups. It is therefore possible that the extent of the loss of endothelial integrity demonstrated by micro-particle release is reparable in both groups, thus leading to no appreciable clinical effects over this time period. However, further research is necessary to validate these findings.

Limitations

In terms of our study, obvious limitations from the small sample size mean that its reliability needs to be confirmed by further research. Other weaknesses arise from the fact that it is a single centre trial, since the method of vein harvesting was affected by numerous factors, such as surgeon preference, practitioner preference, practitioner experience and working hours. The other major difference between the EVH and SOT groups of the current study is the administration of heparin before dissection on EVH patients. However, there is currently no research that has been published exploring the effects of heparin on EMP generation. Since EMPs have a known pro-coagulant potential and recognised functions in the coagulation cascade, it is an important factor to be taken into account.

We have mentioned earlier that MPs are raised in numerous diseases, including diabetes, hypertension and more importantly CAD [10]. Clearly all the patients requiring CABG will have many of these risk factors, as shown in our patient demographic data. Hence different patients may have varying levels of circulating MPs, which could have affected the results.

It is clear that more research needs to be performed to confirm the current results and also to explore the clinical effects in patients. Therefore, future directions need to involve non-invasive techniques that can assess the condition of the EVH grafts *in vivo*. This could involve optical coherence tomography or magnetic resonance angiography. Most crucially, all this research must be followed up by long term clinical data exploring the effects of EVH (splitting up the different CO_2 systems) compared to SOT in vein grafting to ensure the best clinical practice for patients can be ascertained.

Conclusion

The EVH technique causes an increase in the number of apoptotic EMPs released into the harvested conduit, denoting endothelial damage. This could have harmful effects on the graft and could contribute to recent findings that long-term patency rates were inferior in the EVH group. Apoptotic EMPs, marked by Annexin V and CD31 expression, serve as a quantifiable and obtainable measure of endothelial dysfunction, and therefore may have a role as a marker in many cardiovascular disorders. However, no differences were observed between the groups over 3 years with regard to clinical outcomes including repeat angina or re-intervention.

Conflicts of Interest

None.

Source of Funding

There was no fund given by any funders for this study.

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