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The effect of different types of storage solutions on saphenous vein endothelial integrity in diabetic patients undergoing coronary artery bypass grafting

Uticaj različitih tipova prezervacionih rastvora na integritet endotela safenske vene u koronarnoj hirurgiji kod bolesnika sa dijabetesom melitusom podvrgnutih hirurškoj revaskularizaciji miokarda

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Abstract

Background/Aim. Taking into consideration the justified popularity of total arterial revascularization, the saphenous vein graft (SVG) is still one of the most utilized conduits in coronary artery bypass grafting (CABG). One of the determining factors of this conduit's durability is its endothelial integrity at the time of surgery. The aim of the study was to investigate the effect of different storage solutions on SVG endothelial integrity in patients with diabetes mellitus (DM) type 2 (T2DM) and non-T2DM patients undergoing CABG. The solutions under evaluation were heparinized saline solution (0.9% NaCl), heparinized autologous whole blood, Bretschneider (histidine-tryptophan-ketoglutarate – HTK) solution, and fresh frozen plasma (FFP) solution. **Methods.** Forty patients were included in this study and were divided into two groups: group A with 23 T2DM patients and group B with 17 non-T2DM patients. The effects of these solutions were examined by immunohistochemical staining with anti-CD34 antibodies and morphometric comparison in histologic samples of T2DM patients undergoing CABG between July 2021 and September 2022 with samples provided by non-T2DM patients. **Results.** In this study, the FFP solution showed the most prominent positive effect on the preservation of SVG endothelial integrity, with an average cell integrity preservation of 92.2%. HTK solution was found to be the least effective, with an endothelial cell preservation integrity of 26.77%. There was no marked statistically significant difference in results from groups A and B. There was a noticeable contrast in preserving SVG endothelial integrity between the two patient groups, T2DM and non-T2DM patients, although it was not statistically significant. **Conclusion**. The storage solution with the most beneficial effect on SVG endothelial integrity preservation was the FFP solution when harvested *via* the conventional open method in CABG.

Key words:
coronary artery bypass; diabetes mellitus, type 2; histological techniques; organ preservation solutions; saphenous vein; transplants.

Apstrakt

Uvod/Cilj. Uprkos opravdanom porastu popularnosti totalne arterijske revaskularizacije, graft safenske vene (GSV) ostaje jedan od najkorišćenijih provodnika u hirurškoj revaskularizaciji miokarda (*coronary artery bypass grafting* – CABG). Jedan od odlučujućih faktora trajnosti tog provodnika je integritet njegovog endotela u vreme operacije. Cilj rada bio je da se kod bolesnika podvrgnutih CABG sa tip 2 dijabetesom melitusom (T2DM) i kod bolesnika bez T2DM ispita uticaj prezervacionih rastvora na integritet endotelnih ćelija GSV. Rastvori koji su procenjivani bili su: heparinizirani fiziološki rastvor (*sol.* 0,9% NaCl), heparinizirana puna autologna krv, Bretschneider-ov histidin-triptofan-ketoglutarat (HTK) rastvor i rastvor sveže zamrznute plazme (SZP). **Metode**. U studiju je bilo uključeno 40 bolesnika podeljenih u dve grupe: grupu A, u kojoj su bila 23 bolesnika sa T2DM i grupu B, sa 17 bolesnika bez T2DM. Efekti prezervacionih rastvora ispitivani su imunohistohemijskim bojenjem anti-CD34 antitelom i morfometrijskim poređenjem histoloških uzoraka bolesnika sa T2DM, podvrgnutih CABG između jula 2021. i septembra 2022. godine, sa uzorcima koji su dali bolesnici koji nisu imali T2DM. **Rezultati**. Najbolji efekat

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na očuvanje integriteta endotela GSV imao je rastvor SZP, sa prosečnim očuvanjem integriteta endotela od 92,2%. Najnepovoljniji efekat imao je rastvor HTK, sa prosečnim očuvanjem integriteta endotela od 26,77%. Nije bilo statistički značajne razlike rezultata između grupa A i B. Postojao je primetan, mada ne statistički značajan, kontrast u očuvanju integriteta endotela GSV između bolesnika sa T2DM i bolesnika bez T2DM. **Zaključak.** U konvencionalnom metodu CABG, prezervacioni rastvor sa najboljim efektom na očuvanje integriteta endotela GSV bio je rastvor SZP.

Ključne reči:
aortokoronarno premošćavanje; dijabetes melitus, insulin-nezavisni; histološke tehnike; rastvori za čuvanje organa; v. saphena; graftovi.

Introduction

Atherosclerotic coronary artery disease is a leading cause of mortality in the population over 65 years of age worldwide. Risk factors, including diabetic disease, have been a significant contributor to mortality over the two decades of 1990–2010, especially in developing countries. On the contrary, high-income countries are showing a reduction in risk factors due to improved awareness [1].

Total arterial revascularization (TAR) has been gaining momentum worldwide [2]. All things considered, the saphenous vein (SV) graft (SVG) is still the most utilized conduit in coronary surgery, with coronary artery bypass graft (CABG) surgery being one of, if not the most performed type of surgery worldwide. If we consider that only 5–10% of CABG patients will receive TAR, the average number of conduits *per* patient being 3.1 [3], and with more than 500,000 patients every year undergoing CABG with at least one SVG, the number gets only bigger. SVG will stick around in coronary surgery for a long time due to its readiness, ease of harvesting and manipulation, as well as the possibility of creating different graft configurations.

SVG patency is, to this day, the main flaw of this conduit. SVGs are known for their high rate of occlusion (3–12% before hospital discharge, 8–25% in the first year postoperatively, and 50–60% ten years postoperatively) [4] regardless of the advance in surgical technique and pharmacological therapy. Approximately 13% of all CABG patients will be eligible for redo-surgery in the first ten years after the first CABG, while 18% of all percutaneous coronary intervention (PCI) procedures are performed on the CABG patients, and 6% of all PCI procedures are performed on SVGs, indicating the necessity for repeat revascularization [5]. Excluding the harvesting technique [6, 7] and revascularization strategy [8], one of the factors contributing to SVG longevity is the type of storage solution used during surgery. The choice of solution type has shown to have a wide spectrum of effects on the endothelial layer's integrity, both beneficial and disadvantageous [9].

Diabetes mellitus (DM) type 2 (T2DM), is an important cardiovascular risk factor, and the prevalence of T2DM in patients undergoing CABG surgery is nearly 30–40% [10].

The metabolic effects of T2DM alter the walls of blood vessels, beginning with the loss of proper endothelial function, oxidative stress, low-intensity inflammation, and high platelet adhesion rate [11]. Advanced glycation end products and their receptor activation may accelerate SVG smooth muscle cell proliferation in DM patients. Even with adequate glycemic control, the intimal hyperplasia is more pronounced in the SVG of DM patients when compared with patients not affected by this disease [10]. In a study published in 2008, graft occlusions were more common among diabetics compared with non-diabetics. DM was associated with lower vein graft patency but similar arterial graft patency in comparison to non-diabetics. Uncontrolled DM and long duration of disease were found to be significant predictors for graft occlusion [12]. The aim of this study was to determine the difference in SVG endothelial integrity between patients with T2DM and non-T2DM patients undergoing CABG.

Methods

Participants and randomization

Patients with isolated coronary artery disease and the use of at least one SVG were eligible to enroll. Additionally, patients were distributed into two groups according to the presence of T2DM. They were then distributed into four groups according to the type of storage solution used: heparinized saline (0.9% NaCl), heparinized whole autologous blood, fresh frozen plasma (FFP) solution, and Bretschneider's (histidine-tryptophan-ketoglutarate – HTK) solution. Patients with indications for valvular, aortic, redo, and peripheral artery surgery, as well as leg varicosities, postthrombotic syndrome, and state after thrombophlebitis of the veins of lower extremities were excluded from participating in this study. Color duplex scan examination of the great SV was performed preoperatively (Mindray DC-80A X-insight, Shenzhen, China). Randomization was conducted by the method of chance up until the number of required applicants was achieved. The research was conducted in the facilities of the Cardiac Surgery Clinic, the Emergency Center of the University Clinical Center Niš, Serbia, and the Department of Histology and Embryology Laboratory. The study was approved by the Emergency Center of the University Clinical Center Niš Ethics Committee (No 3830/7, from February 4, 2020) and the Faculty of Medicine in Niš Ethical Board (No. 12-15637-2/8, from December 24, 2019). A written informed consent was obtained from every participant in the study at the time of hospital admittance. The total number of patients considered was 200. The number of patients enrolled in this study was 40, all with atherosclerotic coronary disease and indicated for CABG surgery (Table 1).

Table 1

Comparison of T2DM vs. non-T2DM patients

Parameter	Patients	
	T2DM	non-T2DM
Gender		
male	18	13
female	5	4
Age (years)	66 (41–80)	68 (42–80)
EuroSCORE	3.48 (0.73–10.1)	3.39 (0.81–11.2)
BMI	28.3 (23.7–34.5)	26.1 (22.1–33.2)
BSA	2.1 (1.7–2.5)	1.9 (1.6–2.3)
LVEF (%)	44.3 (25–68)	46.1 (27–69)
Current smoker	9 (4 female, 5 male)	10 (4 female, 6 male)
Hypertension	19 (95) using anti-HTN drugs	18 (90) using anti-HTN drugs
HbA1c (%)	6.61 (5.2–8.8)	not measured

T2DM – type 2 diabetes mellitus; BMI – body mass index; BSA – body surface area; LVEF – left ventricular ejection fraction; HbA1c – glycated hemoglobin; HTN – histidine-tryptophan-ketoglutarate.
All values are expressed as numbers, mean (minimum-maximum), or numbers (percentages).

Surgical procedure

The harvesting and storage of the great SV was conducted by two experienced surgeons, both of whom perform more than 100 SVG harvesting procedures annually using the conventional open harvesting technique. The surrounding fat tissue was separated by blunt preparation and sharp dissection from the SV adventitial layer. The initial surgical cut was located 5 cm above the medial malleolus and continued cranially following the anatomic projection of the great SV. The length was determined preoperatively based on the number of planned bypasses. A plastic cannula was placed through the venotomy site and secured with a ligature, with side branches either ligated or clipsed. The SVG was then dilated and checked for leakage using one of the four storage solutions with the help of a 20 mL plastic syringe. The dilation pressure did not exceed 200 mmHg. After this procedure, a sample of the SVG 0.5–2 cm long proximal to the initial incision, 5 cm above the medial malleolus was obtained by sharp section and placed into an inox container (by submersion) filled with the designated storage solution already used for dilation at the room temperature (the operating theater temperature varies from 10–14 ˚C) for an average time period of 32.1 min (12–67 min). The sample was stored accordingly until the proximal anastomosis stage between the coronary artery and the SVG. During this time period, the SV specimen is in a warm ischemic period. The sample is then taken from the container with the solution for temporal simulation purposes and placed into a plastic container filled with 4% buffered water formaldehyde solution. This procedure is done in the setting of full heparinization according to the cardiopulmonary bypass protocol. Using 1 mL of unfractionated heparin containing 5,000 IU, 100 mL of heparinized 0.9% NaCl solution was prepared in an inox container. FFP solution was obtained from the Blood Transfusion Institute in Niš on a daily basis at the time of the patient's arrival at the Operating Room, thawed and ready for use. One unit contains 250 mL of plasma and 500 mg of fibrinogen in a citrate anticoagulant, with an estimated albumin content of 26 g/L and 57 g/L of protein. Using 1 mL of unfractionated heparin containing 5,000 IU Bretschneider's solution, 100 mL of autologous whole blood was obtained *via* the distal part of the central venous catheter placed in a usual manner and prepared in an inox container.

Histological and immunohistochemical procedure

From each of the acquired pieces of SVs, a biopsy sample 5 mm in length was taken and fixated by 4% buffered water formaldehyde solution. These samples were prepared using routine methods of preparation for the illumination microscope analysis. The tissue has undergone dehydration, illumination, and paraffin infiltration and was sliced by microtome into 4 nm sample sizes. These were subject to the methods of histochemical and immunohistochemical marking of specific tissue structures and finally brought to form permanent illumination microscope preparations. For immunohistochemical staining, anti-CD34 monoclonal antibody (mAb) (Dako; cat. No. M716501; dilution 1:50) was used and brought to the state of permanent illumination microscopy preparations in which the specific tissue bond is proven by secondary antibodies and EnVision-Flex visualizing system with a visible chromogen, later counterstained with Mayer hematoxylin. Microscopic preparations have been analyzed using illumination microscope digital camera Olympus BX50 (Olympus, Japan, Tokyo). Microscopic images of SV walls taken from the histochemical and immunohistochemical preparations were captured under constant magnification and stored digitally to be morphometrically processed. Morphometric analysis was achieved using the ImageJ 1.53 version (Wayne Rasband National Institute of Health, USA) program by quantifying the monoclonal antibodies binding rate to SV endothelium CD34 receptors. The degree of preservation of SVG endothelial integrity was determined based on the presence of CD34 positivity on the sections of the examined tissues.

SPSS statistical package (IBM, Armonk, New York, US), version 2.0, was used for statistical data analysis. The standard statistical method for quantitative and qualitative result assessment was used as a basic descriptive statistical parameter. Statistical significance was determined for $p < 0.05$. The statistic hypothesis was tested for a significance risk level value of $\alpha = 0.05$. The minimal sample size required to detect the effect size of 0.05 in the analysis of variance for the four groups and statistical power of 0.9 is 40 respondents.

Results

Table 2 and Figure 1 show the degree of preservation of SVG endothelial integrity, measured based on the CD34 mAb binding rate to the present endothelial cells in the examined tissue in all four groups concerning the type of solution used. Significant statistical difference in the presence of CD34 positivity (binding rate) expressed in percentages (CD34 % value) between the different used solutions groups was: $\chi^2 = 10.71$, df = 3, $p = 0.013 < 0.05$.

When the Shapiro-Wilk test showed deviation in CD34 % value in the whole sample and all tested solution groups, the Kruskal Wallis test was utilized to confirm the statistical significance of CD34 % value between the different solution groups ($\chi^2 = 10.71$, df = 3, $p = 0.013 < 0.05$). The following multiple group comparison, with the use of Bonferroni's correction, has confirmed the statistically significant difference in CD34 % value between the groups with FFP and Bretschneider's solution ($p = 0.008 < 0.01$), meaning that the difference in the CD34 % value is statistically significant in favor of the former.

The presence of T2DM did not make a significant statistical difference between the groups, and in the whole sample, 23 (57.70%) patients were burdened with T2DM (Figure 2).

Table 2

Degree of preservation of SVG endothelial integrity depending on the type of storage solutions

Solutions	CD34 (%)	
	presence	absence
Heparinized saline	73.5	26.5
Heparinized whole autologous blood	82.69	17.31
Fresh frozen plasma	92.9	7.1
Bretschneider's	26.77	73.23

SVG – saphenous vein graft. The number of patients in each tested group of solutions was 10.

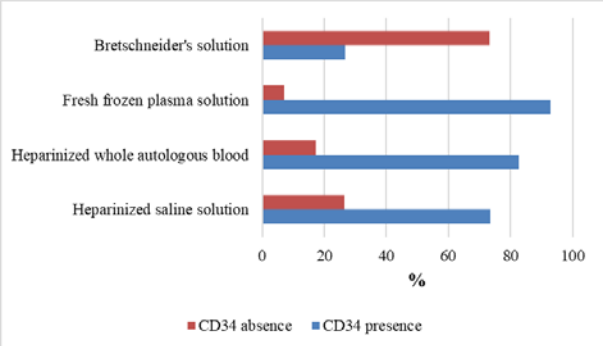

Fig. 1 – Statistical significance of CD34 % value between the different used solutions groups ($\chi^2 = 10.71$, df = 3, $p = 0.013 < 0.05$).

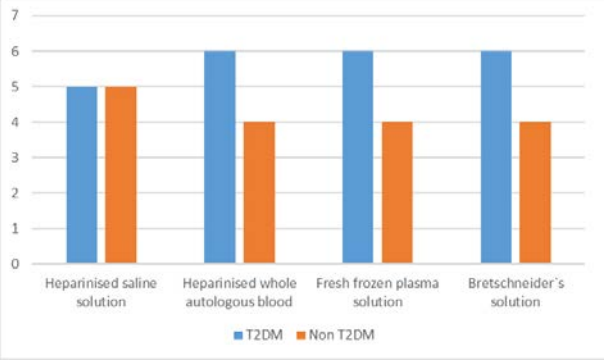

Fig. 2 – The distribution of type 2 diabetes mellitus (T2DM) with respect to the solution type and the entire sample. Data are presented in absolute numbers.

CD34 % value is slightly higher in the group of patients without T2DM, but based on data from the Mann-Whitney *U* test, no statistical significance was obtained (p = 0.7372) (Table 3).

In patients with T2DM, glycated hemoglobin (HbA1c) was recorded on a daily basis. Throughout the whole sample of patients, the value of Spearman's coefficient of correlation showed no significant correlation between the CD34 % value and the HbA1c parameter (ρ = -0.1901, p = 0.3850).

Histological examination

Several samples showed morphological presence of intimal tunic hypertrophy, which is, in some samples, of uniform development throughout the entire luminal circumference, and in others, present as a number of intimal cushions. Immunohistochemical staining with CD34 monoclonal antibodies and Mayer's hematoxylin counterstaining ensured clear visualization and intimal tunic differentiation between the cells with maintained luminal membrane integrity and the ones that showed endothelial layer denudation, as shown in Table 2. Figures 3 and 4 show (in magnification setting ×100) the difference in endothelial layer cell integrity and marked difference between the groups. In both Figures, the dark red-brown lining shows the presence of the endothelial cells, which have been marked using the ImageJ 1.53 version (Wayne Rasband National Institute of Health, USA) program by quantifying the mAbs binding rate to SV endothelium CD34 receptors, enabling morphometric analysis and visualization of endothelial layer integrity.

Table 3

Degree of preservation of SVG endothelial integrity depending on the presence of T2DM

Patients	CD34 (%)	
	presence	absence
T2DM	84.35	15.65
Non-T2DM	78.59	21.41

SVG – saphenous vein graft; T2DM – type 2 diabetes mellitus.

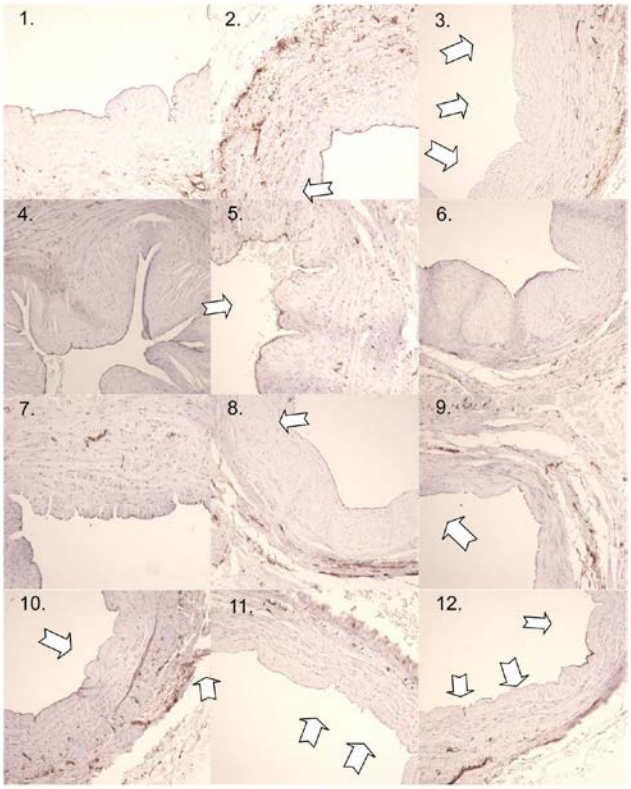

Fig. 3 – Immunohistochemical staining with anti-CD34 on saphenous vein graft (SVG). Counterstaining was performed using Mayer's hematoxylin. Arrows pointing at areas with endothelial layer denudation. Heparinized saline solution (1–3), heparinized whole autologous blood (4–6), fresh frozen plasma solution (7–9), and Bretschneider's solution (10–12) were used in SVG. Patients burdened with type 2 diabetes mellitus (photos 1, 2, 4, 5, 7, 8, 10, 11).

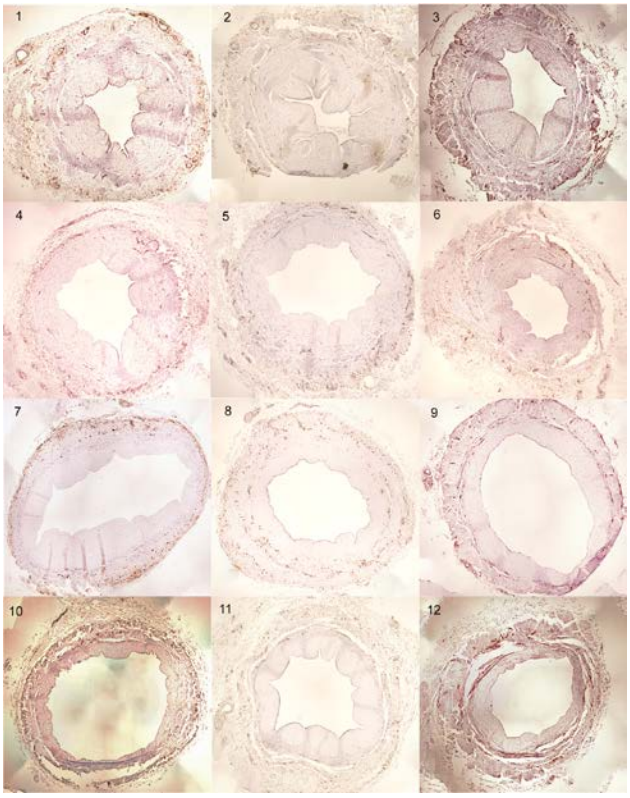

Fig. 4 – Immunohistochemical staining with anti-CD34 on whole cross sections of saphenous vein graft (SVG). Counterstaining was performed using Mayer's hematoxylin. Heparinized saline solution (1–3), fresh frozen plasma solution (4–6), heparinized whole autologous blood (7–9), and Bretschneider's solution (10–12) were used in SVG. Patients burdened with type 2 diabetes mellitus (photos 1, 2, 4, 5, 7, 8, 10, 11).

Discussion

The major findings of the present study are the following: 1) the FFP solution has shown to be superior to Bretschneider's solution when comparing SVG endothelial layer preservation properties ($p < 0.05$); 2) when comparing the two groups of patients, the presence of T2DM did not make a significant statistical difference in endothelial integrity preservation between the groups.

The difference between heparinized saline and heparinized whole autologous blood was not notable in this study in both groups of patients. The effect of the heparin itself remains to be examined further. It is known to distract some of the protective enzymes, relieving the oxidative stress and, therefore, making the endothelium more susceptible to different toxic influences [11, 12]. The first ever conducted study that relates storage solutions and SVG damage was conducted by Gundry et al. [13]. This group compared the effect of heparinized saline and autologous blood in temperatures 4–28 ˚C and dilation pressure from 100–300 mmHg. The use of heparinized saline solution created intramural edema independently from the distension pressure and, in combination with high distension pressure, led to massive endothelial damage. The authors concluded the utilization of heparinized whole blood in SVG storage and manipulation with dilation pressure under 100 mmHg to be an acceptable manner of treatment towards the SVG. Heparinized saline is the most economical solution and also has a reasonably positive effect on endothelial integrity preservation.

Studies have shown that heparinized whole blood may be more effective in preserving graft function compared to saline [14, 15]. Similar results were reported by Lawrie et al. [16] by analyzing SVGs harvested in conventional open technique. SVGs were also stored at room temperature but with higher dilation pressure (200–400 mmHg). Zerkowski et al. [17] compared heparinized autologous whole blood with human albumin, Bretschneider's solution, and heparinized saline and found the best results using autologous whole blood. Wilbring et al. [15] studied SV segments stored in heparinized saline and heparinized autologous whole blood submerged for 30 min at room temperature using Mulvany myograph, concluding heparinized saline is inferior to heparinized autologous whole blood, which does not correlate with the results from this study. These results are not confirmed by the morphometric measurements used in this study in both diabetic and non-diabetic patients; a contrast in results was found in favor of heparinized whole blood but not with statistical significance ($p > 0.05$). Similar to our results,

Chester et al. [18] have compared heparinized saline, heparinized whole autologous blood, and several commercial cardioplegic and storage solutions regarding endothelial reactivity and concluded there was no marked difference between heparinized saline and heparinized autologous whole blood.

In this study, the use of FFP was associated with a greater degree of endothelial preservation compared to the other solutions in both groups of patients using the conventional open-harvesting technique. Considering the FFP constituents match the soluble component of the endothelial glycocalyx, the fact can be utilized to achieve a potential beneficial gain in order to maintain the SVG endothelial cell integrity [19]. In 2010, Weiss et al. [20] continued research, confirming initial results from 2009 [9] and comparing them with ones gained from research with plasma derivative solutions. In both studies, the saline solution was found to be inferior.

Bretschneider's solution is a specialized mixture consisting of different components such as potassium, magnesium, and histidine. It is frequently utilized for the extended preservation of SVG and has the ability to maintain viability for up to 48 hrs [21]. The solution contains nutrients that help sustain the metabolic activity of SVGs, thereby enhancing graft patency. However, the use of Bretschneider's solution may lead to metabolic alkalosis and reduced myocardial contractility. In this study, Bretschneider's solution has been shown to be markedly inferior to the other solutions in the study, comparing the morphometric results obtained. Similar results were reported by Weiss et al. [9] in a study from 2009, showing that with the utilization of an FFP solution, the SVG tissue failed to maintain its integrity. By quantifying the CD34 monoclonal antibodies' binding rate to SV endothelium, the degree of endothelial layer integrity was determined. The clinical consequences of endothelial preservation vs. denudation cannot be foreseen precisely. The non-eventful adaptation of the SVG is not fully described on account of healthy grafts not being excised for ethical reasons. Analyzed samples are usually taken from non-functional grafts [22]. The endothelium's role is, among others, a vasoprotective one, and its dysfunction is recognized as a starting point for atherosclerosis [23]. Denudation is a factor in the development of atherosclerosis and mural thrombosis in animal models, and it also plays a role in plaque development. CABG clinical outcome is more important than graft patency in isolation. Denudation of the endothelium layer is unlikely to cause problems in the short term. The endothelium layer's capacity for regeneration may not, however, be sufficient in the long run [24]. Temperature alone could be a major factor in ischemia-reperfusion injury caused during harvesting. This study's findings are consistent with those of Unal et al. [25] from 2009 in that there was no significant statistical difference between storage solution temperature variations and CD34 binding rates. Although the average amount of time samples spent submerged in the storage solution ranged from 32.1 to 67 min, we did not find any statistically significant differences in the amount of time spent submerged. On the other hand, low storage solution temperature was identified by Lawrie et al. [16] as a risk factor for endothelial cell death.

Study limitations

One unit contains 250 mL of plasma and 500 mg of fibrinogen in a citrate anticoagulant, with an estimated albumin content of 26 g/L and 57 g/L of protein. The variation in FFP composition is present, and the effect of these variations is not known. The effect of FFP shelf life is also unknown. The influence of the harvesting technique is great, if not crucial, and cannot be compensated by even the "perfect" storage solution. Only two cross sections of each SV sample were processed.

Conclusion

There was a noticeable contrast in preserving SVG endothelial integrity between the two patient groups, diabetic (T2DM) and non-diabetic patients, although it was not statistically significant. The storage solution with the most beneficial effect on SVG endothelial integrity preservation was the FFP solution when harvested *via* the conventional open method in CABG.

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