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Complications and grafts in septic vascular surgery

Analysis of the thrombogenicity properties of cryopreserved allografts and the determination of the healthy vascular microbiota

PhD thesis

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List of abbreviations

BMI - body mass index
bp – base pair
BSA - bovine serum albumin
CP - cryopreservation
CPA - cryoprotective agent
CT – computed tomography
DMSO - dimethyl sulfoxide
DNA – deoxyribonucleic acid
ePTFE - expanded polytetrafluoroethylene
ESVS - European Society for Vascular Surgery
FDG-PET/CT -18F-fluoro-D-deoxyglucose positron emission tomography/computed tomography
GSV - great saphenous vein
HIV - human immunodeficiency virus
HLA - human leukocyte antigens
IQR – interquartile range
MAGIC - management of aortic graft infection collaboration
mRNA -messenger ribonucleic acid
PBS – phosphate-buffered saline
PCoA - principal coordinates analysis
PCR - polymerase chain reaction
PERMANOVA - permutational multivariate analysis of variance
RPMI - Roswell Park Memorial Institute
rRNA – ribosomal ribonucleic acid
SCFA - short-chain fatty acid
SD – standard deviation
TBS – Tris-buffered saline
TMAO - trimethylamine-N-oxide
VGI - vascular graft infection

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1. Introduction

The management of vascular infections is one of the significant challenges for vascular surgery. Various bypass operations or surgical patch angioplasties reconstruct the target vessel using autologous grafts, or in their absence, synthetic materials such as expanded polytetrafluoroethylene (ePTFE) or polyethylene terephthalate (Dacron), as well as other biological options like bioengineered, allo-, or xenografts. However, in the case of more widely available artificial grafts, we still have to consider the surgical area and implanted graft infection as a potential complication, despite modern sterilisation techniques, wound treatments and antibiotic therapies. The main danger of vascular graft infection (VGI) is that, in addition to local inflammation, it can cause graft occlusion, sepsis or even life-threatening bleeding. Biological vascular grafts may be a solution to this problem, but their availability and clinical experience are limited, not to mention their very high costs. For the potentially or actually infected surgical area or the absence of a suitable autologous vessel, an infection-resistant, biocompatible, durable, off-the-shelf material would be the key to preventing and treating the above complications. However, the optimal graft material is currently unavailable, although allografts are close.

1.1. Vascular infections

Vascular infection is a relatively rare but potentially life- or extremity-threatening condition because of severe complications such as sepsis, bleeding or thromboembolic events. The incidence of vascular wound infection ranges widely, from 3.5% to 32%, and the risk of prosthetic graft infection is 0.25-6% after implantation. In addition, these numbers may be higher in patients with predisposing factors to infection. (1–5) Incidence and mortality of vascular graft infection depend on multifactorial circumstances (Table 1.); however, the location of the graft basically affects these. (5–7) The incidence and the mortality of VGI affecting the thorax or abdomen-located grafts are 0.2%-6% and 10%-75%, respectively, in the case of VGI with infrainguinally located graft, these numbers are up to 6% and 17%, respectively. In contrast, the VGI of supra-aortic branches is much rarer than the previous ones, the incidence of it is between 0.25%-0.5%, and the mortality is even lower than that. (5–9) Although extracavitary VGI is associated with a lower mortality rate, we have to count on a high extremity amputation rate of up to 40%. (5,6)

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Table 1. Risk factors for vascular graft infection. (5)

Operative risk factors		
pre	intra	post
prolonged pre-operative hospitalisation	breach in aseptic technique	post-operative wound complications (infection, skin necrosis,lymphocoele, seroma, haematoma)
infection in a remote or adjacent site	prolonged operation time	graft thrombosis
recent percutaneous arterial access at the implant site	concomitant gastrointestinal or genitourinary procedure	
emergency/urgent procedure		
re-intervention		
lower limb infection (ulcer, gangrene, cellulitis)		
groin incision		
Patient related risk factors		
malignancy		
lymphoproliferative disorder		
immune disorders		
corticosteroid administration		
chemotherapy		
malnutrition		
diabetes mellitus/ peri-operative hyperglycaemia		
chronic renal insufficiency/end stage renal disease		
liver disease/cirrhosis		
immunosuppression by non-suspended anti-tumour necrosis factor alpha		

In addition, endovascular graft infection is less frequent, with about an incidence of 1%, than of the grafts implanted by open surgery. However, the mortality rate of endovascular graft infections is also high because of the common association with graft-bronchial, -oesophageal or -enteric fistula. (5,7)

Many ways of diagnosing and classifying VGI have been described over the years. Currently, the most accepted classifications are based on the extent of infection, as Szilagyi, Samson and modified Bunt classification. (Table 2.)

Table 2. Classifications for wound and vascular graft infections with respect to wound infection (Szilagyi, Samson) and to the extent of graft involvement (Bunt). (5)

Szilagyi classification:
Grade I: cellulitis involving the wound
Grade II: infection involving subcutaneous tissue
Grade III: infection involving the vascular prosthesis
Samson classification:
Group 1: no deeper than dermis
Group 2: subcutaneous tissue, no direct contact with the graft
Group 3: body of graft but not anastomosis
Group 4: exposed anastomosis, no bleeding, no bacteraemia
Group 5: anastomosis involved, bleeding, bacteraemia
Extent of graft involvement (Bunt classification modified)
Peripheral graft infection:
P0 graft infection: infection of a cavitary graft
P1 graft infection: infection of a graft whose entire anatomical course is non-cavitary
P2 graft infection: infection of the extracavitary portion of a graft whose origin is cavitary
P3 graft infection: infection involving a prosthetic patch angioplasty
Graft-enteric erosion
Graft-enteric fistula
Aortic stump sepsis after excision of an infected aortic graft

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In establishing the diagnosis of VGI, the MAGIC (Management of Aortic Graft Infection Collaboration) criteria developed primarily to identify aortic graft infections can be used with good results. This criterion system is based on the clinical, radiological and laboratory results. (Table 3.)

These classifications and diagnostic methods can guide us in everyday practice to determine VGI and select the appropriate therapy.

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Table 3. The MAGIC criteria. MAGIC=Management of Aortic Graft Infection Collaboration; CT=computed tomography; FDG-PET/CT=18F-fluoro-D-deoxyglucose positron emission tomography/computed tomography (5)

Clinical/surgical	Radiology	Laboratory
Major criteria		
Pus (confirmed by microscopy) around graft or in aneurysm sac at surgery	Perigraft fluid on CT scan ≥ 3 months after insertion	Organisms recovered from an explanted graft
Open wound with exposed graft or communicating sinus	Perigraft gas on CT scan ≥ 7 weeks after insertion	Organisms recovered from an intraoperative specimen
Fistula development, e.g., aorto-enteric or aortobronchial	Increase in perigraft gas volume demonstrated on serial imaging	Organisms recovered from a percutaneous, radiologically guided aspirate of perigraft fluid
Graft insertion in an infected site, e.g., fistula, mycotic aneurysm, or infected pseudo-aneurysm		
Minor criteria		
Localised clinical features of graft infection, e.g., erythema, warmth, swelling, purulent discharge, pain	Other, e.g., suspicious perigraft gas/ fluid soft tissue inflammation; aneurysm expansion; pseudo-aneurysm formation: focal bowel wall thickening; discitis/osteomyelitis; suspicious metabolic activity on FDG-PET/CT; radiolabelled leukocyte uptake	Abnormally elevated inflammatory markers with graft infection as most likely cause, e.g., erythrocyte sedimentation rate, C reactive protein, white cell count
Fever ≥38˚C with graft infection as most likely cause		Blood culture(s) positive and no apparent source except graft infection

1.2. Microorganisms

1.2.1. Detection and identification

For vascular infections, the best outcome is achieved by the timely initiation of appropriate antimicrobial therapy alongside surgical treatment. However, due to empiric antimicrobial therapy already started at the time of sampling or inefficient detection methods, negative culture results are often expected, that makes the selection of an adequate, targeted antimicrobial treatment much more difficult. Although according to the European Society for Vascular Surgery (ESVS) guideline, pathogens responsible for VGI can be detected in approximately 75% of cases (5), it is essential to note that in the majority of cases, 2-5 intraoperative samples or blood cultures are required for success (10,11). Ajdler-Schaeffler et al. found that only 22% of 223 microbiological specimens from 60 patients were culture-positive by conventional methods, allowing microbiological identification of only 26 VGI. However, in addition to the conventional culture method, 16S ribosomal ribonucleic acid (rRNA) gene polymerase chain reaction (PCR) was used to detect pathogens in 12 additional cases. (11) These new molecular-based detection methods, such as 16S rRNA gene PCR (12,13), allow more accurate identification of microorganisms. Moreover, this method may provide better diagnostic results for graft infections and improve the safety of sterility testing of allografts taken during transplantation. However, the disadvantage of this method is that it does not identify only living pathogens, so positive results are obtained even for microorganism fragments or microorganisms present in healthy individuals. The method's strength would be further enhanced by the knowledge of the healthy vascular and perivascular microbiome.

1.2.2. Pathogens

The most common bacteria of VGI are Gram-positive ones such as *Staphylococcus aureus*, coagulase-negative *staphylococci* (*Staphylococcus epidermidis*) and *enterococci*; in more than half of the cases, they can be isolated. These are followed in occurrence by Gram-negative ones such as *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Prevotella* species and *Salmonella* species. In addition, anaerobic bacteria and fungi, such as *Candida* and *Aspergillus* species, can cause VGI. (5,6,14) Infections can be distinguished based on the time since graft implantation, such as early, within four months, and late, after four months. While the former is usually caused by virulent species

such as *Staphylococcus aureus* and Gram-negative bacteria with pronounced inflammatory responses, the latter is usually caused by less virulent ones such as members of the skin flora or fungi with inferior symptoms and negative microorganism isolation. (6)

1.2.3. Vascular microbiota

Thanks to the Human Microbiome Project (15), which enabled a better understanding of the relationship between the human body and microorganisms, in addition to pathogenic ones, it is increasingly important to know the entire ecosystem of the human body to understand infectious and even non-infectious diseases better. Although internal organs and blood are considered sterile, and the pathogens are thought to originate from an external source, via surgical or endovascular procedure or hematogenous way, there is growing evidence of microorganisms in various tissues of healthy and non-healthy individuals. (16–19) Furthermore, some authors could identify biologically relevant living microorganisms from tissues previously thought to be sterile (20,21) or found a link between non-infectious diseases and the microbiome and its metabolites (22). The mapping of the human microbiome opened up new horizons in many areas of medicine, like gastroenterology (23,24), urology (16) and gynaecology (25), thanks to new molecular-based detection methods. However, currently, vascular microbiota is unexplored.

1.3. Vascular grafts

The first vascular grafts to be used in humans were so-called allografts during World War I. (7) In 1948, Kunlin reported the first successful autogenous reversed great saphenous vein (GSV) bypass in a patient with superficial femoral artery occlusion and consequent non-healing foot ulcer. (26) Subsequently, bypass surgery evolved significantly and synthetic grafts have appeared alongside biological grafts. Nowadays, there is increasing research into the development of so-called tissue-engineered grafts, which are expected to combine the positive properties of biological and synthetic grafts. (27) (Table 4.)

However, no graft with biological and biomechanical properties comparable to autologous GSV grafts has been found or developed. Thus, in the absence of GSV or the case of a calibre difference or vascular infection, we are forced to use less suitable graft materials. (5,28,29)

Table 4. Vascular grafts.

Biological grafts		
Autografts	Allografts	Xenografts
	Fresh allografts Cryopreserved allografts	
Synthetic grafts		
Standard synthetic grafts	Impregnated synthetic grafts	
Expanded polytetrafluoroethylene (ePTFE) Polyethylene terephthalate (Dacron) Poliurethan	Silver-impregnated vascular grafts Collagen-impregnated vascular grafts Rifampicin-impregnated vascular grafts	
Tissue-engineered grafts		

1.3.1. Allografts

Vascular allografts are blood vessels, in the case of transplantation of which donor and recipient have different genotypes but belong to the same species. They are also called allogeneic grafts or homografts. They can be derived from cadaveric donations, usually from multiorgan donations of brain-dead donors. The most commonly used allografts are GSV, superficial femoral vein, superficial femoral artery, thoracic and abdominal aorta and aortic bifurcation with iliac arteries.

1.3.1.1. Fresh allografts

Fresh grafts were the first representatives of vascular grafts. These grafts are implanted within a few days after the explantation and stored in different solutions at +4˚C until then. They were tested in several animal experiments, and then, from the beginning of the 20th century, they were also used in human operations. However, they have several disadvantages, such as immunogenicity and storage time of a few days or weeks, significantly affecting their availability. Although many innovations have been made over the years to improve these grafts, however, their poor clinical outcomes and newer storage techniques have overshadowed their use. (7,30)

1.3.1.2. Cryopreserved allografts

After harvesting, these allografts are subjected to a unique freezing process that allows for long-term storage. Although there are many publications on cryopreserved allografts, it is difficult to get a reliable picture of the real advantages and disadvantages of these grafts in the absence of randomised controlled trials or because of the very different aspects of existing studies, such as the site of application, allograft types and the method of preparation used. Regarding the graft type, arterial grafts are generally preferred in the aortoiliac region; however, in the case of lower limb reconstructions, there is no clear position on the relative superiority of each allograft type, some consider cryopreserved GSV grafts more suitable (7), and some prefer arterial allografts (31,32).

Cryopreserved allografts are widely used solutions for either aortic or lower limb vascular infections due to their resistance to infection and low reinfection rate. (5,7,33) Based on meta-analyses, there was no significant difference in reinfection when using autogenous vein (2%), allograft (9%) or impregnated synthetic grafts (11%) for abdominal aortic infection, but all of these were significantly better than ePTFE or Dacron grafts. (5) In addition, their other outstanding feature is that nowadays, cryopreserved allografts are readily available in a wide range of sizes thanks to their long-term storability in well-organized homograft banks. These homograft banks provide the highest quality grafts currently available, with standardised production protocols and a wide distribution among users. (34,35) Despite these advantages and the highly increased production level, allografts are still associated with many complications, such as bleeding, graft degeneration, occlusion or aneurysm formation. (5,7,33) According to the guideline of the ESVS, in the case of cryopreserved allografts for abdominal aortic infection, graft-related complications can range up to 21%. In addition, the five-year reintervention rate can reach up to 55% for the abdominal aorta and 33% for the lower limbs. (5) The exact occurrence of the aneurysm/pseudoaneurysm formation of cryopreserved allografts has yet to be discovered, as in some cases, they are already occluded, but their prevalence is estimated to be between 3-33%. (7,36,37) Early (within 30 days) allograft occlusion prevalence varies widely between 0-17%. (31,38–45) In a meta-analysis comparing different graft materials implanted in the abdominal aortic position, the occlusion rates of cryopreserved allografts, rifampicin-bonded synthetic grafts, standard synthetic grafts, silver-coated synthetic grafts and autologous veins were found 13%, 11%, 10%, 7% and 2%, respectively. (9) Regarding lower extremity vascular infection, when cryopreserved

GSV is used, the one-year primary and secondary patencies are approximately 13-87% and 36-73%, respectively (7). In comparison, Randon et al. reported five-year primary and secondary patency of 17% and 38%, respectively (46). In addition, our study analysing lower limb allograft bypass reconstructions for chronic limb-threatening ischaemia found one-, three- and five-year primary patency rates of 59%, 44% and 41% and secondary patency rates of 60%, 45% and 41%, respectively. (32) Although there are relatively low patency rates of cryopreserved allografts, acceptable amputation rates of 1-14% and limb salvage rates of 71% at two years and 64% at five years are reported. (7) Due to the complications of allografts, their use requires caution. Moreover, regular follow-up of implanted allografts is essential to achieve the best clinical results. (5)

Despite the issues associated with cryopreserved allografts, their important role in the treatment of vascular infections is also shown by the fact that in the ESVS guideline for vascular graft and endograft infections, the cryopreserved allografts are considered the first choice graft material in the case of thoracic or thoracoabdominal aorta infection. In the case of abdominal aorta or lower limb they can serve as a suitable alternative in the absence of autologous venous grafts. (5)

1.3.1.2.1. Cryopreservation

CP, which involves freezing and thawing, is a potentially harmful procedure for grafts. Homograft banks use several different production methods, but the advantages and disadvantages of each still need to be fully understood. (39,41–45,47–49)

During CP, cells or tissues are treated with cryoprotective agents (CPA), the most widely used of which is dimethyl sulfoxide (DMSO), to prevent cryoinjury caused by ice crystal formation or osmotic shock. The cells or tissues are then cooled in a controlled freezing process to a very low temperature below -80 ºC, at which the cell's metabolic processes are arrested to preserve structurally intact cells or tissues for an extended period. The two primary methods of CP are conventional slow freezing and rapid freezing, known as vitrification. These protocols require different CPA concentrations and use different cooling rates. (Table 5.) (50–52)

During slow freezing, the temperature of the biological material is gradually reduced over 2-4 hours using a semi-programmable freezer employing vaporised nitrogen in the presence of a low concentration of CPA so that ice crystals are formed slowly, and cell damage can be minimised. Theoretically, if the cooling is slow enough, the intracellular

water is effluxed rapidly enough to eliminate supercooling and thus prevent intracellular ice formation. As a result of differences in the capacity of different cells to transport water across the plasma membrane, the optimal cooling rate depends on the cell type. The slow cooling process requires a cooling rate of approximately 1 ˚C/min in less than 1.0 M CPA. The advantages of slow freezing include reduced risk of contamination and minimal manipulation skills. However, slow freezing can cause serious freezing damage as ice can accumulate outside the cells. (50–52)

Vitrification is a process whereby a suspension of cells is transferred directly from the aqueous phase to the glass state following direct exposure to liquid nitrogen. The process requires rapid cooling of the cells or tissues to cryogenic temperature with liquid nitrogen after exposure to a high concentration of CPA (40-60% w/V) to avoid ice nucleation. A significant advantage of vitrification is the low risk of freezing injury, thus ensuring a sufficiently high cell survival rate. However, the potential for contamination with pathogenic agents is high, and the technique requires good manipulation skills. (50–52)

Table 5. Comparison of slow freezing and vitrification methods. (50,51)

Characteristics	**Slow freezing**	**Vitrification**
Sample volume (uL)	100-250	1-2
Working time	More than 3 hours	Fast less than 10 min
Cost	Expensive	Comparatively cheaper
Concentration of CPAs	Low	High
Risk of toxicity of CPAs	Low	High
Risk of contamination with pathogenic agents	Low	High
Manipulation skills	Easy	Difficult
Past thaw viability	High	High
Status of system	Closed system only	Opened or closed system

In addition to freezing samples, another essential part of CP, in the case of slow freezing and vitrification, is the pre-use thawing, which involves warming the sample and eliminating CPAs from the biological material. This process can also have adverse effects that can lead to cryoinjury. (50–52) While relatively well-developed protocols are currently available for the freezing processes, there is no clear consensus on the optimal procedure for thawing. Some authors recommend a single-phase fast (52,53) or slow (47) thawing, others suggest a two-phase slow-fast process (54), and others found no difference between the fast and slow methods (55).

It is essential to note that CP procedures were developed to conserve different isolated cells (47), especially reproductive ones. However, in the case of tissues and organs, the size and tissue composition, including different cells and extracellular matrix, of the samples make the CP more complex and fundamentally determine the method and success of it. (47,56–58) Although fragments of reproductive tissues can currently be frozen with good results (59,60), their size and composition cannot be compared with those of other tissues and organs. Now, tissue and organ CP are an unsolved area of medicine; the optimal procedure is not known, although intensive research is in progress. Müller-Schweinitzer's review provides the most comprehensive picture of vascular tissue CP currently available. According to this, the cryomedium should contain a permeable and a non-permeable CPA in non-toxic concentration, for which RPMI (Roswell Park Memorial Institute) medium containing 1.8 M DMSO and 0.1 M sucrose is appropriate. Before freezing, the sample-cryomedium equilibrium rate depends on the tissue's wall thickness and the working temperature but it should be at least 10-20 minutes. Freezing and thawing should be performed slowly, preferably using a controlled-rate freezer at 1 °C/min for arteries and 0.6 °C/min for veins. Once the sample is cooled to approximately -70 °C, it can be stored by vaporised liquid nitrogen at -196°C for a virtually indefinite period. (47)

1.3.1.2.2. Frozen storage time

Although deep freezing can theoretically preserve cells and tissues indefinitely (47), there are no well-designed studies with solid evidence on the upper limit of frozen storage time. The European Homograft Bank sets the shelf life of cryopreserved allografts at five years, according to the European Union Directive (35). Still, as others have highlighted, this time interval is based on convention rather than validated studies (61). According to

experiments with animal jugular veins, although no gross morphological abnormalities were identified during three months of storage, microstructural changes such as focal endothelial cell blebbing, cytoplasmic vacuolisation and disruption of cell-to-cell contacts or mitochondrial swelling of smooth muscle cells, which were associated with preservation time, were observed. (62,63) In addition, Malone et al. described, in parallel to structural changes, a decreasing trend in endothelial cell function - fibrinolytic activity - associated with storage time (63), as well as Cai et al. observed a significant dysfunction of the human umbilical vein endothelial cells after 24 weeks of storage, especially in excretory functions (64). In contrast, Mirabet et al. found no significant loss of cell viability when studying heart valves and their associated vascular wall up to 13 years of storage. (65) In studies of human allografts, there is inconclusive data on the relationship between clinical outcomes and graft storage time. In a univariate analysis, Touma et al. identified the storage time of aortic allografts as a predictive factor for mortality and postoperative complications. However, it was not found to be an independent predictor in a multivariate analysis. (66) In lower limb reconstructions with cryopreserved allografts, Nagy et al. demonstrated an association between allograft storage time and clinical outcomes, similar to the previous study. Their study showed that allografts with less than six months of storage time improved patency and limb salvage. (31) In contrast, Wang and al., who investigated living donor liver transplantation, found no differences in the patency of vascular grafts for outflow reconstruction or the regeneration of liver graft between iliac allografts with less or more than one-year CP storage time. (67)
Given the literature inconsistencies, we need help defining an optimal upper limit of storage time for CP.

1.3.1.2.3. Structural and biomechanical properties

The main functions of the large and medium arteries are to serve as conduits for the distribution of blood to different tissues (conduit function) and to compensate for the pressure and flow pulsatility caused by intermittent ventricular ejections (buffer function). These interrelated functions depend on the geometric and mechanical properties of the arteries. (68) Arteries are complex structures, the arterial wall consisting of three layers. The innermost luminal layer is the tunica intima, which extends from the lumen to the internal elastic lamina. The luminal surface of the intima is lined by endothelium, which is surrounded on its abluminal surface by a connective basement

membrane. The middle layer, the tunica media, is composed mainly of smooth muscle cells, collagen, elastin fibres and glucosaminoglycans and is usually the thickest layer. The outermost layer, the tunica adventitia, anchors the blood vessel to the surrounding tissue. This layer is a connective tissue, containing varying amounts of elastic and collagen fibres. The connective tissue in this layer is quite dense where it is adjacent to the tunica media; this dense layer is called the external elastic lamina. (7) The biomechanical properties of the artery are determined primarily by the individual contribution of the components of the vascular wall, such as collagen, elastin, smooth muscle cells and endothelium, their relative proportions, structural geometry and orientation, and the relationship between them, as well as the integrity and physiological function of these components. (7,68,69) While the collagen and elastin network is generally responsible for the passive mechanics of the artery, smooth muscle cells provide active contraction (70); in addition, the endothelium plays a vital role as a regulator of vasomotor tone. (7) As a central component of biomechanical properties, smooth muscle cells contribute to structural, mechanical and functional changes in the arterial wall through several processes, including cell growth, cell elongation and rearrangement, and changes in the composition of the extracellular matrix. (69)

The viability of smooth muscle cells and endothelium, as well as the integrity of elastin and collagen fibres, contribute to the mechanical behaviour of the arterial wall. The destruction of smooth muscle cells and endothelial cells and the fragmentation of extracellular matrix fibres caused by ice formation can affect the mechanical properties of the artery. (69) Cold storage can result in bulk redistribution of water, damage to the collagen network by ice crystals and disruption of extracellular matrix cross-links. Freezing temperatures can also impair cell functionality or cause complete lysis. These various structural changes negatively affect the mechanical behaviour of the artery. (70) CPAs, such as DMSO, have been used in several studies to protect cells from cryodamage. These CPAs are usually added to the storage solution to reduce ice formation in both the intra- and extracellular space by preventing cryoinjury of the tissue. (69) However, despite CPAs, the changes mentioned earlier may be associated with biomechanical damage, arterial dysfunction, and failure after implantation. (68)

1.3.1.2.4. Thrombogenicity

The endothelium, among its many other functions such as vascular tone, cell adhesion, smooth muscle cell proliferation and vascular wall inflammation regulator, plays a crucial role in maintaining haemostasis. Its role is dual, with healthy endothelial cells expressing platelet aggregation inhibitors and anticoagulants that prevent platelet aggregation and fibrin formation, respectively. However, in the presence of vascular injury or endothelial cell dysfunction, they induce mechanisms that promote fibrin formation, platelet adhesion and aggregation, and pro-fibrinolytic pathways to control these processes. Overall, a functional endothelium is essential to preserve haemostasis and prevent thrombosis. According to the modern approach to coagulation, haemostasis is maintained by the platelets, the vascular wall and the coagulation cascade in association. The subendothelial collagen released when the vascular wall is damaged leads to platelet adhesion and activation via the von Willebrand factor, and the cellular elements of the vascular wall express tissue factor, which triggers the coagulation cascade, ultimately inducing initial, amplification and finally propagation phases of blood coagulation. In addition to these, endothelial cells produce a variety of anticoagulants such as tissue factor pathway inhibitor, thrombomodulin, endothelial protein C receptor, heparin-like proteoglycans and platelet inhibitors, such as nitric oxide and prostacyclin, are expressed and released, keeping the blood clotting process under control. (71) Studies on the effects of CP have reported morphological changes ranging from endothelial cell swelling to complete loss of endothelium and damage of subendothelial layers. (72–76) Despite the morphological alterations, few investigations found extreme thrombogenicity in cryopreserved allografts. (75,76) However, little is known about the valid procoagulant-anticoagulant properties of allografts and the role of the vascular wall layers in these effects.

1.3.1.2.5. Immunogenicity

Although most studies examining the relationship between blood type matching and clinical outcomes suggest no difference between compatible and incompatible vascular allograft transplantations (31,76,77), others found the opposite, raising an immunological origin in the background of graft complications. (77) Increasing evidence suggests that another major cause of allograft degradation/degeneration, beyond the CP procedure, is considered to be immunological processes resulting from recipient-donor interaction.

Studies of allogeneic vascular grafts have revealed several inflammatory and immune phenomena that, due to advanced fibrosis of the media and adventitia, loss and

dysfunction of endothelium and increased proliferation of intimal smooth muscle cells, lead to a deterioration of graft structure and function. (78–82) These structural changes in the allograft are similar to changes in the small vessels of transplanted kidneys and hearts and are attributed to chronic transplant rejection reactions. (78) Previously, it was thought that CP might have an immunosuppressive effect (83), but several studies have disproved this and demonstrated the same antigenicity and immune responses as fresh grafts. This may be due to the preservation of functional living cells and graft viability, by advanced CP techniques, as cellular components, especially endothelial cells as antigen-presenting elements, play a central role in the immune response induced by donor tissue. (81,84) This is confirmed by studies that have achieved lower inflammatory and immune responses with decellularised or devitalised allografts. (79,82) Although several studies have demonstrated a significant early cellular and late humoral immune response induced by donor human leukocyte antigens (HLA) (78,80,84–86), which eventually leads to complete structural remodelling, fibrosis and cell death of the grafts, the role of these processes in graft degeneration/degradation is not clear, as no correlation has been found between immune response-induced rejection and graft complications, clinical outcomes. (86)

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2. Objectives

In this work, we analyse the cryopreservation protocol of the Cardiovascular Biobank of Semmelweis University, focusing on the direct and storage time-dependent effects of CP on the thrombogenicity of arterial allografts. In addition, we analyse the human vascular microbiota by examining arterial allografts as healthy vascular tissues.

Our research sought to answer the following questions:

I. Do cryopreservation and storage time alter the thrombogenicity of arterial allografts?

- fibrin deposition to the arterial wall
- platelet adhesion to the arterial wall

Hypothesis I.: The cryopreservation does not affect the thrombogenicity of arterial allografts.

Hypothesis II.: The six months storage time of cryopreservation does not affect the thrombogenicity of arterial allografts.

II. Does healthy human vascular tissue have a unique microbiota?

- developing a method for analysing the human vascular tissue microbiota
- characterising the healthy composition of the human vascular tissue microbiota

Hypothesis III.: Healthy human vascular wall contains bacterial hereditary material.

Hypothesis IV.: Healthy human arterial wall has a unique microbiota.

3. Methods

In our prospective studies, eleven human femoral arteries were analysed for thrombogenicity, and fourteen human femoral arteries were examined to determine the healthy human vascular microbiota. The arteries were harvested from eleven and fourteen, respectively, different donors in multi-organ donations between October 2019 and February 2021 in Hungary. Samples for thrombogenicity testing were examined at five time points: before the CP as a native sample (BC) and after the CP immediately (C0), on the first (C1), twelfth (C12) and twenty-fourth (C24) week of storage after CP, while native samples were used for the microbiota assay.

3.1. Inclusion and exclusion criteria of arterial allografts

The study's donor inclusion-exclusion criteria were established based on the national guidelines and regulations for multi-organ donation. (87) In summary, the selection process for donors involves assessing the risks associated with tissue usage. These risks are identified through various examinations, including medical, biological, post-mortem, and lifestyle assessments. If any of the following conditions apply, potential donors should be excluded: unknown cause of death, presence of an unknown disease in medical history, malignant disease, transmissible spongiform encephalopathies, untreated infection at the time of donation, human immunodeficiency virus (HIV) infection, autoimmune disease that may affect the transplanted organ, interventions that may impact donor blood test results, potential transmitted diseases, poisoning, previous organ recipient status, and recent vaccination with live virus. Additionally, donors above 65, those with malignancies or positive bacterial-fungal culture or virus serology tests, and allografts with negative evaluations from the performing vascular surgeon (such as significantly injured or calcified allografts) were also excluded.

3.2. Ethical considerations, data collection

The study adhered wholly to the principles outlined in the national multi-organ donation guidelines and the relevant international and national laws. The donor data, which remained anonymous, was collected in advance from the electronic health information system of the donation following the General Data Protection Regulation of the European

Union. The study received approval from the institutional review board, specifically from the Semmelweis University Regional and Institutional Committee of Science and Research Ethics (approval number: 257/2018). Since the vascular tissues were obtained from brain-dead donors as part of multi-organ donations, and the data was analysed anonymously, individual consent was not obtained.

3.3. Harvesting, cryopreservation, storage and thawing of allografts

Femoral arteries were harvested in Budapest from brain-dead donors within multi-organ donations organised by the Organ Coordination Office of the Hungarian National Blood Transfusion Service. Before harvesting, all donors underwent serology tests to confirm the absence of HIV, hepatitis B virus, hepatitis C virus, syphilis, active Epstein-Barr virus, and active cytomegalovirus. The explantation of the femoral arteries (common and superficial femoral artery) was performed under surgical asepsis and using principles of sterile technique. The vascular surgeon, who performed the harvesting, assessed the suitability of the grafts. Subsequently, the harvested arterial allografts were immediately placed in a triple sterile plastic bag (Set of Transplantation Bags–sterile 80 00 61H, Raguse GmbH, Ascheberg, Germany) in 500 ml transport solution (Sodium Chloride 0.9% “Baxter” Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) containing 4 mg/ml cefazolin (Sandoz GmbH, Kundl, Austria) and 0.4 mg/ml fluconazole (Fresenius Kabi Hungary, Budapest, Hungary) at 4˚C. They were transferred to the storage location in an organ transport box (IGL Box Organ Transporter, Institut Georges Lopez, Lissieu, France) at 4˚C. They were stored at a temperature of 4˚C for 12 hours.

3.3.1. Thrombogenicity

The CP procedure occurred within 12 hours after the explantation in a controlled environment. The clean room was classified as "A" with a background classified as "B," and a laminar airflow system was used. (88) After conducting a test to detect bacteria and fungi and reassessing the grafts, five ring samples measuring 0.5 cm in width were taken from each femoral artery. One of the five samples (BC) was placed in a plastic cryotube containing isopentane (Merck Kft, Budapest, Hungary) and stored at -20˚C until the thrombogenicity measurements. The remaining samples (C0, C1, C12, C24) underwent the same CP procedure as the allografts. They were placed in cryo bags (TissueVault Cryogenic Freezing Bag TV1430, Origen Biomedical, Austin, Texas, USA) in a 500 ml

CP solution (Ringer Fresenius, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) containing 20 v/v% DMSO (Molar Chemicals Kft., Halásztelek, Hungary), 4 mg/ml cefazolin (Sandoz GmbH, Kundl, Austria) and 0.4 mg/ml fluconazole (Fresenius Kabi Hungary, Budapest, Hungary) for 10 minutes. After that, these samples were cryopreserved to -80-90˚C according to a controlled freezing method (PC Interface for Thermo ScientificTM CryomedTM Freezers, Version 3.0 and Thermo Scientific CryoMed Controlled-Rate Freezer 7451, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) (Figure 1) using vaporised nitrogen (Messer Hungarogáz Kft., Budapest, Hungary), and they were stored at -80˚C in a deep freezer (Taylor-Wharton K Series Cryogenic Storage System 10K, Taylor-Wharton, Baytown, Texas, USA). After different storage periods, the cryopreserved graft samples were thawed at 37˚C until the ice melted completely. They were washed three times with physiological salt solution (Sodium Chloride 0.9% “Baxter” Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) at 20˚C to remove the CP medium.

3.3.2. Healthy human vascular microbiota

Samples for microbiota analysis were prepared within 24 hours after the explantation under sterile conditions in a clean room classified “A” with a background classified “B” using a laminar air flow system. (88) Three 3 mm3 samples were cut from each femoral artery. The samples were placed into a sterile plastic tube (VWR Low Temperature Freezer Vials, VWR International, LLC, Radnor, PA, USA) in physiological saline solution (sodium chloride 0.9% “Baxter” Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) and transported immediately for microbiota analysis at 4°C.

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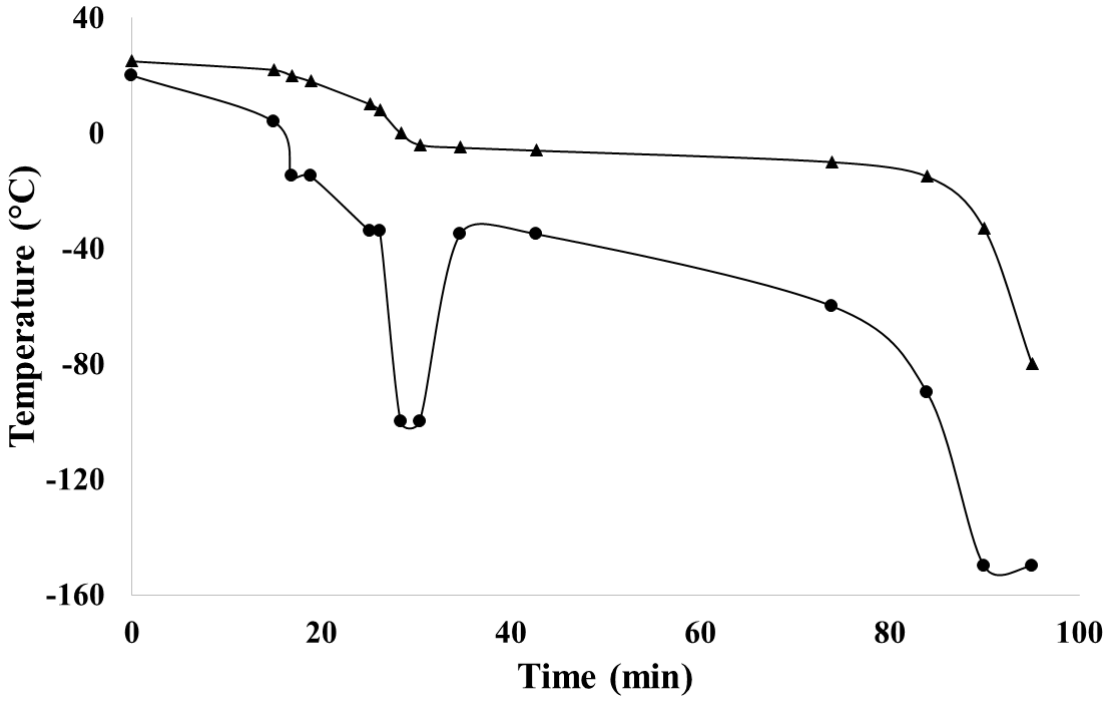

Figure 1. Controlled freezing protocol of the cryopreservation. Continuous line with „●": temperature ramps of controlled-rate freezer's chamber; continuous line with „▲": temperature ramps of sample

3.4. Measurement of thrombogenicity by immunofluorescence imaging

The flow-chamber model was used for testing the allograft samples as thrombogenic surfaces. Frozen cross-sections (10 μm) of allograft samples placed on poly-L-Lys-coated slides were perfused at 0.5 ml/min flow rate in a 0.4-cm-wide and 0.12-mm-high parallel-plate chamber with heparin-anticoagulated blood collected from healthy volunteers. Assuming laminar flow conditions, the shear rate at the surface of the section was 900 s −1, according to the formula 1.03*6 Q/(w*h2), where Q is the flow rate in ml s −1, w and h are the width and the height of the flow path in cm, respectively. This intermediate shear rate was chosen as an adequate model of the rheological situation in medium-sized arteries, where deposition of both platelets and fibrin is enabled. Before the perfusion, the sections were blocked with 2 w/v% bovine serum albumin (BSA) in 0.05 M Tris buffer pH 7.4 containing 0.1 M NaCl and 0.02 w/v% NaN3 (TBS) for 45 minutes, and the 90-s perfusion was followed by a 30-s wash with 1.5 mM KH2PO4, 8.1 mM Na2HPO4 buffer pH 7.4 containing 137 mM NaCl and 2.7 mM KCl (PBS).

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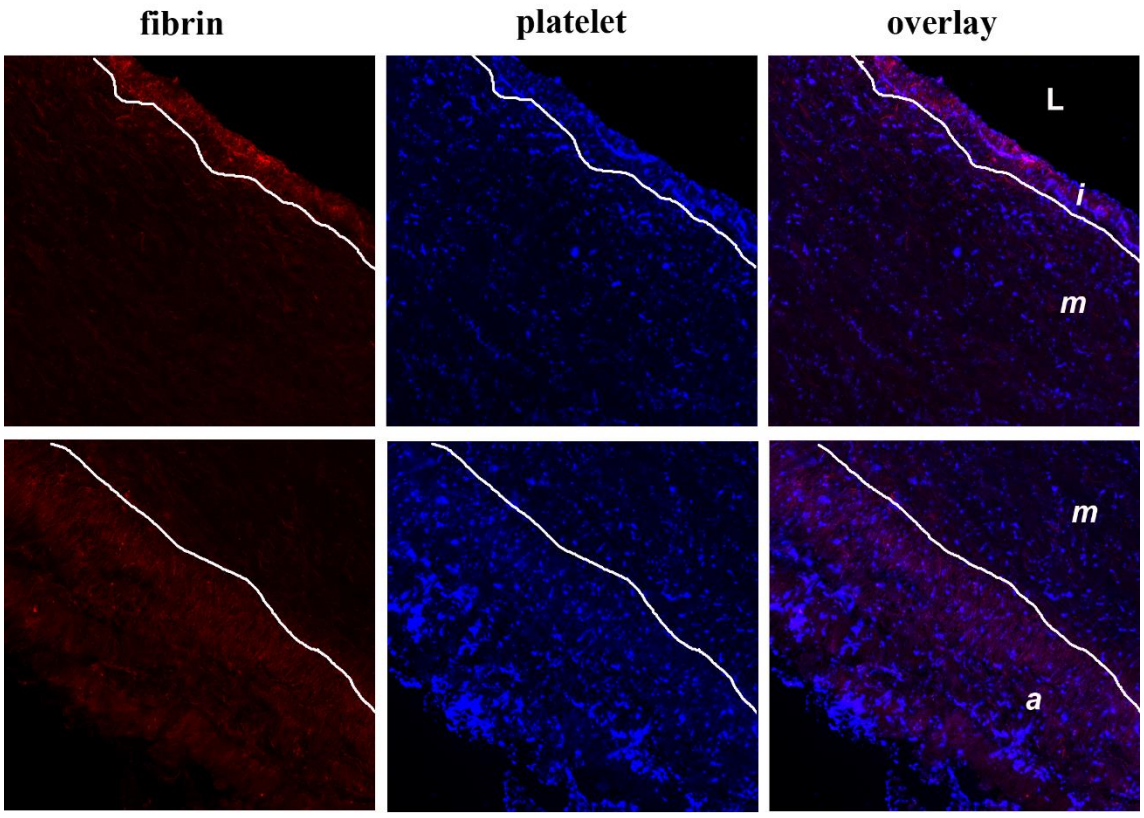

Figure 2. Platelet and fibrin deposition in arterial cross-sections detected by indirect immunofluorescence. Blue colour: distribution of GpIIb/IIIa; red colour: distribution of fibrin; white line: internal and external elastic laminae - indicates the boundaries between the wall layers; L: lumen; i: tunica intima; m: tunica media; a: tunica adventitia (89)

After that, the sections were fixed in acetone at 4˚C for 10 minutes, and the deposited platelets and fibrin were double-stained for indirect immunofluorescence microscopy. Sections were blocked with BSA-TBS for 30 min, followed by platelet staining using the mouse monoclonal antibody against human GpIIb/IIIa (4 μg/ml in BSA-TBS for 30 min, sc-53417, Santa Cruz Biotech), followed by three times 5 min washes in TBS, and 30 min incubation with the Goat-anti-Mouse IgG-Alexa Fluor 633 secondary antibody (2 μg/ml in BSA-TBS, Invitrogen, Budapest, Hungary). Double-staining was continued with the fibrin staining after three times 5 min washes in BSA-TBS: 30 min incubation with the primary rabbit polyclonal antibody developed against the N-terminal part of the gamma chain fibrin(ogen) (20 μg/ml in BSA-TBS, PA5-29734, Invitrogen), followed by three times 5 min washes in TBS, and 30 min incubation with the Goat-anti-Rabbit IgG-Alexa Fluor 546 secondary antibody (2 μg/ml in BSA-TBS, Invitrogen). Following the final

washes in TBS, the stained sections were covered in 50% glycerol in TBS. Confocal images were taken from the slides using a Zeiss LSM710 confocal laser scanning microscope equipped with a 10 × 0.3 lens (Carl Zeiss, Jena, Germany) using 488 nm, 543 nm and 633 nm excitation laser lines, respectively. Emissions were detected in the ranges of 500–530 nm, 565–585 nm and 650–690 nm, respectively. Each allograft vessel at each sampling time point was perfused in duplicates or triplicates, and depending on the section size, 5–10 different images were taken of each perfused cryosection to survey the whole cross-sectional area of the vessel. Quantification of platelet and fibrin(ogen) coverage of the vessel wall was performed with the Image J software (NIH, Bethesda, MD, USA), selecting the region of interest, calculating its surface area in pixels and setting a threshold intensity value for automatic identification of platelets or fibrin(ogen) covered areas in percentage. (89) (Figure 2, Figure 3)

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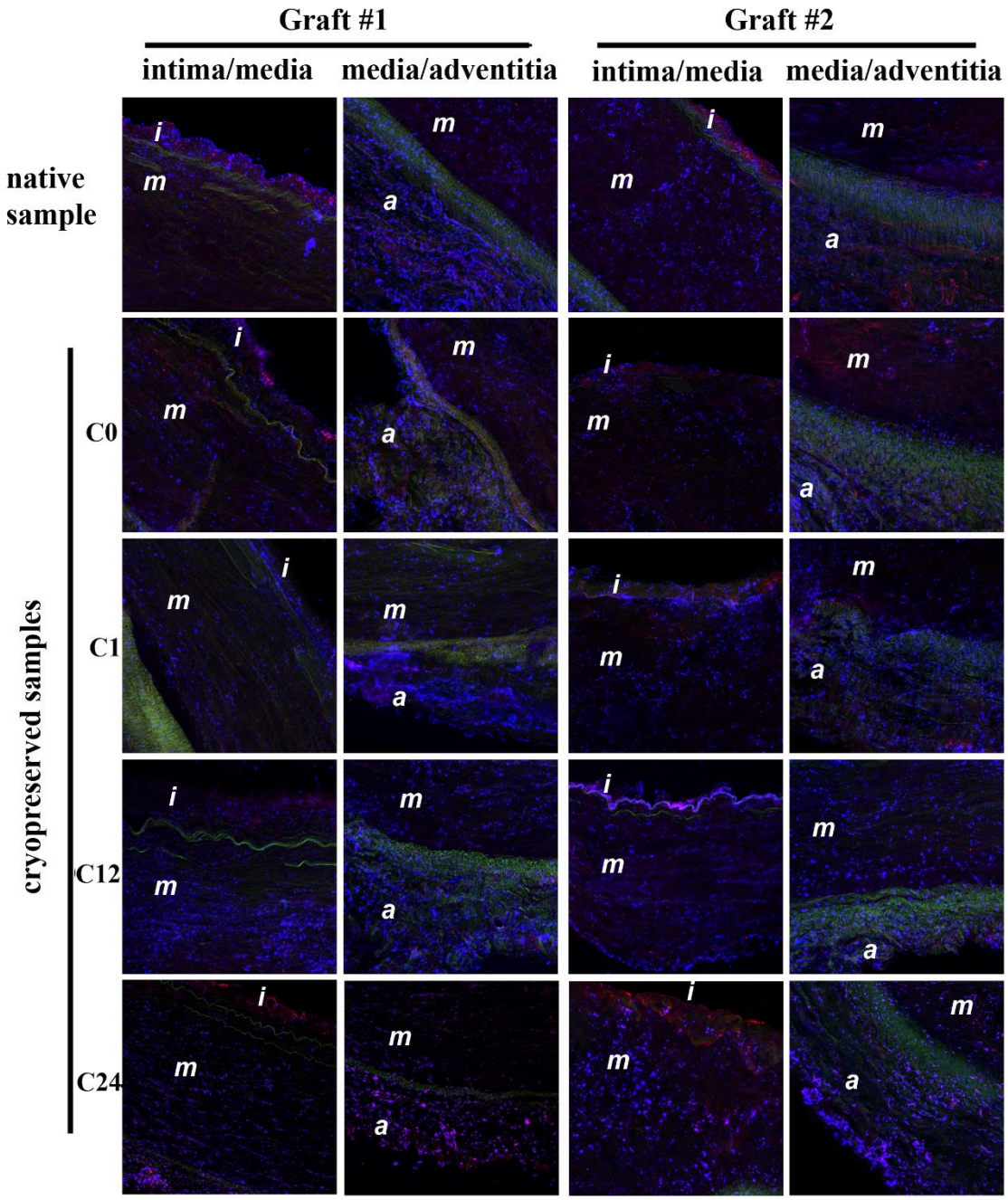

Figure 3. Time sequence of immunofluorescence images monitoring the deposition of fibrin and platelets in different layers of the arterial wall. Images from two different regions of interest in two different allografts are shown for each time point. C0: immediate testing time point after cryopreservation; C1,C12,C24: testing time points one, twelve and twenty-four weeks after cryopreservation, respectively; blue colour: immunostained platelets; red colour: immunostained fibrin(ogen); green colour: autofluorescence of the internal and external elastic laminae - indicates the boundaries between the wall layers; i: tunica intima; m: tunica media; a: tunica adventitia (89)

3.5. Identification of healthy human vascular microbiota: Deoxyribonucleic acid isolation, 16S ribosomal ribonucleic acid gene library preparation and MiSeq sequencing

Following the instructions, the deoxyribonucleic acid (DNA) isolation was performed using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research Corp., Irvine, CA, USA). Before isolation, enzymatic dissolution was performed using ProtK at 56°C for 3 hours. The isolated DNA samples were then stored at -80°C until further processing for PCR amplification. The concentration of genomic DNA was measured using the Qubit2.0 Fluorometer with the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). For amplification of bacterial DNA, PCR was conducted using tagged primers that targeted the V3-V4 region of the bacterial 16S rRNA gene. PCR and DNA purification steps followed Illumina's protocol. The PCR product libraries were evaluated using the Agilent 2100 Bioanalyzer with the DNA 1000 Kit (Agilent Technologies, Waldbronn, Germany). Equimolar concentrations of libraries were pooled and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using MiSeq Reagent Kit v3 (600 cycles PE). Extraction-negative controls and PCR-negative controls were included in each sequencing run to assess the potential contribution of extraneous DNA from reagents. Each vascular tissue sample was independently subjected to DNA extraction to ensure reproducibility. All analysis procedures were performed in triplicate to minimise false results such as contamination using three different samples from each donor. The raw sequencing data were obtained from the Illumina BaseSpace and analysed using the CosmosID bioinformatics platform (CosmosID Metagenomics Cloud, CosmosID Inc. Rockville, MD, USA). The resulting data included taxonomic names, operational taxonomic unit IDs, frequency, and relative abundance. Among the three samples from each donor, the sample with the highest read count was chosen for comparative studies. Figures 8-12 were created based on filtered abundance score matrices at the phyla or genus level from the CosmosID taxonomic analysis, utilising the CosmosID-HUB bioinformatics pipeline. The Jaccard Principal Coordinates Analysis (PCoA) biplot diagram in Figure 12b was generated using the Fathom Toolbox for MATLAB, a multivariate data analysis software.

The results of the current vascular samples were compared with the DNA amounts obtained from faecal and nasopharyngeal samples in our previous studies (90,91). The DNA isolation and 16S rRNA method were consistent across these studies. (92)

3.6. Statistical analysis

3.6.1. Thrombogenicity

Our study involved analysing arterial samples from 11 donors to measure platelet and fibrin content. Since the number of data collected varied among the donors (ranging from 111 to 225), we used a fuzzy sample approach to assess the data. This method allowed us to compare arterial samples from different donors effectively. We employed a specific fuzzy estimation method and a fuzzy version of the invertible cumulative distribution function estimator with the maximum count of nodes. These techniques helped us estimate the data's median, lower, and upper quartile. To evaluate the impact of CP on thrombogenicity, we performed an analysis by comparing coverage values at different time points with those of the native graft. We conducted four Bootstrap one-tailed tests, comparing the quartiles to test the null hypothesis (H0) that the quartiles are equal against the alternative hypothesis (H1) that one quartile is greater than the other. To increase the statistical power of the tests, we adopted a cluster approach to hypothesis testing. We compared the four p-values to a predetermined significance level ($\alpha = 0.05$) and rejected the null hypothesis if at least two p-values were below α.

To analyse the effect of storage time on thrombogenicity, we examined the trends in the median, lower quartile, and upper quartile of the thrombogenic factor abundance over time. We constructed linear regressions for each quartile, using time as the independent variable. These regressions were trained on a fuzzy sample of four triplets (time, quartile, and degree of membership). We used a cluster of four fuzzy Bootstrap procedures to determine the distribution of the predicted regression slope and the probability of the slope being negative or non-negative (or positive or non-positive). We considered the estimated regression slope significant if at least two of the four procedures indicated a probability for non-negativity/non-positivity below the predetermined significance level ($\alpha = 0.05$). These probability-based estimates are more potent than p-values in determining the significance of the slope sign. While p-values only provide the

probability of being wrong when rejecting the null hypothesis, the probability estimates calculate the probability of being right when accepting the alternative hypothesis. (89)

3.6.2. Healthy human vascular microbiota

Categorical variables are represented as counts and percentages, while continuous variables are represented by the median and interquartile range (IQR). To determine statistical significance between different groups of vascular samples, we used the Wilcoxon Rank Sum test for Chao1 Alpha diversity and the permutational multivariate analysis of variance (PERMANOVA) analysis for Jaccard PCoA Beta diversity. These statistical analyses were conducted using the CosmosID bioinformatics platform.

We utilised the Mann-Whitney U test to assess the significance of differences in DNA amounts and amplified PCR products between the vascular and other frequently tested samples (which were not normally distributed variables).

A p-value less than 0.05 was considered to indicate statistical significance. (92)

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4. Results

4.1. Donor characteristics

Table 6. Characteristics of donors included in the thrombogenicity (T) and microbiota (M) studies. Data are presented as number of donors(%) or median(interquartile range); n=number of donors; BMI=body mass index; COPD=chronic obstructive pulmonary disease

Characteristic		T(n=11)	M(n=14)
Cause of death	Trauma	3(27.27)	5(35.71)
	Cerebral ischaemia	2(18.18)	2(14.28)
	Cerebral haemorrhage	6(54.55)	7(50)
Age (year)		45.00(17.50)	49.50(9.50)
Female sex		3(27.27)	3(21.42)
BMI (kg/m2)		26.30(4.15)	27.80(7.05)
Past medical history	Hypertension	3(27.27)	3(21.42)
	Diabetes mellitus	1(9.09)	1(7.14)
	Pulmonary disease (COPD)	1(9.09)	1(7.14)
	Smoking	4(36.36)	7(50)
Blood type	A	5(45.46)	5(35.71)
	B	3(27.27)	2(14.28)
	AB	0(0)	1(7.14)
	0	3(27.27)	6(42.85)
	Rh+	10(90.91)	12(85.71)
	Rh-	1(9.09)	2(14.28)

4.1.1. Thrombogenicity

The median age of the donors was 45.0(33.0;50.5) years. Three donors were female (27.27%). The median body mass index (BMI) was 26.3(23.65;27.8) kg/m2. The leading cause of death was cerebral haemorrhage (6 donors; 54.54%). All essential characteristics of the donors are presented in Table 6..

4.1.2. Healthy human vascular microbiota

The median age of the donors was 49.5 (IQR:10) years. The male/female rate of donors was 11/3. The median BMI was 27.8 (IQR:7.9) kg/m2. The leading cause of death was cerebral haemorrhage (7 donors; 50%). All essential characteristics of the donors are presented in Table 6..

4.2. Thrombogenicity

4.2.1. Fibrin deposition to the arterial wall

The generation of fibrin was evaluated quantitatively by measuring the percentage of area covered by fibrin in the intima, media, and adventitia layers of immunofluorescent images of the graft cross-sections. The statistical analysis showed no significant increase in fibrin deposition in the cryopreserved samples compared to the fibrin levels detected in the native (BC) samples across any of the three layers. Notably, all significant differences between cryopreserved and native graft samples indicated a decrease in fibrin generation (out of the twelve sets of CP data, three median, three bottom quartile, and six top quartile values were lower than their BC counterparts). CP also tended to homogenise the potential for fibrin generation across the artery wall layers, with narrower IQRs observed in eight out of the twelve CP data sets. (Figure 4)

To investigate the hypothesis that storage time affects fibrin deposition in cryopreserved grafts, we conducted a regression analysis of changes in fibrin coverage across the three arterial wall layers over time. In seven out of the nine hypothesis tests performed, which examined the temporal trend of fibrin coverage in the three quartiles of the wall layers, a non-negative trend was excluded at a significance level of 0.05. In the remaining two cases, namely the lower coverage quartile of the media and intima layers, there was a probability of a non-negative trend of 20-22% and 14-28%, respectively. (Figure 5)Therefore, a robust statistical analysis of fibrin coverage data throughout the twenty-four-week storage of arterial grafts definitely rules out the possibility of an increase in

graft thrombogenicity over time and strongly supports a decrease in fibrin generation. (89)

4.2.1. Platelet adhesion to the arterial wall

Platelet adhesion was evaluated by measuring the percentage of area covered by the GpIIb/IIIa-related immunofluorescence signal in the intima, media, and adventitia layers. Like fibrin deposition, the platelet coverage data within the IQR of the cryopreserved grafts largely overlapped with the corresponding range of the BC samples. However, unlike fibrin, in three out of the twelve datasets of cryopreserved samples, either all three (C0 and C1) or two (C12) quartile values of the media significantly exceeded the platelet coverage level of the native samples (BC). In the early cryopreserved samples of the intima (C0, C1, C12), there was a significant upward shift in the median of platelet coverage. However, a significant change in the upper and lower quartiles did not accompany this difference. Based on the data measured at week twenty-four, the values for all three layers no longer exceeded the values of the native sample, and significantly lower quartile values were obtained for the adventitia. (Figure 6)

Regression analysis of the changes in platelet coverage across the three arterial wall layers over time excluded a non-negative trend at a significance level of 0.05 in seven of the nine hypothesis tests performed on the platelet coverage of the three wall layers' three quartiles. The two exceptions were the median and the lower coverage quartile of the intima, where the probability of a non-negative trend was 5-11% and 7-18%, respectively. (Figure 7) Therefore, the regression analysis of the platelet coverage data throughout the twenty-four-week storage of arterial grafts definitely rules out the possibility of a time-dependent increase in graft thrombogenicity and strongly supports a decline in platelet adhesiveness. (89)

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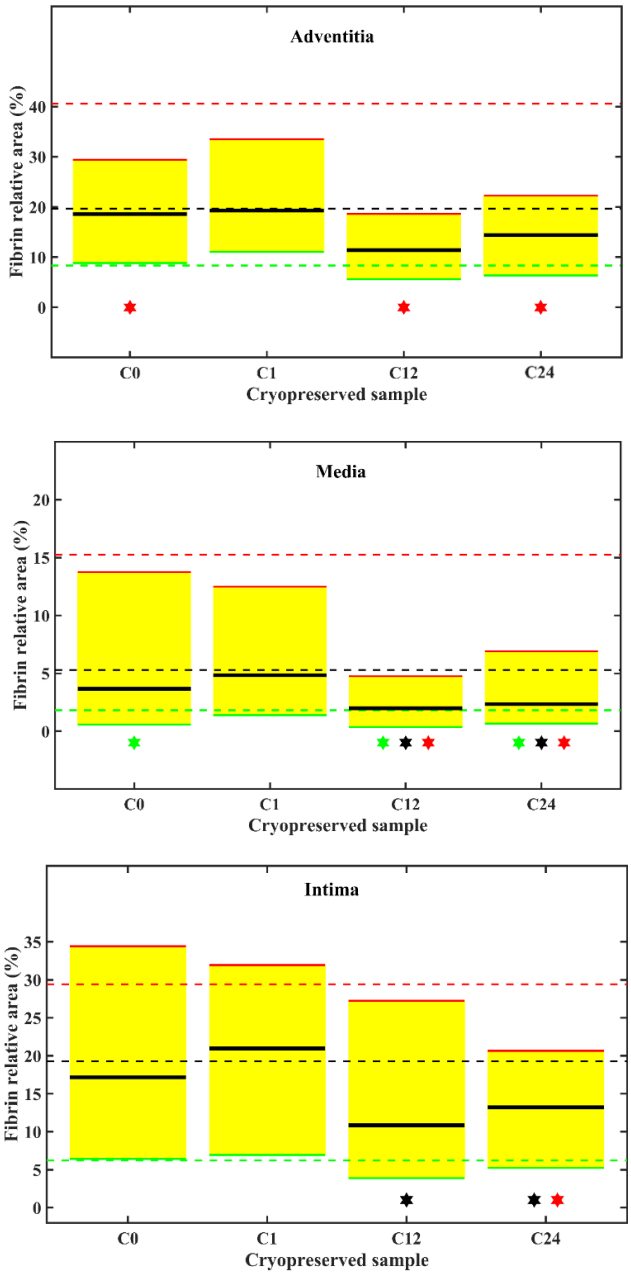

Figure 4. Quartile-wise comparison of the fibrin deposition on cryopreserved and native arterial allografts. C0: immediate testing time point after cryopreservation; C1,C12,C24: testing time points one, twelve and twenty-four weeks after cryopreservation, respectively; dashed and continuous lines: lower (green), median (black) and upper (red) quartiles of coverage data for the native samples and for the cryopreserved samples, respectively; yellow shadow area: the interquartile range of the cryopreserved samples; asterisks: significant differences between the respective quartiles (lower(green), median(black) and upper(red)) of native and cryopreserved samples (89)

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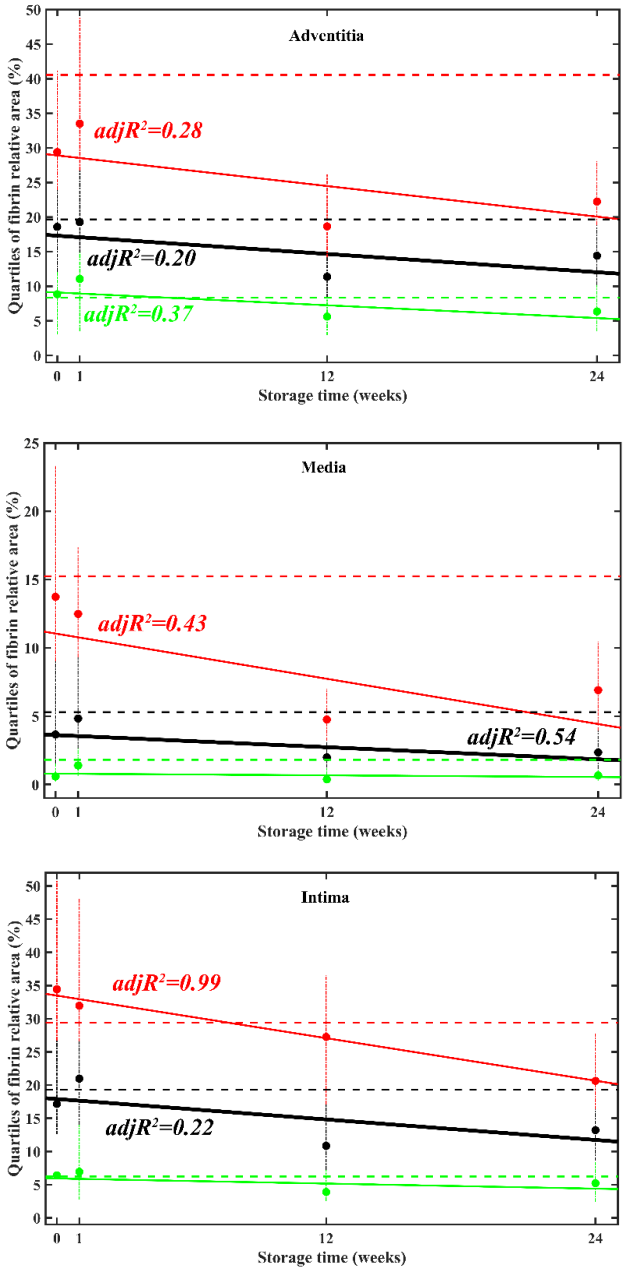

Figure 5. Time course of fibrin deposition in cryopreserved allografts. The explanatory strength of the correlation can be assessed on the basis of the adjusted R2 (adjR^2) values that are shown only for the regression trends proven to be significant at 0.05 level by the statistical approach described in Methods. C0: immediate testing time point after cryopreservation; C1,C12,C24: testing time points one, twelve and twenty-four weeks after cryopreservation, respectively; lower (green), median (black) and upper (red) quartiles of the coverage data are shown with dashed lines for the native samples, circles with error bars (12.5%) for the cryopreserved samples and continuous lines for the linear regression (89)

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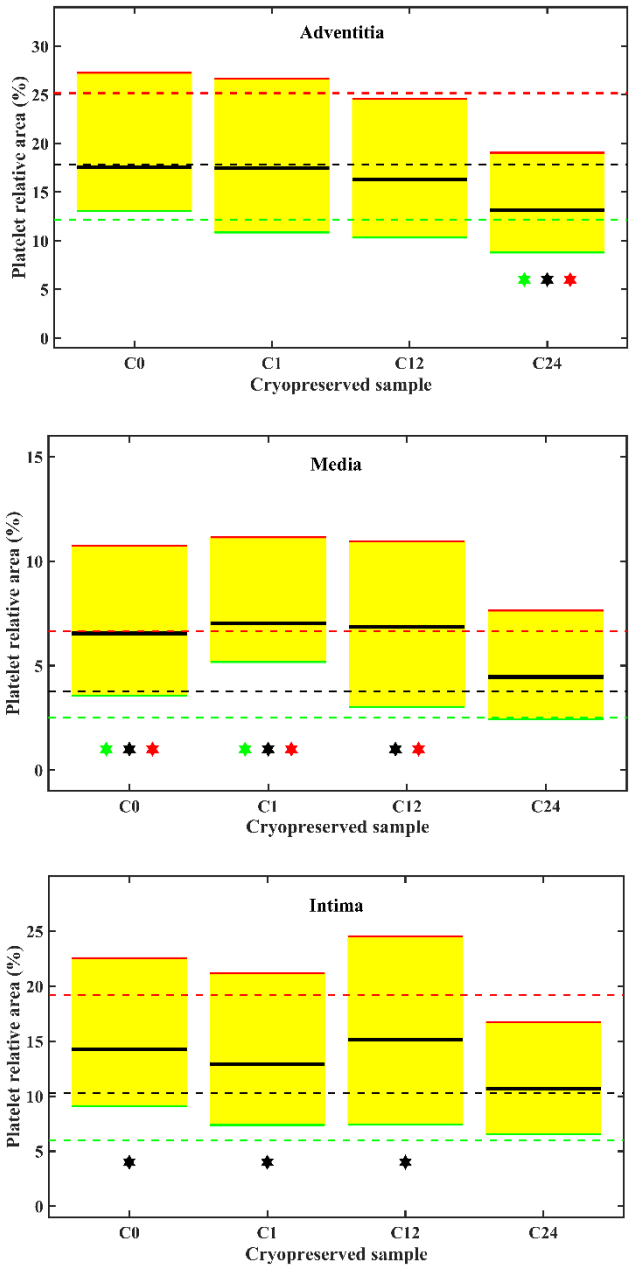

Figure 6. Quartile-wise comparison of the platelet adhesion on cryopreserved and native arterial allografts. C0: immediate testing time point after cryopreservation; C1,C12,C24: testing time points one, twelve and twenty-four weeks after cryopreservation, respectively; dashed and continuous lines: lower (green), median (black) and upper (red) quartiles of coverage data for the native samples and for the cryopreserved samples, respectively; yellow shadow area: the interquartile range of the cryopreserved samples; asterisks: significant differences between the respective quartiles (lower(green), median(black) and upper(red)) of native and cryopreserved samples (89)

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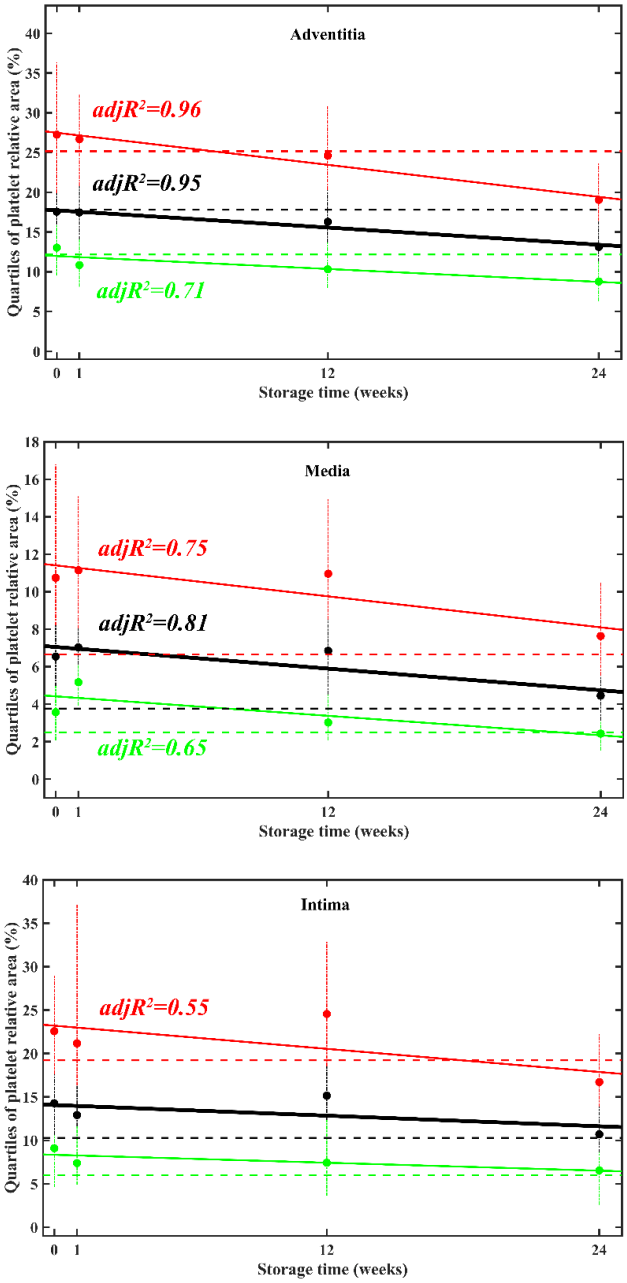

Figure 7. Time course of platelet adhesion to cryopreserved allografts. The explanatory strength of the correlation can be assessed on the basis of the adjusted R2 (adjR^2) values that are shown only for the regression trends proven to be significant at 0.05 level by the statistical approach described in Methods. C0: immediate testing time point after cryopreservation; C1,C12,C24: testing time points one, twelve and twenty-four weeks after cryopreservation, respectively; lower (green), median (black) and upper (red) quartiles of the coverage data are shown with dashed lines for the native samples, circles with error bars (12.5%) for the cryopreserved samples and continuous lines for the linear regression (89)

4.3. Healthy human vascular microbiota

In total, forty-two samples from fourteen patients were tested. The median of isolated DNA from vascular samples was 27.8 ng/µL (IQR: 21.4 ng/µL). From this starting amount, which also contains human DNA, after 16S rRNA PCR, a median DNA amount of 1.546 ng/µL (IQR: 0.762 ng/µL), after indexing PCR, a median DNA amount of 3.947 ng/µL (IQR: 1.996) was amplified. The average length of index PCR products was 667 base pair (bp) (standard deviation (SD): 55 bp). Negative controls and transport buffers did not yield measurable amounts of DNA after DNA isolation or 16S rRNA PCR. 5.8 million valid sequences were obtained, resulting in 3.9 million high-quality reads. One sample's median number of reads was 79,485 (IQR: 24,511).

The most prevalent phyla in the human vascular microbiota were *Proteobacteria* (31.78%), *Firmicutes* (29.18%), and *Actinobacteria* (23.05%). (Figure 8) Based on the

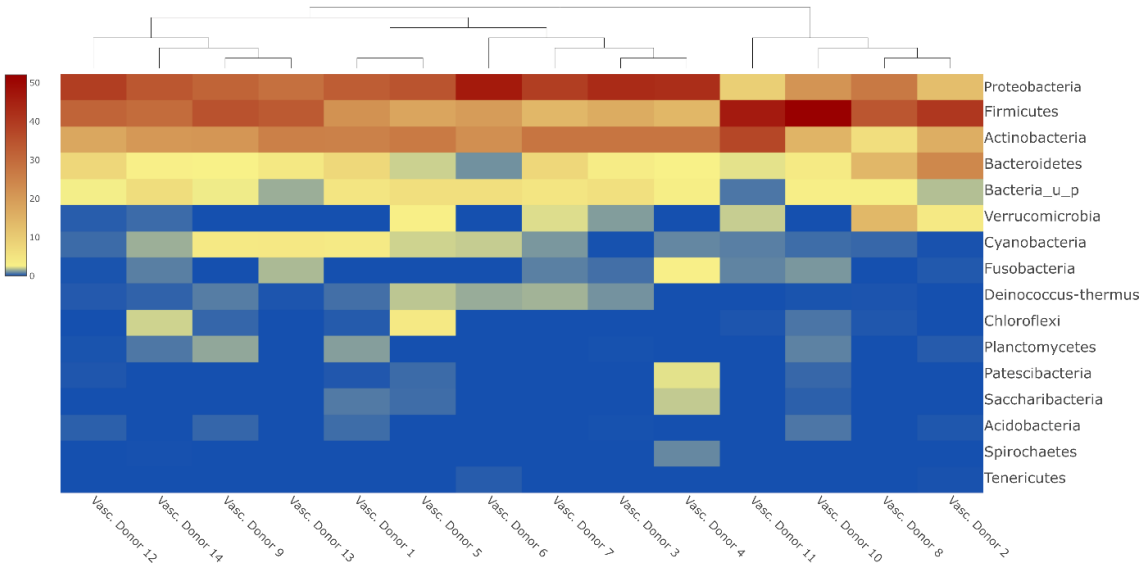

Figure 8. Heatmap of the most abundant phyla in human vascular samples with sample clustering dendogram. (92)

abundance of these phyla, the samples were clustered into three major groups. At the genus level, the most abundant taxa in the human vascular microbiota were *Staphylococcus*, *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Acinetobacter*, and *Propionibacterium*. (Figure 9a) However, the proportions of these genera varied greatly among individual samples, as depicted in the stacked bar graph. (Figure 9b)

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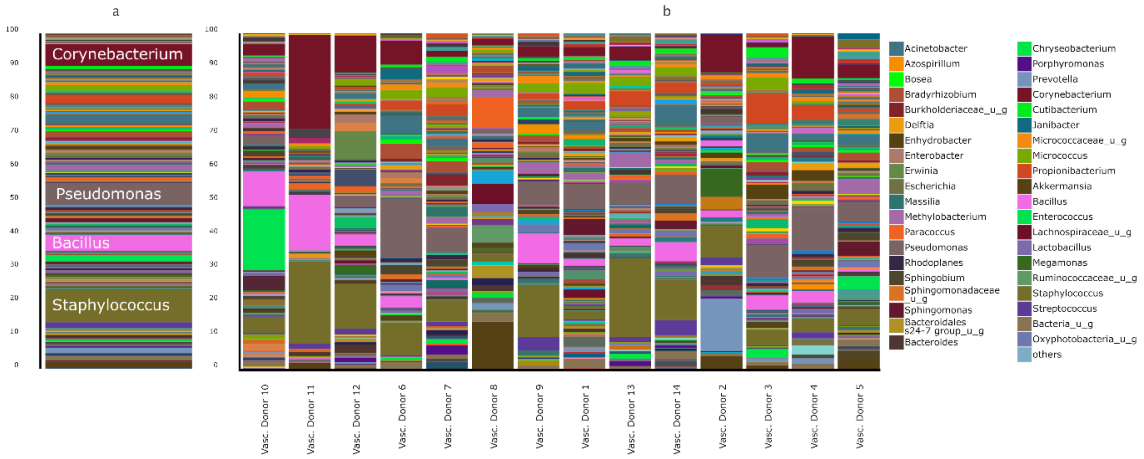

Figure 9. Stacked bar plot of the composition of the human vascular microbiota at the genus level: a. overall results; b. results for each sample. (92)

The median Chao1 alpha diversity of genera was 217.5(IQR: 107), indicating the diversity of bacterial genera. Among the bacterial taxa indirectly associated with atherosclerosis development, *Porphyromonas gingivalis* was found in two samples (0.54%, 0.16%), *Prevotella nigrescens* in one sample (0.1%), and all samples contained varying amounts of *Enterobacteriaceae* spp with a median of 2.25% (IQR: 1.46). Comparatively, bacteria more commonly found in the intestinal flora of healthy individuals than in patients with atherosclerosis included *Roseburia*, which was found in eight samples (median: 0.13%, IQR: 0.08), and *Ruminococcus*, which occurred in thirteen samples (median: 3.07%, IQR: 1.78). *Helicobacter pylori* and *Chlamydophila pneumoniae*, bacteria directly implicated in the development of atherosclerosis, were not detected in any of the samples.

To confirm its uniqueness, the human vascular microbiota was compared with human stool and nasopharyngeal samples. Based on the Jaccard Beta Diversity PCoA analysis (p=0.001), we found significant differences between the samples, suggesting notable distinctions in the microbial composition between the vascular system and the gastrointestinal tracts. (Figure 10)

The composition of the vascular microbiota did not show significant differences when compared by age, sex or smoking habits, as observed in the Three-dimension PCoA pictures. (Figure 11) However, significant differences in the Chao1 alpha diversity of microbiota were found between the O and A blood groups (p=0.0106) and between the O and B blood groups (p=0.045) using the Wilcoxon Rank Sum test. The beta diversity of

the microbiota was assessed using the Jaccard distance measure, and the PCoA biplot was utilised for visualisation. PERMANOVA analysis revealed significant differences in the Jaccard beta diversity values of the microbiotas between the O and A blood groups (p=0.003), between the O and B blood groups (p=0.037), and between the A and B blood groups (p=0.045). (Figure 12) (92)

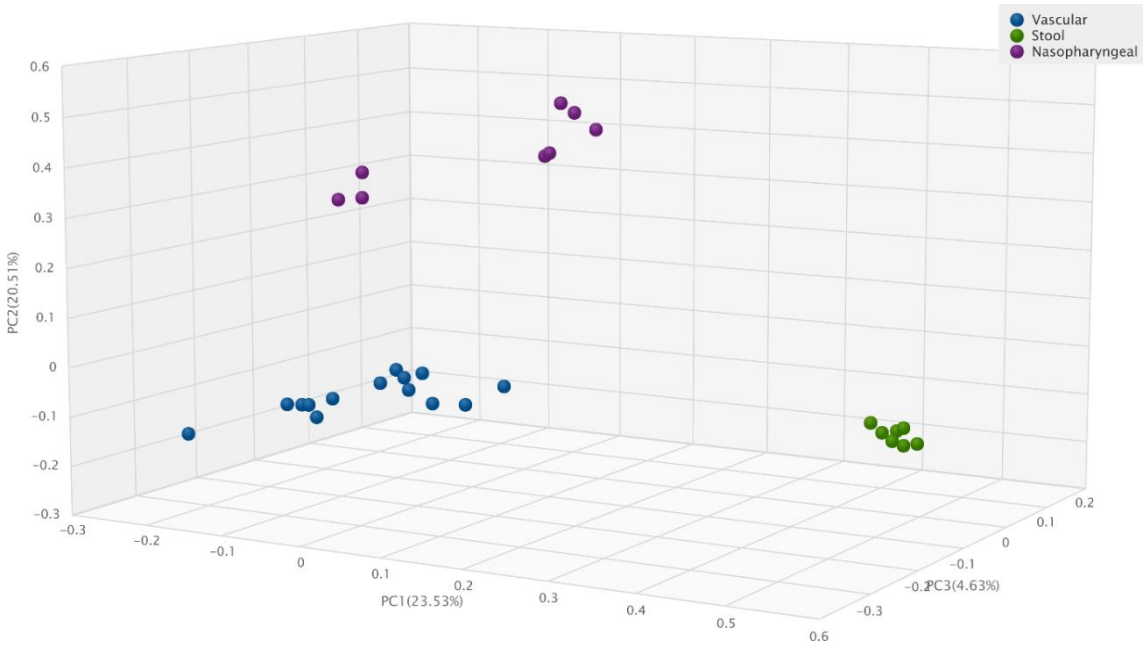

Figure 10. Jaccard Beta Diversity Principal Coordinate Analysis of human vascular, human stool and human nasopharyngeal samples. (92)

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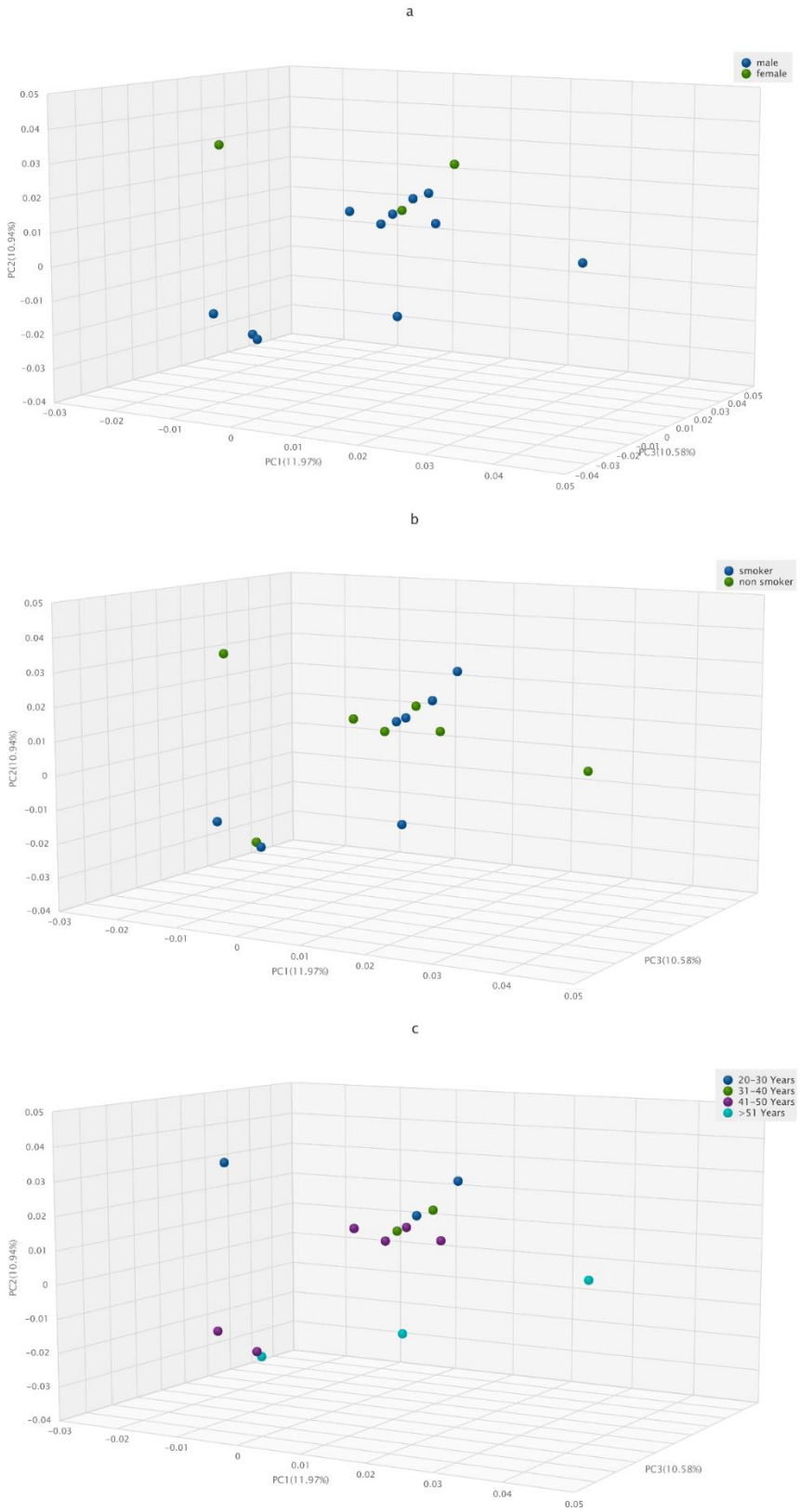

Figure 11. Three-Dimension Principal Coordinate Analysis of human vascular microbiota: a. in male and female patients; b. in smoker and non-smoker patients; c. by age distribution. (92)

DOI:10.14753/SE.2024.3064

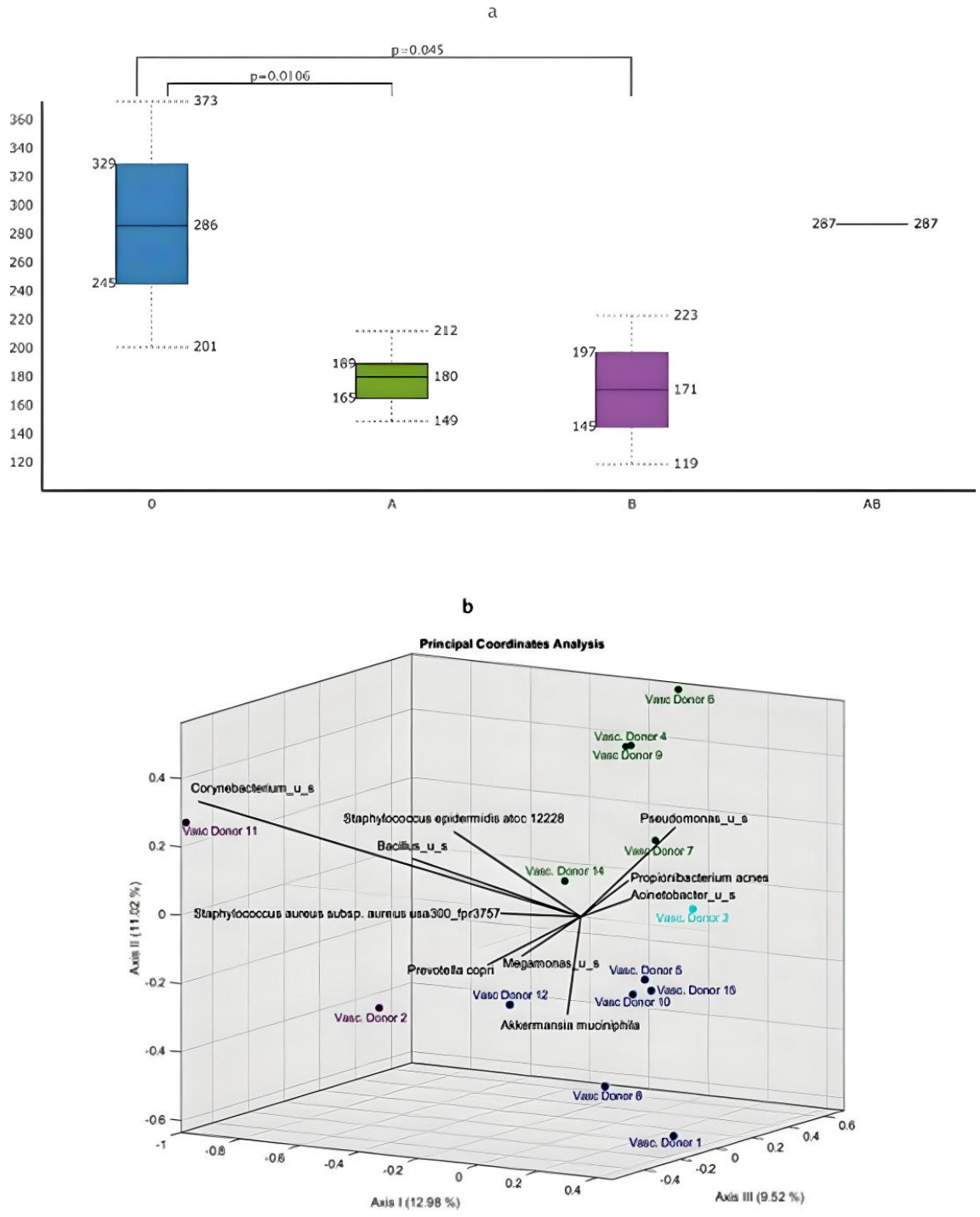

Figure 12. Differences in vascular microbiomic diversity according to A (green)/B(purple)/AB(light blue)/0(dark blue) blood groups. a. Chao1 Alpha Diversity of human vascular microbiome significant differences are indicated with p values; b. bi-plot Jaccard Beta Diversity Principal Coordinate Analysis according to A/B/AB/0 blood groups (92)

5. Discussion

Despite significant advancements in our knowledge of cryopreserved allografts over the past decades, numerous unanswered questions persist.

Many factors determine the success of vascular allograft transplantation, and our aim in the present studies was to obtain information on changes in essential characteristics, such as thrombogenicity, induced by CP and during storage time under in vitro conditions. In addition, using allografts as healthy vascular tissues, another objective was to gain insight into the human vascular microbiota, which, on the one hand, could significantly reduce the risk of transmission of infections during transplantation, and, on the other hand, goes well beyond the allograft issue, allowing a better understanding and treatment of vascular diseases.

The primary role of CP is to preserve cells and tissues in their most intact form, structurally and functionally. In the case of CP of a tissue or even an organ, we face much more complex problems. We have to count on the freezing of much larger samples than cell cultures, and this includes the problem that the CP must work at every single point of the sample at the same time and that the ideal freezing parameters and cryoprotective materials must be found for cells with different properties and even for the extracellular matrix to preserve them and avoid the harmful ice formation. (56) We also have to consider the possible damage to the grafts as the freezing storage time progresses, although there is no clear evidence of it. (31,61–66)

Although Song et al. reported a very high survival of endothelial cells of rabbit common carotid artery above 70% with an appropriate concentration of cryoprotectant and cooling rate, they described morphological changes due to CP with preserved endothelium-dependent relaxation, such as looser endothelium attachment to the basement membrane or intracellularly appearing vacuoles. (73) In contrast, other studies investigating the effects of CP have described significant morphological and functional damage of the endothelium and low cell survival. (72,74) However, some other authors found no significant hemocompatibility differences despite morphological alterations. (75,76) It is essential to add that the role of the endothelium is not fully clarified in cryopreserved allografts, as the donor endothelial lining disappeared within five days after implantation

of vascular allografts, accompanied only by a marked decrease in fibrinolytic activity. (76)

Our findings on thrombogenicity indicated that when arterial allografts were cryopreserved and stored for up to 6 months, they maintained the ability to have haemostatic potential comparable to the fresh native arterial wall. In addition, the fibrin deposition and platelet adhesion to cryopreserved arterial allografts decreased over time, potentially minimising the risk of thrombotic occlusion of allografts. Therefore, the CP method used in our study provided a dual clinical advantage: retained haemostatic and reduced thrombogenic potential of the allografts. The only temporary prothrombotic change was observed in the media layer of the allografts, where platelet deposition exceeded that of fresh native grafts within the initial twelve weeks after CP. When the allografts are used during this early storage period, it may be reasonable to consider antiplatelet therapy to prevent thrombotic events. (89)

Although we have not investigated the mechanism of the decreasing tendency in fibrin generation and platelet adhesion over time, one hypothesised cause may be a reduction in the inducing factors in the walls of the stored grafts. The tissue factor, the main trigger of blood coagulation, is known to lose a significant part of its activity during storage in a frozen state for six months. (93) The practical implication of this result is that graft patency cannot be expected to improve with anticoagulant prophylaxis, as cryopreserved grafts do not induce more robust fibrin generation than native artery walls. This finding is consistent with others that warfarin has no significant effect on the primary patency of cryopreserved saphenous vein allografts. (45) There is also evidence that endothelial von Willebrand factor production decreases significantly with advancing freezing time, which may explain the decrease in platelet adhesion observed in our experiments. (64) Regarding the role of anti-platelet drugs in graft patency, previous studies have reported conflicting results. Some authors have reported a positive effect of these drugs on graft patency (94), while others did not (45). However, our latest results could reconcile these inconsistencies. They suggest that an antiplatelet strategy may only be beneficial within a specific time window, determined by the temporal variation of platelet adhesion to the graft wall. In our study, platelet deposition in the media layer of cryopreserved grafts exceeded that of fresh, non-cryopreserved grafts up to week 12. This suggests that administering anti-platelet therapy during this storage period might be beneficial for

maintaining graft patency. However, platelet deposition remained consistent with pre-CP levels beyond this timeframe and in the intima and adventitia layers at all time points. This indicates that the graft's haemostatic function was expected, and there is no justification for using antiplatelet medication. (89)

Although it may seem logical to examine only the intima for graft thrombogenicity, given the potential for injury during CP and the observation by Street et al. that the endothelium disappears within about five days after implantation (76), examination of the other layers of the vessel wall is essential to obtain a complete picture of the thrombogenicity of the grafts. In our study, therefore, because they are known to have different effects on coagulation and platelet function, we assessed the thrombogenicity of the three arterial wall layers separately. Our data revealed that the adventitia and intima layers showed higher thrombogenic potential. In contrast, the media layer's relative platelet and fibrin coverage was lower than that of the other two layers. (89) On the one hand, the reduced procoagulant potential of the media layer could be explained by lower levels of tissue factor protein and messenger ribonucleic acid (mRNA) in this layer. (95) On the other hand, regarding the relatively lower deposition of platelets in the media, our present results are in agreement with previous studies using the same experimental setup, which demonstrated limited von Willebrand factor-dependent platelet binding to native arterial media and stronger adhesion to the adventitia. (96,97) In another experimental design, the highest and lowest platelet deposition was observed in the adventitia, and the subendothelium of normal, non-atherosclerotic vessel walls, respectively, and the tunica media had intermediate adhesion. (98) Other studies, however, have found that the subendothelium is also capable of rapid and more massive platelet activation than the media (99,100), as we observed in our present work. Regardless of the differences in the relative thrombogenicity of the three layers, its temporal changes after CP were invariably favourable in all of them. Layer-dependent responses to CP were observed only in the media layer, and these were limited to the three earliest storage times. (89)

Although most internal organs and blood are considered sterile, there is growing evidence to disprove this by molecular detection of microorganisms in various tissues of both unhealthy and healthy individuals. (16–19) Furthermore, some authors could identify biologically relevant live microorganisms in traditionally considered sterile environments. (20,21,25) These findings and the discovery of the human microbiome

have fundamentally changed our understanding of certain diseases and their treatment. (16,23–25) The new molecular-based detection methods, such as 16S rRNA gene PCR (12,13), allow more accurate identification of microorganisms. As well as helping to map the healthy human microbiome (15), these techniques can improve diagnostic results for VGI (11) and increase the safety of sterility tests of allografts during transplantation (101,102). However, these methods do not identify only living pathogens, so positive results are obtained even for microorganism fragments or microorganisms in healthy individuals. On the one hand, this can be a problem in cases where the aim is to identify active pathogens. Still, in these cases, the diagnostic value could be significantly increased with a healthy microbiota database initiated in the current research (92). On the other hand, the detection of non-living micro-organisms or fragments thereof may also have great potential as they may play a significant role in triggering certain immunological processes, thus affecting the success of transplantations or may be the cause of pathologies previously thought to be autoimmune or of unknown origin. (22,103,104)

We have a growing knowledge of the microbiome of many tissues and organs. Still, more is needed to know about the local vascular microbiota and its possible role in homeostasis or pathological processes. The main reason is that it is virtually impossible to take adequate vascular tissue samples from healthy people for testing. However, in our study, we successfully developed a method for identifying the human vascular microbiota using arterial allografts, which allowed us to determine the bacterial composition of vascular samples from healthy individuals. Furthermore, we demonstrated the uniqueness of the healthy vascular microbiota by comparing our results with physiological nasopharyngeal and faecal microbiota. (92) Using the same isolation and sequencing methods, our vascular samples contain less microbial DNA than physiological bacterial flora samples. Median bacterial DNA amounts of vascular and stool samples after 16S rRNA PCR are 1.546 ng/μL and 17.4 ng/μL, respectively. However, no significant difference is seen between the mean lengths of the index PCR bacterial products, which are 667 bp and 662 bp, respectively. In addition, the composition of the vascular microbiota is significantly different from physiological nasopharyngeal or faecal microbiota samples. (90,92) These results suggest that the vascular microbiota is unique.

Based on our data, the main phyla of the vascular microbiota are *Proteobacteria* (31.78%), *Firmicutes* (29.18%), and *Actinobacteria* (23.05%). (92) Comparing our results with the skin and gut microbiota, which are considered the two largest microbiota in the body, the main phyla of the former are similar to those of the vascular microbiota: *Actinobacteria* (36-51%), *Firmicutes* (24-34%), *Proteobacteria* (11-16%), and *Bacteroidetes* (6-9%) (105), although their percentage composition shows a significant difference between *Proteobacteria* and *Actinobacteria*, and the main constituents of the gut microbiota show a complete divergence from the vascular microbiota, as *Firmicutes* and *Bacteroidetes* phyla make up about 60-90%, and although *Proteobacteria* and *Actinobacteria* are also present, their proportion is only a few per cent (90,106–108). At the genus level, the most abundant taxa in the human vascular microbiota are *Staphylococcus*, *Pseudomonas*, *Corynebacterium*, *Bacillus*, *Acinetobacter*, and *Propionibacterium*. (92) In contrast, the most abundant genus in the skin and gut are *Propionibacterium, Staphylococcus, Corynebacterium, Cutibacterium* (105,109) and *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, *Ruminicoccus*, *Bacteroides*, *Prevotella*, *Bifidobacterium*, *Faecalibacterium*, *Blautia*, *Roseburia*, *Lachnospiraceae* (90,108,110), respectively. There is no apparent similarity between the microbiota at the genus level. Still, there are several commonalities, such as the presence of a high proportion of *Staphylococcus*, *Corynebacterium* and *Propionibacterium* genus similar to the skin flora or *Bacillus* genus similar to the intestinal flora.

Blood, which is one of the primary cell-to-cell contacts in the body and tissue in direct contact with the vasculature, also has its own microbiota, the most prominent representatives of which are the *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Bacteroidetes* phyla. (17,111) Three of them, *Proteobacteria*, *Firmicutes* and *Actinobacteria* phyla, were identified as the most abundant phyla in the human vascular microbiota. (92) These studies suggest that the two microbiota are related. However, their exact composition may differ. While the main phylum in the blood is *Proteobacteria*, with more than 80% (17), the main phyla in the arterial wall are *Proteobacteria*, *Firmicutes* and *Actinobacteria*, with almost similar proportions. (92) This only partial concordance is consistent with the relationship between the microbiota of other directly contacting tissues and the spaces they border in the body. Several studies have shown that the faecal microbiota only partially reflects the gut mucosa-associated microbiota

(112,113), and the urinary microbiota is also different from the bladder tissue one (16). These examples suggest that transient bacteria in the blood may also contribute to the composition of the blood microbiota. Still, only a fraction of these bacteria may be detected in the vascular wall. In addition, the results of blood microbiota tests can vary widely even in patients who consider themselves healthy, e.g. the presence or absence of periodontitis or other diseases can determine the quantity and quality of viable bacteria in the blood (114,115).

The differences in the microbiota composition suggest that they may be distinct, individual microbiota, but the many commonalities suggest these ecosystems may be related and interact. To better understand the relationship between the human organism and its microbiota and the similarities and differences between microbiota, further studies are needed that simultaneously examine the microbiota of the sampled individuals.

In addition, several studies have found a link between cardiovascular disease and specific bacteria in different body parts. (20,116–124) For example, several epidemiological relationships were identified between cardiovascular disease and periodontal disease, in which the pathogenic behaviour of the oral microbiota was demonstrated. (124) Increased levels of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans*, *Treponema denticola*, Prevotella nigrescens, Fusobacterium nucleatum, Eikenella corrodens and *Parvimonas micra* in the oral cavity may play a role in endovascular pathogenic processes by increasing systemic inflammatory parameters. (125) Only two of our healthy vessel specimens contained *P. gingivalis*, and only one contained *P. nigrescens* of the mentioned bacteria. Furthermore, the gut microbiota may influence cardiovascular physiology through metabolites. The causal effect of trimethylamine-N-oxide (TMAO) on atherosclerosis has been established, and TMAO-producing members of the *Enterobacteriaceae* family are found in higher abundance in the gut microbiota of patients with symptomatic atherosclerosis than in healthy controls. (119,123,124,126) In addition, bacteria of the short-chain fatty acid (SCFA)-producing families Eubacterium, *Roseburia*, and *Ruminococcaceae* are more abundant in the gut microbiota of healthy patients than in those with atherosclerosis. (121,124) All of our samples contained varying small amounts of bacterial DNA from members of the *Enterobacteriaceae* family. *Roseburia* and *Ruminococcaceae* species were also abundant in small quantities in the samples. Assuming that the bacteria may

enter the circulation from the patient's intestinal tract, the DNA detected from the vascular wall provides indirect information that the patient's intestinal flora may contain short fatty acid-producing strains. Bacteria shown to play an indirect role in vascular wall pathology were not detected in the healthy vascular wall microbiome or were present in minimal amounts. (92) The most important bacteria whose DNA was found in the atherosclerotic plaque and whose direct role in atheroma formation has been demonstrated were *P. gingivalis, A. actinomycetemcomitans, C. pneumoniae* and *H. pylori*. (20,122,127) Of these bacteria, only *P. gingivalis* was found in two cases in low abundance in our samples of healthy human vascular wall microbiota. (92)

Even if the exact mechanism of these processes is not known, there is much evidence that the composition of the gut and the additional microbiota influence the proper function of the immune system and the success of organ transplants. (103,104) Certain bacteria can induce allograft rejection by producing metabolites that activate host cell-mediated and humoral immunity. (103,128–130) In addition, a strong correlation between transplant-associated complications such as graft versus host disease development and the gut microbiota and its metabolites was found during allogeneic hematopoietic stem cell transplantation. (104) Vascular allograft transplants have many complications associated with grafts, such as extensive calcification, thrombosis or aneurysmal degeneration. Since the microbiota has been implicated in several organ transplant rejections and the proper function of the immune system (103,104,128–130), it may also play a role in triggering vascular graft failures. Further studies are needed to determine whether bacteria in the vascular wall, their antigens or metabolites affect the outcome of vascular allograft transplantation.

Our study found distinct donor microbiota groups based on the ABO blood grouping system. (92) Although Davenport et al. found no association between ABO antigenic status and gut microbiota composition in a large cohort of 1500 twin pairs (131), Mäkivuokko and Gampa reported similar results to our vascular results for the gut microbiota (132,133).

Based on a 2018 review, there is ample evidence that microorganisms and blood group antigens are linked in the background of some diseases and that glycans that make up antigens are receptors for adhesion molecules of the assembled bacterial community, ultimately determining which bacterial species find a supportive environment and which

do not in the human body. (22) A genome-wide association study of 8956 German individuals also identified an indirect effect of the ABO histo-blood group on the gut microbiome (134). In addition, in a large mosaic pig population, deletion of the gene encoding N-acetylgalactosaminyltransferase was associated with a lower prevalence of Erysipelotrichaceae, bacteria capable of importing and catabolising N-acetylgalactosamine, in the gut microbiome. Since alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase are the genes determining the ABO blood group in humans, the molecular explanation provides evidence for a link between gut microbiome composition and blood groups (135).

All this suggests that the antigenic status of the human body may also affect the vascular microbiota and, consequently, susceptibility to and development of certain diseases. Furthermore, this relationship may also impact the success of vascular transplantation. However, while there is already molecular genetic evidence for a link between the gut microbiota and ABO blood groups, the recently observed correlation between vascular wall microbiota and blood group can only be confirmed after analysis of a more significant number of healthy samples and investigating well-defined vascular diseases.

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6. Conclusions

6.1. Thrombogenicity

Our results showed that when arterial allografts were cryopreserved and stored for up to 6 months, their haemostatic potential remained within the variability range of the fresh native arterial wall. In addition, the fibrin production and platelet adhesion of cryopreserved arterial allografts also decreased over time, minimising the risk of thrombotic occlusion of the grafts. Thus, the CP method showed a dual clinical benefit: retained haemostatic and reduced thrombogenic potential of the grafts. The only transient prothrombotic change was observed in the media of cryopreserved arterial allografts, where platelet deposition exceeded that of fresh native grafts during the first twelve weeks after CP. Antiplatelet treatment may be justified to prevent thrombotic occlusion if grafts are used during this early storage period. The markedly favourable trend of changes in the thrombogenicity of grafts over the six-month follow-up period of the current study justifies a longer-term study of the storage period of cryopreserved arterial allografts to optimise their thrombogenicity. (89)

Thesis I.: The cryopreservation affects the thrombogenicity of arterial allografts. Although there was no difference in fibrin deposition between native and cryopreserved-immediately-thawed samples, however a prothrombotic change was observed in the media of cryopreserved arterial allografts, where platelet deposition exceeded that of fresh native grafts.

Thesis II.: The six months storage time of cryopreservation affects the thrombogenicity of arterial allografts. The fibrin production and platelet adhesion of cryopreserved arterial allografts decreased over time.

6.2. Healthy human vascular microbiota

Our present investigation is the first methodological description suitable for studying the healthy vascular wall microbiota. Based on it, the human arterial wall has a unique microbiota, which differs significantly in composition from other microbiota of the human body. The most important genera are *Staphylococcus*, *Pseudomonas*,

Corynebacterium, *Bacillus*, *Acinetobacter* and *Propionibacterium*, which occur in variable but dominant abundance in all vessel wall samples. In addition, donors were divided into distinct vascular microbiota clusters according to the ABO blood grouping system, suggesting vascular microbiota diversity between individuals with different antigenicity. The pathological or protective role of the microbiome in the background of various diseases can only be demonstrated if we know the microbiota associated with the healthy vascular wall. Our present study provides a basis for future research investigating the direct role of the microbiota in vascular wall abnormalities and the success of allograft transplantation. (92)

Thesis III.: Healthy human vascular wall contains bacterial hereditary material. Our methodology using 16S rRNA sequencing of the arterial samples from brain-dead donors successfully identified bacterial hereditary material in healthy human vascular walls in strict compliance with sterility rules.

Thesis IV.: Healthy human arterial wall has a unique microbiota. Compared to physiological faecal and nasopharyngeal microbiota examined using the same gene sequencing methodology as ours, the microbiota of the healthy vascular wall is significantly different.

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7. Summary

Even with modern infection control strategies, 3.5-32% of vascular surgical procedures are still associated with infection in the surgical site. The main risk of the inflammatory process is the development of life- or limb-threatening complications. There is a long-standing, unfulfilled demand for a surgeon-friendly, infection-resistant, biocompatible, durable, off-the-shelf graft material to prevent or treat these conditions. Although allografts come close to this, vascular allograft transplants are still associated with many complications. The reasons for these are likely found in the processes of graft production on the one hand and in the circumstances of the transplantation on the other.

In our work, we analysed the CP protocol of the Cardiovascular Biobank of Semmelweis University, with particular emphasis on the direct and storage time-dependent effects of CP on the thrombogenicity of arterial allografts. In addition, we analysed the human vascular microbiota by examining arterial allografts as healthy vascular tissue. Our results on thrombogenicity showed that arterial allografts preserved haemostatic properties similar to fresh native arterial walls during CP with storage for six months. In addition, fibrin deposition and platelet adhesion on cryopreserved arterial allografts decreased over time, demonstrating a reduction in their thrombogenic potential. The only transient prothrombotic change was observed in the media layer, where platelet deposition exceeded that of fresh native grafts within the first twelve weeks after CP. In addition, in our study, we successfully developed a method for identifying the human vascular microbiota using arterial allografts, which allowed us to determine the bacterial composition of vascular samples from healthy individuals. Furthermore, we demonstrated the uniqueness of the healthy vascular microbiota by comparing our results with physiological microbiota found elsewhere in the body.

Our thrombogenicity results demonstrate the effect of frozen storage time on allografts. This prompts us to investigate longer storage times further to optimise the production of cryopreserved allografts and the potential need for drug therapy. Besides, our microbiological results, in addition to demonstrating the existence of a healthy vascular microbiota, could form the basis for future research into the direct role of the microbiota in vascular wall disorders and the success of allograft transplantation.

8. References

1. Turtiainen J, Hakala T. Surgical wound infections after peripheral vascular surgery. Scand J Surg. 2014;103:226–31.
2. Turtiainen J, Saimanen E, Partio T, Kärkkäinen J, Kiviniemi V, Mäkinen K, et al. Surgical wound infections after vascular surgery: prospective multicenter observational study. Scand J Surg. 2010;99(3):167–72.
3. Wilson WR, Bower TC, Creager MA, Amin-Hanjani S, O'Gara PT, Lockhart PB, et al. Vascular graft infections, mycotic aneurysms, and endovascular infections: a scientific statement from the American Heart Association. Circulation. 2016;134:e412–60.
4. Bíró G, Szeberin Z, Nemes A, Acsády G. Cryopreserved homograft and autologous deep vein replacement for infrarenal aorto and iliaco-femoral graft infection: Early and late results. J Cardiovasc Surg. 2011;52(2):169–76.
5. Chakfé N, Diener H, Lejay A, Assadian O, Berard X, Caillon J, et al. Editor's Choice – European Society for Vascular Surgery (ESVS) 2020 Clinical practice guidelines on the management of vascular graft and endograft infections. Eur J Vasc Endovasc Surg. 2020;59(3):339–84.
6. Gharamti A, Kanafani ZA. Vascular graft infections: an update. Infect Dis Clin North Am. 2018;32(4):789–809.
7. Sidawy AN, Perler BA. Rutherford's Vascular Surgery and Endovascular Therapy 10th Edition. Canada: Elsevier; 2023. 625-39 and 838-85.
8. O'Connor S, Andrew P, Batt M, Becquemin JP. A systematic review and meta-analysis of treatments for aortic graft infection. J Vasc Surg. 2006;44(1): 38-45.
9. Batt M, Feugier P, Camou F, Coffy A, Senneville E, Caillon J, et al. A meta-analysis of outcomes after in situ reconstructions for aortic graft infection. Angiology. 2018;69(5):370–9.
10. Legout L, D'Elia P V., Sarraz-Bournet B, Haulon S, Meybeck A, Senneville E, et al. Diagnosis and management of prosthetic vascular graft infections. Med Mal Infect. 2012;42(3):102–9.
11. Ajdler-Schaeffler E, Scherrer AU, Keller PM, Anagnostopoulos A, Hofmann M, Rancic Z, et al. Increased pathogen identification in vascular graft infections by

the combined use of tissue cultures and 16s rRNA gene polymerase chain reaction. Front Med. 2018;5:1–9.

12. Church DL, Cerutti L, Gürtler A, Griener T, Zelazny A, Emler S. Performance and application of 16S rRNA gene cycle sequencing for routine identification of bacteria in the clinical microbiology laboratory. Clin Microbiol Rev. 2020;33(4): e00053-19.
13. Wensel CR, Pluznick JL, Salzberg SL, Sears CL. Next-generation sequencing: insights to advance clinical investigations of the microbiome. J Clin Invest. 2022;132(7): e154944.
14. Antonello RM, D'Oria M, Cavallaro M, Dore F, Cova MA, Ricciardi MC, et al. Management of abdominal aortic prosthetic graft and endograft infections. A multidisciplinary update. J Infect Chemother. 2019;25(9):669–80.
15. NIH Human Microbiome Project [Internet]. 2023 [cited 2023 Oct 10]. Available from: https://hmpdacc.org/.
16. Mansour B, Monyók Á, Makra N, Gajdács M, Vadnay I, Ligeti B, et al. Bladder cancer-related microbiota: examining differences in urine and tissue samples. Sci Rep. 2020;10(1):11042.
17. Païssé S, Valle C, Servant F, Courtney M, Burcelin R, Amar J, et al. Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. Transfusion. 2016;56(5):1138–47.
18. Sookoian S, Pirola CJ. Liver tissue microbiota in nonalcoholic liver disease: a change in the paradigm of host-bacterial interactions. Hepatobiliary Surg Nutr. 2021;10(3):337–49.
19. Molina NM, Sola-Leyva A, Haahr T, Aghajanova L, Laudanski P, Castilla JA, et al. Analysing endometrial microbiome: methodological considerations and recommendations for good practice. Hum Reprod. 2021;36(4):859–79.
20. Clifford A, Hoffman GS. Evidence for a vascular microbiome and its role in vessel health and disease. Curr Opin Rheumatol. 2015;27(4):397–405.
21. Potgieter M, Bester J, Kell DB, Pretorius E. The dormant blood microbiome in chronic, inflammatory diseases. FEMS Microbiol Rev. 2015;39(4):567–91.
22. Ewald DR, Sumner SCJ. Human microbiota, blood group antigens, and disease. Wiley Interdiscip Rev Syst Biol Med. 2018;10(3):e1413.

23. Ruan W, Engevik MA, Spinler JK, Versalovic J. Healthy human gastrointestinal microbiome: composition and function after a decade of exploration. Dig Dis Sci. 2020;65(3):695–705.
24. Chang C, Yuan X, Zhang X, Chen X, Li K. Gastrointestinal microbiome and multiple health outcomes: umbrella review. Nutrients. 2022;14(18):3726.
25. Moreno I, Codoñer FM, Vilella F, Valbuena D, Martinez-Blanch JF, Jimenez-Almazán J, et al. Evidence that the endometrial microbiota has an effect on implantation success or failure. Am J Obstet Gynecol. 2016;215(6):684–703.
26. Kunlin J. Le traitement de l'artérite oblitérante par la greffe veineuse. Arch Mal Coeur Vaiss. 1949;42:371-5.
27. Leal BBJ, Wakabayashi N, Oyama K, Kamiya H. Vascular tissue engineering : polymers and methodologies for small caliber vascular grafts. Front Cardiovasc Med. 2021;7: 592361.
28. Conte MS, Bradbury AW, Kolh P, White J V., Dick F, Fitridge R, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. Eur J Vasc Endovasc Surg. 2019;58(1):S1-S109.e33.
29. Aboyans V, Ricco JB, Bartelink MLEL, Björck M, Brodmann M, Cohnert T, et al. 2017 ESC guidelines on the diagnosis and treatment of peripheral arterial diseases, in collaboration with the European Society for Vascular Surgery (ESVS). Eur Heart J. 2018;39(9):763–816.
30. Lomas RJ, Dodd PDF, Rooney P, Pegg DE, Hogg PA, Eagle ME, et al. A standardised protocol for the validation of banking methodologies for arterial allografts. Cell Tissue Bank. 2013;14(3):495–503.
31. Nagy Z, Oláh Z, Kókai J, Molnár AB, Laczkó Á, Szabó GV, et al. Role of the homograft bypass in extremity inferior's reconstructions [Homograftok szerepe az alsó végtagi érrekonstrukciókban]. Magy Seb. 2017;70(1):5-12. [Hungarian]
32. Garbaisz D, Osztrogonácz P, Boros AM, Hidi L, Sótonyi P, Szeberin Z. Comparison of arterial and venous allograft bypass in chronic limb-threatening ischemia. PLoS One. 2022;17(10):e0275628.
33. Garbaisz D, Szeberin Z, Hidi L, Sótonyi P. Use of allografts in vascular surgery infections. Orv Hetil. 2023;164(32):1256–62.
34. Jashari R, Goffin Y, Vanderkelen A, Van Hoeck B, du Verger A, Fan Y, et al.

European Homograft Bank: twenty years of cardiovascular tissue banking and collaboration with transplant coordination in Europe. Transplant Proc. 2010;42(1):183–9.

35. Jashari R, Bouzet V, Alcaraz Blanco MJ, Oleffe A, Lecocq E, Mastrobuoni S. Vascular allografts for clinical application in Europe: assessment of 30 years of experience with vascular tissue banking in Brussels. Cell Tissue Bank. 2023;24(3):613-25.
36. Satam K, Fischer U, Schioppo D, Cardella J, Guzman RJ, Ochoa Chaar CI. Aneurysmal degeneration of the hood of a cryopreserved vein allograft two years after thrombosis. J Vasc Surg Cases Innov Tech. 2022;8(2):300–4.
37. Harlander-Locke MP, Harmon LK, Lawrence PF, Oderich GS, McCready RA, Morasch MD, et al. The use of cryopreserved aortoiliac allograft for aortic reconstruction in the United States. J Vasc Surg. 2014;59(3):669-74.
38. Hartranft CA, Noland S, Kulwicki A, Holden CR, Hartranft T. Cryopreserved saphenous vein graft in infrainguinal bypass. J Vasc Surg. 2014;60:1291–6.
39. Minga Lowampa E, Holemans C, Stiennon L, Van Damme H, Defraigne JO. Late fate of cryopreserved arterial allografts. Eur J Vasc Endovasc Surg. 2016;52:696–702.
40. Singh K, Juneja A, Bajaj T, Voto C, Schor J, Zia S, et al. Single tertiary care center outcomes after lower extremity cadaveric vein bypass for limb salvage. Vasc Endovascular Surg. 2020;54:430–5.
41. Tatar AR, Derycke L, Cochennec F, Jaziri A, Desgranges P, Touma J. Unmet needs in cryopreserved arterial allograft implantation for peripheral vascular graft infections. Eur J Vasc Endovasc Surg. 2020;60:788–9.
42. Guevara-Noriega KA, Lucar-Lopez GA, Pomar JL. Cryopreserved allografts for treatment of chronic limb-threatening ischemia in patients without autologous saphenous veins. Ann Vasc Surg. 2019;60:379–87.
43. Lejay A, Delay C, Girsowicz E, Chenesseau B, Bonnin E, Ghariani MZ, et al. Cryopreserved cadaveric arterial allograft for arterial reconstruction in patients with prosthetic infection. Eur J Vasc Endovasc Surg. 2017;54:636–44.
44. Noel AA, Gloviczki P, Cherry KJ, Safi H, Goldstone J, Morasch MD, et al. Abdominal aortic reconstruction in infected fields: early results of the United

States Cryopreserved Aortic Allograft Registry. J Vasc Surg. 2002;35(5):847–52.

45. Farber A, Major K, Wagner WH, Cohen JL, Cossman D V., Lauterbach SR, et al. Cryopreserved saphenous vein allografts in infrainguinal revascularization: analysis of 240 grafts. J Vasc Surg. 2003;38(1):15–21.
46. Randon C, Jacobs B, Ryck F De, Beele H. Fifteen years of infrapopliteal arterial reconstructions with cryopreserved venous allografts for limb salvage. J Vasc Surg. 2010;51(4):869–77.
47. Müller-Schweinitzer E. Cryopreservation of vascular tissues. Organogenesis. 2009;5(3):97–104.
48. Špaček M, Měřička P, Janoušek L, Štádler P, Adamec M, Vlachovský R, et al. Current vascular allograft procurement, cryopreservation and transplantation techniques in the Czech Republic. Adv Clin Exp Med. 2019;28:529–34.
49. Hruby J, Spunda R, Mericka P, Mlcek M, Pecha O, Splith K, et al. Influence of the new standardized clinical cryopreservation/slow thawing protocol on immunogenicity of arterial allografts in rats. PLoS One. 2020;15(3): e0230234.
50. Jang TH, Park SC, Yang JH, Kim JY, Seok JH, Park US, et al. Cryopreservation and its clinical applications. Integr Med Res. 2017;6(1):12–8.
51. Jaiswal AN, Vagga A. Cryopreservation: a review article. Cureus. 2022;14(11): e31564.
52. Aljaser FS. Cryopreservation Methods and Frontiers in the Art of Freezing Life in Animal Models. In: Bozkurt Y, Numan Bucak M, editors. Animal Reproduction [Internet]. Veterinary Medicine and Science. IntechOpen; 2022 [cited 2023 Oct 10]. Available from: https://www.intechopen.com/chapters/79909
53. Hwang S, Bae JH, Kim IO, Hong JJ. Current vascular allograft procurement, cryopreservation and transplantation techniques in the Asan Medical Center Tissue Bank. Ann Liver Transplant. 2021;1(1):79–85.
54. Gao HH, Li ZP, Wang HP, Zhang LF, Zhang JM. Cryopreservation of whole bovine ovaries: comparisons of different thawing protocols. Eur J Obstet Gynecol Reprod Biol. 2016;204:104–7.
55. Špaček M, Měřička P, Janoušek L, Dalecká M, Benda A, Krs O, et al. Comparison of different thawing protocols in human cryopreserved venous grafts. Ann Vasc Surg. 2020;64:347–54.

56. Pegg DE. The relevance of ice crystal formation for the cryopreservation of tissues and organs. Cryobiology. 2020;93:3–11.
57. Ma X, Liu Y, Sun B, Fan W, Cui Z. Cryopreservation: Organ Preservation. In: Moo-Young M, editor. Comprehensive biotechnology (third edition). Pergamon; 2019. p. 689-708.
58. Arav A. Cryopreservation by directional freezing and vitrification focusing on large tissues and organs. Cells. 2022;11(7):1072.
59. Bahroudi Z, Zarnaghi MR, Izadpanah M, Abedelahi A, Niknafs B, Nasrabadi HT, et al. Review of ovarian tissue cryopreservation techniques for fertility preservation. J Gynecol Obstet Hum Reprod. 2022;51(2):102290.
60. Dolmans MM, von Wolff M, Poirot C, Diaz-Garcia C, Cacciottola L, Boissel N, et al. Transplantation of cryopreserved ovarian tissue in a series of 285 women: a review of five leading European centers. Fertil Steril. 2021;115(5):1102–15.
61. Heng WL, Albrecht H, Chiappini P, Lim YP, Manning L. International Heart Valve Bank Survey: a review of processing practices and activity outcomes. j transplant. 2013;2013:163150.
62. Faggioli GL, Gargiulo M, Giardino R, Pasquinelli G, Preda P, Fini M, et al. Long-term cryopreservation of autologous veins in rabbits. Cardiovasc Surg. 1994;2(2):259–65.
63. Malone JM, Kischer CW, Gorge G. Endothelial fibrinolytic activity. 1980;222:209–22.
64. Cai G, Lai B, Hong H, Lin P, Chen W, Zhu Z, et al. Effects of cryopreservation on excretory function, cellular adhesion molecules and vessel lumen formation in human umbilical vein endothelial cells. Mol Med Rep. 2017;16(1):547–52.
65. Mirabet V, Carda C, Solves P, Novella-Maestre E, Carbonell-Uberos F, Caffarena JM, et al. Long-term storage in liquid nitrogen does not affect cell viability in cardiac valve allografts. Cryobiology. 2008;57(2):113–21.
66. Touma J, Cochennec F, Parisot J, Fialaire Legendre A, Becquemin JP, Desgranges P. In situ reconstruction in native and prosthetic aortic infections using cryopreserved arterial allografts. Eur J Vasc Endovasc Surg. 2014;48(3):292–9.
67. Wang CC, Lopez-Valdes S, Lin TL, Yap A, Yong CC, Li WF, et al. Outcomes of long storage times for cryopreserved vascular grafts in outflow reconstruction in

living donor liver transplantation. Liver Transplant. 2014;20:173–81.

68. Bia D, Pessana F, Armentano R, Pérez H, Graf S, Zócalo Y, et al. Cryopreservation procedure does not modify human carotid homografts mechanical properties: an isobaric and dynamic analysis. Cell Tissue Bank. 2006;7(3):183–94.
69. Virues Delgadillo JO, Delorme S, El-Ayoubi R, DiRaddo R, Hatzikiriakos SG. Effect of freezing on the passive mechanical properties of arterial samples. J Biomed Sci Eng. 2010;03(07):645–52.
70. Chow MJ, Zhang Y. Changes in the mechanical and biochemical properties of aortic tissue due to cold storage. J Surg Res. 2011;171(2):434–42.
71. Yau JW, Teoh H, Verma S. Endothelial cell control of thrombosis. BMC Cardiovasc Disord. 2015;15:130.
72. Novotny R, Mericka P, Chlupac J, Matejka R, Kristek J, Marada T, et al. The effect of different thawing rates on cryopreserved human iliac arteries allograft's structural damage and mechanical properties. Biomed Res Int. 2020;2020:6545190.
73. YC Song, DE Pegg CH. Cryopreservation of the common carotid artery of the rabbit: optimization of the dimethyl sulfoxidee concentration and cooling rate. Cryobiology. 1995;32:405–21.
74. Pegg DE, Wusteman MC, Boylan S. Fractures in cryopreserved elastic arteries. Cryobiology. 1997;34(2):183–92.
75. Huber AJT, Brockbank KGM, Riemann I, Schleicher M, Schenke-Layland K, Fritze O, et al. Preclinical evaluation of ice-free cryopreserved arteries: structural integrity and hemocompatibility. Cells Tissues Organs. 2012;196(3):262–70.
76. Street DL, Russ GA, Ricotta JJ. Platelet avidity of cryopreserved veins: thrombogenicity of cryopreserved veins. J Surg Res. 1988;45(4):363–9.
77. Zehr BP, Niblick CJ, Downey H, Ladowski JS. Limb salvage with CryoVein cadaver saphenous vein allografts used for peripheral arterial bypass: role of blood compatibility. Ann Vasc Surg. 2011;25(2):177–81.
78. Gabriel M, Kostrzewa A, Sobieska M. Immune response after cryopreserved aortic allograft replacement for major vascular infection. Transplant Proc. 2002;34(2):713-4.
79. Muratov R, Britikov D, Sachkov A, Akatov V, Soloviev V, Fadeeva I, et al. New

approach to reduce allograft tissue immunogenicity. Experimental data. Interact Cardiovasc Thorac Surg. 2010;10(3):408–12.

80. Tomita Y, Yoshikawa M, Zhang QW, Uchida T, Nakashima Y, Sueishi K, et al. Immune and non-immune factors in cryopreserved tissues. J Hear Lung Transplant. 2003;22(5):560–7.
81. Solanes N, Rigol M, Khabiri E, Castellà M, Ramírez J, Roqué M, et al. Effects of cryopreservation on the immunogenicity of porcine arterial allografts in early stages of transplant vasculopathy. Cryobiology. 2005;51(2):130–41.
82. Lehr EJ, Rayat GR, Chiu B, Churchill T, McGann LE, Coe JY, et al. Decellularization reduces immunogenicity of sheep pulmonary artery vascular patches. J Thorac Cardiovasc Surg. 2011;141(4):1056–62.
83. Oei FBS, Stegmann APA, Vaessen LMB, Marquet RL, Weimar W, Bogers AJJC. Immunological aspects of fresh and cryopreserved aortic valve transplantation in rats. Ann Thorac Surg. 2001;71(5):S379–84.
84. Carpenter JP, Tomaszewski JE. Human saphenous vein allograft bypass grafts: immune response. J Vasc Surg. 1998;27(3):492–9.
85. Mirelli M, Buzzi M, Pasquinelli G, Tazzari PL, Testi G, Ricchi E, et al. Fresh and cryopreserved arterial homografts: immunological and clinical results. Transplant Proc. 2005;37(6):2688–91.
86. González-Gay M, López-Martínez R, Busto-Suárez S, Riedemann-Wistuba ME, Menéndez-Herrero MÁ, Álvarez-Marcos F, et al. Immunological aspects involved in the degeneration of cryopreserved arterial allografts. Front Surg. 2020;7:616654.
87. Hungarian National Blood Transfusion Service. Legislation of organ and tissue transplantation [Internet]. 2011 [cited 2023 Oct 10]. Available from: https://www.ovsz.hu/en/organ-coordination-office/legislation; https://www.ovsz.hu/hu/oco/jogszabalyok
88. European Comission. EudraLex - The rules governing medicinal products in the european union: Volume 4 - EU guidelines to good manufacturing practice medicinal products for human and veterinary use; Annex 1 - Manufacture of sterile medicinal products (corrected version) [Internet]. 2008 [cited 2023 Oct 10]. Available from: https://www.gmp-

compliance.org/files/guidemgr/annex%2001[2008].pdf

89. Hidi L, Komorowicz E, Kovács GI, Szeberin Z, Garbaisz D, Nikolova N, et al. Cryopreservation moderates the thrombogenicity of arterial allografts during storage. PLoS One. 2021;16(7):e0255114.
90. Szabo H, Hernyes A, Piroska M, Ligeti B, Fussy P, Zoldi L, et al. Association between gut microbial diversity and carotid intima-media thickness. Medicina. 2021;57(3):195.
91. Szabo D, Ostorhazi E, Stercz B, Makra N, Penzes K, Kristof K, et al. Specific nasopharyngeal Corynebacterium strains serve as gatekeepers against SARS-CoV-2 infection. GeroScience. 2023.
92. Hidi L, Kovács GI, Szabó D, Makra N, Pénzes K, Juhász J, et al. Human blood vessel microbiota in healthy adults based on common femoral arteries of brain-dead multi-organ donors. Front Cell Infect Microbiol. 2022;12:1056319.
93. Gökmen BG, Özcan O, Taslak H, Ipar N, Tunali-Akbay T. Effect of freezing time on tissue factor activity and macronutrients of human milk. Protein J. 2020;39(5):591–7.
94. Miller VM, Bergman RT, Gloviczki P, Brockbank KGM. Cryopreserved venous allografts: effects of immunosuppression and antiplatelet therapy on patency and function. J Vasc Surg. 1993;18:216–26.
95. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci USA. 1989;86:2839–43.
96. Komorowicz E, McBane RD, Fass DN. Physical and enzymatic perturbation of the architecture of the tunica media destroys its inherent thromboresistance. Thromb Haemost. 2002;88:827–33.
97. Wohner N, Keresztes Z, Sótonyi P, Szabó L, Komorowicz E, Machovich R, et al. Neutrophil granulocyte-dependent proteolysis enhances platelet adhesion to the arterial wall under high-shear flow. J Thromb Haemost. 2010;8:1624–31.
98. Toschi V, Gallo R, Lettino M, Fallon JT, David Gertz S, Fernández-Ortiz A, et al. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. Circulation. 1997;95:594–9.
99. Vlodavsky I, Eldor A, HyAm E, Atzom R, Fuks Z. Platelet interaction with the

extracellular matrix produced by cultured endothelial cells: a model to study the thrombogenicity of isolated subendothelial basal lamina. Thromb Res. 1982;28:179–91.

100. Fauvel-Lafève F. Microfibrils from the arterial subendothelium. Int Rev Cytol. 1999;188:1–40.
101. SaBTO - Advisory committee on the safety of blood, tissues and organs. Microbiological safety guidelines, previously known as guidance on the microbiological safety of human organs, tissues and cells used in transplantation – Version 2.1 [Internet]. 2023 [cited 2023 Oct 10]. Available from: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1143058/SaBTO-microbiological-safety-guidelines.pdf
102. Fishman JA. Infection in Organ Transplantation. Am J Transplant. 2017;17(4):856–79.
103. Baghai Arassi M, Zeller G, Karcher N, Zimmermann M, Toenshoff B. The gut microbiome in solid organ transplantation. Pediatr Transplant. 2020;24(7):e13866.
104. Chang CC, Hayase E, Jenq RR. The role of microbiota in allogeneic hematopoietic stem cell transplantation. Expert Opin Biol Ther. 2021;21(8):1121–31.
105. Carmona-cruz S, Orozco-covarrubias L, Sa M. The human skin microbiome in selected cutaneous diseases. Front Cell Infect Microbiol. 2022;12:834135.
106. Polidori I, Marullo L, Ialongo C, Tomassetti F, Colombo R, Gaudio F, et al. Characterization of gut microbiota composition in type 2 diabetes patients: a population-based study. Int J Environ Res Public Health. 2022;19(23):15913.
107. Yang X, Xie L, Li Y, Wei C. More than 9,000,000 unique genes in human gut bacterial community : estimating gene numbers inside a human body. PLoS One. 2009;4(6):e6074.
108. Rinninella E, Raoul P, Cintoni M, Franceschi F, Abele G, Miggiano D, et al. What is the healthy gut microbiota composition ? A changing ecosystem across age, environment, diet, and diseases. Microorganisms. 2019;7(1):14.
109. Byrd AL, Belkaid Y, Segre JA. The human skin microbiome. Nat Publ Gr. 2018;16(3):143–55.
110. Piquer-esteban S, Ruiz-ruiz S, Arnau V, Diaz W, Moya A. Exploring the universal healthy human gut microbiota around the World. Comput Struct Biotechnol J.

2022;20:421–33.

111. Castillo DJ, Rifkin RF, Cowan DA, Potgieter M. The healthy human blood microbiome: fact or fiction? Front Cell Infect Microbiol. 2019;9:148.
112. Yang M, Hong G, Jin Y, Li Y, Li G, Hou X. Mucosal-associated microbiota other than luminal microbiota has a close relationship with diarrhea-predominant irritable bowel syndrome. Front Cell Infect Microbiol. 2020;10:515614.
113. Flemer B, Lynch DB, Brown JMR, Jeffery IB, Ryan FJ, Claesson MJ, et al. Tumour-associated and non-tumour-associated microbiota in colorectal cancer. Gut. 2017;66(4):633-643.
114. Damgaard C, Magnussen K, Enevold C, Nilsson M. Viable bacteria associated with red blood cells and plasma in freshly drawn blood donations. PLoS One. 2015;10(3):e0120826.
115. Damgaard C, Sækmose SG, Nilsson M, Kilian M, Nielsen CH, Holmstrup P. Periodontitis increases risk of viable bacteria in freshly drawn blood donations. Blood Transfus. 2021;19(5):376-383.
116. Blanchard T, Bailey R, Holland M, Mabey D. Chlamydia pneumoniae and atherosclerosis. Lancet. 1993;341(8848):825.
117. Ahmed U, Tanwir F. Association of periodontal pathogenesis and cardiovascular diseases: a literature review. Oral Health Prev Dent. 2015;13(1):21–7.
118. Caúla AL, Lira-Junior R, Tinoco EM, Fischer RG. The effect of periodontal therapy on cardiovascular risk markers: a 6-month randomized clinical trial. J Clin Periodontol. 2014;41(9):875–82.
119. Jie Z, Xia H, Zhong SL, Feng Q, Li S, Liang S, et al. The gut microbiome in atherosclerotic cardiovascular disease. Nat Commun. 2017;8(1):845.
120. Jin L, Shi X, Yang J, Zhao Y, Xue L, Xu L, et al. Gut microbes in cardiovascular diseases and their potential therapeutic applications. Protein Cell. 2021;12(5):346–59.
121. Liu H, Chen X, Hu X, Niu H, Tian R, Wang H, et al. Alterations in the gut microbiome and metabolism with coronary artery disease severity. Microbiome. 2019;7(1):68.
122. Kozarov EV, Dorn BR, Shelburne CE, Dunn WA, Progulske-Fox A. Human atherosclerotic plaque contains viable invasive Actinobacillus

actinomycetemcomitans and Porphyromonas gingivalis. Arterioscler Thromb Vasc Biol. 2005;25(3):e17-18.

123. Verhaar BJH, Prodan A, Nieuwdorp M, Muller M. Gut microbiota in hypertension and atherosclerosis: a review. Nutr. 2020;12(10):2982.
124. Rahman MM, Islam F, Mamun AA, Rahaman MS, Islam MM, Meem AFK, et al. The gut microbiota (microbiome) in cardiovascular disease and its therapeutic regulation. Front Cell Infect Microbiol. 2022;12:903570.
125. Zou Y, Huang Y, Liu S, Yang J, Zheng W, Deng Y, et al. Periodontopathic microbiota and atherosclerosis: roles of TLR-mediated inflammation response. Oxid Med Cell Longev. 2022;2022:9611362.
126. Eshghjoo S, Jayaraman A, Sun Y, Alaniz RC. Microbiota-mediated immune regulation in atherosclerosis. 2021;26(1):179.
127. Rosenfeld ME, Campbell LA. Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. Thromb Haemost. 2011;106(5):858–67.
128. Campbell PM, Humphreys GJ, Summers AM, Konkel JE, Knight CG, Augustine T, et al. Does the microbiome affect the outcome of renal transplantation? Front Cell Infect Microbiol. 2020;10:558644.
129. Dery KJ, Kadono K, Hirao H, Górski A, Kupiec-Weglinski JW. Microbiota in organ transplantation: an immunological and therapeutic conundrum? Cell Immunol. 2020;351:104080.
130. Mitchell AB. The lung microbiome and transplantation. Curr Opin Organ Transplant. 2019;24(3):305–10.
131. Davenport ER, Goodrich JK, Bell JT, Spector TD, Ley RE, Clark AG. ABO antigen and secretor statuses are not associated with gut microbiota composition in 1,500 twins. BMC Genomics. 2016;17:941.
132. Gampa A, Engen PA, Shobar R, Mutlu EA. Gut microbiota in health and disease relationships between gastrointestinal microbiota and blood group antigens. Physiol Genomics. 2021;49(11):473–83.
133. Mäkivuokko H, Lahtinen SJ, Wacklin P, Tuovinen E, Tenkanen H, Nikkilä J, et al. Association between the ABO blood group and the human intestinal microbiota composition. BMC Microbiol. 2012;12:94.

134. Rühlemann MC, Hermes BM, Bang C, Doms S, Moitinho-Silva L, Thingholm LB, et al. Genome-wide association study in 8,956 German individuals identifies influence of ABO histo-blood groups on gut microbiome. Nat Genet. 2021;53(2):147–55.
135. Yang H, Wu J, Huang X, Zhou Y, Zhang Y, Liu M, et al. ABO genotype alters the gut microbiota by regulating GalNAc levels in pigs. Nature. 2022;606(7913):358–67.

9. Bibliography of the candidate's publications

9.1. List of publications related to the topic of the thesis

Garbaisz D, Szeberin Z, **Hidi L**, Sótonyi P. Use of allografts in vascular surgery infections [Homograftok használata érsebészeti fertőzésekben]. Orv Hetil. 2023;164(32):1256-1262. [Hungarian]

Hidi L, Kovács GI, Szabó D, Makra N, Pénzes K, Juhász J, Sótonyi P, Ostorházi E. Human blood vessel microbiota in healthy adults based on common femoral arteries of brain-dead multi-organ donors. Front Cell Infect Microbiol. 2022;12:1056319.

Garbaisz D, Osztrogonácz P, Boros AM, **Hidi L**, Sótonyi P, Szeberin Z. Comparison of arterial and venous allograft bypass in chronic limb-threatening ischemia. PLoS One. 2022;17(10):e0275628.

Hidi L, Komorowicz E, Kovács GI, Szeberin Z, Garbaisz D, Nikolova N, Tenekedjiev K, Szabó L, Kolev K, Sótonyi P. Cryopreservation moderates the thrombogenicity of arterial allografts during storage. PLoS One. 2021;16(7):1–16.

9.2. List of publications unrelated to the topic of the thesis

Tóth E, Beinrohr L, Gubucz I, Szabó L, Tenekedjiev K, Nikolova N, Nagy AI, **Hidi L**, Sótonyi P, Szikora I, Merkely B, Kolev K. Fibrin to von Willebrand factor ratio in arterial thrombi is associated with plasma levels of inflammatory biomarkers and local abundance of extracellular DNA. Thromb Res. 2022;209:8-15.

Hidi L, Pomozi E, Boros AM, Legeza P, Bárczi Z, Szeberin Z. Long-term results of open surgical repair of non-infrarenal abdominal aortic aneurysms [Nem infrarenalis lokalizációjú hasi aortaaneurysmák miatt végzett nyitott mutétek hosszú távú adatainak elemzése]. Orv Hetil. 2022;163(37):1472-80. [Hungarian]

Hidi L, Lengyel B, Legeza P, Szeberin Z. Initial experiences with the use of covered stents in endovascular treatment of aortoiliac stenoocclusive diseases [Aortoiliacalis stenoocclusiv betegség fedett sztentekkel végzett endovascularis ellátásával szerzett kezdeti tapasztalataink]. Orv Hetil. 2022;163(39):1553-58. [Hungarian]

Berczeli M, Osztrogonácz P, **Hidi L**, Szeberin Z. Initial experiences with percutaneous endovascular aortic reconstruction [Percutan endovascularis aortarekonstrukcióval szerzett kezdeti tapasztalataink]. Orv Hetil. 2022;163(33):1318-23. [Hungarian]

COVIDSurg Collaborative; GlobalSurg Collaborative. SARS-CoV-2 infection and venous thromboembolism after surgery: an international prospective cohort study. Anaesthesia. 2022;77(1):28-39.

COVIDSurg Collaborative; GlobalSurg Collaborative. Effects of pre-operative isolation on postoperative pulmonary complications after elective surgery: an international prospective cohort study. Anaesthesia. 2021;76(11):1454-64.

COVIDSurg Collaborative, GlobalSurg Collaborative. SARS-CoV-2 vaccination modelling for safe surgery to save lives: data from an international prospective cohort study. Br J Surg. 2021;108(9):1056-63.

Hidi L, Pál D, Boros AM, Kováts T, Menyhei G, Szeberin Z. Analysis of data from the National Vascular Registry on infrarenal aortic aneurysms (2010–2019) [Infrarenalis aortaaneurysma-műtétek országos eredményeinek elemzése a Nemzeti Érsebészeti Regiszter alapján (2010–2019)]. Orv Hetil. 2021;162(31):1233-43. [Hungarian]

COVIDSurg Collaborative; GlobalSurg Collaborative. Timing of surgery following SARS-CoV-2 infection: an international prospective cohort study. Anaesthesia. 2021;76(6):748-58.

Hidi L, Csobay-Novák C, Juhász V, Suhai F, Szeberin Z, Sótonyi P. Endovascular treatment of the persistent false lumen of post-dissection aneurysms with "candy-plug" technique [Postdissectiós aortaaneurysma endovascularis kezelése „candy-plug” technikával perzisztáló állumen esetén]. Orv Hetil. 2020;161(11):437-39. [Hungarian]

Mihály Zs, **Hidi L**, Suhai F, Tarcza Zs, Nemes B, Sótonyi P. Nyaki verőér atípusos, fokális, fibromuscularis dysplasiájának képalkotó diagnosztikai nehézségei. Magy Rad. 2020;94:70-74.

Farkas ÁZ, Farkas VJ, Gubucz I, Szabó L, Bálint K, Tenekedjiev K, Nagy AI, Sótonyi P, **Hidi L**, Nagy Z, Szikora I, Merkely B, Kolev K. Neutrophil extracellular traps in thrombi retrieved during interventional treatment of ischemic arterial diseases. Thromb Res. 2019;175:46-52.

Hidi L, Csobay-Novák C, Nemes A, Nemes B, Oláh Z, Pál D, Sótonyi P. Nellix: New possibilities in the treatment of the aorto-iliac aneurysm - our initial experiences [Nellix: új távlatok az aortoiliacalis aneurysmák kezelésében – kezdeti tapasztalataink]. Magy Seb. 2017;70(1):18-23. [Hungarian]

Németh BT, Mátyás C, Oláh A, Lux Á, **Hidi L**, Ruppert M, Kellermayer D, Kökény G, Szabó G, Merkely B, Radovits T. Cinaciguat prevents the development of pathologic hypertrophy in a rat model of left ventricular pressure overload. Sci Rep. 2016;6:37166.

Oláh A, Németh BT, Mátyás C, **Hidi L**, Lux Á, Ruppert M, Kellermayer D, Sayour AA, Szabó L, Török M, Meltzer A, Gellér L, Merkely B, Radovits T. Physiological and pathological left ventricular hypertrophy of comparable degree is associated with characteristic differences of in vivo hemodynamics. Am J Physiol Heart Circ Physiol. 2016;310(5):H587-97.

Hidi L, Menyhei G, Kováts T, Dobai A, Szeberin Z. Report of the Hungarian Vascular Registry's data of infrarenal aortic aneurysms (2010-2014) [Magyarországon végzett infrarenalis aortaaneurysma-műtétek eredményei az Érsebészeti Regiszter adatai alapján (2010-2014)]. Orv Hetil. 2015;156(49):1991-2002. [Hungarian]

Mátyás C, Németh BT, Oláh A, **Hidi L**, Birtalan E, Kellermayer D, Ruppert M, Korkmaz-Icöz S, Kökény G, Horváth EM, Szabó G, Merkely B, Radovits T. The soluble guanylate cyclase activator cinaciguat prevents cardiac dysfunction in a rat model of type-1 diabetes mellitus. Cardiovasc Diabetol. 2015;14:145.

Oláh Z, **Hidi L**, Entz L, Szeberin Z. A medialis visceralis rotáció szerepe a suprarenalis aorta sebészetében. Magy Seb. 2015;68(4):167-72. [Hungarian]

Oláh A, Németh BT, Mátyás C, Horváth EM, **Hidi L**, Birtalan E, et al. Cardiac effects of acute exhaustive exercise in a rat model. Int J Cardiol. 2015;182:258-66.

Szeberin Z, **Hidi L**, Kováts T, Menyhei G. Report of the 2013 data of the Hungarian Vascular Registry [Az Érsebészeti Regiszter 2013-as adatainak elemzése]. Magy Seb. 2014;67(6):362-71. [Hungarian]

Radovits T, Oláh A, Lux Á, Németh BT, **Hidi L**, Birtalan E, Kellermayer D, Mátyás C, Szabó G, Merkely B. Rat model of exercise-induced cardiac hypertrophy: hemodynamic

characterization using left ventricular pressure-volume analysis. Am J Physiol Heart Circ Physiol. 2013;305(1):H124-34.

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RESEARCH ARTICLE

Cryopreservation moderates the thrombogenicity of arterial allografts during storage

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Abstract

Introduction

Management of vascular infections represents a major challenge in vascular surgery. The use of cryopreserved vascular allografts could be a feasible therapeutic option, but the optimal conditions for their production and use are not precisely defined.

Aims

To evaluate the effects of cryopreservation and the duration of storage on the thrombogenicity of femoral artery allografts.

Methods

In our prospective study, eleven multi-organ-donation-harvested human femoral arteries were examined at five time points during storage at -80˚C: before cryopreservation as a fresh native sample and immediately, one, twelve and twenty-four weeks after the cryopreservation. Cross-sections of allografts were perfused with heparin-anticoagulated blood at shear-rates relevant to medium-sized arteries. The deposited platelets and fibrin were immunostained. The thrombogenicity of the intima, media and adventitia layers of the artery grafts was assessed quantitatively from the relative area covered by fibrin- and platelet-related fluorescent signal in the confocal micrographs.

Results

Regression analysis of the fibrin and platelet coverage in the course of the 24-week storage excluded the possibility for increase in the graft thrombogenicity in the course of time and supported the hypothesis for a descending trend in fibrin generation and platelet deposition

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on the arterial wall. The fibrin deposition in the cryopreserved samples did not exceed the level detected in any of the three layers of the native graft. However, an early (up to week 12) shift above the native sample level was observed in the platelet adhesion to the media.

Conclusions

The hemostatic potential of cryopreserved arterial allografts was retained, whereas their thrombogenic potential declined during the 6-month storage. The only transient prothrombotic change was observed in the media layer, where the platelet deposition exceeded that of the fresh native grafts in the initial twelve weeks after cryopreservation, suggesting a potential clinical benefit from antiplatelet therapy in this time-window.

Introduction

Despite modern sterilisation processes, antibiotic therapies and wound treatments, the management of vascular infections remained a major challenge for vascular surgery. The use of cryopreserved vascular allografts could be a feasible therapeutic option in these cases. However, despite the clinical use, no strong evidence-based guidelines of the production and use of cryopreserved vascular tissues have been issued so far. There are many different cryopreservational strategies, all assuming that the storage time is virtually infinite [1]. Limited data are available concerning the relationship between the quality of allografts and their cryopreservation storage time. Wang and al., who investigated living donor liver transplantation, found no differences in the patency of vascular grafts for outflow reconstruction or the regeneration of liver graft between iliac allografts with less or more than one-year cryopreservation storage time [2]. However, our earlier report on the use of allografts highlighted the superiority in patency and limb salvage of the allografts stored for less than 6 months after cryopreservation in arterial reconstruction of lower extremities [3]. To the best of our knowledge no data on the relationship between allografts and their cryopreservation storage time is available in the literature other than the above two articles.

In addition, the occurrence of early occlusion (within 30 days) of the allografts varies in a broad range between 0–17% [3–11], which could be traced back to effects of multiple factors, such as anatomical location or quality of the allograft. The patency of the implanted grafts strongly depends on the preservation of the inherent thromboresistance of the native arterial wall, i.e. their capacity to prevent intravascular blood clot formation, the key companions of which are platelets and fibrin [12]. However, platelet deposition and fibrin generation by the plasma coagulation system are essential factors of the hemostatic function of the vascular wall to prevent bleeding at the site of the implanted graft. Thus, the implementation of optimal manufacturing and quality control of allografts in vascular surgery requires refined understanding of the balance of thrombogenic and hemostatic potential of the applied allografts.

In line with this timely demand, the aim of this study was to evaluate the effects of cryopreservation and the duration of storage on the deposition of platelets and fibrin in the vessel wall as indicators of the thrombogenicity of femoral artery allografts.

Materials and methods

In our prospective study eleven human femoral arteries, which were harvested from eleven different donors in multi-organ donations, were examined at five time points: before the

cryopreservation as a native sample (BC) and after the cryopreservation immediately (C0), on the first (C1), twelfth (C12) and twenty-fourth (C24) week of storage after cryopreservation.

Donor characteristics

The median age of the donors was 45.0(33.0;50.5) years. Three donors were female (27.27%). The median body mass index (BMI) was 26.3(23.65;27.8) kg/m^2. The leading cause of death was cerebral haemorrhage (6 donors; 54.54%). All essential characteristics of the donors are presented in Table 1.

Inclusion and exclusion criteria of allografts

Donor inclusion criteria of the study were based on the national multi-organ donation criteria and rules [13]. The allografts from donors above 65 years, with malignancy or with positive bacteria-fungal culture or virus serology test and the allografts with negative evaluation of the vascular surgeon, who performed the explantation or cryopreservation, (e.g. significantly injured or calcified allograft) were excluded.

Ethical considerations, data collection

The study was in full compliance with the principles of the national multi-organ donation and the applicable international and national laws. The anonymous data of the donors were collected prospectively from the electronic health information system of the donation according to the General Data Protection Regulation of the European Union. The study was approved by the institutional review board, by Semmelweis University Regional and Institutional Committee of Science and Research Ethics (approval number: 257/2018.). The consents were not obtained, because the vascular tissues were harvested from brain-dead donors in multi-organ donations and the data were analyzed anonymously.

Table 1. Donor characteristics.

Characteristic		n = 11
Cause of death	**trauma**	3(27.27)
	cerebral ischaemia	2(18.18)
	cerebral haemorrhage	6(54.55)
Age (year)		45.00(33.00–50.50)
Female sex		3(27.27)
BMI (kg/m2)		26.30(23.65–27.80)
Past medical history	**Hypertension**	3(27.27)
	Diabetes	1(9.09)
	Pulmonary disease (COPD)	1(9.09)
	Smoking	4(36.36)
Blood type	**A**	5(45.46)
	B	3(27.27)
	AB	0(0)
	0	3(27.27)
	Rh+	10(90.91)
	Rh-	1(9.09)

Data are presented as number of donors(%) or median(lower quartile–upper quartile). n: number of donors; BMI: body mass index; COPD: chronic obstructive pulmonary disease.

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Harvesting, cryopreservation, storage and thawing of allografts

Femoral arteries were harvested in Budapest from brain-dead donors in multi-organ donations organized by the Organ Coordination Office of the Hungarian National Blood Transfusion Service. All donors had negative serology test of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, syphilis, active Epstein-Barr virus and active cytomegalovirus. The explantation of the femoral arteries (common and superficial femoral artery) was performed under surgical asepsis and principles of sterile technique. The suitability of the grafts was evaluated by the vascular surgeon, who performed the harvesting. After the explantation the allografts were placed immediately into a triple sterile plastic bag (Set of Transplantation Bags–sterile 80 00 61H, Raguse GmbH, Ascheberg, Germany) in 500 ml transport solution (Sodium Chloride 0.9% "Baxter" Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) containing 4 mg/ml cefazolin (Sandoz GmbH, Kundl, Austria) and 0.4 mg/ml fluconazole (Fresenius Kabi Hungary, Budapest, Hungary) at 4˚C. They were transferred in an organ transport box (IGLBox Organ Transporter, Institut Georges Lopez, Lissieu, France) at 4˚C to the site of storage and were stored for 12 h at 4˚C.

The cryopreservation was performed within 12 h after the explantation in a clean room classified "A" with a background classified "B" used laminar air flow system [14]. After performing bacteria-fungal culture test and reevaluation of the grafts, five 0.5 cm wide ring samples were cut from each femoral artery. One of the samples (BC) was placed into a plastic cryotube in isopentane (Merck Kft, Budapest, Hungary) and stored at -20˚C. The rest of the samples (C0, C1, C12, C24) underwent the same cryopreservation procedure used for the allografts. They were placed in cryobags (TissueVault Cryogenic Freezing Bag TV1430, Origen Biomedical, Austin, Texas, USA) in a 500 ml cryopreservation solution (Ringer Fresenius, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) containing 20 v/v% dimethyl sulfoxide (Molar Chemicals Kft., Halásztelek, Hungary), 4 mg/ml cefazolin (Sandoz GmbH, Kundl, Austria) and 0.4 mg/ml fluconazole (Fresenius Kabi Hungary, Budapest, Hungary) for 10 minutes. Thereafter, these samples were cryopreserved to -80-90˚C according to a controlled freezing method (PC Interface for Thermo Scientific^{TM} Cryomed^{TM} Freezers, Version 3.0 and Thermo Scientific CryoMed Controlled-Rate Freezer 7451, Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) (Fig 1) using vaporised nitrogen (Messer Hungarogáz Kft., Budapest, Hungary), and they were stored at -80˚C in a deep freezer (Taylor-Wharton K Series Cryogenic Storage System 10K, Taylor-Wharton, Baytown, Texas, USA).

For the thrombogenicity measurements after various storage periods, the cryopreserved graft samples were thawed at 37˚C until the disappearance of the ice components and washed three times with physiological salt solution (Sodium Chloride 0.9% "Baxter" Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) at 20˚C to remove the cryopreservation medium.

Measurement of thrombogenicity with immunofluorescence imaging

The flow-chamber model described previously was used for testing the allograft samples as thrombogenic surfaces [15, 16]. Frozen cross-sections (10 μm) of allograft samples placed on poly-L-Lys-coated slides were perfused at 0.5 ml/min flow rate in a 0.4-cm-wide and 0.12-mm-high parallel-plate chamber with heparin-anticoagulated blood collected from healthy volunteers. Assuming laminar flow conditions, the shear rate at the surface of the section was 900 s^{-1}, according to the formula $1.03^{*}6\ Q/(w^{*}h^{2})$, where Q is the flow rate in ml s^{-1}, w and h are the width and the height of the flow path in cm, respectively. This intermediate shear rate was chosen as an adequate model of the rheological situation in medium-sized arteries, where deposition of both platelets and fibrin is enabled [17]. Before the perfusion the

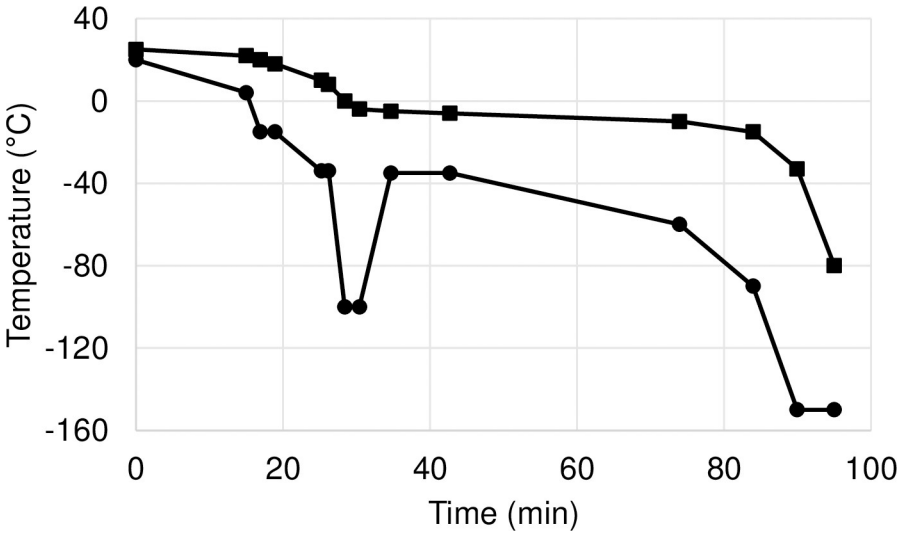

Fig 1. Controlled freezing protocol of the cryopreservation. Temperature ramps of controlled-rate freezer's chamber: continuous line with "•"; temperature ramps of sample: continuous line with "■".

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sections were blocked with 2 w/v% bovine serum albumin (BSA) in 0.05 M Tris buffer pH 7.4 containing 0.1 M NaCl and 0.02 w/v% NaN_3 (BSA-TBS) for 45 minutes and the 90-s perfusion was followed by a 30-s wash with 1.5 mM KH_2PO_4, 8.1 mM Na_2HPO_4 buffer pH 7.4 containing 137 mM NaCl and 2.7 mM KCl (PBS). Thereafter the sections were fixed in acetone at 4˚C for 10 minutes and the deposited platelets and fibrin were double-stained for indirect immunofluorescence microscopy. Sections were blocked with BSA-TBS for 30 min followed by platelet staining using the mouse monoclonal antibody against human GpIIb/IIIa (4 μg/ml in BSA-TBS for 30 min, sc-53417, Santa Cruz Biotech), followed by 3 times 5 min washes in TBS, and 30 min incubation with the Goat-anti-Mouse IgG-Alexa Fluor 633 secondary antibody (2 μg/ml in BSA-TBS, Invitrogen, Budapest, Hungary). Double-staining was continued with the fibrin staining after 3 times 5 min washes in BSA-TBS: 30 min incubation with the primary rabbit polyclonal antibody developed against the N-terminal part of the gamma chain fibrin(ogen) (20 μg/ml in BSA-TBS, PA5-29734, Invitrogen), followed by 3 times 5 min washes in TBS, and 30 min incubation with the Goat-anti-Rabbit IgG-Alexa Fluor 546 secondary antibody (2 μg/ml in BSA-TBS, Invitrogen). Following final washes in TBS, the stained sections were covered in 50% glycerol in TBS. Confocal images were taken from the slides using a Zeiss LSM710 confocal laser scanning microscope equipped with a 10 × 0.3 lens (Carl Zeiss, Jena, Germany) using 488 nm, 543 nm and 633 nm excitation laser lines, respectively, and emissions were detected in the ranges of 500–530 nm, 565–585 nm and 650–690 nm, respectively. Each allograft vessel at each sampling time point was perfused in duplicates or triplicates and depending on the section size, 5–10 different images were taken of each perfused cryosection in order to survey the whole cross-sectional area of the vessel. Quantification of platelet and fibrin(ogen) coverage of the vessel wall was performed with the Image J software (NIH, Bethesda, MD, USA) selecting the region of interest, calculating its surface area in pixels and setting a threshold intensity value for automatic identification of platelets or fibrin(ogen) covered areas in percentage.

Scanning Electron Microscope (SEM) imaging

Two consecutive cryosections (10 μm) of each allograft sample were placed on Thermanox coverslips and perfused with heparinized whole blood as described for the immunofluorescent

imaging above. The perfused sections were cut out of the plastic coverslips with scissors, fixed and processed for scanning electron microscopy. Following repeated washes with 100 mmol/L Na-cacodylate pH 7.2 buffer, samples were fixed in 1 v/v% glutaraldehyde for 16 h. The fixed samples were dehydrated in a series of ethanol dilutions (20–96 v/v%), 1:1 mixture of 96 v/v% ethanol/acetone and pure acetone followed by critical point drying with CO2 in E3000 Critical Point Drying Apparatus (Quorum Technologies, Newhaven, UK). The specimens were mounted on adhesive carbon discs, sputter coated with gold in SC7620 Sputter Coater (Quorum Technologies, Newhaven, UK) and images were taken with scanning electron microscope EVO40 (Carl Zeiss GmbH, Oberkochen, Germany) at 5000x magnification from the intima, media and adventitia layer of each cross-section.

Statistical analysis

Because our immunofluorescence measurements were performed on arterial samples from 11 donors, in each of which we evaluated the platelet and fibrin content from different number of collected data (in the range 111–225), we used a fuzzy sample approach [18] to evaluate the data. This approach allows for the achievement of parity of the arterial samples from different donors, as applied previously [19–21]. Here, we implemented a novel explicit fuzzy estimation method and a novel fuzzy version of the invertible cumulative distribution function estimator with maximum count of nodes [22] for the implicit estimation of the median, lower quartile and upper quartiles of a random variable. A qualitative quartile-wise estimation of the effect of cryopreservation on the thrombogenicity was based on comparisons of coverage values at each time points and the native graft. In this qualitative analysis we tested the null hypothesis H_0 (that the compared quartiles are equal) against the alternative hypothesis H_1 (that one of the quartiles is greater than the other one) using four different Bootstrap one-tailed tests [23] with modifications detailed in S1 File. A cluster approach to hypothesis testing was adopted to increase the statistical power of the tests [24]: each of the four p-values were compared to the predetermined significance level $\alpha = 0.05$ and the null hypothesis was rejected, if at least two out of the four p-values were less than α. A quantitative analysis of the effect of storage time on the thrombogenicity was based on the identification of the trends of changes in the median, lower quartile and upper quartile of the thrombogenic factor abundance in the course of time. For each of the three quartiles we constructed a linear regression with the time as independent variable trained on a fuzzy sample containing four triplets in the form (time, quartile, degree of membership) using a previously described analytical algorithm [25]. A cluster of four fuzzy Bootstrap procedures described in S1 File were used to identify the distribution of the predicted regression slope and the probabilities for the slope to be negative and non-negative (or positive and non-positive). We considered the negativity/positivity of the estimated regression slope to be significant, if at least two of the four fuzzy Bootstrap procedures indicated probability for non-negativity/non-positivity less than the preselected significance level $\alpha = 0.05$. Such estimates based on probabilities are in line, but superior to the p-values of any statistical test in their power to determine the significance of the identified slope sign, because they calculate the probability of being right when accepting the alternative hypothesis, whereas the p-values provide only the probability of being wrong when rejecting the null hypothesis.

Results

We assessed the effects of our allograft-preservation method on the thrombogenicity of the arterial allografts based on the adhesion of platelets and generation of fibrin in the three layers of the grafts (adventitia, media, intima). When cross-sections of the grafts were perfused with heparinized blood at shear rate equivalent to the wall shear rate in medium-size arteries,

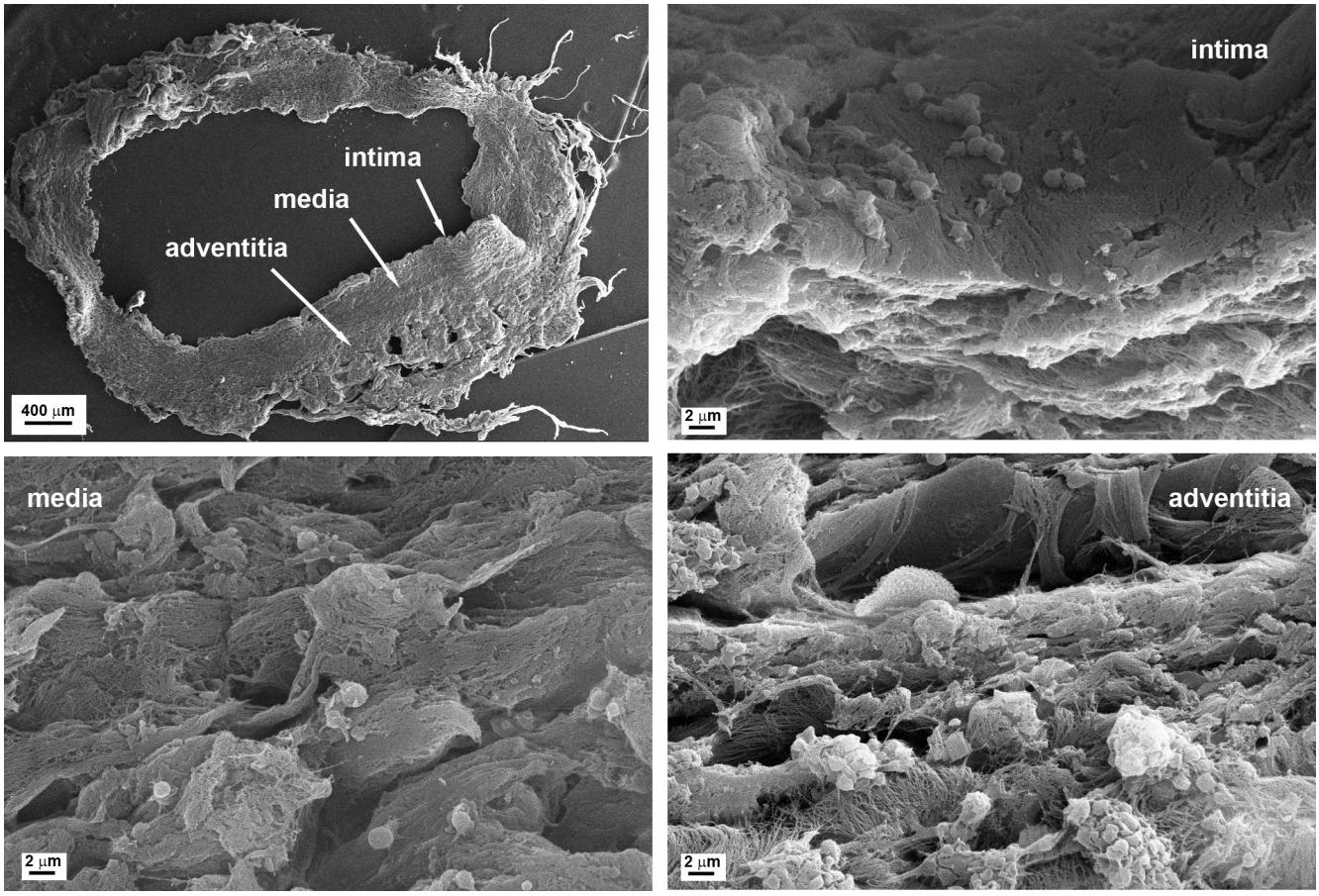

Fig 2. Platelet adhesion to arterial cross-sections. Cross-sections of the arterial grafts were prepared as described in the Materials and Methods (top left panel) and perfused with heparinized blood. Following the perfusion, the cross-sections were fixated and processed for scanning electron microscopic imaging. Isolated adhered platelets are seen in the intima and media layers, whereas larger aggregates are formed in the adventitia.

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platelets adhered to the arterial wall in a highly heterogeneous pattern, distinct in the three layers (Fig 2). In order to account for this heterogeneity in a quantitative manner, we applied a confocal microscopic technique to detect the immunofluorescence signal of a platelet-related antigen (GpIIb/IIIa receptor) on larger areas (lower magnification than SEM) and multiple (5–10) regions of interest in each layer separately (Fig 3). Because the thrombogenic potential of the arterial wall depends not only on the recruitment of platelets, but also on the activation of blood coagulation, we evaluated the deposition of fibrin as an end-product of the blood clotting cascade on the graft cross-sections with anti-fibrin antibody in the same immunofluorescence microscopic assay (Fig 3).

Fibrin deposition to the artery wall

In quantitative terms the generation of fibrin was assessed as percentage of area of the intima, media and adventitia layers covered by fibrin in the immunofluorescent images of the graft cross-sections (Fig 4). The statistical analysis did not support any significant increase of fibrin deposition in the cryopreserved samples over the fibrin level detected in any of the three layers of the native (BC) samples (Fig 5). The subset of fibrin coverage data in the interquartile range of the cryopreserved grafts (yellow shadowed area, Fig 5) largely overlapped with the respective band of the BC samples (the area between the red and green dashed lines, Fig 5) without any

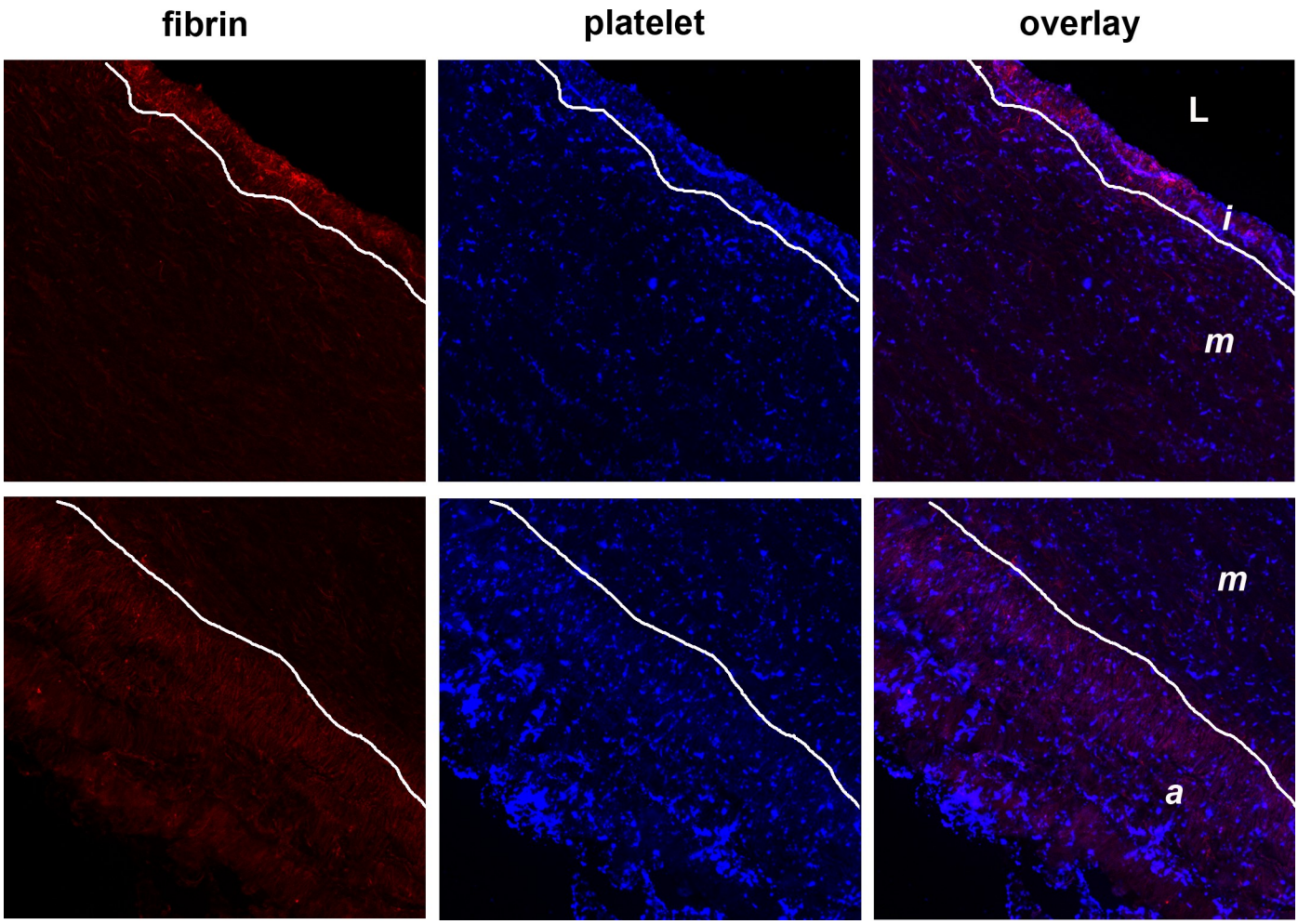

Fig 3. Platelet and fibrin deposition to arterial cross-sections detected by indirect immunofluorescence. Cryosections of allograft samples were perfused with heparinized whole blood and deposited platelets and fibrin were visualized with double immunofluorescence as detailed in Materials and Methods. Representative images illustrate the uneven spatial distribution of GpIIb/IIIa (blue) and fibrin (red) staining in the three layers of the perfused arterial cross-sections. For quantitative evaluation platelet and fibrin surface coverage data were collected from 5–10 such images (original size of the presented images 850 μm × 850 μm) to survey the whole size of the perfused cross-section, and the intrinsic green autofluorescence of elastin (Fig 4, here indicated by the white lines) was used as a guide to encircle the regions of interest and evaluate the three vessel wall layers separately. l: lumen; i: intima; m: media; a: adventitia.

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single significant deviation of a quartile boundary of a cryopreserved sample over a BC sample. It is noteworthy that all significant differences between cryopreserved and native graft samples indicated reduced fibrin generation (out of the 12 sets of cryopreservation data 3 median, 3 bottom quartile and 6 top quartile values were lower than their BC counterparts, Fig 5). Cryopreservation was associated also with a trend to homogenize the fibrin generation potential of the artery wall layers (narrower interquartile range in 8 out of the 12 cryopreservation data sets).

In order to test the hypothesis that the storage time affects the fibrin deposition to the cryopreserved grafts, we performed regression analysis of the changes in fibrin coverage on the three arterial wall layers as a function of time (Fig 6). In seven out of the nine hypothesis tests performed to check the temporal trend of the fibrin coverage in the three quartiles of the three wall layers a non-negative trend was excluded at 0.05 significance level, whereas in the rest two cases–the lower coverage quartile of the media and intima layers–the probability of non-

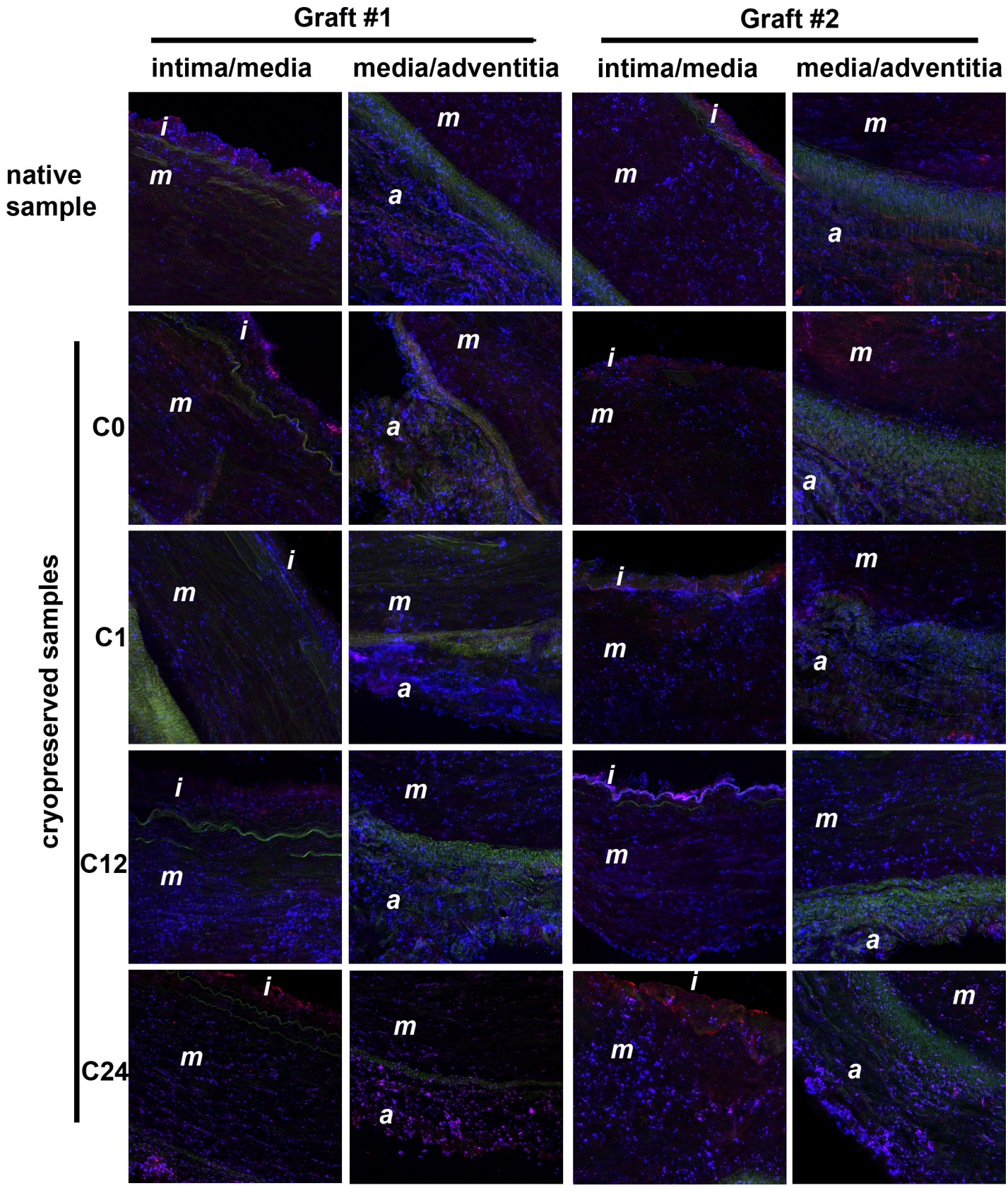
Graft #1
Graft #2
intima/media
media/adventitia
intima/media
media/adventitia
native sample
cryopreserved samples
C0
C1
C12
C24
i
m
a

Fig 4. A time lapse sequence of immunofluorescence images monitoring the fibrin and platelet deposition in different layers of the arterial wall. Cryosections of allografts were immunostained for platelet (blue) and fibrin (red) antigen after whole blood perfusion as in Fig 3. The green autofluorescence of the internal and external elastic lamina indicates the boundaries between the wall layers (i: intima; m: media; a: adventitia). Images from two different regions of interest in two different allografts are shown for each time point.

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negative trend was 20–22% and 14–28%, respectively. Thus, the robust statistical analysis of the fibrin coverage data in the course of twenty-four-week storage of the arterial grafts definitely excludes the possibility for increase in the graft thrombogenicity in the course of time and strongly supports the decline in the fibrin generation.

Platelet adhesion to the artery wall

Platelet adhesion was assessed as percentage of area of the intima, media and adventitia layers covered by the GpIIb/IIIa-related immunofluorescence signal. Similar to fibrin deposition, the platelet coverage data in the interquartile range of the cryopreserved grafts (yellow shadowed area in Fig 7) largely overlapped with the respective band of the BC samples. In contrast to fibrin, however, in 3 out of the 12 datasets of cryopreserved samples all three (C0 and C1 of the media) or two (C12 of the media) of the quartile values exceeded the respective platelet coverage level of the native samples (BC) (Fig 7). In the early cryopreserved samples of the intima (C0, C1, C12), the median of the platelet coverage moved upward, but this difference was not accompanied by a shift in the upper quartile. The interquartile range of these samples largely overlapped with the BC interquartile range. Thus, the early changes in the platelet adhesiveness of the intima can be interpreted as resulting in moderate rearrangements in the platelet coverage within the variability of the non-cryopreserved grafts.

The regression analysis of the changes in platelet coverage of the three arterial wall layers as a function of time excluded a non-negative trend at 0.05 significance level in seven out of the nine hypothesis tests performed on the platelet coverage of the three quartiles of the three wall layers (Fig 8). The two exceptions were the median and the lower coverage quartile of the intima, where the probability of non-negative trend was 5–11% and 7–18%, respectively. Thus, the regression analysis of the platelet coverage data in the course of twenty-four-week storage of arterial grafts definitely excludes the possibility for time-dependent increase in the graft thrombogenicity and strongly supports the decline in the platelet adhesiveness.

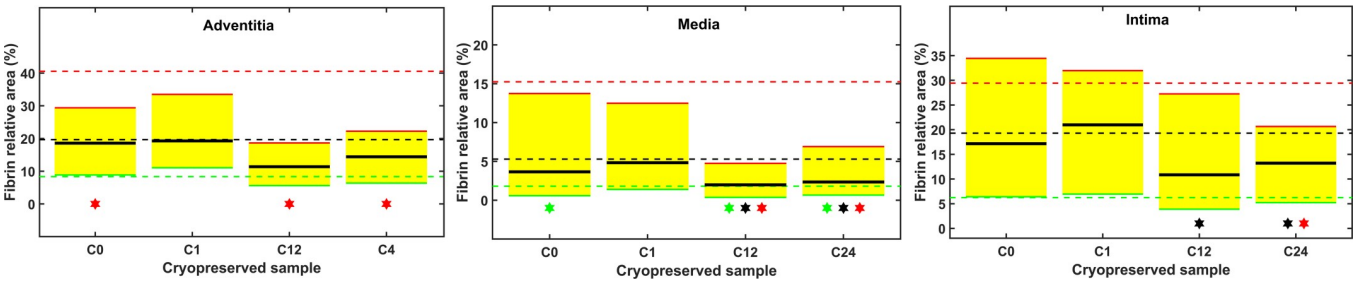

Fig 5. Qualitative quartile-wise comparison of the fibrin deposition on cryopreserved and native arterial allografts. Fibrin coverage of the separate arterial wall layers was measured as illustrated in Fig 3. Lower (green), median (black) and upper (red) quartiles of the measured coverage data are shown with dashed lines for the BC and continuous lines for the cryopreserved samples. The interquartile range of the cryopreserved samples is shadowed in yellow. Symbols indicate significant differences between the respective quartiles (green, lower quartile; black, median; red, upper quartile) of the BC and cryopreserved samples according to the cluster statistical approach described in Materials and Methods.

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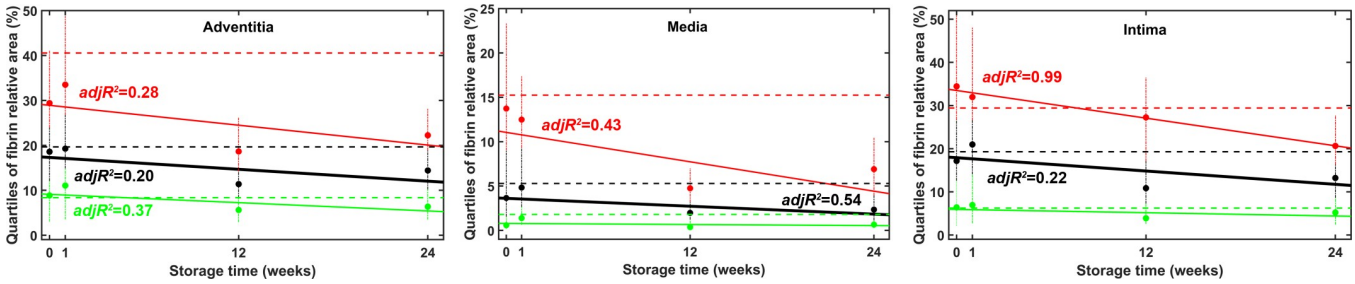

Fig 6. Time course of fibrin deposition in cryopreserved and native arterial allografts. Fibrin coverage of the separate arterial wall layers was measured as illustrated in Fig 3. Lower (green), median (black) and upper (red) quartiles of the measured coverage data are shown with dashed lines for the BC, symbols for the cryopreserved samples and continuous lines for the linear regression performed as described in Materials and Methods. Error bars (vertical dotted lines) indicate the data spanning the range of 12.5% rank below and above the respective quartile value. The explanatory strength of the correlation can be assessed on the basis of the adjusted R^2 ($adjR^2$) values that are shown only for the regression trends proven to be significant at 0.05 level by the statistical approach described in Materials and Methods.

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Discussion

The incidence of vascular surgical wound infection has a wide range, between 3.5% and 32%, and the risk of a prosthetic graft infection is 0.25–6% after the implantation. Moreover, these numbers can be higher in patients with infection-predisposing wound complications and in certain locations of the surgery [26–30]. In these cases, an optimal therapeutic modality would be a biocompatible, infection-resistant, easily accessible vascular graft. Autologous superficial veins are ideal solution for such implantations and they can give the best results for the reconstruction of the lower extremities, but 20–45% of the patients do not have any appropriate veins [7, 28, 31, 32]. In the absence of autologous veins, the cryopreserved allografts can be an alternative solution for the management of the vascular infection. However, because of the lack of evidence-based guidelines for the production and use of cryopreserved vascular tissues, characterization of the biomechanical, biochemical and immunological properties of the allografts are essential for the implementation of optimal manufacturing and quality control processes. The cryopreservation strategy is a crucial point of the investigation of allografts. Harvesting, cryopreservation, thawing are potentially harmful procedures for the grafts. There are many different methods by allograft banks, but their advantages and disadvantages are not fully elucidated [1, 4–6, 8, 10, 11, 33, 34].

The cryopreservation protocol applied in the current study maintained the procoagulant properties of the stored grafts at the level of their fresh native counterparts. In addition, a

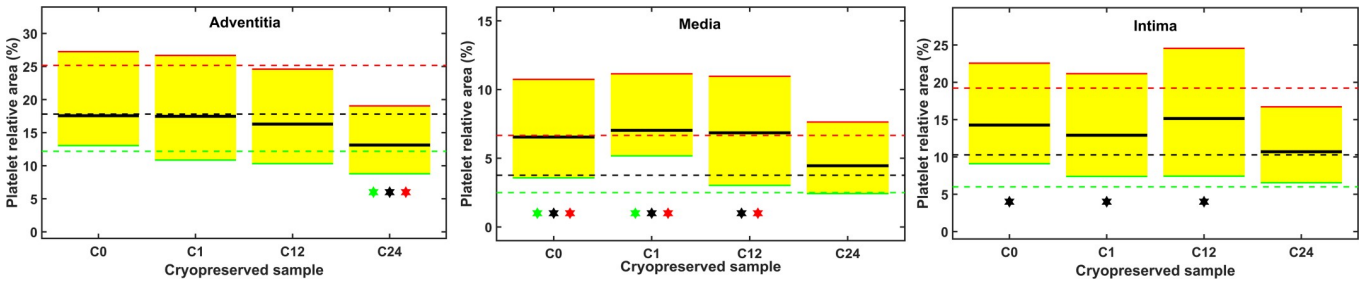

Fig 7. Qualitative quartile-wise comparison of the platelet adhesion on cryopreserved and native arterial allografts. Platelet coverage of the separate arterial wall layers was measured as illustrated in Fig 3. Lower (green), median (black) and upper (red) quartiles of the measured coverage data are shown with dashed lines for the BC and continuous lines for the cryopreserved samples. The interquartile range of the cryopreserved samples is shadowed in yellow. Symbols indicate significant differences between the respective BC and cryopreservation quartiles according to the cluster statistical approach described in Materials and Methods.

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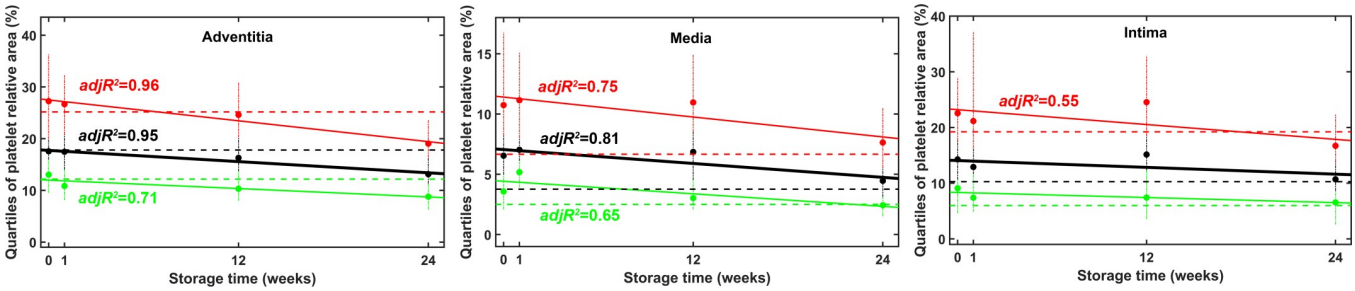

Fig 8. Time course of platelet adhesion to cryopreserved and native arterial allografts. Platelet coverage of the separate arterial wall layers was measured as illustrated in Fig 3. Lower (green), median (black) and upper (red) quartiles of the measured coverage data are shown with dashed lines for the BC, symbols for the cryopreserved samples and continuous lines for the linear regression performed as described in Materials and Methods. Error bars (vertical dotted lines) indicate the data spanning the range of 12.5% rank below and above the respective quartile value. The explanatory strength of the correlation can be assessed on the basis of the adjusted R^2 (adjR^2) values that are shown only for the regression trends proven to be significant at 0.05 level by the statistical approach described in Materials and Methods.

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moderate, but consistent trend for time-related decline in fibrin deposition was observed. Although we did not investigate the mechanism of this anticoagulant trend, a hypothetic cause could be reduction of blood clotting triggers in the wall of stored grafts. Tissue factor, the major trigger of blood coagulation is known to lose a significant fraction of its activity during a six-month storage period in frozen state [35]. A practical implication of this result is that one cannot expect to improve the graft patency with anticoagulant prophylaxis, because the cryopreserved grafts do not trigger more massive fibrin formation than the native artery wall. This finding is in line with the lack of significant effect of warfarin on the primary graft patency of cryopreserved saphenous vein allografts reported by others [11].

Concerning the role of anti-platelet medication in graft patency, controversial results have been reported in previous studies. A definite benefit to patency from the anti-platelet therapy was seen in an animal model with saphenous vein allografts [36], but not in patients undergoing infrainguinal revascularization procedure [11]. Our current findings help the interpretation of this controversy. We propose that an anti-platelet strategy could be of advantage only within a certain time-window determined by the temporal pattern of changes in the platelet-adhesiveness of the stored cryopreserved grafts. In the early storage period in our study (up to 12 weeks after cryopreservation) platelet deposition in the media layer exceeded that of the media in fresh, non-cryopreserved grafts. This is probably the time frame of storage, when anti-platelet medication could be of benefit for the patency of the implanted graft. Out of this time window and in the intima and adventitia layers at all time-points the platelet deposition remained at the level observed prior cryopreservation, which implies a normal hemostatic function of the graft and does not justify any anti-platelet medication.

An important aspect of the current study is that we evaluated separately the thrombogenicity of the three layers of the artery wall, because these are known to exert differential effects on blood clotting and platelet function. According to our data the adventitia and intima layers showed a higher thrombogenic potential, while the media layer's relative platelet and fibrin coverage lagged behind the other two layers. On the one hand, the decreased procoagulant potential of the media layer can be explained by the lower levels of tissue factor protein and mRNA in this layer, as reported earlier [37]. On the other hand, concerning the relatively lower platelet deposition in the media, our current findings are in agreement with earlier studies applying the same experimental setup that showed a limited von Willebrand factor-dependent binding of platelets to native media of arteries and the strongest adhesiveness of the adventitia [15, 16]. In a different experimental setup, the adventitia of normal, non-

atherosclerotic vessel walls was observed similarly with the highest platelet deposition, but the subendothelium had the lowest one and the tunica media had an intermediate adhesiveness [38]. However, other studies established that the subendothelium can also cause a rapid and massive platelet activation more pronounced than the media [39, 40], as seen in our current and earlier work. Independently of the differences in the relative thrombogenicity of the three layers, following cryopreservation its time-related changes were invariably favourable in all of them. Layer-dependent response to cryopreservation was observed only in the media layer and these were restricted to the three earliest times of storage.

The limitations of this study could be the in vitro design, however it is the optimal first part of further investigations at a such multifactorial and undiscovered field for identification of the main issues. The most frequently used allograft material in vascular surgery is the great saphenous vein, especially by revascularization on lower extremities, nevertheless, we have chosen the arteries based on our previous study [3], in which arterial allografts had better patency results, than veins.

Conclusions

Our results revealed that if arterial allografts were cryopreserved and stored for a period of up to 6 months, their hemostatic potential remained within the variability range of the fresh native arterial wall. In addition, both the fibrin generation and the platelet adhesiveness of the cryopreserved artery allografts declined in the course of time, minimizing the risk for thrombotic occlusion of the implanted grafts. Thus, the applied cryopreservation method demonstrated a twofold clinical benefit: retained hemostatic and reduced thrombogenic potential of the grafts. The only transient prothrombotic change was observed in the media of the cryopreserved artery allografts, where the platelet deposition exceeded that of the fresh native grafts in the initial twelve weeks after cryopreservation. If the grafts are to be used in this early storage period, antiplatelet therapy may be justified to prevent thrombotic occlusion.

The definite favorable trend of changes in the thrombogenicity of the grafts during the six-month monitoring period of the current study justifies a longer-term investigation for optimization of the storage period of cryopreserved arterial allografts in terms of thrombogenicity.

Supporting information

S1 File. Detailed statistics.
(PDF)

S1 Data. Original datasets.
(ZIP)

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References

1. Müller-Schweinitzer E. Cryopreservation of vascular tissues. Organogenesis. 2009; 5: 97–104. https://doi.org/10.4161/org.5.3.9495 PMID: 20046671
2. Wang CC, Lopez-Valdes S, Lin TL, Yap A, Yong CC, Li WF, et al. Outcomes of long storage times for cryopreserved vascular grafts in outflow reconstruction in living donor liver transplantation. Liver Transplant. 2014; 20: 173–181. https://doi.org/10.1002/lt.23785 PMID: 24382821
3. Nagy Z, Oláh Z, Kókai J, Molnár AB, Laczkó Á, Szabó GV, et al. Homograftok szerepe az alsó végtagi érrekonstrukciókban. Magy Seb. 2017; 70: 5–12. https://doi.org/10.1556/1046.70.2017.1.1 PMID: 28294663
4. Minga Lowampa E, Holemans C, Stiennon L, Van Damme H, Defraigne JO. Late fate of cryopreserved arterial allografts. Eur J Vasc Endovasc Surg. 2016; 52: 696–702. https://doi.org/10.1016/j.ejvs.2016.08.005 PMID: 27614553
5. Tatar AR, Derycke L, Cochennec F, Jaziri A, Desgranges P, Touma J. Unmet needs in cryopreserved arterial allograft implantation for peripheral vascular graft infections. Eur J Vasc Endovasc Surg. 2020; 60: 788–789. https://doi.org/10.1016/j.ejvs.2020.08.001 PMID: 32912761
6. Lejay A, Delay C, Girsowicz E, Chenesseau B, Bonnin E, Ghariani MZ, et al. Cryopreserved cadaveric arterial allograft for arterial reconstruction in patients with prosthetic infection. Eur J Vasc Endovasc Surg. 2017; 54: 636–644. https://doi.org/10.1016/j.ejvs.2017.07.016 PMID: 28890027
7. Hartranft CA, Noland S, Kulwicki A, Holden CR, Hartranft T. Cryopreserved saphenous vein graft in infrainguinal bypass. J Vasc Surg. 2014; 60: 1291–1296. https://doi.org/10.1016/j.jvs.2014.05.092 PMID: 24997807
8. Guevara-Noriega KA, Lucar-Lopez GA, Pomar JL. Cryopreserved allografts for treatment of chronic limb-threatening ischemia in patients without autologous saphenous veins. Ann Vasc Surg. 2019; 60: 379–387. https://doi.org/10.1016/j.avsg.2019.03.018 PMID: 31200034
9. Singh K, Juneja A, Bajaj T, Voto C, Schor J, Zia S, et al. Single tertiary care center outcomes after lower extremity cadaveric vein bypass for limb salvage. Vasc Endovascular Surg. 2020; 54: 430–435. https://doi.org/10.1177/1538574420925586 PMID: 32489155
10. Noel AA, Gloviczki P, Cherry KJ, Safi H, Goldstone J, Morasch MD, et al. Abdominal aortic reconstruction in infected fields: early results of the United States cryopreserved aortic allograft registry. J Vasc Surg. 2002; 35: 847–852. https://doi.org/10.1067/mva.2002.123755 PMID: 12021697
11. Farber A, Major K, Wagner WH, Cohen JL, Cossman D V., Lauterbach SR, et al. Cryopreserved saphenous vein allografts in infrainguinal revascularization: analysis of 240 grafts. J Vasc Surg. 2003; 38: 15–21. https://doi.org/10.1016/s0741-5214(03)00330-6 PMID: 12844083
12. Wagner DD, Burger PC. Platelets in Inflammation and Thrombosis. Arterioscler Thromb Vasc Biol. 2003; 23: 2131–2137. https://doi.org/10.1161/01.ATV.0000095974.95122.EC PMID: 14500287
13. Hungarian National Blood Transfusion Service. Legislation of organ and tissue transplantation. 2020. Available from: https://www.ovsz.hu/en/organ-coordination-office/legislation; https://www.ovsz.hu/hu/oco/jogszabalyok.

14. European Comission. EudraLex—The rules governing medicinal products in the european union: Volume 4—EU guidelines to good manufacturing practice medicinal products for human and veterinary use; Annex 1—Manufacture of sterile medicinal products (corrected version). 2008. Available from: https://www.gmp-compliance.org/files/guidemgr/annex%2001[2008].pdf.
15. Komorowicz E, McBane RD, Fass DN. Physical and enzymatic perturbation of the architecture of the tunica media destroys its inherent thromboresistance. Thromb Haemost. 2002; 88: 827–833. https://doi.org/10.1055/s-0037-1613310 PMID: 12428102
16. Wohner N, Keresztes Z, Sótonyi P, Szabó L, Komorowicz E, Machovich R, et al. Neutrophil granulocyte-dependent proteolysis enhances platelet adhesion to the arterial wall under high-shear flow. J Thromb Haemost. 2010; 8: 1624–1631. https://doi.org/10.1111/j.1538-7836.2010.03890.x PMID: 20412433
17. Weiss HJ, Turitto VT, Baumgartnet HR. Role of shear rate and platelets in promoting fibrin formation on rabbit subendothelium. Studies utilizing patients with quantitative and qualitative platelet defects. J Clin Invest. 1986; 78: 1072–1082. https://doi.org/10.1172/JCI112663 PMID: 3760183
18. Nikolova N, Chai S, Ivanova SD, Kolev K, Tenekedjiev K. Bootstrap Kuiper testing of the identity of 1D continuous distributions using fuzzy samples. Int J Comput Intell Syst. 2015; 8: 63–75. https://doi.org/10.1080/18756891.2015.1129592
19. Nikolova N, Panayotov P, Panayotova D, Ivanova S, Tenekedjiev K. Using fuzzy sets in surgical treatment selection and homogenizing stratification of patients with significant chronic ischemic mitral regurgitation. Int J Comput Intell Syst. 2019; 12: 1075–1090. https://doi.org/10.2991/ijcis.d.190923.002
20. Farkas ÁZ, Farkas VJ, Gubucz I, Szabó L, Bálint K, Tenekedjiev K, et al. Neutrophil extracellular traps in thrombi retrieved during interventional treatment of ischemic arterial diseases. Thromb Res. 2019; 175: 46–52. https://doi.org/10.1016/j.thromres.2019.01.006 PMID: 30703701
21. Hisada Y, Grover SP, Maqsood A, Houston R, Ay C, Noubouossie DF, et al. Neutrophils and neutrophil extracellular traps enhance venous thrombosis in mice bearing human pancreatic tumors. Haematologica. 2020; 105: 218–225. https://doi.org/10.3324/haematol.2019.217083 PMID: 31048354
22. Nikolova N, Toneva D, Tsonev Y, Burgess B, Tenekedjiev K. Novel methods to construct empirical CDF for continuous random variables using censor data. 2020 IEEE 10th Int Conf Intell Syst IS 2020—Proc. 2020: 61–68. https://doi.org/10.1109/IS48319.2020.9199954
23. Nikolova N, Mihaylova N, Tenekedjiev K. Bootstrap tests for mean value differences over fuzzy samples. IFAC-PapersOnLine. 2015; 48: 7–14. https://doi.org/10.1016/j.ifacol.2015.12.048
24. Tenekedjiev K, Nikolova N, Rodriguez RM, Hirota K. Bootstrap testing of central tendency nullity over paired fuzzy samples. Int J Fuzzy Syst. 2021; Forthcoming.
25. Nikolova N, Rodriguez RM, Symes M, Toneva D, Kolev K, Tenekedjiev K. Outlier detection algorithms over fuzzy data with weighted least squares. Int J Fuzzy Syst. 2021; https://doi.org/10.1007/s40815-020-01049-8
26. Turtiainen J, Saimanen E, Partio T, Kärkkäinen J, Kiviniemi V, Mäkinen K, et al. Surgical wound infections after vascular surgery: prospective multicenter observational study. Scand J Surg. 2010; 99: 167–172. https://doi.org/10.1177/145749691009900312 PMID: 21044935
27. Turtiainen J, Hakala T. Surgical wound infections after peripheral vascular surgery. Scand J Surg. 2014; 103: 226–231. https://doi.org/10.1177/1457496913514384 PMID: 24737857
28. Chakfé N, Diener H, Lejay A, Assadian O, Berard X, Caillon J, et al. Editor's Choice–European Society for Vascular Surgery (ESVS) 2020 clinical practice guidelines on the management of vascular graft and endograft infections. Eur J Vasc Endovasc Surg. 2020; 59: 339–384. https://doi.org/10.1016/j.ejvs.2019.10.016 PMID: 32035742
29. Wilson WR, Bower TC, Creager MA, Amin-Hanjani S, O'Gara PT, Lockhart PB, et al. Vascular graft infections, mycotic aneurysms, and endovascular infections: a scientific statement from the American Heart Association. Circulation. 2016; 134: e412–460. https://doi.org/10.1161/CIR.0000000000000457 PMID: 27737955
30. Bíró G, Szeberin Z, Nemes A, Acsády G. Cryopreserved homograft and autologous deep vein replacement for infrarenal aorto and iliaco-femoral graft infection: Early and late results. J Cardiovasc Surg (Torino). 2011; 52: 169–176. PMID: 21464818
31. McPhee JT, Barshes NR, Ozaki CK, Nguyen LL, Belkin M. Optimal conduit choice in the absence of single-segment great saphenous vein for below-knee popliteal bypass. J Vasc Surg. 2012; 55: 1008–1014. https://doi.org/10.1016/j.jvs.2011.11.042 PMID: 22365176
32. Conte MS, Bradbury AW, Kolh P, White J V., Dick F, Fitridge R, et al. Global vascular guidelines on the management of chronic limb-threatening ischemia. Eur J Vasc Endovasc Surg. 2019; 58: S1–S109. e33. https://doi.org/10.1016/j.ejvs.2019.05.006 PMID: 31182334

33. Hruby J, Spunda R, Mericka P, Mlcek M, Pecha O, Splith K, et al. Influence of the new standardized clinical cryopreservation/slow thawing protocol on immunogenicity of arterial allografts in rats. PLoS One. 2020; 15: 1–12. https://doi.org/10.1371/journal.pone.0230234 PMID: 32155226

34. Špaček M, Měřička P, Janoušek L, Štádler P, Adamec M, Vlachovský R, et al. Current vascular allograft procurement, cryopreservation and transplantation techniques in the Czech Republic. Adv Clin Exp Med. 2019; 28: 529–534. https://doi.org/10.17219/acem/90037 PMID: 30684317

35. Gökmen BG, Özcan O, Taslak H, Ipar N, Tunali-Akbay T. Effect of freezing time on tissue factor activity and macronutrients of human milk. Protein J. 2020; 39: 591–597. https://doi.org/10.1007/s10930-020-09916-x PMID: 32989648

36. Miller VM, Bergman RT, Gloviczki P, Brockbank KGM. Cryopreserved venous allografts: Effects of immunosuppression and antiplatelet therapy on patency and function. J Vasc Surg. 1993; 18: 216–226. https://doi.org/10.1016/0741-5214(93)90601-H PMID: 8350430

37. Wilcox JN, Smith KM, Schwartz SM, Gordon D. Localization of tissue factor in the normal vessel wall and in the atherosclerotic plaque. Proc Natl Acad Sci U S A. 1989; 86: 2839–2843. https://doi.org/10.1073/pnas.86.8.2839 PMID: 2704749

38. Toschi V, Gallo R, Lettino M, Fallon JT, David Gertz S, Fernández-Ortiz A, et al. Tissue factor modulates the thrombogenicity of human atherosclerotic plaques. Circulation. 1997; 95: 594–599. https://doi.org/10.1161/01.cir.95.3.594 PMID: 9024145

39. Vlodavsky I, Eldor A, HyAm E, Atzom R, Fuks Z. Platelet interaction with the extracellular matrix produced by cultured endothelial cells: a model to study the thrombogenicity of isolated subendothelial basal lamina. Thromb Res. 1982; 28: 179–191. https://doi.org/10.1016/0049-3848(82)90260-2 PMID: 7179215

40. Fauvel-Lafève F. Microfibrils from the arterial subendothelium. Int Rev Cytol. 1999; 188: 1–40. https://doi.org/10.1016/s0074-7696(08)61564-8 PMID: 10208009

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Human blood vessel microbiota in healthy adults based on common femoral arteries of brain-dead multi-organ donors

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Discovery of human microbiota is fundamentally changing our perceptions of certain diseases and their treatments. However little is known about the human blood vessel microbiota, it may have important effects on vascular pathological lesions and vascular homograft failure. In our prospective survey study fourteen femoral arteries, harvested from donors in multi-organ donations, were examined using the V3-V4 region 16S rRNA sequencing method. The most abundant phyla in the human vascular microbiota were *Proteobacteria*, *Firmicutes* and *Actinobacteria*. At the genus level, the most abundant taxa were *Staphylococcus*, *Corynebacterium*, *Pseudomonas*, *Bacillus*, *Acinetobacter* and *Propionibacterium*. Of the bacterial taxa that have an indirect effect on the development of atherosclerosis, we found *Porphyromonas gingivalis*, *Prevotella nigrescens* and *Enterobacteriaceae* spp. with different abundances in our samples. Of the bacteria that are more common in the intestinal flora of healthy than of atherosclerosis patients, *Roseburia* and *Ruminococcus* occurred in the majority of samples. The human arterial wall has a unique microbiota that is significantly different in composition from that of other areas of the body. Our present study provides a basis for ensuing research that investigates the direct role of the microbiota in vascular wall abnormalities and the success of vascular allograft transplantations.

KEYWORDS

vascular, blood vessel, microbiota, allograft, 16S rRNA

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Introduction

Traditionally, most of the internal organs and blood are considered sterile. However, recent evidence has challenged this dogma by molecular-based detection of microorganisms in different tissues of healthy and non-healthy individuals (Paisse et al., 2016; Mansour et al., 2020; Molina et al., 2021; Sookoian and Pirola, 2021). Moreover, some authors could identify biologically relevant living microorganisms in these "sterile" regions (Clifford and Hoffman, 2015; Potgieter et al., 2015; Moreno et al., 2016). These insights, and the discovery of human microbiota, can fundamentally change our perceptions of certain diseases and their treatments.

The vascular system has been also considered a sterile environment. However, microorganisms can be detected in some vascular diseases in the arterial wall (aneurysm, atherosclerosis, vasculitis) (Blanchard et al., 1993; Kozarov et al., 2005; Rosenfeld and Campbell, 2011; Clifford and Hoffman, 2015). While microbiota research of gut and other traditionally non-sterile areas is in an advanced stage, little is known about the vascular wall tissue microbiota. We have collected a growing pool of knowledge about the cardiovascular effects of intestinal microbiota's metabolites (Jie et al., 2017; Liu et al., 2019; Verhaar et al., 2020; Jin et al., 2021). However, little information is available on the local vascular microbiota and its possible role in homeostasis or pathological processes. It is often argued that detected bacterial DNA in sterile areas of the human body is just a residual fragment of dead bacteria. However, biologically active metabolites and antigens derived from the same bacteria also present may play important roles in various pathological or autoimmune processes. In addition, the relationship between outcome of liver or renal transplantation and the gut, oral or urinary microbiota has been repeatedly reported (Campbell et al., 2020; Dery et al., 2020). At this point, we have no information about relationship of vascular allografts and any microbiota, although vascular graft failures are common and have no clear explanation (Chakfe et al., 2020). On one hand, knowledge about the human vascular microbiota can help understand the pathoetiology of the vascular diseases in more detail. On the other, the information gathered through this process can increase the success of vascular transplantation.

In reviewing current reports, we have not found any example that would describe a possible method for identifying healthy human vascular wall tissue microbiota or results characterizing the composition of the human blood vessel microbiota. The aim of our study was to develop a hands-on method for analysing the human vascular wall tissue microbiota and to characterize its healthy composition.

Methods

In our prospective survey study fourteen human femoral arteries, harvested from fourteen different donors in multi-organ donations between October 2019 and February 2021 in Hungary, were examined to identify the healthy human vascular microbiota.

Donor characteristics

The median age of the donors was 49.5 (IQR:10) years. Male/female rate of donors was 11/3. The median body mass index (BMI) was 27.8 (IQR:7.9) kg/m^2. The leading cause of death was cerebral haemorrhage (7 donors; 50%). All essential characteristics of the donors are presented in Table 1.

Inclusion and exclusion criteria of allografts

Donor inclusion criteria of the study were based on the national multi-organ donation criteria and rules (Hungarian National Blood Transfusion service., 2020). Briefly, the selection criteria for donors are based on an analysis of the risks associated with the use of tissues. Signs of these risks must be identified through a medical examination, medical history, lifestyle, biological examination, post-mortem examination and any other suitable examination. Donors should be excluded from

TABLE 1 Donor characteristics.

Characteristic		n = 14
Cause of death	trauma	5 (35.71)
	cerebral ischaemia	2 (14.28)
	cerebral haemorrhage	7 (50)
Age (year)		49.5 (IQR:10)
Female sex		3 (21.42)
BMI (kg/m2)		27.8 (IQR:7.9)
Past medical history	Hypertension	3 (21.42)
	Diabetes	1 (7.14)
	Smoking	7 (50)
Blood type	A	5 (35.71)
	B	2 (14.28)
	AB	1 (7.14)
	O	6 (42.85)
	Rh+	12 (85.71)
	Rh-	2 (14.28)

Data are presented as number of donors (%) or median (IQR). n, number of donors; BMI, body mass index.

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donating if any of the following criteria apply:The cause of death is unknown., Unknown disease in medical history., Malignant disease., Transmissible spongiform encephalopathies., Any untreated infection at the time of donation., HIV, Any autoimmune disease that may affect the transplanted organ., Any intervention that may modify the results of the donor's blood tests., Any potencial transmitted disease., Poisoning., The donor was a former recipient., Vaccination with live virus. The allografts from donors above 65 years, with malignancy or with positive bacterial-fungal culture or virus serology tests and the allografts with negative evaluation of the vascular surgeon, who performed the explantation (e.g. significantly injured or calcified allograft), were excluded.

Ethical considerations, data collection

The study was in full compliance with the principles of the national multi-organ donation and the applicable international and national laws. The anonymous data of the donors were collected prospectively from the electronic health information system of the donation according to the General Data Protection Regulation of the European Union. The study was approved by the institutional review board, by Semmelweis University Regional and Institutional Committee of Science and Research Ethics (approval number: 257/2018).

Harvesting of allografts and preparing of samples

Femoral arteries were harvested in Budapest from brain-dead donors in multi-organ donations organized by the Organ Coordination Office of the Hungarian National Blood Transfusion Service. All donors had negative serology test of human immunodeficiency virus, hepatitis B virus, hepatitis C virus, syphilis, active Epstein-Barr virus and active cytomegalovirus. The explantation of the femoral arteries (common and superficial femoral artery) was performed under surgical asepsis and sterile techniques. The suitability of the grafts was evaluated by the vascular surgeon, who performed the harvesting. Immediately after explantation, the allografts were placed into a triple sterile plastic bag (Set of Transplantation Bags – sterile 80 00 61H, Raguse GmbH, Ascheberg, Germany) in 500 mL transport solution (Sodium Chloride 0.9% "Baxter" Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) containing 4 mg/mL cefazolin (Sandoz GmbH, Kundl, Austria) and 0.4 mg/mL fluconazole (Fresenius Kabi Hungary, Budapest, Hungary) at 4°C. They were transferred in an organ transport box (IGLBox Organ Transporter, Institut Georges Lopez, Lissieu, France) at 4°C to the site of storage and were stored for 12 h at 4°C.

The samples for microbiota analysis were prepared within 24 h after the explantation under sterile conditions in a clean room classified "A" with a background classified "B" used laminar air flow system (European Commission, 2022). From each femoral artery three 3 mm^3 samples were cut. The samples were placed into a sterile plastic tube (VWR Low Temperature Freezer Vials, VWR International, LLC, Radnor, PA, USA) in physiological saline solution (sodium chloride 0.9% "Baxter" Intravenous Infusion in Viaflo, Baxter Hungary, Budapest, Hungary) and transported immediately for microbiota analysis at 4°C.

DNA isolation, 16S rRNA gene library preparation and MiSeq sequencing

DNA isolation was performed by ZymoBIOMICS DNA Miniprep Kit (Zymo Research Corp., Irvine, CA, USA) according to the manufacturer's instructions, after enzymatic dissolution with ProtK (56°C, 3 h). Isolated DNA samples were placed at −80°C until polymerase chain reaction (PCR) amplification. Concentration of genomic DNA was measured using a Qubit2.0 Fluorometer with Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA). Bacterial DNA was amplified with tagged primers covering the V3-V4 region of the bacterial 16S rRNA gene. PCR and DNA purifications were performed according to Illumina's protocol. PCR product libraries were assessed using DNA 1000 Kit with Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany). Equimolar concentrations of libraries were pooled and sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) using MiSeq Reagent Kit v3 (600 cycles PE).

In order to evaluate the contribution of extraneous DNA from reagents, extraction negative controls and PCR negative controls were included in every run. To ensure reproducibility, each vascular tissue sample was independently extracted. To avoid false results (e.g.: contamination) and to increase the reliability of the study, all analysis procedures were done in triplicate from 3 different samples of each donor. Raw sequencing data were retrieved from the Illumina BaseSpace and the data were analysed by the CosmosID bioinformatics platform (CosmosID Metagenomics Cloud, app.cosmosid.com, CosmosID Inc. Rockville, MD, USA, www.cosmosid.com) described elsewhere (Yan et al., 2019). Briefly: The raw reads from paired-end fastq files were processed through read trimming (DADA2) to remove adapters as well as reads and bases of low quality. The forward and reverse overlapping pairs were joined together; and with the supplementing unjoined R1 and R2 reads were than used as input for OTU picking. OTUs are identified against the CosmosID curated 16S database using a closed-reference OTU picker and 97% sequence similarity through the QIIME framework. The final results contain taxonomic names, OTU ids, frequency, and relative abundance. Of the 3 samples from each donor, the sample with highest read quantity were selected for

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comparative studies. Figures 1–5 were generated from the phyla or genus-level filtered abundance score matrices from the CosmosID taxonomic analysis using the CosmosID-HUB bioinformatics pipeline (app.cosmosid.com/comparative). Software for multivariate data analysis of Fathom Toolbox for MATLAB was used to create the Jaccard PCoA biplot diagram of Figure 4B (Jones, 2017).

Statistical analysis

Categorical variables are described as counts of cases and percentages. Continuous variables are described as median with interquartile range. Statistical significance between cohorts of vascular samples were implemented Wilcoxon Rank Sum test for Chao1 Alpha diversity and PERMANOVA analysis for Jaccard PCoA Beta diversity using the statistical analysis support application of CosmosID bioinformatics platform.

We compared the results of current vascular samples with the DNA amounts of faecal and pharyngeal samples from our previous studies (Szabo et al., 2021), obtained using the same DNA isolation and 16S rRNA method. The levels of statistical significance for the differences between the amounts of isolated DNA or amounts of amplified and tagged PCR products – not normally distributed variables – measured in the vascular samples or other frequently tested samples was calculated by the Mann-Whitney U test.

A value of $p<0.05$ was considered significant.

Results

From each common-superficial femoral artery samples three smaller pieces were processed separately, for a total of forty-two tested samples from fourteen patients. The median of isolated DNA from vascular samples was 27.8 ng/μL (IQR: 21.4 ng/μL). From this starting amount, which also contains human DNA, after 16S rRNA PCR a median DNA amount of 1.546 ng/μL (IQR: 0.762 ng/μL), after indexing PCR a median DNA amount of 3.947 ng/μL (IQR: 1.996) was amplified. The average length of index PCR products was 667 bp (SD: 55 bp). From the simultaneously processed transport buffers of samples, as from negative controls neither DNA isolation nor 16S rRNA PCR resulted in measurable amounts of DNA.

A total of 5.8 million valid sequences were obtained, resulting in 3.9 million high-quality reads; the median number of reads within one sample was 79485 (IQR: 24511).

The most abundant phyla in the human vascular microbiota were *Proteobacteria* (31.78%), *Firmicutes* (29.18%) and *Actinobacteria* (23.05%) (Figure 1). The samples are clustered into three major groups according to the rate of the three most relevant phyla. At the genus level, the most abundant taxa in the human vascular microbiota were *Staphylococcus, Pseudomonas, Corynebacterium, Bacillus, Acinetobacter* and *Propionibacterium* (Figure 2A). However, the stacked bar graph clearly shows that the individual samples contained very different proportions of these genera (Figure 2B). The median of the Chao1 alpha diversity of genera was 217.5 (IQR: 107).

Of the bacterial taxa that have an indirect effect on the development of atherosclerosis, we found *Porphyromonas gingivalis* in two samples (0.54%, 0.16%), *Prevotella nigrescens* in one sample (0.1%) and all samples contained different amounts of *Enterobacteriaceae spp* with a median of 2.25% (IQR: 1.46). Of the bacteria that are more common in the intestinal flora of healthy people than in patients with atherosclerosis, *Roseburia* was found in 8 samples (median: 0.13%, IQR: 0.08) and *Ruminococcus* occurred in 13 samples (median: 3.07%, IQR: 1.78). *Helicobacter pylori* and *Chlamydophila pneumoniae*, bacteria that can play a

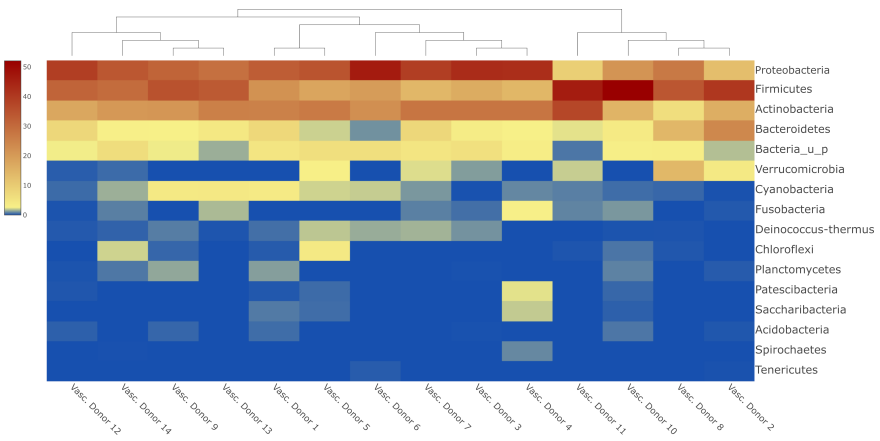

FIGURE 1
Heatmap about the most abundant phyla in human vascular samples with sample clustering dendogram.

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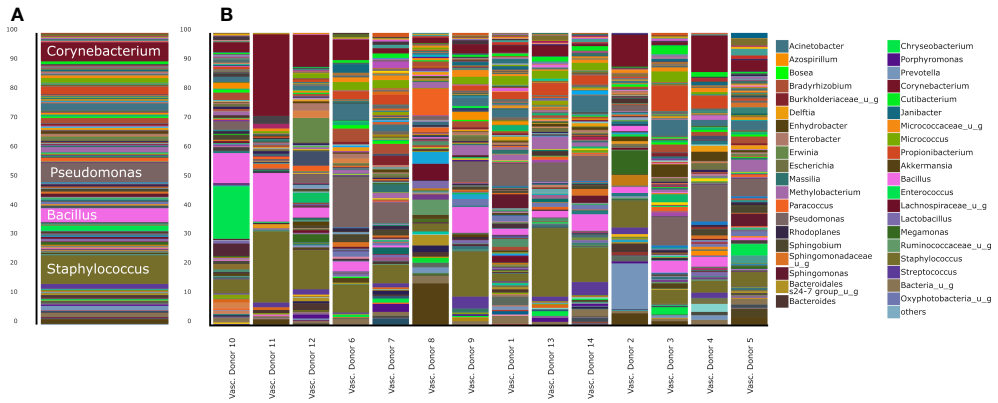

FIGURE 2
Stacked bar representation of human vascular microbiome composition at genus level. **(A)**, aggregated results and **(B)**, results of separated samples.

direct role in development of atherosclerosis, were not detected in any of the samples.

Due to the low number of cases, we grouped donors based on only a few individual traits and examined differences in microbiota composition. Three-Dimension Principal Component Analysis (3D PCoA) pictures fails to show any significant difference i) between the vascular microbiota of male and female patients (Figure 3A), ii) in smoker and non-smoker patients (Figure 3B), iii) according to age distribution (Figure 3C).

There were significant differences in the Chao1 alpha diversity of microbiota between the O and A blood groups (p=0.0106) and between the O and B blood groups (p=0.045) (Figure 4A) using the Wilcoxon Rank Sum test. Jaccard distance measure was used for assessing the beta-diversities of the microbiota and Principal Coordinate Analysis (PCoA) biplot for visualizing microbiotas (Figure 4B). There were significant differences using PERMANOVA analysis in the Jaccard beta-diversity values of the microbiotas between O and A blood groups (p= 0.003), between O and B blood groups (p=0.037) and between A and B blood groups (p=0.045).

Compared to previous results of our study group (Szabo et al., 2021), Figure 5 shows significant differences with Jaccard Beta Diversity PCoA between human vascular and human stool (p=0.001) or human nasopharyngeal (p=0.001) samples.

Discussion

In the case of visceral tissues previously considered sterile, it has been repeatedly shown that they contain microbial genetic material (Aagaard et al, 2014; Ghaemi et al., 2021; Sookoian and Pirola, 2021). Indeed, hereby we could detect these from healthy arterial wall. We managed to develop a usable protocol for the identification of healthy vascular microbiota by which we were able to characterize the composition of the human blood vessel microbiota.

It is impossible to collect an artery wall sample from a living, healthy person for research purposes, so no one has previously characterized the microbiome composition of a healthy blood vessel wall. Access to this special, unique test material was only possible by processing a small piece of a blood vessel section removed from a dead person intended for vascular allograft transplantation.

Our vascular samples contain less microbial DNA than samples containing physiological bacterial flora. Using the same isolation and sequencing methods the difference is significant - in contrast with our previous results (Szabo et al., 2021) - after 16S rRNA PCR the median bacterial DNA amount is 1.546 ng/μL from vascular samples and 17,4 ng/μL in stool samples (p= 0,002). However, there is no significant difference between the average length of index PCR bacterial products 667 bp in vascular samples and 662 bp in stool samples (Szabo et al., 2021). The microbiota of the human vessel forms a well-defined cluster that is significantly distinct from the microbiota of other human samples. The composition of the vascular microbiota is uniquely and significantly different from either nasopharyngeal or faecal microbiota samples.

Recent accounts report on the blood microbiota of healthy donors with *Proteobacteria*, *Actinobacteria, Firmicutes* and *Bacteroidetes* phyla as the largest representatives (Paisse et al., 2016; Castillo et al., 2019). Three of them, *Proteobacteria, Firmicutes*, and *Actinobacteria* phyla were also identified by us as the most abundant phyla in the human vascular microbiota. Based on these studies, we can assume that the two microbiotas are related to each other. However, their exact composition could be different. While the main phylum of the blood was *Proteobacteria*, over 80% (Paisse et al., 2016), the main phyla of the arterial wall were *Proteobacteria, Firmicutes*, and *Actinobacteria* with almost similar proportions. The result, that there can be a difference between the microbiome of the blood vessel wall and the microbiome of the circulating blood is

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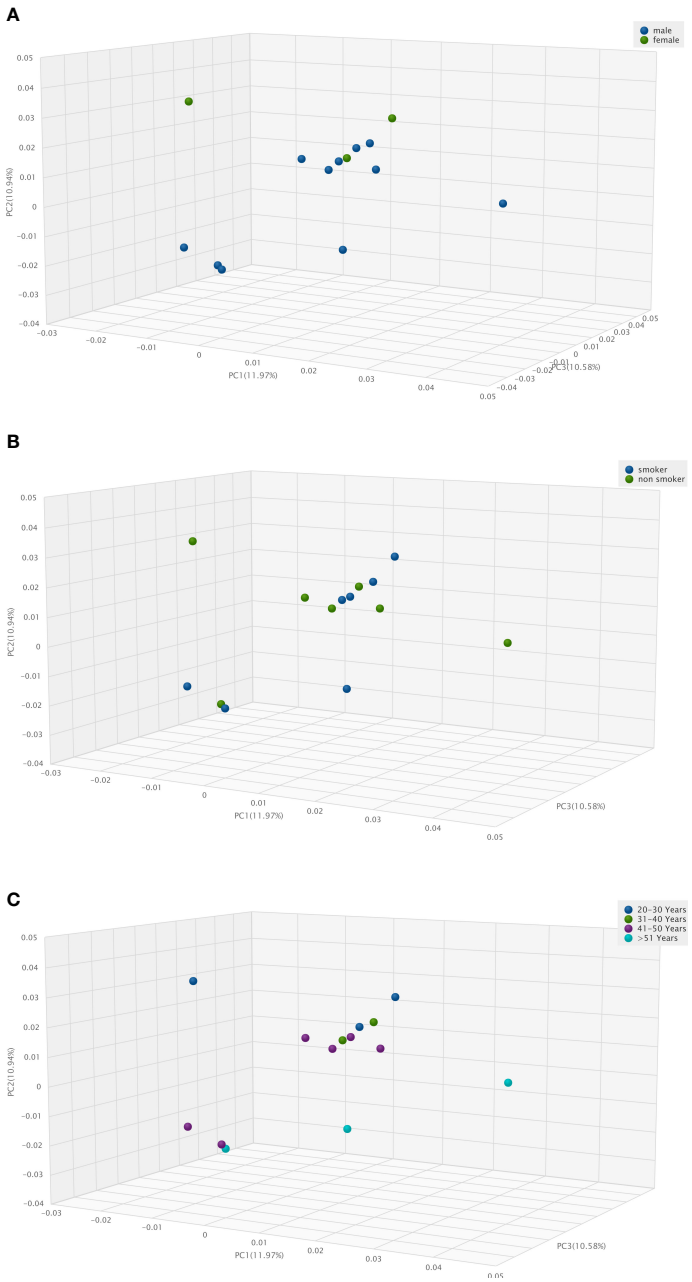

FIGURE 3
Three-Dimension Principal Component Analysis (3D PCA) of human vascular microbiome. **(A)**, in male and female patients, **(B)**, in smoker and non-smoker patients, **(C)**, according to age distribution.

not surprising. Numerous studies have proven that faecal microbiota only partially reflected the gut mucosal-associated microbiome (Flemer et al., 2017; Yang et al., 2020), and the urine microbiome also differs from the bladder tissue microbiome (Mansour et al., 2020). Based on these examples, we can assume that bacteria transiently present in the blood are also added to the blood microbiome composition, but only a part of them can be detected in the vessel wall. Furthermore, the results of blood microbiome tests can be very different even for patients who consider themselves to be healthy, the presence or absence of e.g. periodontitis or other disorders determines the quantity and quality of viable bacteria in the blood (Damgaard et al., 2015; Damgaard et al., 2021). The vessels we examined did not contain any viable bacteria, because samples with positive culture results were excluded from the study. All of these can result in differences in blood and blood vessel wall microbiome results.

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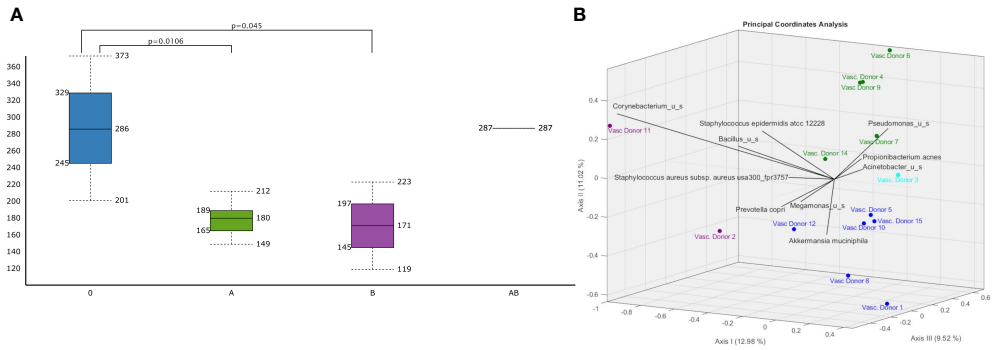

FIGURE 4
Differences in vascular microbiomic diversity according to A (green)/B(purple)/AB(light blue)/O(dark blue) blood groups. **(A)** Chao1 Alpha Diversity of human vascular microbiome significant differences are indicated with p values and **(B)** bi-plot Jaccard Beta Diversity Principal Coordinate Analysis according to A/B/AB/O blood groups..

In addition to the microbes circulating in the blood, those microbes whose DNA content can be detected in the vessel wall can have a direct effect on the function or pathological changes of the vessel wall with their metabolic products or antigens derived from them.

Numerous studies have found association between cardiovascular diseases (coronary, cerebrovascular or peripheral) and specific bacteria in different sites of body (Blanchard et al., 1993; Kozarov et al., 2005; Caula et al., 2014; Ahmed and Tanwir, 2015; Clifford and Hoffman, 2015; Jie et al., 2017; Liu et al., 2019; Verhaar et al., 2020; Jin et al., 2021). Increased amounts of *Porphyromonas gingivalis*, *Tannerella forsythia*, *Prevotella intermedia*, *Aggregatibacter actinomycetemcomitans, Treponema denticola, Prevotella nigrescens, Fusobacterium nucleatum, Eikenella corrodens* and *Parvimonas micra* in the oral cavity may play a role in endovascular pathogenic processes by enhancing systemic inflammatory parameters (Caula et al., 2014; Tapashetti et al., 2014; Ahmed and Tanwir, 2015; Damgaard et al., 2017). Only two of our healthy vascular samples contained *P. gingivalis* and only one sample contained *P. nigrescens* from the bacteria mentioned above. Gut microbiota can also impact the cardiovascular physiology with the produced metabolites that enter the blood (Verhaar et al., 2020). A causal effect of Trimethylamine-N-Oxide (TMAO) on atherosclerosis has been established (Eshghjoo et al., 2021), and the TMAO producer members of *Enterobacteriaceae* family have higher abundance in the gut microbiota of patients with symptomatic atherosclerosis compared to healthy controls (Jie et al., 2017). Nevertheless, Short Chain Fatty Acid (SCFA) - producing bacteria from the *Eubacterium, Roseburia*, and *Ruminococcaceae* families

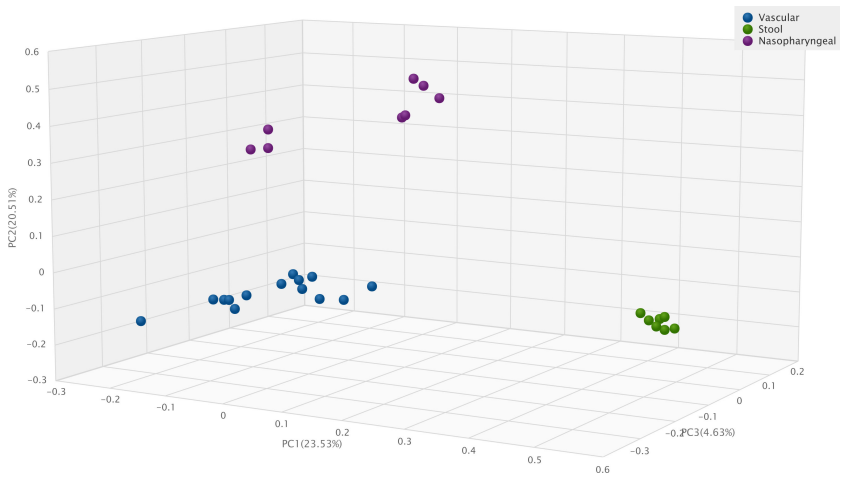

FIGURE 5
Jaccard Beta Diversity Principal Coordinate Analysis of human vascular, human stool and human nasopharyngeal samples.

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have higher abundance in the gut microbiota of healthy patients than in atherosclerosis ones' (Liu et al., 2019). Each of our samples contained different low amounts of bacterial DNA from members of the *Enterobacteriaceae* family, and the occurrence of *Roseburia* and *Ruminococcaceae* species in the samples was also common in small amounts. Assuming that bacteria may enter the circulation from the intestinal tract of the patients, the DNA detected from the vascular wall is an indirect information that the intestinal flora of the patient may contain short fatty acid-producing strains. The bacteria that have been proven to play indirect role in the pathological changes of the vessel wall were not detectable, or only in minimal amounts, in the healthy vessel wall microbiome.

The most important bacteria whose DNA have been found in the atherosclerotic plaque and whose direct role in the development of atheromas has been demonstrated are *P. gingivalis, A. actinomycetemcomitans, C. pneumoniae* and *H. pylori (*Kozarov et al., 2005; Rosenfeld and Campbell, 2011; Clifford and Hoffman, 2015*)*. Of these bacteria, only *P. gingivalis* occurred in 2 cases in low amounts in our healthy human vessel wall microbiota samples.

In our study, the donors were divided into well-separated vascular microbiota clusters according to ABO blood group system. Mäkivuokko and Gampa reported similar results for gut microbiota (Makivuokko et al., 2012; Gampa et al., 2017). However, Davenport et al. found no association between ABO antigen status and gut microbiota composition in a large cohort of 1500 twins (Davenport et al., 2016). In a genome-wide association study in 8956 German individuals an indirect influence of ABO histo-blood groups on gut microbiome was also identified (Ruhlemann et al., 2021). In a large mosaic pig population, the deletion in the gene encoding N-acetyl-galactosaminyl-transferase was associated with lower abundance of *Erysipelotrichaceae*- bacteria that can import and catabolize N-acetyl-galactosamine -in gut microbiome. Since alpha 1-3-N-acetylgalactosaminyltransferase and alpha 1-3-galactosyltransferase are the genes determining the ABO blood group in humans, a molecular explanation proves the connection between the gut microbiome composition and the blood groups (Yang et al., 2022). There is already molecular-genetic proof of the relationship between the gut microbiome and ABO blood groups, but the recently detected blood vessel wall microbiome - blood group correlation can only be confirmed after examining a larger number of samples.

The composition of the intestinal and additional microbiotas influences the success of organ transplantations. Certain bacteria could precipitate allograft rejection by producing metabolites that activate host cell-mediated and humoral immunity (Mitchell, 2019; Baghai Arassi et al., 2020; Campbell et al., 2020; Dery et al., 2020). Vascular allograft transplantation has a high number of graft-related complications such as extended calcification or aneurysmal degeneration, the exact cause of which is unknown despite extensive research of the last decades (Chakfe et al., 2020). However, as the role of the microbiota has arisen in case of a series of organ-graft rejection, it could be also one to induce vascular transplantation-failures. Further studies are needed to determine whether antigens or metabolites of bacteria in the blood vessel wall affect the outcomes of vascular allograft transplantation.

The limitations of this study could be the low number of the donors, however the number of multi-organ donations are limited and there is no other ethical way for harvesting healthy vascular samples. The concept of "healthy" is a difficult one to define. However, multi-organ donations have very strict criteria, which can ensure the structural and functional health of the harvested organs despite possible comorbidities. Nonetheless, future studies investigating subgroups of donors with different comorbidities could be useful.

Summarizing the results of our research the human arterial wall has a unique microbiota that is significantly different in composition from that of other areas of the human body. The most important genera were *Staphylococcus, Pseudomonas, Corynebacterium, Bacillus, Acinetobacter* and *Propionibacterium*, which were found in variable but dominant quantities in all vessel wall samples. In addition, the donors were divided into well-separated vascular microbiota clusters based on ABO blood group system. Follow-up studies with a larger number of samples are needed to reveal the relationship between the microbiome composition of the vessel wall and the success of transplantation. Our present study is the first methodological description that can be used to examine the microbiome of the vessel wall. The pathological role of a microbe in the damaged vessel wall can only be proven if we know the microbiome associated with the healthy vessel wall. Our present study provides a basis for following research that investigates the direct role of the microbiota in vascular wall disorders and the success of allograft transplantations.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://www.ncbi.nlm.nih.gov/, PRJNA748211, PRJNA224116.

Ethics statement

The studies involving human participants were reviewed and approved by Semmelweis University Regional and Institutional Committee of Science and Research Ethics (approval number: 257/2018). Written informed consent for participation was not

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required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

Conceptualization: PS, DS, EO, LH, Data curation: GK, LH, EO, Funding acquisition: PS, LH, DS, Investigation: NM, KP, EO, LH, Methodology: PS, LH, EO, NM, KP, Project administration: LH, NM, EO, Resources: EO, DS, LH, JJ, Software: EO, DS, JJ, Supervision: PS, EO, DS, Validation: PS, LH, EO, DS, Visualization: EO, DS, JJ, Writing - original draft: EO, LH, Writing – review and editing: PS, DS, EO, JJ. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

Aagaard, K., Ma, J., Antony, K. M., Ganu, R., Petrosino, J., and Versalovic, J. (2014). The placenta harbors a unique microbiome. *Sci. Transl. Med.* 6, 237ra65. doi: 10.1126/scitranslmed.3008599

Ahmed, U., and Tanwir, F. (2015). Association of periodontal pathogenesis and cardiovascular diseases: a literature review. *Oral. Health Prev. Dent.* 13, 21–27. doi: 10.3290/j.ohpd.a32823

Baghai Arassi, M., Zeller, G., Karcher, N., Zimmermann, M., and Toenshoff, B. (2020). The gut microbiome in solid organ transplantation. *Pediatr. Transplant.* 24, e13866. doi: 10.1111/petr.13866

Blanchard, T., Bailey, R., Holland, M., and Mabey, D. (1993). Chlamydia pneumoniae and atherosclerosis. *Lancet* 341, 825. doi: 10.1016/0140-6736(93)90597-A

Campbell, P. M., Humphreys, G. J., Summers, A. M., Konkel, J. E., Knight, C. G., Augustine, T., et al. (2020). Does the microbiome affect the outcome of renal transplantation? *Front. Cell Infect. Microbiol.* 10. doi: 10.3389/fcimb.2020.558644

Castillo, D. J., Rifkin, R. F., Cowan, D. A., and Potgieter, M. (2019). The healthy human blood microbiome: Fact or fiction? *Front. Cell Infect. Microbiol.* 9. doi: 10.3389/fcimb.2019.00148

Caula, A. L., Lira-Junior, R., Tinoco, E. M., and Fischer, R. G. (2014). The effect of periodontal therapy on cardiovascular risk markers: a 6-month randomized clinical trial. *J. Clin. Periodontol* 41, 875–882. doi: 10.1111/jcpe.12290

Chakfe, N., Diener, H., Lejay, A., Assadian, O., Berard, X., Caillon, J., et al. (2020). Editor's choice - European society for vascular surgery (ESVS) 2020 clinical practice guidelines on the management of vascular graft and endograft infections. *Eur. J. Vasc. Endovascular Surg.* 60, 958–958. doi: 10.1016/j.ejvs.2020.07.080

Clifford, A., and Hoffman, G. S. (2015). Evidence for a vascular microbiome and its role in vessel health and disease. *Curr. Opin. Rheumatol.* 27, 397–405. doi: 10.1097/BOR.0000000000000184

Damgaard, C., Magnussen, K., Enevold, C., Nilsson, M., Tolker-Nielsen, T., Holmstrup, P., et al. (2015). Viable bacteria associated with red blood cells and plasma in freshly drawn blood donations. *PloS One* 10, e0120826. doi: 10.1371/journal.pone.0120826

Damgaard, C., Reinholdt, J., Enevold, C., Fiehn, N. E., Nielsen, C. H., and Holmstrup, P. (2017). Immunoglobulin G antibodies against porphyromonas gingivalis or aggregatibacter actinomycetemcomitans in cardiovascular disease and periodontitis. *J. Oral. Microbiol.* 9, 1374154. doi: 10.1080/20002297.2017.1374154

Damgaard, C., Saekmose, S. G., Nilsson, M., Kilian, M., Nielsen, C. H., and Holmstrup, P. (2021). Periodontitis increases risk of viable bacteria in freshly drawn blood donations. *Blood Transfus* 19, 376–383. doi: 10.2450/2021.0336-20

Davenport, E. R., Goodrich, J. K., Bell, J. T., Spector, T. D., Ley, R. E., and Clark, A. G. (2016). ABO antigen and secretor statuses are not associated with gut microbiota composition in 1,500 twins. *BMC Genomics* 17, 941. doi: 10.1186/s12864-016-3290-1

Dery, K. J., Kadono, K., Hirao, H., Gorski, A., and Kupiec-Weglinski, J. W. (2020). Microbiota in organ transplantation: An immunological and therapeutic conundrum? *Cell Immunol.* 351, 104080. doi: 10.1016/j.cellimm.2020.104080

Eshghjoo, S., Jayaraman, A., Sun, Y., and Alaniz, R. C. (2021). Microbiota-mediated immune regulation in atherosclerosis. *Molecules* 26 (1), 179. doi: 10.3390/molecules26010179

Flemer, B., Lynch, D. B., Brown, J. M., Jeffery, I. B., Ryan, F. J., Claesson, M. J., et al. (2017). Tumour-associated and non-tumour-associated microbiota in colorectal cancer. *Gut* 66, 633–643. doi: 10.1136/gutjnl-2015-309595

Gampa, A., Engen, P. A., Shobar, R., and Mutlu, E. A. (2017). Relationships between gastrointestinal microbiota and blood group antigens. *Physiol. Genomics* 49, 473–483. doi: 10.1152/physiolgenomics.00043.2017

Ghaemi, F., Fateh, A., Sepahy, A. A., Zangeneh, M., Ghanei, M., and Siadat, S. D. (2021). Blood microbiota composition in Iranian pre-diabetic and type 2 diabetic patients. *Hum. Antibodies* 29, 243–248. doi: 10.3233/HAB-210450

Jie, Z., Xia, H., Zhong, S. L., Feng, Q., Li, S., Liang, S., et al. (2017). The gut microbiome in atherosclerotic cardiovascular disease. *Nat. Commun.* 8, 845. doi: 10.1038/s41467-017-00900-1

Jin, L., Shi, X. M., Yang, J., Zhao, Y. Y., Xue, L. X., Xu, L., et al. (2021). Gut microbes in cardiovascular diseases and their potential therapeutic applications. *Protein Cell* 12, 346–359. doi: 10.1007/s13238-020-00785-9

Jones, D. L. (2017). Fathom Toolbox for MATLAB: software for multivariate ecological and oceanographic data analysis. (College of Marine Science, University of South Florida, St. Petersburg, FL, USA).

Kozarov, E. V., Dorn, B. R., Shelburne, C. E., Dunn, W. A.Jr., and Progulske-Fox, A. (2005). Human atherosclerotic plaque contains viable invasive actinobacillus actinomycetemcomitans and porphyromonas gingivalis. *Arterioscler. Thromb. Vasc. Biol.* 25, e17–e18. doi: 10.1161/01.ATV.0000155018.67835.1a

Liu, H., Chen, X., Hu, X., Niu, H., Tian, R., Wang, H., et al. (2019). Alterations in the gut microbiome and metabolism with coronary artery disease severity. *Microbiome* 7, 68. doi: 10.1186/s40168-019-0683-9

Makivuokko, H., Lahtinen, S. J., Wacklin, P., Tuovinen, E., Tenkanen, H., Nikkila, J., et al. (2012). Association between the ABO blood group and the human intestinal microbiota composition. *BMC Microbiol.* 12, 94. doi: 10.1186/1471-2180-12-94

DOI:10.14753/SE.2024.3064

Mansour, B., Monyok, A., Makra, N., Gajdacs, M., Vadnay, I., Ligeti, B., et al. (2020). Bladder cancer-related microbiota: examining differences in urine and tissue samples. *Sci. Rep.* 10, 11042. doi: 10.1038/s41598-020-67443-2

Mitchell, A. B. (2019). The lung microbiome and transplantation. *Curr. Opin. Organ Transplant.* 24, 305–310. doi: 10.1097/MOT.0000000000000631

Molina, N. M., Sola-Leyva, A., Haahr, T., Aghajanova, L., Laudanski, P., Castilla, J. A., et al. (2021). Analysing endometrial microbiome: Methodological considerations and recommendations for good practice. *Hum. Reprod.* 36, 859–879. doi: 10.1093/humrep/deab009

Moreno, I., Codoner, F. M., Vilella, F., Valbuena, D., Martinez-Blanch, J. F., Jimenez-Almazan, J., et al. (2016). Evidence that the endometrial microbiota has an effect on implantation success or failure. *Am. J. Obstet Gynecol* 215, 684–703. doi: 10.1016/j.ajog.2016.09.075

Paisse, S., Valle, C., Servant, F., Courtney, M., Burcelin, R., Amar, J., et al. (2016). Comprehensive description of blood microbiome from healthy donors assessed by 16S targeted metagenomic sequencing. *Transfusion* 56, 1138–1147. doi: 10.1111/trf.13477

Potgieter, M., Bester, J., Kell, D. B., and Pretorius, E. (2015). The dormant blood microbiome in chronic, inflammatory diseases. *FEMS Microbiol. Rev.* 39, 567–591. doi: 10.1093/femsre/fuv013

Rosenfeld, M. E., and Campbell, L. A. (2011). Pathogens and atherosclerosis: update on the potential contribution of multiple infectious organisms to the pathogenesis of atherosclerosis. *Thromb. Haemost.* 106, 858–867. doi: 10.1160/TH11-06-0392

Ruhlemann, M. C., Hermes, B. M., Bang, C., Doms, S., Moitinho-Silva, L., Thingholm, L. B., et al. (2021). Genome-wide association study in 8,956 German individuals identifies influence of ABO histo-blood groups on gut microbiome. *Nat. Genet.* 53, 147–155. doi: 10.1038/s41588-020-00747-1

Sookoian, S., and Pirola, C. J. (2021). Liver tissue microbiota in nonalcoholic liver disease: A change in the paradigm of host-bacterial interactions. *Hepatobi Surg. Nutr.* 10, 337–349. doi: 10.21037/hbsn-20-270

Szabo, H., Hernyes, A., Piroska, M., Ligeti, B., Fussy, P., Zoldi, L., et al. (2021). Association between gut microbial diversity and carotid intima-media thickness. *Medicina (Kaunas)* 57 (3), 195. doi: 10.3390/medicina57030195

Tapashetti, R. P., Guvva, S., Patil, S. R., Sharma, S., and Pushpalatha, H. M. (2014). C-reactive protein as predict of increased carotid intima media thickness in patients with chronic periodontitis. *J. Int. Oral. Health* 6, 47–52.

Verhaar, B. J. H., Prodan, A., Nieuwdorp, M., and Muller, M. (2020). Gut microbiota in hypertension and atherosclerosis: A review. *Nutrients* 12 (10), 2982. doi: 10.3390/nu12102982

Yang, M., Hong, G., Jin, Y., Li, Y., Li, G., and Hou, X. (2020). Mucosal-associated microbiota other than luminal microbiota has a close relationship with diarrhea-predominant irritable bowel syndrome. *Front. Cell Infect. Microbiol.* 10, 515614. doi: 10.3389/fcimb.2020.515614

Yang, H., Wu, J., Huang, X., Zhou, Y., Zhang, Y., Liu, M., et al. (2022). ABO genotype alters the gut microbiota by regulating GalNAc levels in pigs. *Nature* 606, 358–367. doi: 10.1038/s41586-022-04769-z

Yan, Q., Wi, Y. M., Thoendel, M. J., Raval, Y. S., Greenwood-Quaintance, K. E., Abdel, M. P., et al. (2019). Evaluation of the CosmosID bioinformatics platform for prosthetic joint-associated sonicate fluid shotgun metagenomic data analysis. *J. Clin. Microbiol.* 57 (2), e01182–18. doi: 10.1128/JCM.01182-18

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RESEARCH ARTICLE

Comparison of arterial and venous allograft bypass in chronic limb-threatening ischemia

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Abstract

Introduction

Femoro-popliteal bypass with autologous vascular graft is a key revascularization method in chronic limb-threatening ischemia (CLTI). However, the lack of suitable autologous conduit may occur in 15–45% of the patients, necessitating the implantation of prosthetic or allogen grafts. Only little data is available on the outcome of allograft use in CLTI.

Aims

Our objective were to evaluate the long term results of infrainguinal allograft bypass surgery in patients with chronic limb-threatening ischemia (CLTI) and compare the results of arterial and venous allografts.

Methods

Single center, retrospective study analysing the outcomes of infrainguinal allograft bypass surgery in patients with CLTI between January 2007 and December 2017.

Results

During a 11-year period, 134 infrainguinal allograft bypasses were performed for CLTI [91 males (67.9%)]. Great saphenous vein (GSV) was implanted in 100 cases, superficial femoral artery (SFA) was implanted in 34 cases. Early postoperative complications appeared in 16.4% of cases and perioperative mortality (<30 days) was 1.4%. Primary patency at one, three and five years was 59%, 44% and 41%, respectively, while secondary patency was 60%, 45% and 41%, respectively. Primary patency of the SFA allografts was significantly higher than GSV allografts (1 year: SFA: 84% vs. GSV: 51% p = 0,001; 3 years: SFA: 76% vs. GSV: 32% p = 0,001; 5 years: SFA: 71% vs. GSV: 30% p = 0.001). Both primary and secondary patency of SFA allograft implanted in below-knee position were significantly higher than GSV bypasses (p = 0.0006; p = 0.0005, respectively). Limb salvage at one, three and five years following surgery was 74%, 64% and 62%, respectively. Long-term survival was 53% at 5 years.

Conclusion

Allograft implantation is a suitable method for limb salvage in CLTI. The patency of arterial allograft is better than venous allograft patency, especially in below-knee position during infrainguinal allograft bypass surgery.

Introduction

Infrainguinal autologous conduit bypass surgery is a key revascularization method in chronic limb threatening ischemia (CLTI). The most suitable autologous vascular graft is the ipsilateral single-segment great saphenous vein (GSV), but contralateral GSV, upper limb vein, or small saphenous vein are also suitable for use. Some 15–45% of patients [1–3] require an alternative vascular graft for surgery, due to the lack (earlier coronary or lower limb bypass surgery, varicectomy) or inadequacy (varicosity, insufficient graft diameter or length, structural alterations, earlier thrombophlebitis) of the autologous conduit. An alternative vascular graft may be a prosthetic graft or an allograft.

Following the implantation of prosthetic grafts, lower patency rates were reported, compared to autologous venous bypass, particularly in below-knee bypasses. Alternative autologous veins–such as arm vein conduit–emerges as a potential choice for infrainguinal bypass, however its primary patency is low, 23% at 5 years [4]. The five-year primary patency rate was 38% following below-knee heparin-bonded polytetrafluoroethylene (PTFE) bypasses. [5]. Considering the poor patency rates of synthetic grafts, the high risk of synthetic graft infection due to ulcer or gangrene commonly occurring in CLTI, the use of allografts in limb salvage surgery may be promising.

An allograft is a biological vascular graft, which is explanted during multiple organ donations. In most cases, GSV and/or superficial femoral artery (SFA) are explanted during the donation. Since allografts are classified as biological grafts, using them is more advantageous than prosthetic grafts in situations such as infection, septic condition, ischemic ulcer, and gangrene, since it has appropriate resistance to infection. Moreover, allograft has other advantages, such as very similar compliance and flow dynamics as autologous conduit.

The results following allograft implantation in cases of CLTI are still unclear.

Our aim was to conduct a retrospective analysis of the outcomes of infrainguinal allograft bypass surgery in patients with CLTI over a period of 11 years, and to compare SFA and GSV allografts from the perspectives of graft patency, limb salvage, as well as survival.

Materials and methods

We conducted a retrospective single-centre observational study of patients with CLTI exposed to infrainguinal allograft bypass surgery between January 2007 and December 2017 with the help of a computerized patient record system and patient follow-up.

Allograft implantation was performed if there was a lack or inadequacy of the ipsilateral or contralateral GSV autologous conduit, taking into account the recommendations for graft selection based on international guidelines [6].

Our study inclusion criteria:

- Infrainguinal femoro-popliteal bypass
- Graft material: allograft
- Indication for surgery: CLTI (Fontaine stage III-IV; Rutherford stage 4-5-6)

- TASC C or D femoro-popliteal lesions

Our study exclusion criteria:

- Indication for surgery: septic condition, graft infection
- Graft material: composit (autologous vein + allograft)

Patients undergoing allograft implantation due to graft infection were excluded.

During femoro-popliteal allograft bypass surgery, the proximal anastomoses were placed on the common femoral artery, and the distal anastomoses on the above- or below-knee position.

In every case, the implanted allograft was single-segment SFA or GSV. All of the GSV allografts implanted in reverse position. SFA or GSV was choosen by the surgeon intraoperatively depending on the available grafts that matched the required diameter and length. Patients with composite graft (prosthesis+allograft) implantations were excluded.

The implanted allografts were removed from brain-dead donors—with complete donor anonimity—who were found suitable for multi-organ donation conducted by the Hungarian National Blood Transfusion Service, Organ Coordination Office in accordance with the international rules and asepsis guidelines. None of the transplant donors were from a vulnerable population. The consents were not obtained, because by Hungarian law, any person who dies of brain death and fits in the criterias of multi-organ transplantation is considered as a donor and does not require his or her consent in his or her lifetime.

Following explantation by an experienced vascular surgeon, the grafts were placed into a special transport solution [500 ml transport solution: Sodium Chloride 0.9% "Baxter" Intravenous Infusion in Viaflo, (Baxter Hungary, Budapest, Hungary); 4 mg/ml cefazolin (Sandoz GmbH, Kundl, Austria), 0.4 mg/ml fluconazole (Fresenius Kabi Hungary, Budapest, Hungary)] into a triple sterile plastic bag (Set of Transplantation Bags–sterile 80 00 61H, Raguse GmbH, Ascheberg, Germany), kept at 4˚C, and delivered to the Allograft Tissue Bank of the Semmelweis University, Department of Vascular and Endovascular Surgery, Budapest, Hungary, where they were prepared carefully and frozen within 24 hours [7].

During graft conservation, the cryopreservation was performed in a clean room classified "A" with a background classified "B" used laminar air flow system and the grafts were frozen in a cryopreservation solution (500 ml cryopreservation solution (Ringer Fresenius, Fresenius Kabi Deutschland GmbH, Bad Homburg, Germany) containing 20 v/v% dimethyl sulfoxide (Molar Chemicals Kft., Halásztelek, Hungary), 4 mg/ml cefazolin (Sandoz GmbH, Kundl, Austria) and 0.4 mg/ml fluconazole (Fresenius Kabi Hungary, Budapest, Hungary), and stored at -80˚C. At the beginning of the surgery, the graft to be implanted was thawed in a water bath at 20–25˚C and then implanted after preparation. We did not consider blood type and we did not administer any immunosuppressive drugs.

Regarding limb salvage, only major amputations at the below-knee or above-knee levels were taken into consideration. Graft patency were investigated only in cases where the follow-up time was complete. Primary and secondary patency were defined according to the Society for Vascular Surgery (SVS) definitions [8].

Long term patient follow-up was performed by the operating surgeon at 1, 6, and 12 months and then annually after the surgery. If there was suspicion for stenosis or occlusion of the allograft during the clinical examination, duplex US was performed.

Graft infection was definied according to Szilagyi classification [9].

Statistical analysis of the data was performed using the GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA). Student t-test, Fisher's method, multivariable Cox-regression and Kaplan-Meier analysis were used. We checked the distribution of the variables

by using the Shapiro-Wilk normality test and presented continuous data as mean with standard deviation or median with interquartile ranges, as appropriate. Categorical data were described as event numbers with percentages. A 95% confidence interval was considered to be statistically significant ($p < 0.05$).

During the course of our study, data were handled in accordance with the valid legal regulations and the work was carried out with the permission of the Regional, Institutional Scientific and Research Ethics Committee of Semmelweis University (#SE TUKEB 132/2015). All data handling and data processing met the requirements of Act LXIII of 1992 on the Protection of Personal Data and Disclosure of Data of Public Interest and Act XLVII of 1997 on the Processing and Protection of Medical and Other Related Personal Data. Before surgery, all patients received adequate information about both the surgery and the use of their personal data in medical research. Written and oral medical information was given to all patients. Written informed consent was obtained from all patients participating in the study, in accordance with the approved Institutional Review Board (IRB) protocols.

Results

Patient characteristics, operative details

Between January 2007 and December 2017 a total of 134 cases of infrainguinal allograft implantations due to CLTI were performed. The mean age of the patients was 66.4 ± 9.9 years, with the majority of them being males (91 patients, 67.9%).

GSVs were implanted in 100 cases (74.6%), SFAs in 34 cases (25.4%).

Grafts were implanted at above-knee position in 35 cases (26.1%) and at below-knee position (popliteal, tibioperoneal trunk) in 99 cases (73.9%).

The main cardiovascular risk factors detailed in Table 1.

Postoperative outcomes

The mean length of hospital stay was 13.6 ± 5.6 days.

Early postoperative complications (<30 days) occurred in 16.4% of the cases, among which significant cardiovascular complications occurred in 2 cases (1.4%): stroke in one patient and acute myocardial infarction in another patient. Hematoma and wound infection requiring reoperation occured in 4 (2.9%) and 6 (4.4%) cases, respectively. (Table 2).

Reoperations (open surgery) were performed in 26.1% of cases. (Table 3) Graft infections developed in 2 cases (1.4%). Both graft infections were Grade III infections according tot he

Table 1. Demographic data and comorbidities.

Features	*Mean±SD*	
Age (years)	66.4±9.9	
	Cases (N)	**%**
Male	91	67.9
Comorbidities		
Smoking	107	79.8
Hypertension	121	90.2
Hyperlipidemia	106	79.1
Coronary diseases	49	36.5
Diabetes	56	41.7
Stroke	16	11.9
COPD	14	10.4
Renal failure	2	1.4

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Table 2. Early postoperative complications.

Complication	*Cases (N)*	*%*
Occlusion	10	7.4
Hematoma	4	2.9
Wound infection	6	4.4
Stroke	1	0,7
AMI	1	0.7

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Szilagyi classification. In one case, graft ligation was performed due to septic bleeding followed by combined antibiotic therapy. In the other case, wound exploration, lavage and necrectomy were performed, followed by combined antibiotic therapy.

Neither early postoperative complications nor reoperations were significantly affected by homograft type and the location of the distal anastomosis (early postoperative complications vs. location of the distal anastomosis: p = 0.21; vs. allograft type: p = 0.14; early reoperations vs. location of the distal anastomosis: p = 0.55; vs. allograft type: p = 0.68; late reoperations vs.location of the distal anastomosis: p = 0.34; vs. allograft type: p = 0.97.

Structural changes (aneurysm or dilatation) occurring in the graft, which were distant from the anastomosis, were described in 2 cases (1.4%). In one case, the entire segment of the allograft was dilated, resulting in prosthetic graft replacement. In the other case, an aneurysm (d = 22mm) formed on the distal segment of the allograft, which was occluded and no surgery was performed.

The perioperative mortality rate was 1.4% (2 cases). Long-term survival at one, three and five years after surgery was 85%, 68% and 53%, respectively (Fig 1).

Graft patency, limb salvage

Primary patency of the entire group was 59% after one year, 44% after three years, and 41% after 5 years. Secondary patency of the entire group after 1, 3 and 5 years was 60%, 45% and 41%, respectively (Fig 2). SFA allograft patency rate was significantly higher compared to the GSV allograft patency rate (1 year: SFA: 84% vs. GSV: 51% p = 0.001; 3 years: SFA: 76% vs. GSV: 32% p = 0.001; 5 years: SFA: 71% vs. GSV: 30% p = 0.001) (Fig 3). Both primary and secondary patency rate of SFA allografts were significantly higher than GSV allografts in the below-knee position (primary patency at 1 year: SFA: 89% vs. GSV: 46% p = 0.0006; 3 years: SFA: 84% vs. GSV: 32% p = 0.0006; 5 years: SFA: 78% vs. GSV: 32% p = 0.0006). The secondary patency results at 1 year: SFA: 89% vs. GSV: 46% p = 0.0005; 3 years: SFA: 84% vs. GSV: 32% p = 0.0005; 5 years: SFA: 78% vs. GSV: 32% p = 0.0005) (Fig 4). Neither primary, nor secondary patency rate of SFA allografts were significanty different from GSV allografts implanted at the above-knee position (p = 0.78; p = 0.79).

Limb salvage rates at one, three and five years after surgery were 74%, 64% and 62%, respectively (Fig 5). Neither the allograft type (3 years: SFA: 35% vs. GSV: 38%; p = 0.679) nor the

Table 3. Reoperations.

Early (<30 days) (n = 14)	***Late (>30 days) (n = 21)***
Graft occlusion (n = 10)	Graft occlusion (n = 18)
Graft lesion–bleeding, hematoma (n = 3)	Graft dilatation (n = 1)
Wound infection (n = 1)	Proximal anastomosis pseudoaneurysm (n = 1)
	Graft infection (n = 1)

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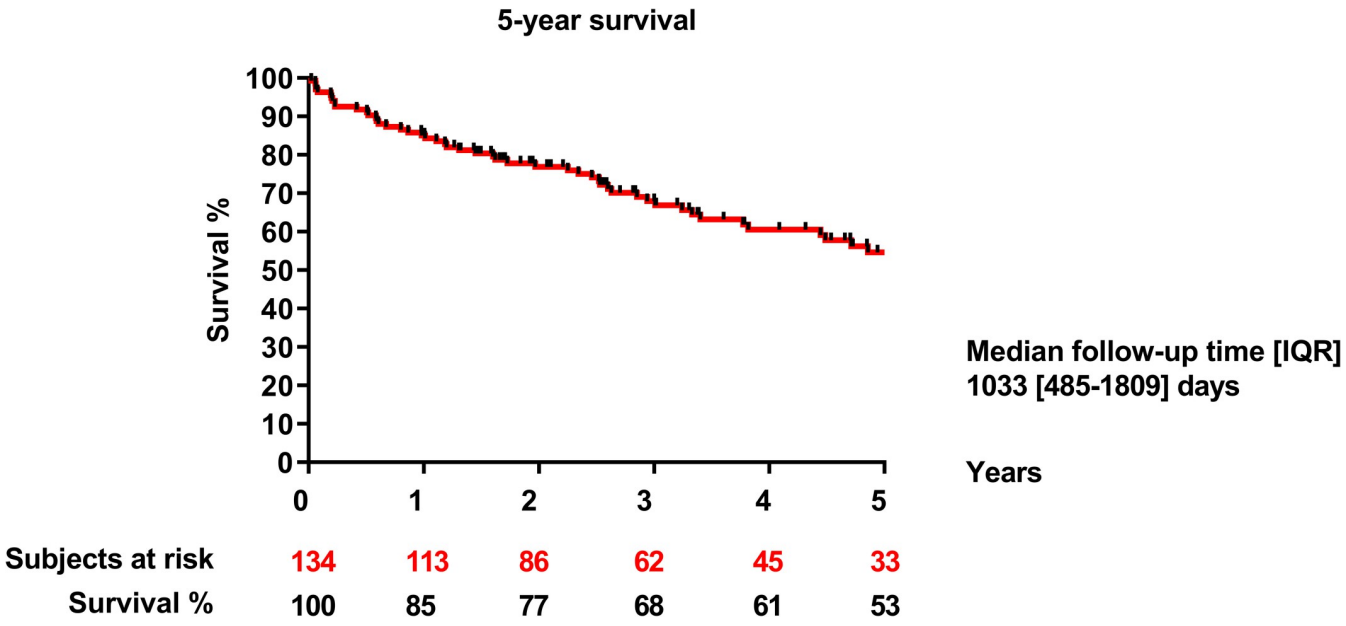

Fig 1. 5-year survival.

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location of the distal anastomosis (3 years: below-knee: 38% vs. above-knee: 40%; p = 0.907) affected limb salvage significantly.

Discussion

Even nowadays there are a significant number of patients with CLTI who need to undergo vascular surgery. The estimated annual incidence is 220–3500 cases per 1 million people with a prevalence of 1% in the adult population [10,11]. Twenty percent of patients require amputation and 25% die within a year after the onset of critical limb ischemia [12]. The best method of revascularization, meaning either open or endovascular intervention, is key to limb salvage.

In case of open surgery, the first method of choice is autologous GSV femoro-popliteal bypass, which has the most favorable patency compared to alternative graft types [13,14]. In the absence of an autologous conduit or in case of unsuitability, an alternative vascular graft has to be chosen, which, in most cases, would be a prosthetic graft or an allograft.

Prosthetic bypass grafts are not ideal for patients with Fontaine IV gangrene or ulcer due to the potential risk of infection and in addition such grafts have poor patency rates [15]. Uhl et al. [16] investigated the patency of below-knee femoro-popliteal bypass surgery with autologous vein compared to heparin-bound PTFE grafts, and found that the use of an autologous vein continues to be the first choice for revascularization surgery below the inguinal ligament.

In the absence of autologous conduit allograft is an alternative option for a vascular graft. Most ot the available literature on femoro-popliteal allograft bypass surgeries involve the use of allografts for graft infection. There are only a few data available on the results of allograft implantations in CLTI.

In this retrospective analysis, we studied the data of patients who underwent femoro-popliteal allograft bypass surgery due to CLTI, and we compared the results of the two most commonly used allograft types, SFA and GSV, in terms of graft patency, limb salvage, and survival. There are relatively few centers in Europe using allografts and most of their earlier reports present a small number of cases.

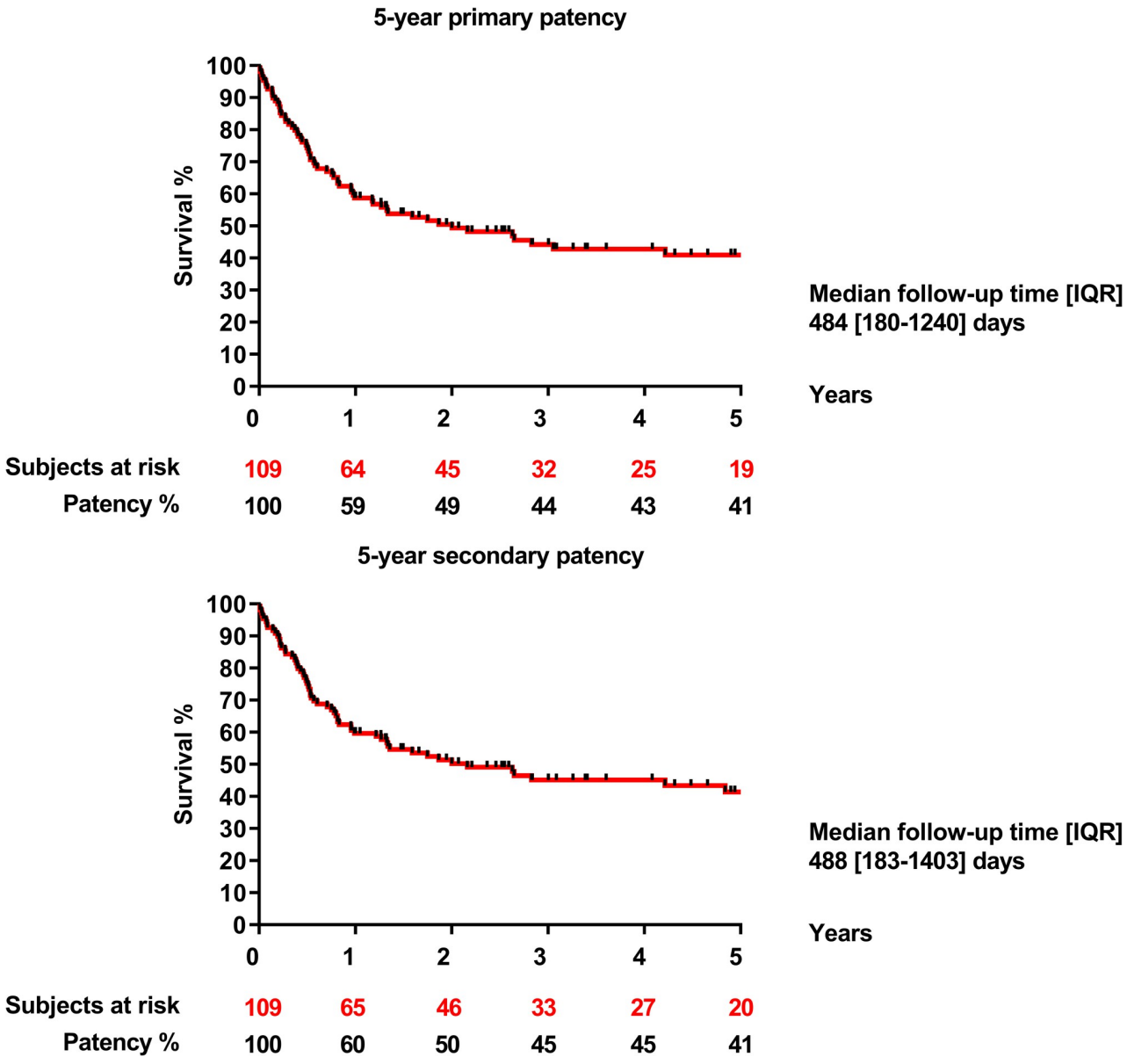

Fig 2. 5-year primary and secondary patency.

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The majority of the implanted allografts were GSVs (74.6%), SFAs were implanted in 25.4% of the cases. There are hardly any reports that compare the results of venous and arterial allografts. The vast majority of publications report the results of venous allografts [17–21], with fewer studies presenting the results of arterial allograft implantations [22,23].

The possibility of graft degeneration is important when considering the use of an allograft, which may question its use as opposed to prosthetic graft. In our study, graft degeneration was detected in only 2 out of the 134 cases. Masmejan et al. [22] reported graft degeneration in one of 42 cases, and Albertini et al. [24] found 4 cases among 165 bypasses. Our results support the literature data, according to which there are a negligible number of degenerations in case of allografts. Careful explantation of grafts from the donor, cautious tissue conservation and

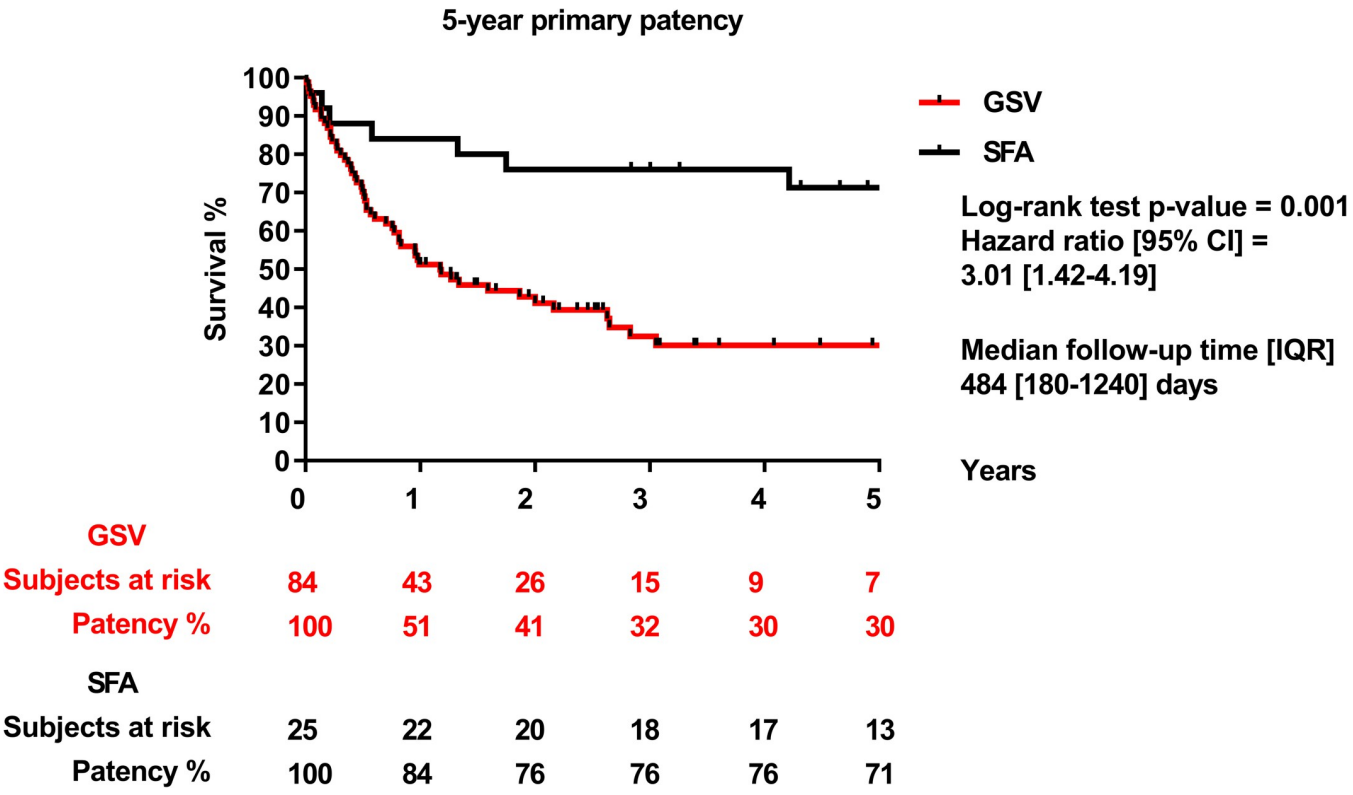

Fig 3. 5-year primary patency of GSV (great saphenous vein) and SFA (superficial femoral artery).

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preoperative preparation, as well as strict adherence to basic vascular surgical principles are important issues in relation to the prevention of graft degeneration.

One year after the implantation, the allograft showed a primary patency rate of 59%, and 41% at five years. Secondary patency rates did not differ significantly from the primary patency rates. The reason for the high number of occlusions was the high occlusion rate of venous grafts, we therefore examined the patency of GSV and SFA allografts. One, three, and five years after surgery, the patency of SFA allografts was significantly higher as compared to the GSV allografts. Masmejan et al. [22] studied 42 cases of arterial allografts during a 10-year period, finding graft patency rates of 60% at 1 year and 26% at 5 years. Lejay et al. [25] reported a primary patency rate of 59% at 5 years when studying 28 arterial allograft implantations. Investigating the outcomes of infrainguinal allograft bypass surgeries after one year, O'Banion et al. [18] reported a primary patency rate of 35%, Ziza et al. [20] of 47% and Randon et al. [17] of 56%. Our results are in accordance with the literature findings, and are in line with previous studies on the graft patencies of venous and arterial allograft types. Better patency rates of SFA allograft owing to the below-knee patency results, where SFA allograft shows significantly higher patency rates than GSV allograft.

The higher occlusion rate of venous allografts may be explained by the smaller vessel diameter, the weaker structure of the vessel wall–thus being liable to degeneration–and the more vulnerable intimal layer, making it prone to thrombosis. In their study of 1404 patients who underwent lower limb bypass surgery due to CLTI, Schanzer et al. [13] found that grafts with smaller vessel diameters had negative effects on subsequent graft patencies.

Arterial allografts may be anatomically and physiologically more suitable than venous grafts, owing to the more resistant mechanical properties when exposed to arterial blood flow

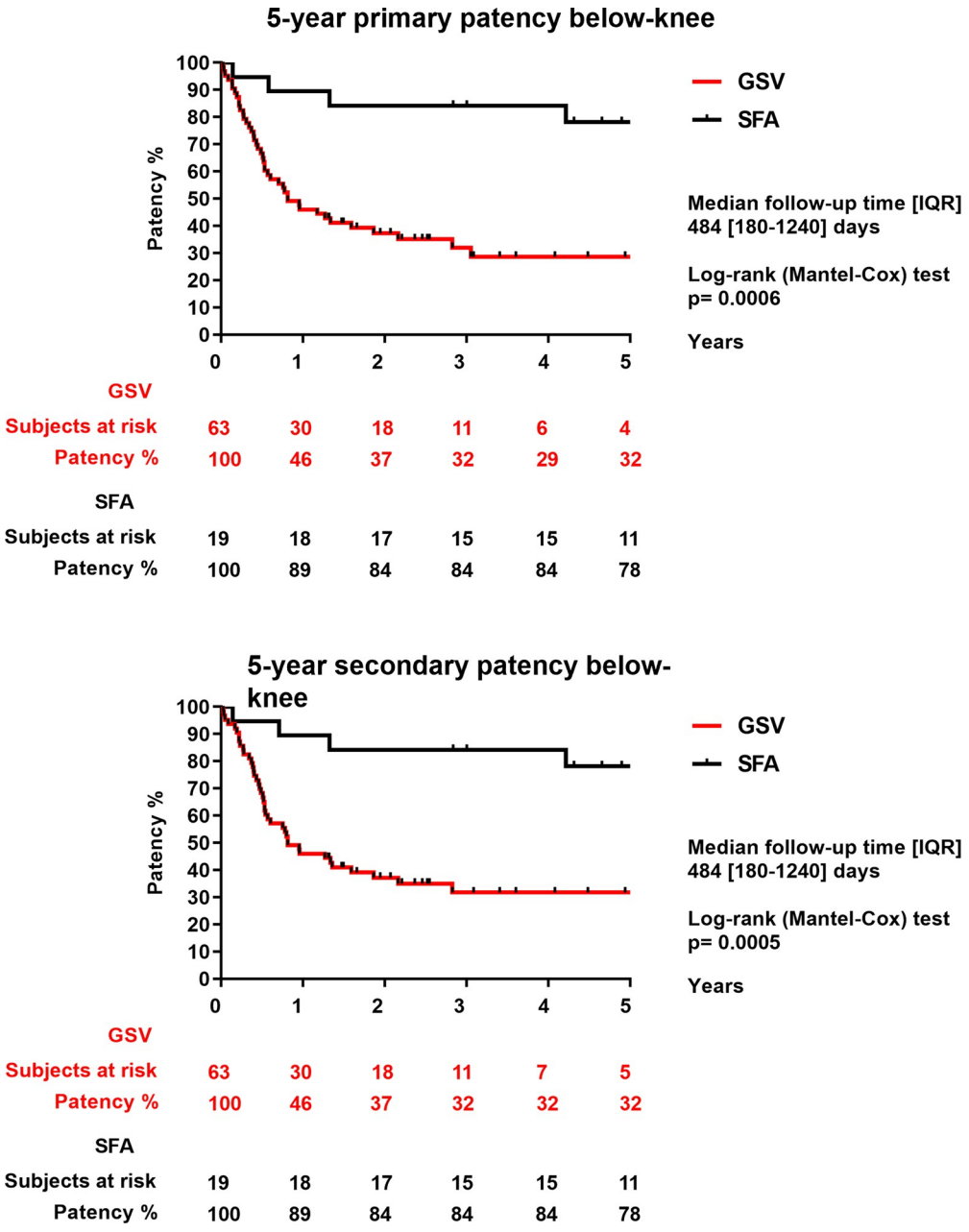

Fig 4. 5-year primary and secondary patency of GSV (great saphenous vein) and SFA (superficial femoral artery) implanted in below-knee position.

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and blood pressure. Walden et al. [26] denoted that matching graft and artery elastic properties resulted in higher patency rates. According to Pukacki et al. [27], cryopreservation maintains the elastic properties of arterial allografts, which is mostly the same as the elasticity of the recipient vessel.

The site of distal anastomosis–above-knee or below-knee–may have a considerable impact on graft patency. Earlier studies proved that below-knee distal anastomosis showed lower patency rates than in case of proximal bypass [13,28]. In our analysis, although below-knee bypasses showed somewhat lower patency rates, we could not detect significant differences

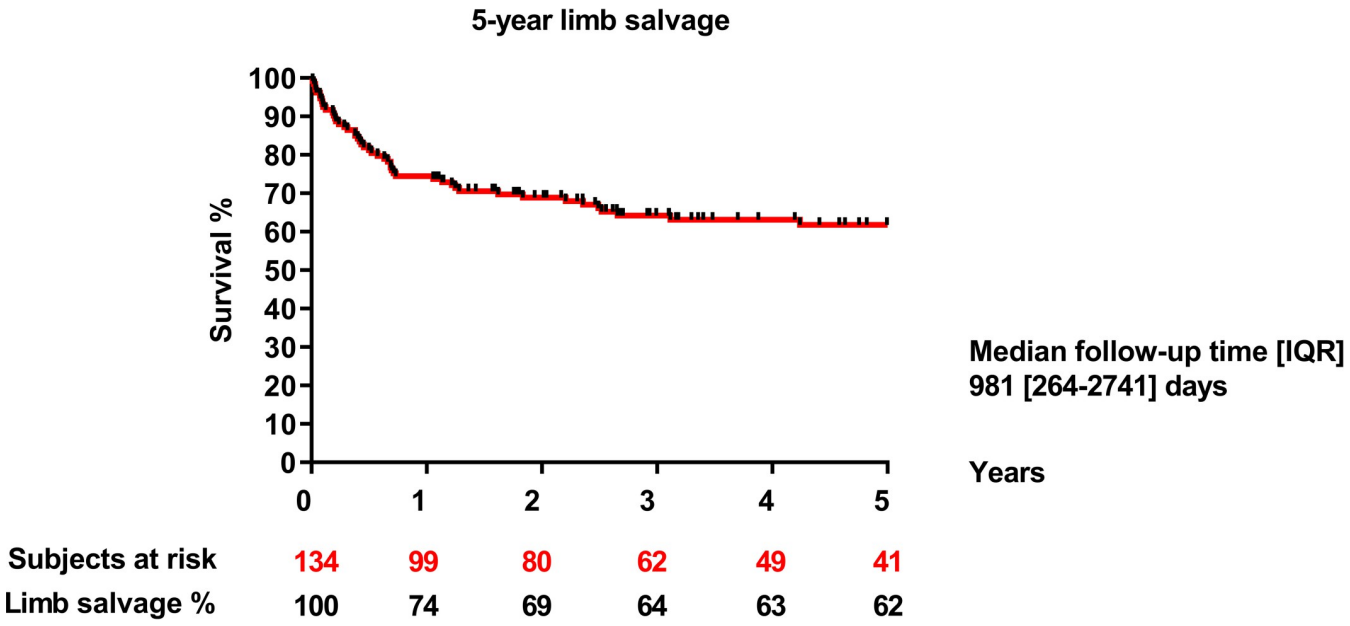

Fig 5. 5-year limb salvage.

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between the patencies of above- and below-knee anastomoses. O'Banion et al. [18] reported similar results. Better patency rates have been described in allograft bypasses for the superficial femoral artery or the above-knee section of the popliteal artery than for the below-knee section of the popliteal artery or for distal anastomosis located at the crural arteries, however, no significant differences could be confirmed.

In our study, the allograft occlusion rate was found to be high, but limb salvage showed an acceptable rate. Five years after surgery, 62% of the operated limbs were still viable. Similarly to our results, Randon et al. [17] reported a limb salvage rate of 65% at 5 years with a primary patency rate of 11.1% and a secondary patency rate of 38.5%. The reason for the acceptable long-term amputation rate is that during bypass graft patency, the distal limb tissues in even Fontaine stage IV patients received a sufficient amount of blood flow for the ischemic ulcer or gangrene to heal. In the event of early graft occlusion, wound healing contributes to long-term limb survival.

The low rate of long-term survival– 53% at 5 years–is in accordance with the literature data according to which patients with CLTI have a 5 year survival rate of 50–60% [29,30]. The high mortality rate is not directly caused by the peripheral vascular disease or surgery, but rather by comorbidities and risk factors related to diffuse cardiovascular disease with negative effects on survival.

Our study has its limitations, which may influence the obtained results and restrict actual prediction outcomes. In this retrospective nonrandomized study, all pre-, intra-, and postoperative assessments, methods and decisions were at the discretion of the operating surgeon, we did not follow a specific, strict protocol. Implanted arterial and venous allografts were not randomized due to the nature of the retrospective study and the limited availability of allografts.

Conclusions

In our retrospective analysis involving a large number of cases, we analysed the results of infrainguinal allograft bypass surgery in patients with CLTI.

Allograft implantation is known to be a good alternative to prosthetic graft, showing promising results in case of risk of infection, which is a common morbidity after bypass procedures for CLTI.

Our results showed the patency rates to be low, but acceptable. Our study is the first to report that SFA allograft is a better option than GSV at below-knee position in patients with CLTI, since both primary and secondary patency of SFA graft was found to be significantly higher.

Further prospective randomized studies are needed in order to evaluate allograft bypass surgeries performed in case of CLTI.

Our results call attention to the fact that during femoro-popliteal bypass surgery in patients with CLTI, allograft implantation–often as a last resort for limb salvage–seems to be a suitable method for revascularization in the absence of an autologous conduit.

Supporting information

S1 Database.
(XLSX)

Author Contributions

Conceptualization: Dávid Garbaisz, Zoltán Szeberin.

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Formal analysis: Dávid Garbaisz, András Mihály Boros.

Investigation: Dávid Garbaisz, Péter Osztrogonácz, András Mihály Boros, Zoltán Szeberin.

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Project administration: Dávid Garbaisz.

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References

1. Taylor LM, Jr., Edwards JM, Porter JM. Present status of reversed vein bypass grafting: five-year results of a modern series. J Vasc Surg. 1990; 11(2):193–205; discussion -6. Epub 1990/02/01. https://doi.org/10.1067/mva.1990.17235 PMID: 2299743.
2. McPhee JT, Goodney PP, Schanzer A, Shaykevich S, Belkin M, Menard MT. Distal anastomotic vein adjunct usage in infrainguinal prosthetic bypasses. J Vasc Surg. 2013; 57(4):982–9. Epub 2013/02/05. https://doi.org/10.1016/j.jvs.2012.10.098 PMID: 23375606; PubMed Central PMCID: PMC3930457.
3. Moreira CC, Leung AD, Farber A, Rybin D, Doros G, Siracuse JJ, et al. Alternative conduit for infrageniculate bypass in patients with critical limb ischemia. J Vasc Surg. 2016; 64(1):131–9.e1. Epub 2016/06/28. https://doi.org/10.1016/j.jvs.2016.01.042 PMID: 27345506.
4. Avgerinos ED, Sachdev U, Naddaf A, Doucet DR, Mohapatra A, Leers SA, et al. Autologous alternative veins may not provide better outcomes than prosthetic conduits for below-knee bypass when great saphenous vein is unavailable. J Vasc Surg. 2015; 62(2):385–91. Epub 2015/05/07. https://doi.org/10.1016/j.jvs.2015.03.025 PMID: 25943451.

5. Dorigo W, Fargion A, Bassoli G, Di Domenico R, Giacomelli E, Piffaretti G, et al. Autologous saphenous vein and heparin-bonded expanded polytetrafluoroethylene as graft materials for below-the-knee femoro-popliteal bypass in patients with critical limb ischemia: A propensity score-matched analysis. Surgeon. 2022; 20(2):85–93. Epub 2021/03/10. https://doi.org/10.1016/j.surge.2021.02.001 PMID: 33685832.
6. Conte MS, Bradbury AW, Kolh P, White JV, Dick F, Fitridge R, et al. Global Vascular Guidelines on the Management of Chronic Limb-Threatening Ischemia. Eur J Vasc Endovasc Surg. 2019; 58(1s):S1–S109.e33. Epub 2019/06/12. https://doi.org/10.1016/j.ejvs.2019.05.006 PMID: 31182334; PubMed Central PMCID: PMC8369495.
7. Biro G, Szeberin Z, Nemes A, Acsady G. Cryopreserved homograft and autologous deep vein replacement for infrarenal aorto and iliaco-femoral graft infection: early and late results. J Cardiovasc Surg (Torino). 2011; 52(2):169–76. Epub 2011/04/06. PMID: 21464818.
8. Stoner MC, Calligaro KD, Chaer RA, Dietzek AM, Farber A, Guzman RJ, et al. Reporting standards of the Society for Vascular Surgery for endovascular treatment of chronic lower extremity peripheral artery disease. J Vasc Surg. 2016; 64(1):e1–e21. Epub 2016/06/28. https://doi.org/10.1016/j.jvs.2016.03.420 PMID: 27345516.
9. Chakfé N, Diener H, Lejay A, Assadian O, Berard X, Caillon J, et al. Editor's Choice—European Society for Vascular Surgery (ESVS) 2020 Clinical Practice Guidelines on the Management of Vascular Graft and Endograft Infections. Eur J Vasc Endovasc Surg. 2020; 59(3):339–84. Epub 2020/02/10. https://doi.org/10.1016/j.ejvs.2019.10.016 PMID: 32035742.
10. Nehler MR, Duval S, Diao L, Annex BH, Hiatt WR, Rogers K, et al. Epidemiology of peripheral arterial disease and critical limb ischemia in an insured national population. J Vasc Surg. 2014; 60(3):686–95. e2. Epub 2014/05/14. https://doi.org/10.1016/j.jvs.2014.03.290 PMID: 24820900.
11. Farber A, Eberhardt RT. The Current State of Critical Limb Ischemia: A Systematic Review. JAMA Surg. 2016; 151(11):1070–7. Epub 2016/08/24. https://doi.org/10.1001/jamasurg.2016.2018 PMID: 27551978.
12. Hirsch AT, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, et al. ACC/AHA 2005 guidelines for the management of patients with peripheral arterial disease (lower extremity, renal, mesenteric, and abdominal aortic): executive summary a collaborative report from the American Association for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (Writing Committee to Develop Guidelines for the Management of Patients With Peripheral Arterial Disease) endorsed by the American Association of Cardiovascular and Pulmonary Rehabilitation; National Heart, Lung, and Blood Institute; Society for Vascular Nursing; TransAtlantic Inter-Society Consensus; and Vascular Disease Foundation. J Am Coll Cardiol. 2006; 47(6):1239–312. Epub 2006/03/21. https://doi.org/10.1016/j.jacc.2005.10.009 PMID: 16545667.
13. Schanzer A, Hevelone N, Owens CD, Belkin M, Bandyk DF, Clowes AW, et al. Technical factors affecting autogenous vein graft failure: observations from a large multicenter trial. J Vasc Surg. 2007; 46(6):1180–90; discussion 90. Epub 2007/12/25. https://doi.org/10.1016/j.jvs.2007.08.033 PMID: 18154993.
14. Almasri J, Adusumalli J, Asi N, Lakis S, Alsawas M, Prokop LJ, et al. A systematic review and meta-analysis of revascularization outcomes of infrainguinal chronic limb-threatening ischemia. J Vasc Surg. 2018; 68(2):624–33. Epub 2018/05/29. https://doi.org/10.1016/j.jvs.2018.01.066 PMID: 29804736.
15. Albers M, Battistella VM, Romiti M, Rodrigues AA, Pereira CA. Meta-analysis of polytetrafluoroethylene bypass grafts to infrapopliteal arteries. J Vasc Surg. 2003; 37(6):1263–9. Epub 2003/05/24. https://doi.org/10.1016/s0741-5214(02)75332-9 PMID: 12764274.
16. Uhl C, Grosch C, Hock C, Topel I, Steinbauer M. Comparison of Long-term Outcomes of Heparin Bonded Polytetrafluoroethylene and Autologous Vein Below Knee Femoropopliteal Bypasses in Patients with Critical Limb Ischaemia. Eur J Vasc Endovasc Surg. 2017; 54(2):203–11. Epub 2017/06/08. https://doi.org/10.1016/j.ejvs.2017.05.001 PMID: 28587797.
17. Randon C, Jacobs B, De Ryck F, Beele H, Vermassen F. Fifteen years of infrapopliteal arterial reconstructions with cryopreserved venous allografts for limb salvage. J Vasc Surg. 2010; 51(4):869–77. Epub 2010/03/30. https://doi.org/10.1016/j.jvs.2009.11.062 PMID: 20347683.
18. O'Banion LA, Wu B, Eichler CM, Reilly LM, Conte MS, Hiramoto JS. Cryopreserved saphenous vein as a last-ditch conduit for limb salvage. J Vasc Surg. 2017; 66(3):844–9. Epub 2017/05/16. https://doi.org/10.1016/j.jvs.2017.03.415 PMID: 28502546.
19. Hartranft CA, Noland S, Kulwicki A, Holden CR, Hartranft T. Cryopreserved saphenous vein graft in infrainguinal bypass. J Vasc Surg. 2014; 60(5):1291–6. Epub 2014/07/07. https://doi.org/10.1016/j.jvs.2014.05.092 PMID: 24997807.

20. Ziza V, Canaud L, Gandet T, Molinari N, Alonso W, Chastan R, et al. Outcomes of cold-stored venous allograft for below-knee bypasses in patients with critical limb ischemia. J Vasc Surg. 2015; 62(4):974–83. Epub 2015/07/05. https://doi.org/10.1016/j.jvs.2015.04.437 PMID: 26141692.

21. Buckley CJ, Abernathy S, Lee SD, Arko FR, Patterson DE, Manning LG. Suggested treatment protocol for improving patency of femoral-infrapopliteal cryopreserved saphenous vein allografts. J Vasc Surg. 2000; 32(4):731–8. Epub 2000/09/30. https://doi.org/10.1067/mva.2000.110049 PMID: 11013037.

22. Masmejan S, Deslarzes-Dubuis C, Petitprez S, Longchamp A, Haller C, Saucy F, et al. Ten Year Experience of Using Cryopreserved Arterial Allografts for Distal Bypass in Critical Limb Ischaemia. Eur J Vasc Endovasc Surg. 2019; 57(6):823–31. Epub 2019/05/28. https://doi.org/10.1016/j.ejvs.2018.11.020 PMID: 31130420.

23. Guevara-Noriega KA, Lucar-Lopez GA, Pomar JL. Cryopreserved Allografts for Treatment of Chronic Limb-Threatening Ischemia in Patients Without Autologous Saphenous Veins. Ann Vasc Surg. 2019; 60:379–87. Epub 2019/06/15. https://doi.org/10.1016/j.avsg.2019.03.018 PMID: 31200034.

24. Albertini JN, Barral X, Branchereau A, Favre JP, Guidicelli H, Magne JL, et al. Long-term results of arterial allograft below-knee bypass grafts for limb salvage: a retrospective multicenter study. J Vasc Surg. 2000; 31(3):426–35. Epub 2000/03/10. PMID: 10709053.

25. Lejay A, Delay C, Girsowicz E, Chenesseau B, Bonnin E, Ghariani MZ, et al. Cryopreserved Cadaveric Arterial Allograft for Arterial Reconstruction in Patients with Prosthetic Infection. Eur J Vasc Endovasc Surg. 2017; 54(5):636–44. Epub 2017/09/12. https://doi.org/10.1016/j.ejvs.2017.07.016 PMID: 28890027.

26. Walden R, L'Italien GJ, Megerman J, Abbott WM. Matched elastic properties and successful arterial grafting. Arch Surg. 1980; 115(10):1166–9. Epub 1980/10/01. https://doi.org/10.1001/archsurg.1980.01380100018004 PMID: 6448593.

27. Pukacki F, Jankowski T, Gabriel M, Oszkinis G, Krasinski Z, Zapalski S. The mechanical properties of fresh and cryopreserved arterial homografts. Eur J Vasc Endovasc Surg. 2000; 20(1):21–4. Epub 2000/07/25. https://doi.org/10.1053/ejvs.2000.1120 PMID: 10906292.

28. McPhee JT, Barshes NR, Ozaki CK, Nguyen LL, Belkin M. Optimal conduit choice in the absence of single-segment great saphenous vein for below-knee popliteal bypass. J Vasc Surg. 2012; 55(4):1008–14. Epub 2012/03/01. https://doi.org/10.1016/j.jvs.2011.11.042 PMID: 22365176.

29. Fowkes FG, Aboyans V, Fowkes FJ, McDermott MM, Sampson UK, Criqui MH. Peripheral artery disease: epidemiology and global perspectives. Nat Rev Cardiol. 2017; 14(3):156–70. Epub 2016/11/18. https://doi.org/10.1038/nrcardio.2016.179 PMID: 27853158.

30. Long-term mortality and its predictors in patients with critical leg ischaemia. The I.C.A.I. Group (Gruppo di Studio dell'Ischemia Cronica Critica degli Arti Inferiori). The Study Group of Criticial Chronic Ischemia of the Lower Exremities. Eur J Vasc Endovasc Surg. 1997; 14(2):91–5. Epub 1997/08/01. PMID: 9314849.

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ÖSSZEFOGLALÓ KÖZLEMÉNY

Homograftok használata érsebészeti fertőzésekben

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Az érsebészeti graftinfekciók és a fertőzött szöveti környezetben végzett érrekonstrukciók is jelentős morbiditással és mortalitással járó állapotok, melyek komoly kihívást jelentenek az operáló érsebész számára. A szeptikus érsebészetben nincs egységes álláspont sem az operatív stratégiát, sem az érpótlásra használt graft anyagát illetően. Az ajánlások alapján az elsőként választandó a beteg saját szervezetéből származó, ún. autológ graft használata, azonban ennek hiánya vagy alkalmatlansága esetén ígéretes lehet a szervdonorból explantált, ún. homograft alkalmazása. Célunk az elmúlt 7 év során megjelent, érsebészeti szeptikus kórállapotokban felhasznált homograftokkal foglalkozó szakirodalmi közlemények áttekintése volt. Az adatgyűjtés a 2016. január 1. és 2022. december 31. közötti időszakot áttekintve történt. A PubMed és Medline adatbázisokban szisztematikus keresés során az érsebészeti szeptikus kórállapotokban alkalmazott, krioprezervált homograft beültetésének eredményeiről beszámoló közleményeket választottuk ki és elemeztük. A publikációk eredményeinek elemzése a következő végpontok alapján történt: a vizsgálat jellege, demográfiai adatok, mortalitási arány, a grafthoz köthető szövődmények és a reoperáció aránya, a graft nyitva maradása, végtagmentési arány, graft-újrafertőződési arány és túlélési arány. A szisztematikus keresést követően 16 közlemény adatait dolgoztuk fel. A publikációkat két csoportra osztottuk: aortán végzett érműtétek (aorta) és perifériás ereken végzett érműtétek (perifériás). Az aortacsoportba 12 közlemény került, melyek 542 beteg adatait dolgozták fel. A korai halálozási arány (<30 nap) 2,8% és 42,8% közötti, a homografttal kapcsolatos reoperációs arány 5,9% és 29% közötti, míg a graft-újrafertőződési arány 10% alatti volt. A perifériás csoportba 4 vizsgálat került, amelyek 252 beteg adatait fedik le. A korai halálozási arány (<30 nap) 2,0% és 38% közötti, az allografttal kapcsolatos reoperációs arány 4,0% és 55% közötti (korai és késői), míg a graft-újrafertőződési arány 4% körüli volt. Összefoglaló közleményünk adatai alapján az érsebészeti fertőzések során felhasznált homograftokkal végzett beavatkozások mortalitása és reoperációs aránya nem ítélhető meg egyértelműen, azonban az alacsony újrafertőződési arányból és az előnyös biológiai tulajdonságokból adódóan megfelelő választás lehet érrekonstrukciók során.
Orv Hetil. 2023; 164(32): 1256–1262.

Kulcsszavak: érgraft, allograft, homograft, krioprezervált, infekció

Use of allografts in vascular surgery infections

With the growing number of patients with vascular endografts, the number of patients with graft infections has also increased. Septic conditions and the choice of grafts are an important challenge in vascular surgery. The aim of this study was to review the literature of the last 7 years showing allograft use in septic conditions in vascular surgery which helps provide insight into the current results of vascular allografts. Data were collected between 1st January 2016 and 31st December 2022. A systematic search was conducted for publications of cryopreserved allograft usage for vascular infection in PubMed and Medline databases. The results of the publications were reviewed based on the following key endpoints: study design, patient's characteristics, mortality rate, graft related complication and reintervention rate, graft patency, limb salvage, graft reinfection rate and survival rate. After a systematic search, 16 publications were included. The articles were divided into two groups: aortic and peripheral. The aortic group included 12 studies covering the data of 542 patients. Early mortality rate (<30 days) was between 2.8% and 42.8%. Allograft-related reintervention rates ranged between 5.9% and 29% (early and late). The rate of graft reinfection was below 10%. 4 studies were included in the peripheral group covering the data of 252 patients. Early mortality rate (<30 days) was between 2.0% and 38%. Allograft-related reintervention rates ranged between 4.0% and 55% (early and late). Reinfection rate was around 4%, but only poor quality data were available. Infections in vascular surgery remain a challenging problem, however, cryopreserved allografts show low reinfection rate and reasonable durability, thus, allografts may be an acceptable option for reconstruction.

Keywords: vascular, allograft, homograft, cryopreserved, infection

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Rövidítések

ESVS = (European Society for Vascular Surgery) Európai Érsebészeti Társaság; MESH = (Medical Subject Headings) orvostudományi tárgyszógyűjtemény; PRISMA = (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) metaanalízisek és rendszeres áttekintések javasolt kiértékelési szempontjai

Az érsebészeti graftinfekciók kezelése és a fertőzött szöveti környezetben végzett érrekonstrukciók a legnehezebb feladatok közé sorolhatók az érsebészetben. Érműtéteket követően a graftinfekció incidenciája 0,5–6% között változik, a graft anyagától és a beültetett graft lokalizációjától függően [1, 2]. A szeptikus vascularis szövődmények magas morbiditású és mortalitású kórképek, ezenkívül a nehéz helyzetet gyakran súlyosbítja a műtétre kerülő betegcsoport rossz általános állapota és a számos kísérő betegség egyidejű fennállása. Habár a probléma igen jelentős, napjainkig nincs egységes szakmai álláspont az ideális műtéti kezelésről. A fertőzött érprotézis teljes vagy részleges kimetszése, a széles körű débridement, a drenázs és az *in situ* vagy extra anatomikus rekonstrukció a szeptikus állapot fajtájától és súlyosságától függően mind elfogadott kezelési lehetőségek. Az *in situ* rekonstrukció további veszélyekkel járhat, mivel fennáll a graft vagy a környező szövetek újrafertőződésének veszélye, mely akár életveszélyes szövődményekhez (az érgraft elzáródása, szeptikus vérzés anastomosiselégtelenség miatt, generalizált szepszis, szeptikus sokk) vezethet, azonban sok érgyógyászati központban ez az általánosan elfogadott kezelési módszer.

A hasi főverőérrel kapcsolatos fertőzés a szeptikus érsebészet egyik legsúlyosabb állapota, melynek kezelése komplex, nagy szakmai jártasságot igénylő feladat. Az aortafertőzésnek két fő megjelenési formája ismert: a szeptikus natív aortaaneurysmák (incidencia: 0,7–2,6%) és a hasi aortagraft vagy endovascularis graft fertőzései (incidencia: 0,2–3,0%; 0,1–1,2%) [3, 4]. Az infekciós (mycoticus) aneurysmák mortalitása magas [5]. Az aortagraft-fertőzések rendkívül rossz prognózisú kórállapotok, melyek kiemelkedően nagy morbiditási és 10–50%-os mortalitási aránnyal járnak [6]. Az aortafertőzés – akár műér-, akár mycoticus aneurysma – a fertőzött aneurysma vagy műér excíziójával/explantációjával, majd extraanatomikus bypass képzésével vagy – a fertőzésnek ellenálló grafttal – *in situ* rekonstrukcióval kezelhető. A kezelési stratégiát limitálja, hogy aortapozícióban – a jelentős érátmérő-különbség miatt – az általánosan elfogadott autológ vena (v.) saphena magna érpótlás nem lehetséges.

A szeptikus érsebészet másik, jelentős nehézséggel járó területét a perifériás vascularis infekciók jelentik, amelyek többnyire az alsó végtagi érrendszert, ritkán a felső végtagi vagy nyaki ereket is érinthetik. Az artériás érrekonstrukció fertőzött szövetek között és a műérfertőzés egyaránt komoly kihívás elé állítják az érsebészt. A definitív sebészi terápia minden fertőzött szövet kiterjedt kimetszését, drenázst és a keringés folytonosságának helyreállítását jelenti akár extraanatomikus, akár *in situ* rekonstrukcióval. A perifériás graftinfekció előfordulása femorofemoralis műérrel végzett bypasst követően 2,5%, femoropoplitealis műérrel végzett bypasst követően 2,8% [7–9].

Érsebészeti infekciók esetén nincs egységes szakmai álláspont az ideális graft anyagát illetően. Többféle graft-típus áll rendelkezésre az *in situ* rekonstrukció elvégzéséhez, habár számos irodalmi adat alátámasztja, és a szakmai álláspont is egységes annak tekintetében, hogy a beteg saját érrendszeréből származó autológ érgraft (autograft) a legjobb választás. Autograft-beültetésre alkalmas lehet az azonos vagy ellenoldali v. saphena magna, v. saphena parva, v. femoralis és akár a felső végtagi vénák. Az autograft elégtelensége (nem megfelelő anatómia, visszérbetegség) vagy hiánya (korábbi visszérműtét, korábbi bypassműtét – cardialis/vascularis) esetén alternatív graftként szolgálhat a rekonstrukció elvégzéséhez az ezüstionokkal impregnált, ún. 'silver' műérgraft, antibiotikummal (rifampicin) kezelt műérgraft, állati eredetű, ún. xenológ graft (xenograft – marhapericardium) és a krioprezervált allogén graft (allograft, homograft).

A homograft a biológiai érgraftok közé sorolható, amely többszervi transzplantáció során kerül explantálásra szervdonorból. Az érsebészetben a homograftok a 20. század második felétől kezdtek megjelenni. Az első humán aortahomograft-beültetést *Gross* végezte [10]. *DeBakey és mtsai* először alkalmaztak aortahomograftot okkluzív aortabetegségben [11].

A transzplantáció során a v. saphena magna, az arteria femoralis superficialis és az aortoiliacalis rendszer is explantálható és graftként használható. A graftkivételt követően a graftok konzerválása érdekében krioprezerváció történik, melynek eredményeként a graftok –80 °C-os mélyhűtőben kerülnek tárolásra egy speciálisan erre a feladatra létrehozott érhomograftbankban.

Mivel a homograft biológiai graftnak minősül, tulajdonságaiból adódóan ellenállóbb lehet a fertőzésekkel szemben. Alkalmazása előnyösebb lehet a műérgraftok beültetésénél olyan helyzetekben, mint a graftinfekció, szeptikus állapot, ischaemiás fekély vagy gangraena jelenléte. Ezenkívül a homograftnak más előnyei is vannak, mint például az autológ grafthoz nagyon hasonló felépítés és áramlási dinamika [12].

A jelen tanulmány célja az elmúlt 7 év során megjelent, érhomograftok szeptikus állapotokban történő alkalmazásával foglalkozó szakirodalmi közlemények áttekintése, amely segíthet betekintést nyújtani a vascularis homograftok jelenlegi eredményeibe. Vizsgálatunk segíthet az egységes konszenzus kialakításában a grafttípusok szeptikus érsebészetben történő felhasználásáról, amelyről jelenleg nincs egységes álláspont.

Irodalomkutatás

Az adatgyűjtés a 2016. január 1. és 2022. december 31. közötti időszakot áttekintve történt. A PubMed és Medline adatbázisokban szisztematikus keresés során az érsebészeti szeptikus kórállapotokban alkalmazott, krioprezervált homográft beültetésének eredményeiről beszámoló közleményeket választottuk ki és elemeztük. Az irodalmi összefoglaláshoz felhasznált közlemények kiválasztása a PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) irányelv szabályai alapján történt.

Az adatbázis-elemzés a MESH (Medical Subject Headings) szójegyzék kifejezései szerint, a következő kulcsszavak felhasználásával történt: „vascular" [All Fields], „allograft" [All Fields], „homograft" [All Fields] „cryopreserved" [All Fields], „infection" [All Fields]. A keresési beállításokban a következő szűrőket állítottuk be: 7 éves időszak, teljes szöveg és absztrakt elérhető, angol nyelvű közlemény, eredeti közlemény.

Az elemzés során azokat a közleményeket választottuk ki, amelyekben az aorta vagy a perifériás alsó végtagi erek, natív vagy már korábban beültetett graft fertőzése miatt történt rekonstrukció krioprezervált aorta vagy artériás/vénás homográftok felhasználásával. Kizártuk azokat a publikációkat, amelyek egy vizsgálaton belül több grafttípus felhasználását és együttes eredményeit mutatják be. További kizárási kritériumok voltak: közlemények, amelyeknek kizárólag az absztraktjuk érhető el, nem angol nyelvű közlemények, összefoglaló (review) tanulmányok, konferenciaabsztraktok, kutatói levelek (research letter) és esetbemutatások. A vizsgálatba nem vontuk be azokat a közleményeket sem, amelyekben a homográft-felhasználás indikációja nem egyértelműen az infekció volt.

A publikációk eredményeinek elemzése a következő végpontok alapján történt: a vizsgálat jellege (retrospektív/prospektív), demográfiai adatok, mortalitási arány, grafthoz köthető szövődmények és a reoperáció aránya, graft-nyitvamaradás, végtagmentési arány, graft-újrafertőződési arány és túlélési arány.

A közlemények keresését, kiválasztását és adatelemzését két vizsgáló végezte.

Az adatbázis-keresés – a korábban részletezett kulcsszavakkal és szűrőbeállításokkal – 65 potenciálisan alkalmas közleményt eredményezett. A további cím- és absztraktáttekintést követően 27 felelt meg összefoglaló közleményünk keresési kritériumkövetelményeinek. A kizárási kritériumaink alapján történő újabb elemzés során 11 közleményt zártunk ki a következő okok miatt: összefoglaló közlemény, esetbemutatás, több grafttípus eredményeinek együttes elemzése. A fennmaradó 16 közleményt vontuk be az elemzésbe, melyek teljes mértékben megfeleltek a bevonási kritériumoknak. A vizsgált publikációkat két csoportra osztottuk a cikkek témáját tekintve: aorta és perifériás. 2 cikk alkalmas volt mindkét csoportba történő besorolásra, mindkét esetben a vizsgálat felépítése alapján történt a besorolás. A bevont közlemények adatait táblázatban foglaltuk össze, melyet háttéradatbázisban helyeztünk letétbe.

Az adatbázis-keresés során talált kéziratok kis száma azt mutatja, hogy a krioprezervált homográftok alkalmazása érsebészeti fertőzések során az érsebészetnek egy kevésbé kutatott területe. A rendelkezésre álló közlemények többnyire egyetlen központ eredményeit dolgozzák fel retrospektíven, kis betegszámmal. Nagy randomizált, multicentrikus vizsgálatok nem állnak rendelkezésre. Ez lehet az egyik oka, hogy a mai napig nincs erős bizonyíték és ennek megfelelően szakmai irányelv szintjén megvalósuló egységes álláspont az érsebészeti fertőzések során alkalmazott graft típusáról azokban az esetekben, amikor nem áll rendelkezésre autológ ér a rekonstrukcióhoz. A homográft-beültetés korábbi változó eredményei továbbra is szakmai vita tárgyát képzik, ráadásul a világszerte kevés homográftbank miatt a homográftokhoz még mindig nehéz hozzáférni. Tovább nehezíti a homográftok elérhetőségét a megfelelő, speciális infrastruktúrát és szakértelmet igénylő begyűjtésük, konzervásuk, kezelésük és logisztikájuk, melyek a homográft-beültetést összességében költséges terápiás eszközzé teszik. A fentiekben részletezett nehézségeket tükrözi a szakmailag irányadó Európai Érsebészeti Társaság (ESVS) legfrissebb irányelve is az érsebészeti fertőzéseket illetően [9]. A szakmai irányelv akár centrális (aorta), akár perifériás szinten előforduló érsebészeti fertőzés esetén krioprezervált homográft használatát IIa/IIb ajánlással, „C" evidenciaszinttel ajánlja. Ennek megfelelően az irányelv a hatékonyságot ellentmondásos bizonyítékok és/vagy eltérő vélemények szerint értékeli, és az ajánlások a szakértők véleményének konszenzusán és/vagy kis elemszámú tanulmányokon, retrospektív vizsgálatokon, adatbázisokon alapulnak.

Aortainfekció

Az aortacsoportba 12 közleményt vontunk be, amelyek mindegyike a krioprezervált homográft beültetésének eredményeit tárgyalja infekciós eredetű hasi aortaaneurysma rekonstrukciója vagy aortagraft/endográft infekció során. A többnyire retrospektív adatgyűjtésre épülő vizsgálatok döntő hányada egy központ eredményét dolgozza fel. 4 közlemény prospektíven gyűjtött adatokat mutat be. A publikációk által vizsgált időszak az 1997 és 2022 közötti időben változik. Az értékelt cikkek összességében 511 beteg eredményeit dolgozzák fel. A legtöbb tanulmány alacsony betegszám alapján elemez, habár 3 kutatás nagyobb volumenű beteganyagot mutat be: 71 [13], 96 [14] és 200 [6] beteg.

A cardiovascularis betegségek demográfiai adataival összhangban a betegek döntő hányada férfi, átlagéletkoruk 64,2 és 72,8 év közé tehető.

A korai halálozási arány (<30 nap) 2,8% és 42,8% közé tehető. 5 tanulmány 10% alatti korai halálozásról számol be [13, 15–17], 3 vizsgálat 10% és 25% közötti arányt

említ [18, 19], és 1 közlemény írja le a halálozási arányt 40% felettinek [20]. Az utóbb említett publikáció korai halálozási eredménye extrém magas, az irodalmi adatokban azonban találunk ezzel összhangban lévő, hasonlóan magas halálozási adatokról beszámoló cikket. *Coselli és mtsai* polietilén-tereftalát (Dacron) műérgraft és krioprezervált homograft implantációját végezték thoracalis aortainfekcióban, és hasonlóan magas mortalitási adatokról számoltak be (42%) [21]. Fontos megállapítani, hogy a homograft használatának eredményeit mindkét vizsgálat thoracalis aortainfekció állapotában mutatja be: erről önmagában is ismert, hogy nagyon magas mortalitású kórkép, mely a klinikai megjelenésétől függően akár 75% lehet [9]. A késői halálozás hozzávetőlegesen megegyezik a korai halálozási adatokkal, mely 2,8% és 42% közötti. 6 közlemény is 20% vagy ezen érték feletti késői halálozási adatokat ír le, mely igen magasnak tekinthető. A magas mortalitás magyarázata, hogy az aorta/aortagraft infekció önmagában jelentős halálozással járó, nagy kihívást jelentő, igen rossz prognózisú kórkép. A halálozási adatokat befolyásolja, hogy a szeptikus érsebészeti betegek gyakran multimorbid, rossz általános állapotú betegek, és a szeptikus állapot önmagában a halálozás egy független rizikófaktora. Az aorta/aortagraft infekciók sokszor sürgősségi, akár életmentő beavatkozást jelentenek, mely szintén növeli a halálozás esélyét. Aortoenteralis, aortooesophagealis vagy légúti fistula jelenléte – mely gyakran áll a szeptikus aortagraft-fertőzés hátterében – igen rossz prognosztikai faktornak tekinthető [22]. A magas virulenciájú, rezisztens vagy multirezisztens kórokozók – melyek gyakran megjelennek aortagraft-infekciók során – szintén csökkentik a túlélési esélyeket.

Azok a publikációk, amelyek a homografthoz köthető reoperációs arányt említik, annak értékét 5,9% és 29% közé teszik (korai és késői). *Ben Ahmed és mtsai* 3%-os korai letális homograftanastomosis-elégtelenségi arányról számolnak be, viszont nem találtak homograft-degenerációt egyik beteg esetén sem [13]. Ezen adatokhoz hasonlóan, *Bossi és mtsai* nem írnak le homograft-degenerációt vagy -reinfekciót [18]. *Weiss és mtsai* relatíve magas, homografthoz köthető szövődményarányt írnak le [16]. Vizsgálatukban a korai szövődményarány 12%, a késői pedig 29%, mely adatok magukban foglalják a graftstenosist (16%), -elzáródást (13%) és az álaneurysma-képződést (10%). A homografttal összefüggő szövődmények megjelenését következtetéseik szerint riasztónak gondolják, azonban a szövődmények megjelenésével kapcsolatban meghatározónak tartják a homograftkonzerváló eljárás típusát. *Couture és mtsai* közleménye alapján a homografthoz köthető szövődmények megjelenése 7,5% (korai) és 12,5% (késői), mely adatokból arra következtetnek, hogy a homografthoz köthető középtávú szövődményráta gyakori [6]. A legtöbb publikáció szerint a homograft-explantáció és a konzerváló eljárás technikája és folyamata, valamint a preoperatív kezelési technika kiemelt jelentőségű a homografthoz köthető szövődmények elkerülésében. A tanulmányok többsége arra a következtetésre jutott, hogy a grafthoz köthető szövődmények kisebb arányban fordulnak elő krioprezervációt követően. Egységes az álláspont azt illetően, hogy a krioprezerváció egyfajta védőfaktort jelent a beültetendő graft eredményességére és tartósságára nézve. A megfelelő technikával krioprezervált homograftok jobb kollagénszerkezettel rendelkeznek, jobb a mechanikai stabilitásuk [23, 24]. Ezenfelül a krioprezerváció nem befolyásolja a muscularis artériák viszkoelaszticitását és az elasztikus artériák falszerkezetét [25].

Az elemzett közlemények mindegyike megközelítően azonos túlélési adatokat közölt, fókuszálva az 1 és 3 éves túlélési arányra. 1 évet követően 70–80%, 3 évet követően 60–70% a túlélés. Néhány cikk közöl 5 éves túlélési adatokat, melyek 54% és 66% közötti értékeket mutatnak. A túlélési arány értékelésekor szintén figyelembe kell venni azt a tényt – mely a halálozási adatokat is meghatározza –, hogy az érsebészeti szeptikus fertőzésben szenvedő betegek gyakran rossz általános állapottal és több társbetegséggel rendelkeznek.

A graftinfekció/graftreinfekció rettegett és jelentős szövődmény a szeptikus érsebészetben. Az irodalmi adatok alapján – melyben a szakmai álláspont is egységes – a legjobb újrafertőződési arány autológgraft-pótlással érhető el [9, 26], azonban a korábban részletezett okoknak megfelelően autograft nem érhető el minden esetben, tehát ezekben az esetekben alternatív grafttípus felhasználása szükséges. Műérgraft fertőzése során antibiotikummal impregnált Dacron graft cseréjét követően 11%-os reinfekciós arányról számolnak be, míg homograftcserét követően ugyanez az arány 4%-ra tehető [14]. Az összefoglaló közleményünkben elemzett cikkek alapján a reinfekciós arány minden esetben 10% alattinak bizonyult az aortaszakaszon előforduló fertőzések tekintetében. Több közlemény is arról számol be, hogy egyik vizsgált esetben sem fordult elő reinfekció [17–20]. Összesen 1 publikáció említ magasabb reinfekciós arányt, mely 8,5% volt [6].

A homograft-beültetést követő graft-újrafertőződés minimalizálására a publikációk multimodális sebészeti megközelítést javasolnak: a fertőzött graft teljes eltávolítását, a graft körüli fertőzött szövetek agresszív débridement-jét, a beültetett homograft lehetőség szerinti fedését csepleszlebennyel, majd szoros utánkövetést és mikrobiológiai tenyésztés alapján végzett tartós, célzott antibiotikumterápiát. Ez a kezelési stratégia összhangban áll az ESVS ajánlásaival is. A homograft a biológiai graftok közé sorolható grafttípus, így a műérrel ellentétben a felszíne nem kedvez a patogén mikrobák megtelepedésének, tehát fertőzött környezetbe beültetve a reinfekció kockázata is jóval alacsonyabb lehet.

A vizsgált publikációk egyetértenek abban, hogy a homograft-beültetés megfelelő választás lehet aorta(graft-)fertőzés esetén, mely elfogadható és viszonylag alacsony morbiditási és mortalitási kimenetellel rendelkezik. Csak 1 cikk számol be gyakrabban előforduló, homografttal

összefüggő szövődményekről, mely ezáltal konklúzióként felhívja a figyelmet arra, hogy a homograft-beültetést fenntartásokkal kell kezelni [16]. Általánosságban elmondható, hogy krioprezervált homograft beültetését követően alacsony a graftdegeneráció előfordulása és a grafttal összefüggő szövődmények fellépése. Számos tanulmány említi a homograft-konzerválás, -kezelés (tárolás, előkészítés) és -beültetés során alkalmazott műtéti technika fontosságát a jobb műtéti eredmények elérése érdekében.

Az aortacsoportba sorolt közlemények egységesen említik azt az általános problémát, hogy világszerte alacsony számú homograftbank található, és a legtöbb érsebészeti centrum számára nehéz a homograftok elérhetősége.

Perifériás érinfekció

A perifériás csoportba 4 tanulmányt vontunk be, melyek megfeleltek minden kritériumnak a perifériás vascularis graftinfekció vagy a fertőzött területen végzett érműtét során beültetett krioprezervált homograft témájában.

Az elemzett közlemények közül 1 vizsgálat támaszkodik prospektíven gyűjtött, retrospektíven elemzett adatokra, és 3 tanulmány teljes mértékben retrospektív adatokat közöl. Az összes publikáció egycentrumú vizsgálatot mutat be. A publikációk által vizsgált időszak az 1992 és 2018 közötti időben változik. Az értékelt cikkek összességében 252 beteg eredményeit dolgozzák fel. A közlemények döntő hányada kis betegszámmal dolgozik, 1 vizsgálat közli több, 118 beteg [27] bevonását.

A cardiovascularis betegségek demográfiai adataival összhangban a betegek döntő hányada férfi, átlagéletkoruk 61 és 69 év közé tehető.

Ebben a betegcsoportban az elemzés kritériumainak megfelelő kisszámú közlemény miatt a vizsgálatok eredményeit a következőkben külön tárgyaljuk, majd következtetéseiket együttesen értékeljük.

Lejay és mtsai műérgraft fertőzése miatt beültetett krioprezervált artériás homograftokat vizsgáltak [28]. A beteganyagot két csoportra osztották. Az első csoportba az aortoiliacalis szinten végzett rekonstrukció betegei kerültek (47%), a második csoportba a lágyékszalag alatti, perifériás szinten végzett műtéteket sorolták (53%). Tanulmányuk alapján hangsúlyozzák, hogy az eredményeik nem olyan kedvezőek, mint ahogyan azt remélték. Az 5 éves, homografthoz köthető reoperációs arány az első csoportban 55%, míg a második csoportban 33% volt. Kiemelték, hogy a homografttal kapcsolatos reoperációs adatok ellentmondásosak, ami a krioprezervált artériás homograftkezelés során fellépő technikai különbségeknek tudható be. A homograft konzerválása, a graft kezelése és a műtéti technika befolyásolhatja a hosszú távú operatív eredményeket. Hasonlóképpen ellentmondó eredményekről számolnak be a homograft-reinfekció kapcsán. Hangsúlyozzák, hogy a graftfertőzés diagnosztizálásában és kezelésében nagyon fontos a korai multidiszciplináris döntéshozatal.

Furlough és mtsai krioprezervált artériás homografttal végzett perifériás artériás rekonstrukció eredményeit tanulmányozták infekciós esetekben [29]. Kiemelik, hogy a homograft-beültetés egyik fő előnye az alacsony újrafertőződési arány. Vizsgálatukban a graftreinfekció aránya 3,5% volt, szemben a műérgraftokkal, amelyeknél ez az irodalmi adatok alapján 10% [30, 31]. A graftreinfekció megelőzésére olyan addicionális sebésztechnikai eljárásokat végeztek, mint a szelektív izomlebenyfedés és negatív nyomású sebkezelő rendszer felhelyezése. Az utóbbi módszerek széles körben használatosak a szeptikus érsebészetben, habár hatékonyságukat nagy, randomizált, multicentrikus tanulmányok nem bizonyították. Az ESVS ajánlásai között IIa/C–B ajánlással és evidenciaszinttel rendelkeznek [9]. Kiemelten alacsony amputációs arányról számoltak be, a közleményükben összesen egy, grafthoz köthető amputációt említenek. A homograft-konzerváló eljárásnak és a graftkezelésnek nagy jelentőséget tulajdonítanak, melyek alapvetően meghatározzák a grafthoz köthető szövődmények megjelenését. Két, homografthoz köthető szövődményt írnak le, melyek egyike graftthrombosis, a másik pedig graftdegeneráció volt. Összegezve, eredményeik nagyon kedvezőek, de figyelembe kell venni, hogy következtetéseik kis betegszámon alapulnak.

Mestres és mtsai húszéves tapasztalatról számolnak be a krioprezervált allograftok alkalmazását illetően érsebészeti infekciós esetekben [27]. Közleményükben 38%-os rövid távú mortalitást írnak le, amely magasabb az irodalmi adatokban említett számoknál [31, 32]. Rossz eredményüknek az a magyarázata, hogy a műtétek fele sürgős műtéti indikáció alapján történt, és a betegek jelentős részének olyan további társbetegségei is voltak, amelyek a perioperatív időszakban végzett, nem vascularis beavatkozásokat is igényeltek. Következtetéseikben két fő nehézséget emelnek ki. Az egyik, hogy igen nehéz a homograftokhoz történő hozzájutás, a másik pedig, hogy nincs egységesen elfogadott konszenzus az érfertőzések kezeléséről. Sok vascularis központban nem áll rendelkezésre a homograft, főleg sürgősségi esetekben.

Heinola és mtsai krioprezervált vénás homograftokat vizsgáltak suprainguinalis rekonstrukció során [33]. Tanulmányukban csak a vénás homograftokat vizsgálták, mivel korábbi tapasztalataik alapján az artériás homograftok kapcsán több szövődmény jelentkezett. Akut graftthrombosis és atheroscleroticus degeneráció az artériás homograftok esetén sokkal gyakrabban fordult elő. A kutatócsoport hangsúlyozza, hogy a krioprezervációs eljárás során az endothelfunkció nem marad meg, ezáltal sikerül megelőzni a különböző immunológiai reakciókat, melyek hozzájárulhatnak az érelzáródáshoz. Fontos kiemelni, hogy szintén hangsúlyozzák az alacsony graftreinfekciós arányt, mely eredményeikben 4%. Arra a következtetésre jutottak, hogy a krioprezervált vénás ho-

mograftok a fertőzésekkel szemben ellenálló és biztonságos rekonstrukciós graftanyagnak tűnnek.

Az érhomograftok használata perifériás szeptikus érsebészeti kórképekben még kevésbé vizsgált területe a szeptikus érsebészetnek, a centrális – aorta- – infekciókhoz viszonyítva. Ez abból a tényből is adódhat, hogy a perifériás vascularis infekciók száma nagyobb, mint az aortainfekciók előfordulása, viszont kevésbé vezetnek közvetlenül életet veszélyeztető állapothoz. Több közlemény is említi, hogy a homografthoz való hozzájutás nehézségekbe ütközik, tehát a nagyobb számú, de az aortafertőzéshez viszonyítva általában kevésbé súlyos perifériás ér/graft fertőzések esetén alacsonyabb számban alkalmaz homografthot a szakma, így a vizsgálatukra is kevésbé fókuszál. A vascularis graftinfekció előfordulása femorofemoralis műér-bypassműtéteket követően 2,5% [7], femoropoplitealis műér-bypassműtétek után 2,8% [8]. Ezzel szemben a hasi aortagraft-infekció incidenciája nyitott műtétet követően 0,19%, endovascularis műtétet követően 0,16% [34]. Nehézségként merül fel a cikkek alapján, hogy nincs egységes álláspont a kezelési stratégiát illetően, amit a szakmai irányelv gyengébb alapokon álló ajánlásai is alátámasztanak. Szinte minden közlemény kiemeli a homograft-konzerválás és -tárolás jelentőségét és a precíz perioperatív kezelés fontosságát. A halálozási adatokat nagymértékben befolyásolja a szeptikus érsebészeti kórállapotokban szenvedő betegek rossz általános állapota és sokszor a kísérő betegségek jelenléte.

Következtetés

Az irodalmi áttekintés alapján következtetésként levonható, hogy a fertőzések továbbra is súlyos problémát jelentenek az érsebészet számára. Erős evidencián alapuló ajánlás nem áll rendelkezésünkre, így nincs egységes szakmai álláspont a szeptikus esetek kezelését illetően, különösen a rekonstrukciók során használt graft anyagát és típusát tekintve. Ideális grafttípus/graftanyag az autológ érgraftokon kívül mind a mai napig nem létezik. A krioprezervált homograft elfogadható és ígéretes választás lehet mind aortafertőzések, mind perifériás szeptikus érszövődmények esetén, habár az elemzett vizsgálatok alapján az eredmények ellentmondásosak. A publikációk eredményei alapján a fő következtetések, hogy a krioprezervált homograftoknak alacsony az újrafertőződési arányuk és elfogadható a szerkezeti tartósságuk. A graft-nyitvamaradás eredményei – mely az érsebészeti rekonstrukciók egy kiemelt indikátora –, illetve a mortalitási adatok ellentmondásosak.

A közlemények döntő hányada kiemeli a krioprezervált homograftok konzerválási és kezelési technikáinak fontosságát, amelyek alapvetően meghatározhatják a grafttal kapcsolatos szövődmények előfordulását. Jelenleg nincs egységesen elfogadott tartósítási módszer, mely napjainkban is intenzív kutatás tárgyát képezi. A jelen összefoglaló közlemény egyik fontos megállapítása, hogy a konzerválási-krioprezerválási módszerek és a graftkezelési technika fejlesztése és szabványosítása fontos feladat a jövőre nézve, mellyel tovább javíthatók a homograft-beültetés eredményei.

A homograft-beültetés eredményességét nagyban javítja, hogy az elmúlt évek kutatási eredményei hozzájárultak a graftkezelési és -konzerválási technikák finomodásához, de még mindig sok a megválaszolatlan kérdés.

Az egyik fő nehézség – amelyet a legtöbb közlemény említ – a világszerte nehezen hozzáférhető homograftbankok hiánya és így a homograftok nehéz elérhetősége. Ennek következménye, hogy kevés kutatás folyik a krioprezervált homograftokkal kapcsolatban, és kevés a jó minőségű publikáció, melyekből egyértelmű következtetéseket lehetne levonni. Ráadásul a legtöbb tanulmány kis esetszámú vizsgálatokon alapul, ami megnehezíti a szilárd alapokra helyezhető következtetések levonását. Tanulmányunk felhívja a figyelmet a homograftbankok és ezáltal a tudományos kutatások fejlesztésének fontosságára.

Az egységes szakmai konszenzus létrejöttének további fontos akadálya, hogy a legtöbb tanulmány egy-egy központ kutatási eredményeit foglalja magában. Fontos lenne multicentrikus, randomizált vizsgálatok végzése, melyek segítenék az egységes szakmai álláspont kialakítását, a nemzetközi szakmai irányelvek fejlődését. Összefoglaló tanulmányunkkal hangsúlyozzuk a nemzetközi érsebészeti szakmai együttműködés kialakításának fontosságát ezen az érsebészeti területen.

Anyagi támogatás: A közlemény megírása és a kapcsolódó kutatómunka anyagi támogatásban nem részesült.

Szerzői munkamegosztás: G. D.: A kézirat, a táblázatok és az ábra elkészítése és szerkesztése. G. D., H. L.: Irodalomkutatás. G. D., S. P., Sz. Z.: A tanulmány megtervezése. S. P., Sz. Z., H. L.: A kézirat szakmai véleményezése, végleges formájának kialakítása. A közlemény végleges változatát valamennyi társszerző elolvasta és jóváhagyta.

Érdekeltségek: A szerzőknek nincsenek érdekeltségeik.

Irodalom

[1] Debus ES, Diener H. Reconstructions following graft infection: an unsolved challenge. Eur J Vasc Endovasc Surg. 2017; 53: 151–152.
[2] Lorentzen JE, Nielsen OM, Arendrup H, et al. Vascular graft infection: an analysis of sixty-two graft infections in 2411 consecutively implanted synthetic vascular grafts. Surgery 1985; 98: 81–86.
[3] Kahlberg A, Melissano G, Tshomba Y, et al. Strategies to treat thoracic aortitis and infected aortic grafts. J Cardiovasc Surg. (Torino) 2015; 56: 269–280.
[4] Cronenwett JL, Wayne Johnston K. Rutherford's vascular surgery. Elsevier, Philadelphia, PA, 2014.
[5] Oderich GS, Panneton JM, Bower TC, et al. Infected aortic aneurysms: aggressive presentation, complicated early outcome, but durable results. J Vasc Surg. 2001; 34: 900–908.

[6] Couture T, Gaudric J, Du Montcel ST, et al. Short and mid term outcomes of cryopreserved abdominal aortic allografts used as a substitute for infected prosthetic grafts in 200 patients. Eur J Vasc Endovasc Surg. 2021; 62: 89–97.
[7] Eiberg JP, Røder O, Stahl-Madsen M, et al. Fluoropolymer-coated Dacron versus PTFE grafts for femorofemoral crossover bypass: randomised trial. Eur J Vasc Endovasc Surg. 2006; 32: 431–438.
[8] Exton RJ, Galland RB. Major groin complications following the use of synthetic grafts. Eur J Vasc Endovasc Surg. 2007; 34: 188–190.
[9] Chakfé N, Diener H, Lejay A, et al. Editor's choice – European Society for Vascular Surgery (ESVS) 2020 clinical practice guidelines on the management of vascular graft and endograft infections. Eur J Vasc Endovasc Surg. 2020; 59: 339–384. Erratum: Eur J Vasc Endovasc Surg. 2020; 60: 958.
[10] Gross RE. Treatment of certain aortic coarctations by homologous grafts; a report of nineteen cases. Ann Surg. 1951; 134: 753–768.
[11] DeBakey ME, Creech O Jr., Cooley DA. Occlusive disease of the aorta and its treatment by resection and homograft replacement. Ann Surg. 1954; 140: 290–310.
[12] Pukacki F, Jankowski T, Gabriel M, et al. The mechanical properties of fresh and cryopreserved arterial homografts. Eur J Vasc Endovasc Surg. 2000; 20: 21–24.
[13] Ben Ahmed S, Louvancourt A, Daniel G, et al. Cryopreserved arterial allografts for in situ reconstruction of abdominal aortic native or secondary graft infection. J Vasc Surg. 2018; 67: 468–477.
[14] Minga Lowampa E, Holemans C, Stiennon L, et al. Late fate of cryopreserved arterial allografts. Eur J Vasc Endovasc Surg. 2016; 52: 696–702.
[15] Masabni K, Weaver MR, Kandagatla P, et al. Cryopreserved allograft in the management of native and prosthetic aortic infections. Ann Vasc Surg. 2019; 56: 1–10.
[16] Weiss S, Bachofen B, Widmer MK, et al. Long-term results of cryopreserved allografts in aortoiliac graft infections. J Vasc Surg. 2021; 74: 268–275.
[17] Heo SH, Kim YW, Woo SY, et al. Recent results of in situ abdominal aortic reconstruction with cryopreserved arterial allograft. Eur J Vasc Endovasc Surg. 2017; 53: 158–167.
[18] Bossi M, Tozzi M, Franchin M, et al. Cryopreserved human allografts for the reconstruction of aortic and peripheral prosthetic graft infection. Ann Vasc Dis. 2017; 10: 391–397.
[19] Arasu R, Campbell I, Cartmill A, et al. Management of primary mycotic aneurysms and prosthetic graft infections: an 8-year experience with in-situ cryopreserved allograft reconstruction. ANZ J Surg. 2020; 90: 1716–1720.
[20] Couture T, Gaudric J, Davaine JM, et al. Results of cryopreserved arterial allograft replacement for thoracic and thoracoabdominal aortic infections. J Vasc Surg. 2021; 73: 626–634.
[21] Coselli JS, Köksoy C, LeMaire SA. Management of thoracic aortic graft infections. Ann Thorac Surg. 1999; 67: 1990–1993. Discussion: 1997–1998.
[22] Batt M, Jean-Baptiste E, O'Connor S, et al. Early and late results of contemporary management of 37 secondary aortoenteric fistulae. Eur J Vasc Endovasc Surg. 2011; 41: 748–757.
[23] Gournier JP, Adham M, Favre JP, et al. Cryopreserved arterial homografts: preliminary study. Ann Vasc Surg. 1993; 7: 503–511.
[24] Vogt PR. Arterial allografts in treating aortic graft infections: something old, something new. Semin Vasc Surg. 2011; 24: 227–233.
[25] Rosset E, Friggi A, Novakovitch G, et al. Effects of cryopreservation on the viscoelastic properties of human arteries. Ann Vasc Surg. 1996; 10: 262–272.
[26] Dorweiler B, Neufang A, Chaban R, et al. Use and durability of femoral vein for autologous reconstruction with infection of the aortoiliofemoral axis. J Vasc Surg. 2014; 59: 675–683.
[27] Mestres CA, Quintana E, Kopjar T, et al. Twenty-year experience with cryopreserved arterial allografts for vascular infections. Eur J Cardiothorac Surg. 2019; 55: 358–365.
[28] Lejay A, Delay C, Girsowicz E, et al. Cryopreserved cadaveric arterial allograft for arterial reconstruction in patients with prosthetic infection. Eur J Vasc Endovasc Surg. 2017; 54: 636–644.
[29] Furlough CL, Jain AK, Ho KJ, et al. Peripheral artery reconstructions using cryopreserved arterial allografts in infected fields. J Vasc Surg. 2019; 70: 562–568.
[30] Brown KE, Heyer K, Rodriguez H, et al. Arterial reconstruction with cryopreserved human allografts in the setting of infection: a single-center experience with midterm follow-up. J Vasc Surg. 2009; 49: 660–666.
[31] Vogt PR, Brunner-La Rocca HP, Carrel T, et al. Cryopreserved arterial allografts in the treatment of major vascular infection: a comparison with conventional surgical techniques. J Thorac Cardiovasc Surg. 1998; 116: 965–972.
[32] Kieffer E, Gomes D, Chiche L, et al. Allograft replacement for infrarenal aortic graft infection: early and late results in 179 patients. J Vasc Surg. 2004; 39: 1009–1017.
[33] Heinola I, Kantonen I, Mattila I, et al. Cryopreserved venous allografts in supra-inguinal reconstructions: a single centre experience. Eur J Vasc Endovasc Surg. 2019; 58: 912–919.
[34] Vogel TR, Symons R, Flum DR. The incidence and factors associated with graft infection after aortic aneurysm repair. J Vasc Surg. 2008; 47: 264–269.

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