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Morphologic comparison of blood vessels used for coronary artery bypass surgery

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ABSTRACT

Background: Aim of this study was to evaluate morphologic features of healthy saphenous vein and internal thoracic artery used in coronary artery bypass surgery and compare results.

Materials and methods: Ten specimens of saphenous veins and ten of internal thoracic arteries used for coronary artery bypass graft were obtained from 20 patients. Histological routine and immunohistochemical staining was performed with: endothelin (ET), tissue inhibitor of metalloproteinase 2 (TIMP2), metallomembranoproteinase 2 (MMP2), transforming growth factor beta (TGF β), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), protein gene product 9.5 (PGP9.5), vascular cell adhesion molecule (VCAM), intercellular adhesion molecule (ICAM). A semiquantitative evaluation method was used.

Results: There was found: moderate positive endothelin-containing cells in both blood vessel types; moderate positive MMP2 cells and moderate to numerous positive TIMP2 cells in veins. In arteries – occasionally marked positive MMP2 cells and negative TIMP2; moderate to numerous positive VEGF endothelial cells on small blood vessels in vein wall and occasional in artery wall; numerous TGFβ structures in veins and abundance of VCAM, ICAM positive cells, few in arteries; few HGF positive structures in veins, negative in arteries; In veins few PGP9,5 positive nerve fibres, in arteries - moderate. Moderate TUNEL reaction positive apoptotic cells in veins and few to moderate in arteries.

Conclusions: Vsaphena magna grafts are characterized by more intensive modelation plasticity. Number of VEGF, VCAM and ICAM found in v.saphena magna proves the
possible tendency of graft failure on basis of local blood supply intensification. Appearance of endothelin positive cells indicate the similar homeostasis condition in endotheliocytes in both – vein and artery grafts.

**Key words: saphenous vein, internal thoracic artery, immunohistochemistry**

**INTRODUCTION**

Arteries (*arteria thoracica interna*) and veins (*vena Saphena magna*) are often used for coronary artery bypass surgery. Morphologic architecture of relatively healthy blood vessel wall is known however qualitative data on morphologic features and possibly changes in blood vessels actually used in coronary artery bypass surgery is lacking. Therefore, it was important to conduct morphologic study and comparison of both blood vessel types that might lead us to reasons that influence grafts post-operative sustainability.

There are differences and similarities in morphologic characterisation of blood vessel wall; it is composed of three layers, the intima, media, and adventitia. In arteries the internal elastic layer further separates the intima and media, and the external elastic layer separates the media and adventitia [1, 2].

The intimal luminal surface is lined by the endothelium which is continuous layer of flat polygonal endothelial cells in direct contact with blood flow. In venous system these cells produce vasorelaxants, such as prostacyclin and nitric oxide, that prevent platelet activation, adhesion, and aggregation. Nitric oxide also negatively affects the expression of chemical mediator secretion and inflammatory cell adhesion molecules including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) [1, 3, 4].

In arteries, the thickness of the intima increases with age (phlebosclerosis). The earliest signs of atherosclerosis appear in the areas of sheer stress and increased intimal thickness [4, 5, 6]. In case of inflammation, the glycocalyx is sheared off, permitting the attachment of leukocytes and the transport of water from microvessels, and possibly initiating the development of atherosclerotic lesions [7].

Intimal layer in arteries ends with internal elastic lamina that may function as a barrier to macromolecular accumulation in the vascular wall. Structural defects within the internal elastic lamina are directly implicated in the onset of intimal thickening in human arteries [8].

Media is a porous heterogeneous medium consisting of an extracellular matrix with embedded smooth muscle cells. In larger veins, such as the saphenous vein, there are coarse
bundles of irregular muscle, partially organized into longitudinal and circular layers [1, 5, 9]. Arteries are classified as either elastic or muscular according to the proportion of cellular and fibrous components in this layer or transitional if they have features of both elastic and muscular arteries [10]. The internal thoracic artery is an artery of the transitional (mixed) type. The nonfenestrated internal elastic lamina of the internal thoracic artery may inhibit cellular migration, perhaps preventing the initiation of intimal hyperplasia and the initiation of atherosclerosis [11]. Nonparallel branching elastin strands in media increase the capacity to change diameter under neurohumoral stimulation. However, in general it is less well developed in veins than that of the arterial system. This thin media in veins may contribute to the development of varicosities in lower extremities [1, 5, 9]. The tunica media contains extracellular connective elements (elastin, collagen types I and III, proteoglycans, and glycosaminoglycans), transforming growth factor-β downregulates their mitogenesis and stabilizes the extracellular matrix against smooth muscle cell migration. In addition, heparin and heparin-like molecules neutralize fibroblast growth factor to downregulate cell proliferation. This process is important because these factors keep the normal vessel wall in a state of low cell turnover with low rates of proliferation and apoptosis. Injury or changes to the environment, as when veins are exposed to arterial flow, can increase rates of proliferation or apoptosis [1, 12, 13].

Adventitia extends from the external elastic lamina to an ill-defined boundary usually contiguous with the perivascular connective tissue. The adventitia varies in thickness and organization. It is generally the thickest layer in large veins. Bundles of longitudinally oriented smooth muscle cells are interspersed with collagen and elastic fibres in this layer. Compared with vasa vasorum of corresponding arteries, vasa vasorum are much more extensive in venous adventitia and penetrate into deeper regions of the adventitia as well. Lower oxygen tension in venous blood is a possible explanation for this phenomenon. In thick-walled arteries, mural stresses and deformations may affect the vasa vasorum [1, 3, 13].

In general, veins have thinner walls than their corresponding arteries because their cellular and fibrous components are typically more limited than those of the arterial system. This wall composition leads to the properties of veins as “capacitance vessels” and arteries as “resistance vessels.” It should be noted that the composition of vein walls is also different, with a relative abundance of collagen fibres, particularly in large veins, and a relative paucity of elastic fibres—as might be assumed from the diminished internal and external elastic
laminae. Researchers increasingly have recognized the vein graft tunica adventitia as an important repository of progenitor cells which subsequently can migrate and proliferate; and as a source of vascular wall inflammatory cells, cytokines, and chemokines [1, 14, 15, 16].

**Specific factors for blood vessel wall**

Endothelin-1 (ET-1) is the endogenous agonist for ET receptors [17]. ET-1 carries out its effects through two types of membrane–G protein–coupled receptors (ETA and ETB). ETB receptor function seems to differ between a similarly sized arterial and venous pair [18]. Many important functions are mediated by the activation of these receptors, such as cardiovascular remodelling, vasoconstriction, cell proliferation and differentiation, production of extracellular matrix, and water and sodium secretion control. ET receptors can be found on vascular smooth muscle cells, adventitial fibroblasts, and mostly endothelial cells [19, 20, 21].

Matrix metalloproteinases (MMPs) are a family of endopeptidases whose primary function is the cleavage and degradation of extracellular matrix components are involved in wound healing, tissue repair and remodelling in response to injury and vasoconstriction. It is believed that MMPs are induced in the vessel wall in response to increased blood flow and are involved significantly in arterial wall remodelling. MMPs are produced by smooth muscle cells and macrophages to actively modify the matrix. Activities of those proteins are regulated by tissue inhibitors of metalloproteinases (TIMPs). In addition to an inhibitory role against metalloproteinases, they also directly suppress the proliferation of endothelial cells maintaining tissue homeostasis by suppressing the proliferation of quiescent tissues in response to angiogenic factors, and by inhibiting protease activity in tissues undergoing remodelling of the extracellular matrix [1, 21, 22, 23].

Transforming growth factor beta (TGFβ) encoded protein regulates cell proliferation, differentiation and growth, and can modulate expression and activation of other growth factors. TGFβ critically regulates the development of neointima formation following vascular injury [24, 25, 26, 27].

Hepatocyte growth factor (HGF) is an angiogenic, cardioprotective factor important for tissue and vascular repair. High levels of HGF are associated with chronic inflammatory diseases, such as coronary artery disease (CAD) and are suggested as a marker of the ongoing atherosclerotic event in patients with CAD. Pleiotropic growth factor has potential angiogenic, anti-apoptotic, antifibrotic and anti-inflammatory benefits [26, 27].
Vascular endothelial growth factor (VEGF) is a secreted glycoprotein believed to be a multifunctional regulator of endothelial cell growth whose biological activities are mediated via receptors which are expressed predominantly on vascular endothelial cells, induces proliferation and migration of vascular endothelial cells and is essential for both physiological and pathological angiogenesis. Stretch-induced modulation of genes involved in myogenic differentiation contributes to the vascular remodelling that underlies pathologic complications, such as neointima development and atherosclerosis, of the vein grafts [9, 28, 29, 30].

Protein Gene Product 9.5 (PGP 9.5) is also known as ubiquitin C-terminal hydrolase 1 (UCHL-1). It is highly specific to be expressed in neurons and in cells of the diffuse neuroendocrine system. Originally isolated as a neuron-specific protein, also play important roles in the nonlysosomal proteolytic pathway. In the vascular system PGP 9.5-immunoreactivity occurs in an extensive plexus of fine perivascular nerve fibres and fascicles running around and along both arteries and veins, mainly at the adventitial-medial border [31, 32].

Vascular cell adhesion molecule 1 (VCAM-1) encodes a cell surface protein expressed by cytokine-activated endothelium. It is not expressed under baseline conditions but is rapidly induced by proatherosclerotic conditions [33].

Intercellular adhesion molecule 1 (ICAM-1) is a member of the immunoglobulin superfamily of adhesion receptors. It is a cell surface protein which is typically expressed on endothelial cells and cells of the immune system and are involved in the binding of a cell to another cell or to the extracellular matrix. Levels of endothelial ICAM-1 expression greatly increase after stimulation by cytokines (e.g., IL-1, TNF-α, IFN-γ), or bacterial endotoxin. They have roles in cell proliferation, differentiation, motility, trafficking, apoptosis and tissue architecture [34, 35].

Increased shear stress upregulates ICAM-1, steady shear stress upregulates VCAM-1, and oscillatory stress decreases levels of both molecules [1].

Terminal deoxynucleotidyl transferase (TdT) dUTP Nick-End Labeling (TUNEL) assay has been designed to detect apoptotic cells that undergo extensive DNA degradation during the late stages of apoptosis [36].

Aim of this study was to evaluate morphologic features of healthy saphenous vein and healthy internal thoracic artery used in coronary artery bypass surgery and compare results.
MATERIALS AND METHODS

Twenty blood vessel samples were acquired in Pauls Stradins clinical university hospital, Heart surgery centre from patients who were admitted to the hospital for coronary artery bypass surgery. Patients records were retrieved and analyzed according to a predetermined protocol.

Ten specimens of saphenous veins used for coronary artery bypass graft were obtained from 10 patients (seven males and three females) age ranged from 55 to 81 years old and ten specimens of internal thoracic arteries used for coronary artery bypass graft were obtained from 10 patients (eight males and two females) age ranged from 54 to 75 years old.

Hospitals Ethics committee and local Committee of Ethics at Riga Stradins University approved the research on 22nd February of 2018. Before the surgery all patients were informed about the procedure of collecting the sample, possible risks and agreed on the procedure by signing patents consent.

Data on vascular risk factors (age, sex, BMI, smoking habits, physical activities, pregnancies, use of hormonal drugs) were collected from all patients.

Methods

During the surgery after obtaining a blood vessel used as bypass, a size of 2-3 cm tissue samples from that blood vessel were removed for the study and taken to the laboratory.

Tissue samples delivered to the laboratory were immersed for 24 hours in Stefanini liquid – a mixture consisting of 2% formaldehyde and 0.2% picric acid in 0.1-M phosphate buffer (pH 7.2) for fixation [37]. Afterwards they were washed for 12 hours in phosphate buffer (pH 7). Then, tissue samples were embedded in paraffin and using microtome cut into 3-4 µm thick sections. Xylene was used to clear off paraffin, and alcohol 96° to dehydrate tissue sections. The slides were prepared for histological routine staining and immunohistochemical staining using the HiDef Detection™ HRP Polymer System to identify the following markers in tissue samples: ET (mouse, ab-2786, 1:250, Abcam), MMP2 (mouse, sc-53630, 1:100, Santa Cruz Biotechnology, Inc), TIMP2 (mouse, sc-21735, 1:200, Santa Cruz Biotechnology, Inc), TGF β (rabbit, sc-82, 1:100, Santa Cruz Biotechnology, Inc), HGF (goat, f-21, 1:300, RD Systems), VEGF (rabbit, orv-191500, 1:100, Biorbyt), PGP9.5 (rabbit, 439273a, 1:200, Invitrogen), VCAM (goat, cd-106, 1:200, RD Systems), ICAM (goat, cd-54, 1:300, RD Systems).
Next step included rinsing of tissue samples in wash buffer (TRIS) (Lot 0713513, Diapath S.p.A., Italy) twice for five minutes, then placing them in a microwave oven for up to 20 minutes in boiling EDTA buffer (Lot 0713311, Diapath S.p.A., Italy) and then cooling down until 65°C (approximately 20 minutes). The specimen was placed in a TRIS wash buffer, and blocking with peroxidase block (Lot 1213603A, Cell Marque, USA) was performed for ten minutes. After rinsing twice for five minutes it was once more rinsed in TRIS for five minutes.

Different staining systems were used taking into account the origin of the antibodies. When obtained from goat, LSAB system (Santa Cruz Biotechnology, Inc., USA) was used. Primary antibody was introduced for two hours. Before and after introducing to secondary antibody (biotin) for 30 minutes, it was washed in TRIS for five minutes. Next step was introducing with horseradish peroxidase-streptavidin complex for 30 minutes followed by washing with TRIS for five more minutes.

When obtained from mouse or rabbit, the EnVision staining system (Lot 1528902C, Cell Marque, USA) was used. Primary antibody was introduced for one hour. Before and after the En Vision+/binding for 30 minutes, it was washed in TRIS twice, each for five minutes.

To stain any of these tissues, they were covered with 3,3′-diaminodbenzidine sensitive colorimetric substrate and left in room temperature for ten minutes and the washed in distilled water for five minutes. At this point positive structures stained brown. To stain negative structures haematoxylin (Mayer’s haematoxylin, Bio Optica Milano S.p.A., Italy) was used for two minutes.

To detect and quantify apoptotic cell death at single cell level in cells and tissues in situ Cell Detection Kit was used. Dewaxation and rehydration of paraffin embedded tissue according to standard protocol by heating at 60°C followed by washing in xylene and rehydration through a graded series of ethanol and double distilled water. In next phase incubation of tissue sections for 15-30 minutes at +21°C to +37°C with Proteinase K takes place. TUNEL mixture is prepared immediately before use and is kept on ice until use. Meanwhile slides are rinsed twice with phosphate buffered saline (PBS) and area around the sample is dried. TUNEL reaction mixture is added on the sample. It is covered with a lid and left for incubation for 60 minutes at +37°C in a humidified atmosphere in the dark. Reaction is finished with rinsing it with PBS.
For the analysis of the positive structures detected by immunohistochemistry, a semiquantitative evaluation method was used [38]. The designations were as follows: 0 – negative reaction; 0/+ - occasionally marked structures in the view field; + - a few positive structures in the view field; +/+ - a few to moderate number of positive structures in the view field; ++ - a moderate number of positive structures found in the view field; ++/+++ - moderate to numerous positive structures; +++ - numerous positive structures in the view field; ++++ - abundance of positive structures in view field. Ten view fields for each slide were analyzed at magnification of x200 by semiquantitative method. The evaluation was performed with Leica microscope by two independent researchers with following comparison of the results later. Median of the results for each slide was then processed further. IBM SPSS program was used for statistical analysis. Spearman correlation test was performed for finding correlations. For statistical comparison Mann-Whitney U test was performed.

RESULTS

Routine morphology

Vein wall as well as arterial wall was composed of three tunicae evaluated in routine staining. The first tunica from the luminal side was the intima, that was fully or partially covered with endothelial cells. Tunica intima in all vein specimens was thinnest from all the layers (Figure 1-A) with one exception, where tunica intima was thick, wider than tunica media (Figure 1-B). Even though arterial specimens also showed all three tunicae (Fig. 1-C) in half of the specimens tunica intima revealed atheromatous deposits (Figure 1-D). The media in veins was thick and filled with smooth muscle cells with one exception, where tunica media was thin. Tunica media in all arteries was thick and filled with smooth muscle cells. Tunica adventitia in both arteries and veins consisted of bundles of collagen fibres, fibroblasts, vasa vasorum from which few were sclerotic in all vein specimens and only in two artery specimens.

Immunohistochemistry results

Moderate (++) positive endothelin-containing endothelial cells were found in veins as well as in arteries (Table 1).

Moderate (++) positive MMP2 endothelial cells, smooth muscle cells and fibroblasts (Figure 2-A) and variable - mainly moderate to numerous (+/+++) positive TIMP2
endothelial cells, smooth muscle cells and fibroblasts (Figure 2-B) were found in veins. Despite variability, in all cases positive structures were evaluated as equal with both MMP2 and TIMP2 with one exception, where MMP2 positive structures were evaluated as moderate (++), but TIMP2 positive structures were evaluated as few (+). Comparing to arteries – variable, but mostly occasionally marked (0/+) positive MMP2 endothelial cells, smooth muscle cells and fibroblasts (Figure 2-C) and variable, but mostly negative (0) TIMP2 reaction on endothelial cells, smooth muscle cells and fibroblasts (Figure 2-D) were found. In all cases there were more positive structures for MMP2 than TIMP2 with one exception, where they were evaluated as equal.

Moderate to numerous (++/+++) positive VEGF endothelial cells were found on small blood vessels in vein wall (Figure 3-A), however only occasional (0/+ ) positive VEGF endothelial cells were found on small blood vessels in artery wall (Figure 3-B).

All vein specimens were rich with TGFβ, VCAM and ICAM: numerous (+++) TGFβ structures, abundance (++++) of VCAM and ICAM positive endothelial cells were also found. All arterial specimens had few (+) TGFβ, VCAM and ICAM positive structures.

HGF expression was not characteristic in veins nor in arteries: only few (+) positive structures were found in tunica intima of veins and in arteries no (0) positive structures were found.

In tunica adventitia of veins few (+) PGP9.5 positive nerve fibres were found, comparing to the same layer of arteries it was slightly more innervated, where moderate (++) PGP9.5 positive nerve fibres were found.

Moderate (++) TUNEL reaction positive apoptotic cells were found in veins and few to moderate (+/++) TUNEL reaction positive apoptotic cells were found in arteries.

There was found significant difference in number of positive MMP2, TIMP2, TGFβ, VCAM, ICAM and HGF-containing structures between arteries and veins (U=0.000, p<0.001).

There was also found significant difference in number of positive VEGF-containing structures between arteries and veins (U=4.000, p<0.001) and significant difference in number of TUNEL reaction positive apoptotic cells between arteries and veins (U=12.000, p=0.003).

Results for positive structures with endothelin and PGP9.5 did not show significant difference between arteries and veins.
There was found significant correlation between number of HGF positive structures and the number of TIMP2 positive structures in veins. The correlation level is medium positive (r=0.731; p=0.016).

**DISCUSSION**

Saphenous vein grafts remain the most commonly implanted surgical conduits during coronary artery bypass grafting (CABG), yet they are prone to accelerated atherosclerosis and subsequent failure [39]. Internal thoracic artery grafts exhibit a striking absence of occlusive lesions, and has far superior patency rates compared to the saphenous veins following CAGB surgery. The reason for these unique artery qualities has not been clearly determined, but is most likely multifactorial [40].

Even though our study showed the amount of endothelin expressed in veins and arteries were similar, reasons behind this result may be different. As saphenous vein grafts are exposed to arterial blood flow and pressure, they might generally exhibit unfavourable vascular remodelling afterwards [40, 41]. Studies have suggested that competitive flow is an important factor in early internal thoracic artery graft failure. Flow competition from minimally diseased native coronary vessels has been implicated in the failure of these grafts, but it was not thought to affect saphenous vein graft patency [42, 43]. In a study (Meng, 2013) that established a swine model of coronary artery bypass grafting with a left internal mammary artery graft to the left anterior descending coronary artery, in order to investigate the influence of competitive flow on left internal mammary artery graft flow, it was tested that plasma concentration of the endothelin in left internal mammary artery after grafting was significantly higher than that before grafting [43]. This shows that changes of blood flow in both types of blood vessels used as coronary artery bypass grafts impact the patency of a graft.

Expression of MMPs and TIMPs should always be evaluated together as MMPs are involved in tissue repair and remodelling, but activities of those proteins are regulated by TIMPs by inhibition and also suppression of the proliferation of endothelial cells maintaining tissue homeostasis [22, 23]. The extra cellular matrix is a dynamic structure that requires constant synthesis and degradation by MMPs [44, 45]. We found that in saphenous vein grafts the number of MMP and TIMP2 positive structures was moderate to numerous and mostly equal and this might be the basic level of expression of these factors in the vein. Or this could
be explained by sudden change in blood flow and pressure requiring active change of extra cellular matrix as normally venous blood pressure is lower and does not require such levels of these proteins. In internal thoracic arteries both proteins were found to be variable, but MMP2 were mostly occasionally marked positive, but TIMP2 mostly negative. To provide constant change of extra cellular matrix in higher blood pressure, expression of these proteins in internal thoracic artery grafts should be expected high to maintain a normal architecture of an arterial wall. Since in half of arterial specimen an atheromatous plaque was observed in routine staining that indicate a failure of maintaining such architecture. Furthermore, our study shows almost an absence of TIMP2 that normally would supress enzymes that degrade internal elastic membrane, resulting in vascular smooth muscle cells and collagen fibres entering tunica intima that was described as intimal hyperplasia.

We found numerous TGFβ positive structures in saphenous vein wall, but all arterial specimens had few TGFβ positive structures. TGFβ - a signaling family with essential functions in the physiologic homeostasis of the vascular endothelium and smooth muscle, as well as other tissues. TGFβ family includes a structurally diverse set of more than 33 cytokines that regulate the differentiation, proliferation, migration and survival of diverse cell types [46]. As Shi-Min Yuan et al. (2011) states that severe vascular wall degeneration and collagen deposition together with overexpressed TGF-β signalling cytokines may provide preliminary evidence for the failure of the saphenous vein grafts [47]. This might indicate internal thoracic artery grafts longer durability.

Allen S. et al. (1998) states that adhesion molecules can increase the binding of leukocytes to the vascular endothelium, which is thought to be an important factor in the early development of atherosclerosis [48]. Also harvesting the vein graft is known to activate the graft endothelium [49]. Our study showed abundance of VCAM and ICAM positive endothelial cells in saphenous vein grafts, although arterial specimens had only few VCAM and ICAM positive structures, which implies higher possibility for vein graft failure.

Physiological remodelling of blood vessels before and after birth has been shown to be the result of a balance between apoptosis and cell proliferation. The role of apoptosis has been investigated in vessel remodelling that occurs as arteries adapt to changes in cardiovascular function after birth [50]. Cho et al. (1995) have demonstrated that apoptosis significantly contributes to postpartum arterial remodelling and that changes in cell death rates alone may be sufficient to induce profound changes in the vessel wall mass [51].
Use of TUNEL reaction for detection of apoptotic cardiomyocytes in patients who underwent coronary artery bypass surgery had been done before by Kovacević M. and colleagues (2007) [52]. However, apoptosis in myocytes of grafts haven’t been researched. Our study shows that there is significant difference in number of apoptotic cells between arteries and veins. As moderate TUNEL reaction positive apoptotic cells were found in veins and less TUNEL reaction positive apoptotic cells were found in arteries, what suggests higher plasticity of vein wall in comparison to arterial one.

VEGF plays a fundamental role in physiological and pathological angiogenesis and also induces endothelium derived vasorelaxation [53]. More positive VEGF endothelial cells were found on small blood vessels in vein wall than on small blood vessels in artery wall. This might indicate that more active angiogenesis takes place in the wall of a vein graft.

As it is known that extensive plexus of fine perivascular nerve fibres and fascicles are positioned around and along both arteries and veins, mainly at the adventitial-medial border [31]. This shows that only the fascicles of nerve fibres enter the wall of a blood vessel. That explains why we found only few PGP9,5 positive nerve fibres in veins, and the same layer of arteries was slightly more innervated. These results did not significantly differ between arteries and veins.

CONCLUSIONS

Vsaphena magna grafts are characterized by relatively higher number of MMP2, TIMP2, HGF and TGFβ positive structures than the artery graft that suggest more seemingly the intensification of modelation plasticity of the vein grafts.

Notably higher expression of VEGF, VCAM and ICAM in v.saphena magna, but not a.thoracica interna graft proves the possible tendency of graft failure on basis of local blood supply intensification.

A.thoracica interna graft is characterized by moderate neuropeptide-containing innervation what is much more indistinct in the v.saphena magna graft, while similar appearance of endothelin positive and apoptotic cells indicate the similar homeostasis condition in endotheliocytes and equal expression of programmed cell death in both – vein and artery grafts.

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**Table 1.** Shows median of evaluation results for IHC.

| Blood vessel                  | Endothelin | MMP 2  | TIMP 2  | TGF β | HG F | VEGF | PGP9.5 | VCA M | ICA M | TUNEL |
|-------------------------------|------------|--------|---------|-------|------|------|--------|-------|-------|-------|
| Vena Saphena magna            | ++         | ++     | +++/+   | +++   | +    | ++++ | +++    | ++++  | ++    |
| Arteria thoracica interna     | ++         | 0/+    | 0       | +     | 0    | 0/+  | +++    | +     | +     | +++   |

**Figure 1.** Note classic picture of *v.saphena magna* wall with three intact layers–tunica intima, tunica media and tunica adventitia (A), with one exception (B) of hyperplasia of tunica intima (arrow), where it is thick and wider than tunica media. Haematoxylin and eosin, X 100. In comparison note a classic picture of *a.thoracica interna* wall with three layers–tunica intima, tunica media and tunica adventitia (C) and atheromatous deposit in tunica intima (arrow), which was found in half of the *a.thoracica interna* specimens (D). Haematoxylin and eosin, X 200

**Figure 2.** Throughout all layers of *v.saphena magna* there can be seen moderate (++) MMP2 positive endothelial cells, smooth muscle cells and fibroblasts (A), MMP2 IHC, X 100 and numerous (+++) TIMP2 positive endothelial cells, smooth muscle cells and fibroblasts (B), TIMP2 IHC, X 200. In comparison throughout all layers of *a.thoracica interna* there can be seen few (+) MMP2 positive endothelial cells, smooth muscle cells and fibroblasts (C), MMP2 IHC, X 200 and occasionally marked (0+/+) TIMP2 positive endothelial cells, smooth muscle cells and fibroblasts (D), TIMP2 IHC, X 200.

**Figure 3.** Note adventitial layer of *v.saphena magna* with abundance (++++) of VEGF positive endothelial cells found in small blood vessels (A) in comparison with adventitial layer of *a.thoracica interna* with few (+) VEGF positive endothelial cells found in small blood vessels (B). VEGF IHC, X 400
