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Cytokeratin 8 in venous grafts: A factor of unfavorable long-term prognosis in coronary artery bypass grafting patients

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Abstract

Background: *Smooth muscle cells, present in the saphenous vein (SV) tunica media, may contribute to late occlusion of venous aortocoronary grafts. The aim of present study was to evaluate expression of selected cytoskeletal proteins in tunica media of SV grafts obtained from patients undergoing coronary artery bypass grafting (CABG) and correlate procured results to late venous graft failure observed in these patients.*

Methods: *The study involved 218 patients (mean age of 62.5 ± 8.7 years) who underwent primary isolated CABG with the use of at least one venous aortocoronary bypass graft. Expressions of α-smooth muscle actin, smooth muscle-myosin heavy chain, calponin and cytokeratin 8 in SV wall were estimated by means of immunohistochemistry. The primary clinical endpoint was defined as the presence of any coronary artery disease (CAD) progression symptom while angiographic one as significant stenosis in the venous graft.*

Results: *Thirty-eight (18.1%) patients have reached the primary clinical endpoint. Freedom from clinical CAD deterioration was 0.95 ± 0.02, 0.87 ± 0.03 and 0.83 ± 0.04, for 12-, 24-, 36-month follow-up, respectively. Forty-four study participants have reached the angiographic endpoint. Multivariate logistic regression analysis revealed an increased expression of cytokeratin 8 accompanied by calponin underexpression in SV tunica media were independent risk factors for venous graft failure.*

Conclusions: *An increased expression of cytokeratin 8 and weak of calponin in tunica media of SV grafts might be useful markers of unfavorable long-term prognosis in CABG patients. In the future, assessment of their expression may enable to select the most appropriate candidates for SV grafts.* (Cardiol J 2013; 20, 6: 583–591)

Key words: coronary artery bypass grafting, saphenous vein, calponin, cytokeratin 8, outcome

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Introduction

Coronary artery bypass grafting (CABG) is a method of choice in treatment of patients with severe multi-vessel coronary artery disease (CAD) [1]. In majority of cases, it significantly improves the quality of life, alleviating the patient from coronary symptoms [2, 3]. A long-term outcome in CABG patients depends, however, on graft patency rate as well as atherosclerotic progression in native coronary arteries. It is commonly accepted arterial grafts, particularly left internal mammary artery implanted to the left anterior descending artery, result in higher late patency rates as compared to the venous conduits [4]. Unfortunately, the adequate arterial conduits are not always available, thus more easily harvested saphenous vein (SV) grafts are still widely used.

There are at least 3 principal mechanisms leading to venous graft failure. They include thrombosis, intimal hyperplasia and accelerated atherosclerosis [5]. Among others, vascular smooth muscle cells (SMCs) are believed to play a crucial role in pathogenesis of the late graft occlusion [6]. Secondary to locally distributed stimulating agents, they are able to migrate through internal elastic lamina to tunica intima, proliferate and secrete extracellular matrix [7]. It results in progressive neointimal formation that might lead to the decisive narrowing of the grafts and clinically CAD progression or angina recurrence and, in some cases, acute coronary syndromes (ACSs) [8].

On the other hand, the populations of both arterial and venous SMCs were reported to be heterogeneous and manifest at least 2 phenotypes: elongated/spindle-like cells or epithelioid/rhomboid shaped cells that expressed a variant proliferative and chemotactic activity [9, 10]. Subtypes of SMCs were also characterized by altered expression of smooth muscle proteins such as smooth muscle myosin heavy chain (SM-MHC), calponin (CALP) and cytokeratin 8 (CK8) that were associated with various stages of cellular differentiation [10]. SM-MHC and CALP are expressed predominantly in the mature SMCs while CK8 expression is typical for fetal and poorly differentiated cells [10–12].

In line with the above, the aim of the present study was to evaluate expression of selected cytoskeletal proteins in tunica media of SV grafts obtained from CABG patients and correlate procured results to late venous graft fate observed in these patients.

Table 1. Pre- and intraoperative clinical data

Parameters	Patients (n = 218)
Age	62.5 ± 8.7
Gender (male/female)	168/50
Body mass index (BMI)	29.3 ± 4.1
Obesity (BMI > 30)	92 (42.2%)
Coronary artery disease:	
Stable angina	180 (82.6%)
Unstable angina	38 (17.4%)
History of infarct	140 (64.2%)
Previous PCI	66 (30.3%)
Arterial hypertension	162 (74.3%)
Diabetes mellitus	98 (45.4)
Hyperlipidemia	92 (42.4%)
Peripheral vascular disease	50 (22.9%)
Neurological events	18 (8.3%)
CCABG/OPCAB	130/88
Number of grafts/patient	2.6 ± 0.6
Grafts type (distal anastomoses):	
Arterial (LIMA, RIMA, RA)	235
Venous	349
Complete revascularization*	154 (70.6%)

*Means all severely stenotic or occluded native coronary arteries were bypassed; CCABG — conventional coronary artery bypass (in the extracorporeal circulation); LIMA — left internal mammary artery; OPCAB — off-pump coronary artery bypass; PCI — percutaneous coronary intervention; RA — radial artery; RIMA — right internal mammary artery

Methods

Study group

Local Ethics Committee has approved a research protocol (No.1201/08) and an informed written consent from each study participant was obtained. In total, 218 consecutive patients (168 [77.1%] males and 50 [22.9%] females) at the mean age of 62.5 ± 8.7 years (46–85 years) who underwent primary isolated CABG with the use of at least one venous aortocoronary bypass graft between August 2008 and February 2009 were enrolled in the prospective study. Patients who needed emergent operations and with any peripheral veins pathologies were excluded from the study. Basic preoperative demographics and clinical history data are summarized in Table 1.

Operation procedure and sample collection

All surgeries were performed from median sternotomy. A part of them were accomplished on the beating heart (off-pump coronary artery bypass,

Table 2. Immunohistochemical analysis of cytoskeletal expression using Remmele scale [15]

Percentage of positive cells	Points	Intensity of color reaction	Points
No positive cells	0	No reaction	0
< 10% positive cells	1	Weak color reaction	1
10–50% positive cells	2	Moderate color reaction	2
51–80% positive cells	3	Strong color reaction	3
> 80% positive cells	4		

OPCAB) with the routine use of tissue stabilizer (Octopus 4) and intracoronary shunts (ClearView) (both, Medtronic Inc., USA). The others were carried out in the extracorporeal circulation in the moderate hypothermia (conventional CABG) (Table 1).

SV was obtained through a full-length thigh incision over its course [13]. Key points included minimal manipulation of the graft ("no-touch" technique), avoiding of extensive dilation of the conduits, using low-intensity electrocautery and control of the branches with stainless-steel vascular clips. In all the cases, the distal part of harvested SV segment (at least 1.5–2.0 cm in length) was saved for subsequent laboratory studies.

Immunohistochemistry

The venous segments were carefully rinsed with 0.9% NaCl at the room temperature, slightly dilated and fixed in Bouin's solution for 2–3 h. They were dehydrated and then embedded in paraffin blocks by means of routine procedure. Eventually, they were cut into 5-μm sections on a semi-automatic rotary microtome (Leica RM 2145, Leica Microsystems, Nussloch, Germany). Each sample of the SV (taken from separate patients) was cut into approximately 30 paraffin sections. All of the immunohistochemical (ICC) analyses employed StreptABComplex/HRP method modified by use of biotinylated tyramide (Dako Catalyzed Signal Amplification System, Peroxidase, K1500, DakoCytomation A/S, Glostrup, Denmark) as previously described [14]. After deparaffinization with xylene and gradual rehydratation the endogenous peroxidase activity was blocked with 10% hydrogen peroxide (v/v) treatment for 10 min. At the consecutive stages of ICC, the indirect ABC technique was performed, with the following steps: (1) preincubation with the appropriate normal goat serum in phosphate buffered solution for 30 min at room temperature; (2) incubation with the first specific antibody, overnight at 4°C in a hybridization chamber; (3) incubation with the secondary antibody for 60 min at room temperature; (4) final antigen-antibody complexes staining using 0.5% 3-3' diaminobenzidine (DAB; Sigma Chemical Co., St. Louis, MO).

The following monoclonal mouse anti-human specific antibodies were used: anti-α-smooth muscle actin (α-SM actin) (M0851), anti-SM-MHC (MAB13431), anti-CALP (M3556) (all from Dako-Cytomation A/S, Glostrup, Denmark) and anti-CK8 (ab9023; Abcam, Cambrigde, UK).

The intensity of cytoplasmic protein expression was assessed using the semi-quantitative immuno-reactive score (IRS) scale according to Remmele and Stenger [15]. It takes into account the percentage of positive cells (scale from 0 to 4) and the intensity of the color reaction (scale from 0 to 3) and final score that ranges from 0 to 12 is a product of points given for individual traits (Table 2). On the base of IRS, cytoplasmic protein expression was defined as negative (IRS 0–1), positive weak (IRS 2–3), positive moderate (IRS 4–6) or strong (IRS 7–12) [16]. All tissue sections were analyzed under AxioImager Z.1 light microscope and selected pictures were captured with attached AxioCam MRc5 digital camera (Carl Zeiss MicroImaging GmbH, Göttingen, Germany).

ICC analysis of proteins expression in each vessel section was done within 10 representative microscopic fields (× 200 magnification). Intensity of ICC reaction was evaluated independently by 2 pathologists on coded samples. Additionally, all of ICC analyses included the negative controls that consisted of specimens incubated with non-immune IgG1 (X-0931, DakoCytomation A/S, Glostrup, Denmark) and sections in which the primary or secondary antibody was omitted.

Evaluation of late coronary outcome

The primary clinical endpoint of this prospective study included any clinical form of CAD progression that occurred throughout post-discharge follow-up. It confines all ACSs cases (unstable angina, myocardial infarction (MI) with or without ST seg-

ment elevation, respectively STEMI or NSTEMI) as well as recurrence or progression of stable angina according to Canadian Cardiovascular Society (CCS) classification. Patients CCS status was assessed at the end of postoperative rehabilitation (usually 4 weeks after surgery) and then at the end of follow-up. Additionally, at the end of follow-up, all aortocoronary bypass grafts were examined by means of coronary angiography (n = 187) or computed tomography angiography (CTA) with multi-slice detector (n = 23). CTA was offered only to subject who withdrew consent for follow-up invasive coronary examination. Hemodynamically significant SV disease was defined as graft lumen restriction exceeded 70% (the primary angiographic endpoint).

Clinical data and SMCs protein expression in patients with lesions in venous bypass grafts

Selected preoperative clinical and intraoperative data were compared between groups of patients with and without significant venous graft disease in the last follow-up coronary angiography: SVGD(+) vs. SVDG(–), respectively. Moreover, expression of SMCs proteins such as SM-MHC, CALP and CK8 in SV segments obtained from patients of both subgroups was also recalculated and analyzed.

Data management and statistical analysis

Initially, all continuous variables were estimated for normality with the Shapiro-Wilk W test. When they complied with a normal distribution they were expressed as the mean ± standard deviation and then they were compared using un-paired t test. Non-parametric continuous variables expressed as median (25^{th}–75^{th} percentile) were compared using Mann-Whitney U test. Categorical data were expressed as number (n) and percent (%) or median (25^{th}–75^{th} percentile) and were compared employing Pearson χ^2 test or Kruskal-Wallis test. A p value < 0.05 was considered to be as statistically significant.

The univariate followed by multivariate logistic regression analysis of the several variables (demographic, clinical, immunohistochemical) were performed to identify predictors for accelerated venous graft disease development (angiographic endpoint of the study). Only variables with a p value of < 0.2 by means of univariate regression model were entered into a multivariate logistic regression one. Variables revealed to be significant ($p < 0.05$) at the latter model were considered to be independent predictors for accelerated graft failure. The p values and odds ratios (ORs) with corresponding 95% confidence intervals (95% CI) were presented. The following variables, including risk factors of atherosclerosis development (advanced age [> 70 years], gender, obesity [BMI > 30], preoperative unstable angina, previous MI and previous percutaneous coronary intervention [PCI], arterial hypertension, diabetes mellitus treated with insulin, hyperlipidemia, peripheral vascular disease), severity of CAD (younger age of CABG necessity [≤ 50 years], left main stenosis, three vessels disease) and these derived from ICC analysis of SMCs proteins (both weak and strong expressions of SM-MHC, CALP and CK8) were entered into logistic regression analysis.

Freedom from CAD progression was estimated using Kaplan-Meier method. All statistical analysis were carried out using Statistica 9.0 for Windows (StatSoft, Inc., Tulsa, OK, USA).

Results

Clinical observation: Late coronary outcome

Eight individuals enrolled to the study protocol were discarded from both clinical and angiographic analysis of late coronary outcome. Six of them died either soon after surgery (due to postcardiotomy low cardiac output syndrome [n = 2], gastro-intestinal complications [n = 1]) or during follow-up for non-cardiac-related causes [n = 3]). Other 2 developed perioperative MI (troponin-positive with ST segment elevation and evident *de novo* segmental pathologies in left ventricular myocardial contractility).

During follow-up period, lasting 45.4 ± 5.7 months and completed by 100% of the study participants, the primary clinical endpoint of the study was reached by 38 of them (38/210; 18.1%). It confined deterioration of stable angina according to CCS classification in 30 and ACSs in 8 cases (STEMI [n = 4], NSTEMI [n = 2] and unstable angina [n = 2]). Comparison of stable angina status according CCS classification before and after surgery is presented graphically in Figure 1. Freedom from CAD progression estimated by means of Kaplan-Meier method was 0.95 ± 0.02, 0.87 ± 0.03 and 0.83 ± 0.04, respectively for 12-, 24-, 36-month follow-up. Forty four individuals reached the primary angiographic endpoint of the study (Fig. 2), including 37 patients with clinical symptoms of CAD progression. SVGD development was usually accompanied by progression of atherosclerosis in the native coronary arteries. Only in 1 case culprit lesions in the native vascular bed but not in bypass grafts were regarded as reason of marked clinical CAD deterioration.

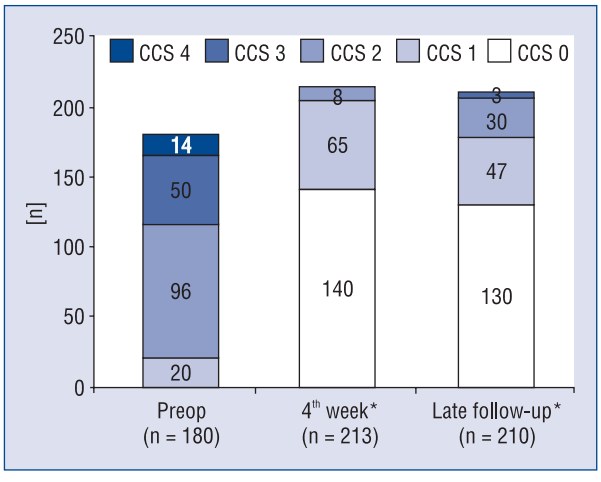

Figure 1. Canadian Cardiovascular Society (CCS) classification before and after surgery. Note: CCS classification applies only to stable angina patients; *p < 0.05 preop vs. 4th week and preop vs. late follow-up; preop — preoperative.

Expression of SMCs proteins

ICC expression of α-SM actin was estimated in all the studied SV grafts. Its expression was present within SMCs cytoplasm and referred to IRS 9 or 12 regardless postoperative clinical course of CABG patients (data not shown). Interestingly SM-MHC was not expressed in 27.5% of SV segments (n = 60). In 10 (4.6%) cases its expression was recognized as positively strong (IRS ≥ 7). In the rest of cases, SM-MHC expression was accepted as weakly or moderately positive. If preent, SM-MHC was localized regularly within the whole SMC, except of nucleus territory.

CALP was expressed in all studied SV transplants. In majority of cases (n = 200, 91.3%), its cytoplasmic presence within SMCs was regarded as moderately or strongly positive. It was not seen within tunica adventitia or loose connective tissue present in tunica media.

In more than 50% of analyzed SV transplants, there were no expression of CK8 (IRS 0–1). Otherwise, only in 34 cross-sections of SV grafts (15.6%) it was assessed as strongly positive in tunica media. Similarly to SM-MHC expression, CK8 occupied the whole SMCs cytoplasm except of nucleus.

Comparison of patients with and without venous graft failure

Generally, SVGD(+) patients at the time of surgery were significantly younger as compared to SVGD(–) subjects (57.8 ± 8.3 vs. 63.6 ± 8.5, p < 0.001). No significant differences in prevalence of commonly accepted risk factors for atherosclerosis development (obesity, arterial hypertension, diabetes) as well as postoperative medical therapy

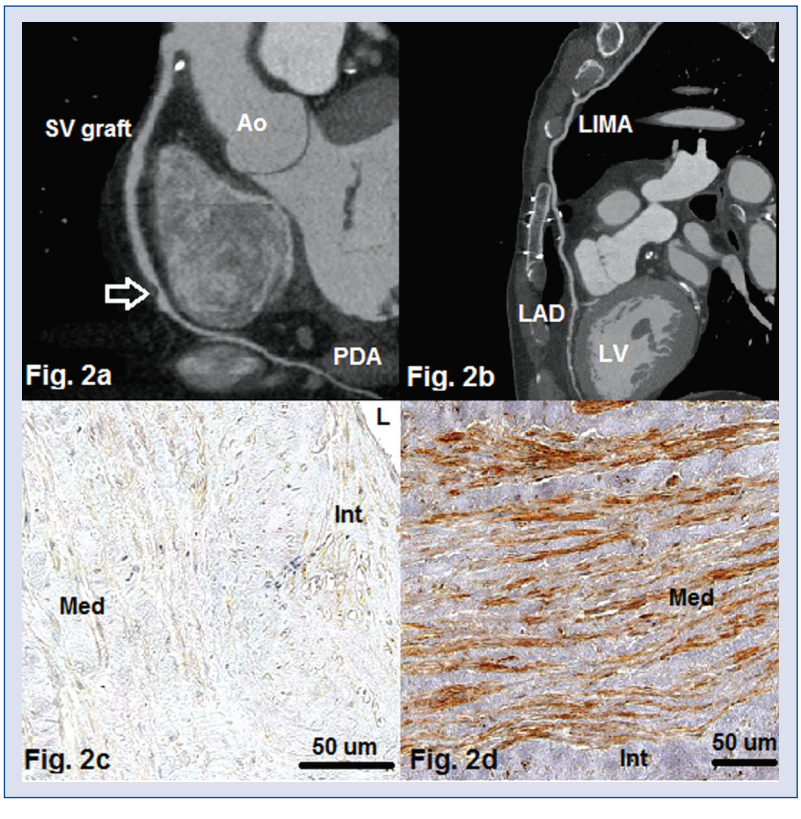

Figure 2. Final follow-up computed tomography angiography of 49-year-old man with severe angina deterioration (CCS class III at the end of follow-up) revealed significant stenosis (arrow) in saphenous vein (SV) graft to the right coronary artery (**A**) and proper angiographic appearance of left internal mammary artery (LIMA) implanted to left anterior descending artery (LAD) (**B**). Note almost no expression of smooth muscle myosin heavy chain (IRS 1) (**C**) but strong immunoreactivity of cytokeratin 8 (IRS 12) (**D**) on the cross-sections of the harvested SV segments; Ao — aorta; Int — SV tunica intima; L — SV lumen; LV — left ventricle; Med — SV tunica media; PDA — posterior descending artery.

Table 3. A comparison of selected clinical data and expression of cytoskeletal smooth muscle cells proteins between SVGD(+) and SVGD(–) patients.

	SVGD(+); n = 44	SVGD(–); n = 174	P
Age [years]	57.8 ± 8.3	63.6 ± 8.5	< 0.001
Age ≤ 50 years	18 (40.9%)	22 (12.6%)	< 0.001
Age > 70 years	6 (13.6%)	42 (24.1%)	0.092
Obesity (BMI > 30)	20 (45.4%)	72 (41.3%)	0.632
Arterial hypertension	34 (77.3%)	128 (73.6%)	0.609
Diabetes treated with insulin	10 (22.7%)	32 (18.4%)	0.540
Hyperlipidemia	16 (36.3%)	76 (43.7%)	0.378
Peripheral vascular disease	8 (18.2%)	42 (24.1%)	0.379
Postoperative statins	43 (97.7%)	170 (97.7%)	0.998
Postoperative ASA/clopidogrel	44 (100.0%)	174 (100.0%)	1.000
Median SM-MHC IRS score	1 (1–4)	2 (1–4)	0.032
No or weak SM-MHC expression	28 (63.3%)	112 (64.3%)	0.929
Strong SM-MHC expression	0 (0%)	10 (5.7%)	0.001
Median CALP IRS score	6 (2–6)	8 (6–9)	< 0.001
Weak CALP expression	12 (27.3%)	6 (3.4%)	0.001
Strong CALP expression	6 (13.6%)	74 (42.5%)	< 0.001
Median IRS score	7.5 (4–9)	0 (0–4)	< 0.001
No or weak CK8 expression	8 (18.2%)	128 (73.6%)	< 0.001
Strong CK8 expression	22 (50.0%)	12 (6.9%)	< 0.001

IRS score are presented as medians (25^{th}–75^{th} percentile); ASA — acetylsalicylic acid; BMI — body mass index; CALP — calponin; CK8 — cytokeratin 8; IRS — immuno-reactive score; SM-MHC — smooth muscle myosin heavy chain; SVGD — saphenous vein graft disease

(statins, acetylsalicylic acid [ASA]) were found between groups (Table 3). Statistical analysis of SMCs cytoskeletal expression pattern predicting development of atherosclerotic plaques in SV grafts revealed the risk of critical transplant occlusion is higher in CK8-positive (Fig. 3) and CALP-weak positive study participants. All the detailed information are summarized in Table 3.

Regression analysis

Nine variables (age ≤ 50 years, gender, previous PCI, three vessels disease, SM-MHC IRS ≥ 7, weak CALP expression, CALP IRS ≥ 7, no or weak CK8 expression and CK8 IRS ≥ 7) reached $p < 0.2$ in the univariate model and were then analyzed in the multivariate one. That latter one revealed a strong expression of CK8 (OR 1.90, 95% CI 1.11––7.76, $p = 0.037$) and weak of CALP (OR 1.40, 95% CI 1.04–6.57, $p = 0.042$) were the independent risk factors for SVGD development. Otherwise, the strong expression of both CALP and SM-MHC as well as lack of CK8 were associated with low risk for restricting lesions development in venous aortocoronary grafts and, in consequence, favorable CABG long-term outcome. The results of logistic regression model to indentify independent risk factors for late SVGD development are presented in Table 4.

Discussion

The main purpose of CABG is to bridge over lesions compromising adequate flow in the coronary arteries and thus to improve myocardial perfusion. Clinically CABG patients usually experience marked improvement in quality of life, better physical performance and relief from angina [17]. This effect persists unless development of graft disease or rapid progression of atherosclerosis in the native coronary arteries [18]. In our study it was shown the majority of CABG patients presented significant improvement in CAD symptom control at least 3–4 years after surgery. However, a subset of CABG individuals with post-discharge CAD deterioration had been growing systematically by the end of follow-up period (Fig. 1). The last follow-up angiographic examination revealed CAD symptoms recurrence and progression were associated with SVGD. However, it must be stressed that 7 patients reached the primary angiographic endpoint without any clinical manifestation of CAD progression. All of them had diabetes treated with insulin for at least 5 years. Thus it is likely that the clinical and angiographic endpoints discrepancy may have resulted from diabetes-related cardiac autonomic neuropathy [19].

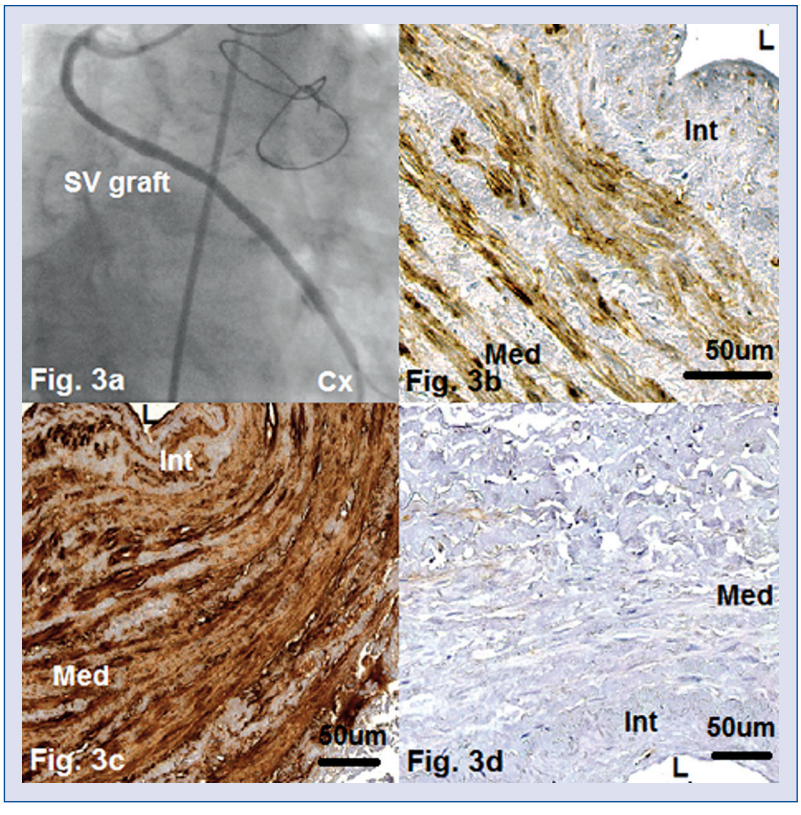

Figure 3. Final follow-up angiographic normal appearance of saphenous vein (SV) transplant to marginal branch (**A**) in 77-year-old male patient without any clinical symptoms of coronary artery disease deterioration throughout post--discharge follow-up. On the cross--sections of the SV segments obtained intraoperatively, strong immunoreactivity of both smooth muscle myosin heavy chain (IRS 9) (**B**) and calponin (IRS 12) (**C**) but no expression (IRS 0) of cytokeratin 8 (**D**) were visualized in tunica media; Cx — circumflex artery; Int — SV tunica intima; L — SV lumen; Med — SV tunica media.

Table 4. The results of uni- and multivariate logistic regression analysis.

Variable	Univariate	Multivariate	OR (95% CI)
Age ≤ 50 years	0.001	0.289	–
Age > 70 years	0.384	–	–
Gender	0.059	0.961	–
Obesity	0.843	–	–
Unstable angina	0.364	–	–
Previous MI	0.874	–	–
Previous PCI	0.146	0.239	–
Arterial hypertension	0.484	–	–
Diabetes mellitus	0.636	–	–
Hyperlipidemia	0.634	–	–
Peripheral vascular disease	0.827	–	–
Left main disease	0.248	–	–
Three vessel disease	0.047	0.181	–
No or weak SM-MHC expression	0.595	–	–
SM-MHC IRS ≥ 7	0.027	0.037	0.52 (0.05–0.97)
Weak CALP expression	<0.001	0.042	1.40 (1.04–6.57)
CALP IRS ≥ 7	0.001	0.006	0.21 (0.07–0.64)
No or weak CK8 expression	< 0.001	0.021	0.11 (0.02–0.72)
CK8 IRS ≥ 7	< 0.001	0.037	1.90 (1.11–7.76)

BMI — body mass index; CALP — calponin; CI — confidential interval; CK8 — cytokeratin 8; IRS — immuno-reactive score; MI — myocardial infarction; OR — odds ratio; PCI — percutaneous coronary intervention; PVD — peripheral vascular disease; SM-MHC — smooth muscle myosin heavy chain

Statistical analysis revealed that SVGD(+) subjects were markedly younger than SVGD(–). It may be related to more aggressive atherosclerosis in younger CAD patients [20]. Relatively young age was found significantly predictive of angina recurrence not only after CABG but also after PCI [21]. Moreover, we have shown recently, in the elderly CABG individuals both SV wall and tunica media were hypotrophic [22]. However, SVGD(+) and SVGD(–) patients did not differ to one another with respect to prevalence of such factors for atheroma formation as obesity, diabetes mellitus, hyperlipidemia, arterial hypertension or peripheral vascular disease. It could not rule out that aforementioned factors would be of importance in longer follow-up (e.g. 10–15 years after surgery). Moreover, the same postoperative medical management, including statins and ASA was applied. Thus it is likely that other undisclosed factors may also have a potent impact on long-term performance of venous aortocoronary bypass grafts.

It was proved previously, a long-term clinical and angiographic success of SV as aortocoronary bypass graft was determined by the inevitable biological process called "arterialization" [23, 24]. SMCs and fibroblasts are the main effector cells of arterialization, and in the consequence crucial for long-term fate of grafts. In our study we evaluated expression of cytoskeletal SMCs proteins such as α-SM actin, SM-MHC, CALP and CK8 in the SV tunica media. α-SM actin was employed to identify SMCs within SV tunica media as it usually detected in all stages of SMCs development [10]. In all SV specimens α-SM actin positive cells within tunica media were found. SM-MHC and CALP are usually expressed in well differentiated, mature, contractile phenotype SMCs [10, 12]. In our study we found SVGD(+) patients had no strong SM-MHC expression and only 6 (14%) cases of them presented strong expression of CALP. Contrary to them, among SVGD(–) individuals more than 40% of medial SMCs manifested strong CALP immunostaining. Another examined cytoskeletal SMCs protein, CK8 is not usually detected in normal, well differentiated contractile SMCs. It has been found previously in both immature, fetal or in synthetic SMCs that could migrate from tunica media into the neointima [11, 25]. Interestingly, although we examined SV segments obtained from the adults we detected positive strong expression of CK8 in 50% (!!!) of SVGD(+) individuals while in much less than 10% of SVGD(–) subjects. Additionally, aforementioned findings were supported by logistic regression model that revealed different expressions of venous medial smooth muscle proteins associated with various stages of differentiation were of paramount importance for long-term CABG outcome. Strong expression of CK8 in medial SMCs and weak expression of CALP were confirmed to be the independent risk factors for unfavorable outcome after CABG with the use of SV conduits. Thus degree of SMCs differentiation within SV tunica media has impact on their ability to respond to various aforementioned biologically active chemo-attractant and mitogenic molecules. Higher representation of poorly differentiated, probably synthetic SMCs is linked to accelerated development of culprit lesions in venous aortocoronary bypass grafts. This finding should provoke the following question, why in some adult patients expression of CK8, fetal cytoskeletal protein, is so strong while CALP and SM-MHC markedly diminished. It was proved the contractile, mature SMCs may undergo dedifferentiation in synthetic phenotype under pathological circumstances such as ischemia, repetitive injuries (mechanical, inflammatory etc.), prolonged exposure to growth factors (e.g. VEGF) in the experimental models but also in atherosclerotic lesions [26, 27]. This phenotypic modulation is usually accompanied by a loss of contractile function together with progressively impaired expression of cytoskeletal markers of maturity. This process of SMCs change seems to be reversible (reversibly synthetic SMCs) but if causative factor persists SMCs may become incapable of return or return to the contractile state is incomplete (irreversibly synthetic cells) [27]. Thus, it might be some of CK8 strong positive and CALP weak positive SMCs presented irreversibly synthetic phenotype that featured high potential to biological activity (i.e. proliferation, migration etc.).

Our study supports earlier findings that medial SMCs in SV wall do not represent homogenous population in terms of not only morphology (different shapes of both cells and their nuclei) but also their biological properties (variations in response to potent mitogenic molecules) [10, 28]. We have also proved this biological variability had significant impact on CABG outcome with the use of SV grafts.

The findings of this study should encourage surgeons in cooperation with histologists to work out a simple test to identify SV segments with high likelihood of the accelerated venous graft disease and unfavorable outcome after CABG. Positive result of the screening method would disqualify SV segment from use and encourage surgeon to employ alternative sources of the conduits.

Conclusions

Strong expression (IRS ≥ 7) of CK8 and weak of calponin (IRS 2–3) in tunica media of SV transplants may be useful markers in identification of venous segments exposed to accelerated atheroma formation and, in consequence, venous graft failure. In the future, assessment of these proteins expression it will enable to select the groups of CABG patients who will benefit particularly from SV aortocoronary grafts.

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Conflict of interest: none declared

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