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Fibrin sealant injection through the drain with adjuvant compression as a treatment of groin lymphatic complications after vascular operation

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Abstract

Introduction: *Groin lymphatic complications after femoral artery operations are rare, but if developed, they could be a source of severe complications that could be potentially fatal. The novel technique of combining compression and application of Tisseel sealant (Baxter AG, Vienna, Austria) to treat lymphatic fistulas in the groin region after common femoral artery exposure was evaluated.*

Material and methods: *Twelve groins from eleven patients with groin lymphatic complications were enrolled into the study. Patients had femoral artery exposure during one of the following procedures: endovascular aortic aneurysm repair, thrombendarterectomy or extra-anatomic by-pass. Postoperatively, average lymphatic drainage through the drain was 140 ± 60 (range 48–300) mL per day. Intervention was performed at a median of 12 (9–23) days after operation. The drain was cut off close to the skin, a thin double-channel catheter was introduced as deep as possible through the drain and sealant was injected into the wound. Simultaneously, the remaining part of the drain was removed, the orifice in the skin was sutured and a compression dressing was kept in place for 24 hours. The median patient observation period was 13 months (range 2.5–23) and surveillance of the groin was performed using ultrasonography.*

Results: *Early outcomes showed full technical success. None of the patients were readmitted due to lymphorrhea, infection or poor wound healing during follow-up. No lymphoceles were detected by ultrasonography.*

Conclusion: *Fibrin glue injection augmented by compression is an effective method for treating postoperative lymphatic fistulas and to prevent lymphorrhagia and lymphocele formation in the groin region after femoral artery exposure.*

Key words: vascular surgery, lymphatic fistula, mini-invasive treatment, tissue sealant, femoral artery

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Article highlights

Fibrin sealant injection through the groin drain with adjuvant compression after vascular operation stopped the leakage and prevented lymphorrhagia and lymphocele formation in median 13 months follow-up in all 11 patients.

Introduction

Groin lymphatic complications after vascular operations were estimated to be from 1 up to 11.5% [1, 2], and involved 92% of all wound complications after common femoral artery endarterectomies [2]. Groin lymphatic complications have several clinical scenarios.

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At the beginning prolonged lymphatic outflow from the Redon drain can be noticed. After drain removal, a lymphocutaneous fistula through the channel of the drain or lymphorrhea from a disrupted or not completely healed wound can occur. After proper wound healing a lymphocele can be formed as a cystic collection of lymphatic fluid from open lymphatic vessels that form a pocket in the soft tissue. All of the above complications disturb wound healing and could lead to severe infections followed by septic bleeding and death. Prolongation of the postoperative stay raises the costs of treatment [3–5]. For each kind of groin lymphatic complication, many therapies have been described, though none have emerged as the best management. Thus, the application of preventive approaches seems to be the most appropriate solution. At first incision and careful ligation of the dissected tissue is extremely important [3, 6]. In a randomized study, Swinnen et al. [7] showed a significant decrease in lymphatic leaks after femoral artery surgery using a transverse (12.7%) rather than vertical incision (27.9%). Giovannacci et al. [8] also presented in a prospective randomized study, a significant reduction of 19% to 10% in lymphatic complications after the prophylactic application of fibrin glue to the inguinal wound before closure. Proper management with a groin drain can prevent subsequent uncontrolled lymphorrhea or lymphocele formations.

Objective

The goal of this study is to determine the efficacy of a fibrin sealant injection through a drain augmented by compression as a treatment of prolonged lymphatic discharge from a Redon drain to avoid lymphorrhea or lymphocele formation after common femoral artery exposure.

Material and methods

Twelve groins with prolonged lymphatic drainage from a Redon drain after femoral artery exposure in eleven patients were assessed. Subjects were enrolled between November 2016 and March 2018. The median age was 71 years (range 58–85). In the presented group, six patients (50%) had undergone endovascular aortic aneurysm repairs, two (16%) thrombendarterectomies of the common femoral artery and three had extra-anatomic by-pass grafts: aorto-bifemoral, subclavian-bifemoral and femorofemoral crossover by-pass graft.

The standardized surgical technique for wound closure consisted of accurate hemostasis, installation of a Redon drain near the femoral artery and sutures to the subcutaneous tissue and skin. The drain was conducted to the skins surface through a separate incision.

Prolonged lymphatic outflow from the Redon tube was defined as a lymphatic drainage of at least 30 mL per day through a 14 or 16 French drain with a negative pressure for more than 7 days. The amount and character of the exudate was assessed every day. Median time from the primary vascular operation to the procedure was 12 (9 to 23) days. For all patients, informed consent was taken before the procedure. This study was approved by the institutional review board. Characteristics of the patients and drainage are presented in Table 1.

The procedures were performed in an operation theatre [9]. No bacterial culture samples were taken prior the procedure due to a lack of clinical signs of surgical site infection — wounds healing process was uneventful. The procedure started by cutting off the drain 5 cm above the skin. Through the drain, a thin dual-channel catheter was introduced to the end. The catheter was connected to two syringes with a ready to use Tisseel sealant (Baxter AG, Vienna, Austria), prepared according to the prescribing information [10]. Then, the tissue glue was injected alongside the simultaneous removal of the remaining part of the drain tip of the drain was sent for bacterial culture. Glue was injected as long as it came out through the drain's hole. No more than 10 mL of the glue was necessary. Subsequently, a single skin stitch was placed to close the area of drain removal and a compression dressing was applied with immobilization of the patient introduced for 24 hours.

Patient surveillance lasted for nearly two and a half months to over 22 months. Ultrasound examinations of the groin, performed 1–3 months after glue injection, were undertaken for each patient to exclude lymphocele.

Results

There was 100% technical success. All lymphatic fistulas stopped immediately after the procedure. Seven (63%) patients were discharged in the 2 days after the procedure, and the rest of the study group was discharged at 4 to 6 days. None of the patients developed lymphorrhea during the median 13 month follow-up. There were no readmissions due to poor healing of the wound, skin necrosis or surgical site infection. No signs of lymphocele were observed under ultrasound control during follow-up. Outcomes, follow-up and patient survival are presented in Table 2.

Discussion

Prolonged lymphatic discharged from a Redon drain after femoral artery exploration is a nuisance for both

Table 1. Demography, risk factors, types of operation, duration and amount of drainage

No.	Sex	Age	Important comorbidities	Type of operation	Type of incision	Postoperative duration of lymph drainage (days)	Drainage Mean (max–min) ± sd (ml)
1	F	58	No	EVAR raaa	Transverse	14	300 (40,500) ± 151
2	M	58	Tobacco abuse	Aortobifemoral by-pass	Longitudinal	11	91 (50–140) ± 26
3	F	83	Diabetes mellitus	TEA femoral artery	Longitudinal	9	151 (60–200) ± 49
4	M	84	Anticoagulant therapy, diabetes mellitus, heart failure	EVAR AAA	Transverse	17	122 (30–270) ± 68
5	M	71	Diabetes mellitus	Aortobifemoral by-pass	Longitudinal	13	130 (75–200) ± 53
6	M	71	Tobacco abuse	Suprapubic by-pass	Longitudinal	12	136 (90–200) ± 31
7	F	77	Diabetes mellitus	EVAR AAA	Transverse	23	172 (30–340) ± 73
8	M	64	No	Mbevar TAAA	Transverse	14	157 (40–800) ± 191
9	M	85	COPD, tobacco abuse	TEA femoral artery	Longitudinal	8	48 (20–90) ± 23
10 — left groin	M	68	Obesity, diabetes mellitus, tobacco abuse	Fevar jaaa	Transverse	13	149 (40–250) ± 65
11 — right groin					Transverse		140 (40–350) ± 100
12	M	68	No	EVAR AAA	Transverse	10	105 (50–150) ± 38

EVAR: endovascular aneurysm repair; rAAA: ruptured abdominal aortic aneurysm; TEA: thrombendarterectomy of femoral artery; mbEVAR: multibranched endovascular aneurysm repair; TAAA: thoracoabdominal aneurysm repair; fEVAR: fenestrated endovascular aneurysm repair; jAAA: juxtarenal abdominal aneurysm repair; COPD: chronic obstructive pulmonary disease

Table 2. Patient follow-up

No.	Follow-up (months)	Appearance of lymphorea or lymphocele	Alive	Reason for death
1	21	No	Yes	
2	23	No	Yes	
3	23	No	Yes	
4	3	No	No	Circulatory insufficiency*
5	13	No	Yes	
6	15	No	Yes	
7	18	No	Yes	
8	3	No	Yes	
9	2,5	No	Yes	
10 — left groin	4	No	No	MOF*
11 — right groin		No		
12	6	No	Yes	

*Death not linked to the sealing procedure or prior vascular operation; MOF: multiorgan failure

the surgeon and the patient. While there is currently no precise definition for this complication, two factors have to be taken into account according to the literature: duration of drainage and amount of lymph discharge in mL per day. Dietl et al. and Toepel defined this pathology as a secretion of a minimum of 50 mL per day for more than 4 and 7 days after surgery, respectively [2, 11]. In this study, according to Giovannacci et al. [8], leakage above 30 mL per day was established as incorrect. The median duration of leakage was 12 days, in opposite of the 3 days due in Giovannacci's definition [8]. In our series, the waiting time from the operation to drain removal with simultaneous sealing was longer, because spontaneous disappearance of the leakage was assumed day by day. Prolongation of drain removal can be justified by Van den Brande et al. [12] findings. They showed an 82.6% healing rate for postoperative lymphocutaneous fistulas within 3 weeks of drainage; however, a 4% infection rate was observed. Shermak [13] showed that the mean time to identification of groin lymphatic complications after vascular surgery was 14 days. Thus, determining the right time of drain removal is crucial to preventing lymphorrhagia and lymphocele. Removing the drain too early can cause leakage through the wound and as a consequence, further measurements of the lymphatic liquid would not be possible. Moreover, it would necessitate the replacement of sodden compresses several times a day and increase the threat of infection. In this situation, application of vacuum-assisted closure therapy has been shown to be an appropriate management technique [14]. The method of successful injection of fibrinogen and thrombin through the wound drain with simultaneous pressure for 48 hours was introduced, with success, to treat lymphatic fistulas after the resection of lymphangiomatous tumor of the abdominal wall by Giberson [15]. Moreover fibrin glue is effective in the prevention of lymph leakage when used during wound closure and to treat lymphoceles of the groin [16, 17]. In this study, Tisseel a two-component fibrin sealant made from pooled human plasma was used. The first component was thrombin with calcium chloride, and the second consisted of fibrinogen and aprotinin. Thrombin is a highly specific protease that transforms fibrinogen into fibrin. Aprotinin is a protease inhibitor that increases the resistance of the fibrin sealant clot to degradation in a fibrinolytic environment. When the two components are combined, the final stage of the blood coagulation cascade is mimicked and thus, hemostasis and sealing or gluing of tissues are achieved. Tisseel is also effective in heparinized patients and in patients medicated with anti-platelet drugs [10].

It has been shown that prothrombin, factor V, fibrinogen and plasminogen are present in lymph in a concentration of about 20–60% of the plasma levels. Lymph also contains only small quantities of the various inhibitors of the fibrinolytic system [18, 19]. Thus, lymph clotting is slower, but its fibrinolytic activity is considerably higher than that of the blood [20, 21]. Although the lymph does not contain any thrombocytes, thromboplastin formation is sufficient for clot formation [22, 23]. Thus, it can be assumed that the mechanism of action of the sealant is to simultaneously increase coagulation and decrease the fibrinolytic lymph properties. In this study, the action of the sealant was combined with a pressure dressing for 24 hours. Compression decreases lymphatic flow, which may wash out the fibrin glue before it can adhere to a tissue surface. In this study, a skin stitch was placed to close the hole after drain withdrawal to stop the glue leaking out. Thus, the necessity and duration of the pressure dressing application should be evaluated.

In conclusion, in this study, for the first time, drain removal with subsequent sealing to treat prolonged lymphatic drainage from a Redon tube was described. This method appears to be noninvasive, simple and highly effective. Moreover, it prevented the development of lymphorrhea and lymphoceles over the one year period of observation. There were no incidences of infection as a result of the drain removal with subsequent sealing and this was performed in an operating theater under antibiotic prophylaxis.

This study has several limitations due to its nonrandomized character and small cohort. The following issues are open for further research: What is the best point in time to remove the drain with sealing and whether it would be safe to carry out such procedures in an outpatient clinic. To solve those problems further randomized studies should be undertaken.

Conflict of interest

None.

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Clinical symptoms and signs and severity of venous disease are not associated with non-thrombotic iliac vein lesions in patients with primary varicose veins

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Abstract

Introduction: *The purpose of this study was to determine relationship between non-thrombotic iliac vein lesions and symptomatology of primary varicose veins (PVV). The identification of such association would be helpful in selecting patients with PVV for further diagnostic evaluation.*

Material and methods: *Thirty-two patients with unilateral PVV scheduled for great saphenous vein high ligation and stripping were enrolled in the study. There were 25 (78%) women. The mean age of the patients was 48 years. The patients were asked about pain, oedema, night cramps, heaviness and a history of superficial thrombophlebitis in PVV limb. A clinical stage of CEAP classification was determined and Venous Clinical Severity Score (VCSS) was calculated. During the surgery right and left iliac venous axes were interrogated with an intravascular ultrasound with Volcano s5 Imaging System (Volcano Corporation, Rancho Cordova, CA, USA) and catheters Visions PV .035 minimal lumen area (MLA) and percentage of stenosis (%S) of examined veins were calculated. An association between clinical symptoms and signs in PVV limb and %S of ipsilateral common iliac vein (CIV) and external iliac vein (EIV) was statistically analysed.*

Results: *Pain, oedema, night cramps, heaviness and history of superficial thrombophlebitis were reported by 14 (44%), 17 (53%), 11 (34%), 19 (59%) and 6 (19%) of patients, respectively. Twenty-five (78%) limbs were classified as C2 and 7 (22%) limbs as C4a according to CEAP classification. The median VCSS was 4. The mean MLA and %S was 92.9 mm^2 and 47% and 74.2 mm^2 and 48% for CIV and EIV, respectively. Neither smaller MLA nor greater %S of CIV and EIV were associated with symptoms, more advanced stage of CEAP classification or higher VCSS.*

Conclusions: *Neither clinical symptoms nor severity of venous disease can identify non-thrombotic iliac vein lesions in patients with primary varicose veins.*

Key words: non-thrombotic iliac vein lesions, primary varicose veins, clinical symptoms and signs

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Introduction

A stenting of the deep venous system, especially in ilio-caval segment has gain a great interest, recently. This was followed by the development of new, dedicated venous stents and refinements of the venous stenting technique. Nowadays a lot is known about how to stent; however, there is still not enough knowledge about who to stent especially in case of May-Turner syndrome. As originally described by May and Turner and later by

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Cockett and Thomas, the syndrome is caused by the compression of the left common iliac vein by the right common iliac artery [1, 2]. Nowadays it is known that the compression may affect not only left common iliac veins but also right common and external iliac veins on both sides. A term non-thrombotic iliac vein lesions (NIVL) has been coined to name these compression related obstructions. It has been observed, however, that NIVLs are frequent in the population and on many occasions are asymptomatic [3]. They are more difficult to diagnose than infrainguinal venous lesions and usually for that purpose computed tomography or magnetic resonance venography or intravascular ultrasound is required. The latter is considered the most sensitive tool for diagnosis of NIVLs [4]. Taking into account costs, invasiveness and availability of these imaging studies, a preselection of patients is necessary. Undoubtedly in the developed countries, primary varicose veins are the most frequent disorder of the lower limb venous system. They are often associated with symptoms that may also result from the presence of venous outflow obstruction. The purpose of this study was to investigate if there is any relationship between clinical symptoms and occurrence and severity of NIVLs in patients with primary varicose veins. Should there be any relationship it might provide an indication for different diagnostic work-up and treatment of these patients.

Material and methods

The protocol of the study was approved by the institutional bioethical committee and all patients signed an informed consent prior to the inclusion. Patients with primary varicose veins (PVV) scheduled for varicose vein surgery were invited to participate in the study. In all invited patients a clinical examination and a duplex Doppler of lower limb venous system was performed. The main inclusion criterion was the presence of unilateral PVV associated with great saphenous vein incompetence but with competent deep venous system. The exclusion criteria were as follows: history or ultrasound signs of proximal deep venous thrombosis, age < 18 years, pregnancy and breastfeeding, severe chronic venous insufficiency defined as the C4b-C6 class of CEAP (clinical-etiological-anatomical-pathophysiological) classification [5], chronic and acute lower limb ischaemia, known thrombophilia or other coagulation disorders, lymphoedema, any acute or chronic inflammatory disease, active cancer or history of chemo- or radiotheraphy, symptomatic coronary artery disease, history of major pelvic or retroperitoneal surgery or trauma, aortic or iliac aneurysms or portal hypertension.

In all enrolled patients, a thorough chronic venous disorders-oriented history-taking and physical examination of the limb with PVV were performed. If the symptoms or signs were present in both limbs their severity in PVV limb was recorded. The patients were asked about pain, oedema, night cramps, heaviness and a history of superficial thrombophlebitis. Based on the history taking and physical examination a clinical stage of CEAP classification was determined and Venous Clinical Severity Score was calculated [6]. At duplex Doppler GSV reflux was graded according to Hach's classification [7].

During the varicose vein surgery, inferior vena cava and right and left iliac venous axes were interrogated with an intravascular ultrasound with Volcano s5 Imaging System (Volcano Corporation, Rancho Cordova, CA, USA) and catheters Visions PV .035 of maximum imaging diameter of 60 mm (Volcano Corporation, Rancho Cordova, CA, USA). The technique of IVUS examination has been described in the literature [8]. In short, in the operated limb the 9Fr introducer sheath was inserted by the direct puncture of great saphenous vein close to the sapheno-femoral junction and in the opposite limb by ultrasound guided percutaneous puncture of the femoral vein. IVUS catheter was introduced over the guide wire to the level of right atrium and during the continuous manual withdrawal the IVUS image was recorded. The pull-back record was stored and then archived on a DVD disc for a further analysis.

The morphometric analysis of inferior vena cava and left and right, common iliac veins (CIV) and external iliac veins (EIV) was performed with the Volcano s5 Imaging System (Volcano Corporation, Rancho Cordova, CA, USA). In each analysed vein a cross sectional area (CSA) of a non-stenosed segment was measured and denominated as the reference CSA (ref-CSA). CSA of the most stenosed segment was determined and was denominated a Minimal Lumen Area (MLA). Percentage of stenosis (S%) of each analysed vessel was calculated according to the following formula:

$$S\% = (\text{ref-CSA} - \text{MLA})/\ \text{ref-CSA} \times 100$$

An association between clinical symptoms and signs in PVV limb and presence of the ipsilateral venous stenosis was studied. In order to examine the additive effect of stenosis on the occurrence of clinical symptoms, four degrees of narrowing of the examined veins were established: grade 0 — stenosis ≤ 30%, grade 1 — stenosis 31% to 50%, grade 2 — stenosis 51% to 70% and grade 3 — stenosis > 70% and the venous axis stenosis score (VASS) was calculated by adding the grade of stenosis. In further analysis an association between clinical symptoms and signs in PVV limb and VASS was studied.

Statistical analysis

Normality of distribution of the variables was checked with Shapiro-Wilk test.

Quantitative variables of normal distribution are described by mean and standard deviation (SD) or by median and quartiles otherwise.

For qualitative variables absolute numbers and percentage distributions were shown.

Quantitative variables were compared between two groups by the means of Mann-Whitney test. Correlations between quantitative variables were analysed with Spearman's correlation coefficient. Significance level was set at 0.05.

Results

Thirty-two patients were enrolled in the study. There were 25 (78%) women. The mean age of the patients was 48 years. The mean BMI was 27.3 kg/m^2. Thirteen patients (40%) had varicose veins of the left lower extremity and 19 (60%) patients had varicose veins of the right lower extremity. The symptoms reported by the patients in PVV limb are presented in Table 1. Twenty-five limbs were classified as C2 and 7 limbs as C4a according to CEAP classification. The median VCSS was 4.0 (3.0–5.0). The reflux in GSV was graded as I in 12 %, as II in 32%, as III in 40% and as IV in 16% of limbs.

The median values of MLA and S% of the examined veins of PVV limb were 87.4 mm^2 (51–135.2 mm^2) and 44.0% (27.0–62.0%) for CIV and 74.8 mm^2 (49.8–96.0 mm^2) and 46.5% (35.5–63.0%) for EIV.

Table 1. The symptoms reported by the patients in primary varicose veins limb

Symptom	Number of patients	% of patients
Pain	14	44
Oedema	17	53
Night cramps	11	34
Heaviness	19	59
Hx of superficial thrombophlebitis	6	19

There were not any statistically significant differences in degree of stenosis of ipsilateral veins in relation to presence of symptoms and clinical CEAP class in PVV limb. The details are presented in Table 2. There were not any significant correlations between vein stenoses and VCSS, Spearman correlation coefficient and p value was 0.1 and 0.58 for CIV and –0.2 and 0.32 for EIV, respectively. The percentage of stenosis also did not correlate with degree of reflux in GSV, Spearman correlation coefficient and p value was 0.2 and 0.24 for CIV and 0.2 and 0.39 for EIV, respectively.

Similarly, there were no differences in MLA of iliac veins between patients with and without symptoms and between patients in class II and IVa according to the CEAP classification (Table 3). The MLA of CIV did not correlate with VCSS and but there was a positive correlation between MLA of EIV and VCSS. Spearman correlation coefficient and p value was 0.01 and 0.95 for CIV and 0.47 and 0.006 for EIV, respectively. The MLA

Table 2. Comparison of stenosis of examined veins in primary varicose veins limbs in relation to the presence of symptoms and stage of clinical-etiological-anatomical-pathophysiological (CEAP) classification; CIV: common iliac vein; EIV: external iliac vein

	CIV % of stenosis		EIV % of stenosis	
	Median (Q1–Q3)	p	Median (Q1–Q3)	p
Pain	57.28 (35.1–75.47)	p = 0.158	38.84 (36.31–58.65)	p = 0.88
Without pain	40.58 (23.07–57.56)		53.34 (18.8–66.41)	
Oedema	54.43 (24.44–62.6)	p = 0.983	39.32 (36.05–62.8)	p = 0.913
Without oedema	43.82 (28.39–61.32)		50.94 (29.61–66.11)	
Night cramps	24.65 (20.83–43.83)	p = 0.068	38.36 (32.17–61.65)	p = 0.774
Without night cramps	57.28 (35.17–63.04)		49.97 (35.62–62.77)	
Heaviness	54.43 (28.45–62.89)	p = 0.377	45.47 (35.72–62.74)	p = 0.839
Without heaviness	36.33 (22.8–53.5)		47.86 (24.13–66.2)	
Hx of superficial thrombophlebitis	50.31 (32.71–59.69)	p = 0.882	54.13 (40.86–73.01)	p = 0.219
Without hx of superficial thrombophlebitis	43.83 (23.71–65.27)		38.34 (26.07–63.07)	
CEAP4	56.67 (28.68–59.3)	p = 0.533	36.42 (34.53–46.33)	p = 0.405
CEAP2	44.71 (26.69–71.51)		54.46 (35.4–62.68)	

Table 3. Comparison of minimal lumen area (MLA) of examined veins in primary varicose veins limbs in relation to the presence of symptoms and stage of clinical-etiological-anatomical-pathophysiological (CEAP) classification; CIV: common iliac vein; EIV: external iliac vein

	CIV MLA		EIV MLA	
	Median (Q1–Q3)	p	Median (Q1–Q3)	p
Pain	85.4 (40.42–103.82)	p = 0.88	90.8 (60.62–104.03)	p = 0.217
Without pain	80.2 (51.95–135.2)		68.8 (35.95–92)	
Oedema	86.5 (46–109.3)	p = 0.879	94.7 (50.8–106.5)	p = 0.152
Without oedema	82.25 (52.58–140.05)		63.65 (43.95–79.95)	
Night cramps	109.3 (73.4–146.6)	p = 0.092	75.5 (54.65–109.4)	p = 0.521
Without night cramps	55.25 (42.67–100.53)		75.6 (45.47–94.62)	
Heaviness	56.8 (45.35–117.35)	p = 0.266	75.5 (46.55–95.1)	p = 0.636
Without heaviness	94.75 (81.23–132.93)		76.45 (51.65–114)	
Hx of superficial thrombophlebitis	95.7 (60.13–130.6)	p = 0.929	51.95 (29.72–84.9)	p = 0.421
Without hx of superficial thrombophlebitis	82.25 (44.5–118.5)		72.95 (50.27–99.07)	
CEAP4	103 (56.6–117.35)	p = 0.678	95.5 (82–117.1)	p = 0.126
CEAP2	80.2 (46–139.8)		68.8 (50.8–94.4)	

Table 4. Comparison of venous access stenosis score (VASS) of examined veins in primary varicose veins limbs limbs with and without symptoms; CEAP: clinical-etiological-anatomical-pathophysiological classification

	VASS	
	Median (Q1–Q3)	p *
Pain	4 (3–5,75)	p = 0,203
Without pain	3 (3–5)	
Oedema	3 (3–5)	p = 0,821
Without oedema	4 (3–5)	
Night cramps	3 (2,5–5)	p = 0,27
Without night cramps	4 (3–5)	
Heaviness	4 (3–5,5)	p = 0,622
Without heaviness	4 (3–5)	
Hx of superficial thrombophlebitis	3 (3–4,5)	p = 0,95
Without hx of superficial thrombophlebitis	4 (3–5)	
CEAP2	4 (3–5)	p = 0,052
CEAP4	3 (3–3)	

did not correlate with degree of reflux in GSV, Spearman correlation coefficient and p value was -0.15 and 0.48 for CIV and –0.04 and 0.84 for EIV, respectively.

The analysis of the additive effect of stenosis did not demonstrate any significant difference in venous axis stenosis score between patients with and without symptoms. Patients with CIVa class had even lower VASS than CII class patients and the difference was at the border of statistical significance (Table 4).

The VASS did not correlate with VCSS (Spearman correlation coefficient –0.1, p = 0.58) but there was a moderate, positive correlation of borderline statistical significance between VASS and degree of reflux in GSV (Spearman correlation coefficient 0.375, p = 0.06).

Discussion

Primary varicose veins are dilated and tortuous superficial veins of lower extremity. The exact patomechanism of their development is not fully known. Moreover, even large PVV may be completely asymptomatic and small ones may give symptoms such as oedema, heaviness and pain. It has been shown that non-thrombotic iliac vein lesions are not associated with the occurrence of primary varicose veins [8]. The purpose of this study was to determine if there is any relationship between non-thrombotic iliac vein lesions and symptomatology of primary varicose veins. The patients constituted the representative sample of primary varicose vein population with female sex predominance and presented whole range of venous symptoms. The presence of iliac vein stenosis was assessed with an intravascular ultrasound which is the most accurate imagine modality for detecting of these lesions [4, 9, 10, 11]. The impact of stenosis on clinical symptoms was analysed in three aspects: the relative stenosis that was the percentage

of stenosis, the absolute stenosis that was the minimal lumen area and additive stenosis that was the venous axis stenosis score.

The main finding of this study is that clinical symptoms and signs and severity of venous disease are not associated with non-thrombotic iliac vein lesions in patients with primary varicose veins and without a severe chronic venous insufficiency. Even some inverse relations have been observed, such as higher venous axis stenosis score in lower CEAP class or greater minimal lumen area in higher VCSS. It may seem to disagree with the data from venous stenting studies.

In the studies on stenting of NIVLs the most prevalent symptoms reported by patients and also indications for the invasive treatment were pain and swelling in more than 70% of patients [12]. However, both pain and swelling reoccurred in 18–23% and 47–53% of patients, respectively despite widely patent stents [12]. Even less optimistic data come from a recently published study of 109 patients with chronic venous insufficiency that underwent iliac venous stenting for IVUS detected stenosis greater than 50% that were compared to 63 patients with iliac vein stenosis < 50% that were treated conservatively. At least moderate persistent pain or discomfort post-procedure was reported by 43% stented patients and 58% non-stented patients and the quality of life measured by CIVIQ-20 did not differ between the groups [13]. These data may point to other than NIVL aetiology of symptoms and also to some degree of placebo effect at least in relation to pain.

In the of 68 patients who underwent stenting of NIVLs it was shown that the clinical benefit may be expected if the stenosis measured by IVUS is greater than 64% [14]. In this study we did not find any correlation between the occurrence of symptoms and degree of stenosis though the stenosis of iliac veins exceeded 62% in one quarter of patients.

It should be noted that there are not any randomized placebo-controlled studies on venous stenting and the quality of evidence is weak [15]. The population of patients referred for venous stenting is a mixture of non-thrombotic and thrombotic lesions and consist of individuals that have more severe symptoms [16]. The patients from reports on venous stenting had median VCSS 9 while the median VCSS in this study was 4 [16]. So definitely the patients from venous stenting studies are preselected in some way and may be different from the patients from our study.

It should be also remembered that pathophysiology of chronic venous disease is very complex and not straight forward. The symptoms are supposed to result from reflux and/or obstruction. So, most probably in PVV the symptoms are caused by reflux rather than obstruction and the reflux is the consequence of weakness of the venous wall of superficial venous system [17, 18]. The symptoms may result from the disturbances at the level of microcirculation not necessarily from the venous obstruction or reflux what explains a significant relief of symptoms and signs of chronic venous disease observed after treatment with venotonic drugs [19]. That is why we often see patients without varicose veins or venous reflux but with significant symptoms.

The patients with primary varicose veins and without severe chronic venous insufficiency should not be investigated towards NIVL because even if found iliac vein stenosis will not have any clinical importance. That is in agreement with a recently published by several American vascular societies appropriate use criteria which consider iliac vein or inferior vena cava stenting for iliac vein compression as an incidental finding by imaging with minimal or no symptoms or signs, and incentivizing sonographers to find reflux highly inappropriate [20].

Although generally no associations between the severity of venous disease and iliac vein stenosis have been observed in this study, one of the tendencies may require further evaluation. There has been a borderline, positive correlation between venous axis stenosis score and the degree of axial reflux in GSV that might mean that outflow obstruction predisposes to development and progression of such reflux.

Undoubtedly the limitations of this study are a relatively small number of patients and absence of patients with severe chronic venous insufficiency; therefore, the conclusion cannot be applied to the patients with venous leg ulcers.

Conclusions

It can be concluded that though IVUS-detected stenoses of iliac veins are common in patients with primary varicose veins and without severe chronic venous insufficiency, they are not associated with severity of symptoms. Thus, these patients do not require routine imaging studies to diagnose non-thrombotic iliac vein lesions.

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Conflict of interest

None.

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Investigation of clinical and genetic factors in patients with deep vein thrombosis. A retrospective study

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Abstract

Introduction: *Generally, risk factors for deep vein thrombosis (DVT) include advanced age, surgery, fractures, pregnancy, puerperium, use of oral contraceptives, hormone replacement therapy, cancer and genetics associated with the hemostatic system in influencing thrombotic risk. The aim of this study was to evaluate routine complete blood count parameters, the clinical and genetic data of patients with diagnosis of DVT in our hospital and to contribute to the literature with our results.*

Material and methods: *This retrospective study included a total of 152 patients (67 males and 85 females) diagnosed with DVT between January 2016 and September 2019. The history, clinical findings, venous doppler ultrasonography and genetic analysis results of patients were evaluated.*

Results: *The study included 152 DVT patients. When the lower extremity venous doppler ultrasonography (VDU) results were evaluated, venous insufficiency was detected in 126 patients (82.9%), 57 of whom were male and 69 were female. Genetic results of F2 G20210A, FVL G1691A, MTHFR C677T and MTHFR A1298C were examined. FLV gene distributions were statistically different between genders (38.8% of men; 16.5% of women). This difference was also statistically significant ($p = 0.003$).*

Conclusion: *The hemogram parameters were found to be insufficient markers. VDU was seen to be a clinically necessary marker in diagnosis; and genetic outcomes were important in initiating appropriate treatment in the early period. These results also show that there are differences according to gender in determining thrombotic risk.*

Key words: thrombosis, deep vein thrombosis, genetics, factor V Leiden

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Introduction

Venous thromboembolism (VTE) is the general term for all pathological thrombosis arising in the venous circulation, which clinically includes pulmonary embolism (PE) and deep vein thrombosis (DVT). DVT is usually defined as a clot that forms in the deep veins of the lower extremities, impairing the circulation by blocking the vessel [1]. Deep vein thrombosis is an important cause of mortality and morbidity since it is a cause of pulmonary embolism and post-thrombotic syndrome. Advanced age, immobility, trauma, history of surgical operation and malignancy are conditions that increase the tendency to develop DVT [2]. The diagnostic tools for the diagnosis of DVT are limited in primary care. The patient's history and classic clinical findings on physical examination alone are not sufficient to diagnose or exclude DVT. In order to reliably exclude DVT, a pre-test probability scoring of DVT, called the Wells' scoring developed by Wells et al. [3], is used for

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the diagnosis. Following the Wells' scoring, DVT is diagnosed by D-dimer and venous doppler ultrasonography performed on patients.

Venous doppler ultrasonography (VDU) is the standard imaging test for patients suspected of having lower extremity DVT. The sensitivity of VDU is 97% for proximal DVT, while it is 73% for calf veins, and may remain inadequate for calf veins in asymptomatic patients. Compression VDU performed at certain intervals allows imaging of all leg and calf veins [4]. VDU makes it easier for the physician to diagnose or exclude the disease. In addition, another important factor is genetic findings that allows for easier treatment of DVT for the physician. The genetic factors include factor V Leiden (FVL) G1691A, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C, and coagulation factor II (FII) G20210A polymorphism regions [5]. FVL is the most common cause of hereditary thrombophilia and is resistant to protein C. The risk of thrombosis is increased 5-fold in heterozygotes and 50-fold in homozygotes. FII G20210A mutation is also among the causes of hereditary thrombophilia. Patients with FVL G1691A and FII G20210A compound heterozygotes carry a twenty-fold increased risk of thrombosis compared to those with both mutations [5, 6]. Hyperhomocysteinemia (elevation of homocysteine level) may be both genetic and acquired. MTHFR is an enzyme involved in homocysteine metabolism. The most common genetic causes of hyperhomocysteinemia are the C677T and A1298C mutations that lead to MTHFR enzyme deficiency. Although hyperhomocysteinemia is an independent risk factor for atherosclerotic vascular diseases, it has been reported that it increases the risk of venous thrombosis and is associated with DVT [5, 7]. Evaluation of these factors is important in diagnosis and treatment of the disease.

In our study, we retrospectively evaluated demographic characteristics, reasons for admission to hospital, physical examination findings, DVT localization (determined by VDU) and genetic findings of patients, who received a diagnosis of DVT, and aimed to determine whether an acute attack can be predicted in the diagnosis, treatment and/or whether an acute attack had an effect on markers.

Material and methods

The study included a total of 152 patients, 85 females (55.9%) and 67 males (44.1%), diagnosed with lower extremity DVT. Patients who had a DVT attack for the first time were included in the study. The patients were diagnosed by clinical symptoms and VDU results. Patients with known heritable diseases were excluded. The routine hemogram results, clinical findings and genetic results of these patients were retrospectively evaluated. The ethical approval for our study was obtained from the Ethics Committee of Bozok University, Faculty of Medicine, Turkey (document no: 2017-KAEK-189_2019.06.26_07). The study was conducted in accordance with the principles of the Helsinki Declaration of the World Medical Association. All participants gave informed written consent.

Statistics

SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analysis of the data. Categorical measurements were expressed as number and percentage, and continuous measurements were expressed as mean and standard deviation (median and minimum-maximum, where necessary). The Chi-square test or Fisher's exact test were used to compare categorical variables. Normality distributions were checked in the comparison of continuous measurements between groups; the Mann-Whitney U test was used since the parametric distribution did not provide precondition assumption. The correlation between the variables was determined by Spearman's correlation coefficient. The level of statistical significance was taken as 0.05 in all tests.

Results

Of the 152 DVT patients participated in the study, 85 were female and 67 were male. The mean age of the patients was 48.6 ± 12.2 years, 46.9 ± 11.8 years for females, and 50.8 ± 12.4 years for males. In general, the admission complaints were pain, swelling and accompanying redness in 87% of the patients. When the hemogram results of the DVT patients included in the study were examined, all values were found to be within the normal range in both males and females. When the results were statistically evaluated by gender, it was found that there was a significant difference between the male and female patients in terms of eosinophil, erythrocyte, hematocrit, hemoglobin, mean cell hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and procalcitonin (PCT) values (Table 1). When we evaluated the hemogram results of our patients in the light of the data obtained, we found a significant difference between the female and male patients depending on gender in eosinophil ($p = 0.025$), erythrocyte ($p = 0.003$), hematocrit ($p = 0.000$), hemoglobin ($p = 0.000$), MCH ($p = 0.010$), MCHC ($p = 0.014$) and PCT ($p = 0.009$) values. When the results of lower extremity VDU were evaluated, the VDU findings were found to be normal in 26 patients (17.1%), 13 of whom were male and 13 of whom were female. However, venous insufficiency was

detected in 126 patients (82.9%), 57 of whom were male and 69 of whom were female. Thirty (23.8%) patients had deep venous insufficiency in at least one vein. Of the patients, 61 (48.4%) had venous thrombosis in the left lower extremity, 47 (37.3%) in the right lower extremity, and 18 (14.3%) had venous thrombosis both in the right and left lower extremities. It was found that fifty-four patients (42.8%) had DVT in the great saphenous vein (GSV), 47 (37.3%) in the femoral vein, 28 (22.2%) in the popliteal vein, 19 (15%) in the tibial vein, and 7 (5.5%) had DVT in the iliac vein.

Genetic results of FII, FVL and MTHFR genes were also evaluated to help early treatment. When the FII gene G20210A polymorphism region was evaluated, it was found that of the 152 patients, 142 (93.4%) had GG normal genotype (WT) and 10 had GA heterozygous mutant (6.6%) (HT) genotype. None of the patients had AA homozygous mutant (HM) genotype. When the FVL gene G1691A polymorphism region was evaluated, it was found that of the 152 patients, 106 (69.7%) had GG (WT), 40 (26.3%) had GA (HT) and 6 (3.9%) had AA (HM) genotypes. When the MTHFR gene C677T polymorphism region was evaluated, it was found that of the 152 patients, 82 (53.9%) had CC (WT), 57 (37.5%) had CT (HT) and 13 (8.6%) had TT (HM) genotypes. When the MTHFR gene A1298C polymorphism region was evaluated, it was found that of the 152 patients, 51 (33.5%) had AA (WT), 81 (53.3%) had AC (HT) and 20 (13.2%) had CC (HM) genotypes. When the genetic findings were statistically evaluated by gender, it was found that there was a significant difference in male heterozygous individuals in terms of FVL G1691A polymorphism region ($p = 0.003$). 38.8% of men were heterozygous while 16.5% of women were heterozygous. In addition, the proportion of individuals who were normal is higher in women than in men (Table 1, Fig. 1). Moreover, it was found that 11 of the 152 patients had no genotypic change, while 68 patients had more than one mutation. It was determined that 38 patients had heterozygous mutations in two genes, 17 patients had heterozygous mutations in three genes, 10 patients had one heterozygous and one homozygous mutations, 2 patients had two heterozygous mutations, and 1 patient had two homozygous mutations.

Discussion

It is known DVT affects approximately 0.1% of people annually and the incidence rises with increasing age. It is a disease with a high morbidity and mortality rate when not diagnosed and treated early. It may cause pulmonary embolism, venous gangrene, chronic venous insufficiency, venous hypertension and post-thrombotic syndromes [6]. The signs and symptoms of DVT are nonspecific and specific tests are required for the diagnosis [8]. In the etiology of DVT, the findings of hemogram, VDU and genetic analysis are helpful in the diagnosis and treatment process and in preventing post-treatment complications. In our study, we evaluated whether an acute attack could be predicted in the diagnosis and treatment by using hemogram, clinical and genetic parameters of DVT patients and/or whether an acute attack had an effect on markers.

In DVT, hemogram findings may provide important data at the time of initial diagnosis, but are insufficient alone. Some studies in the literature have reported that there might be a correlation between certain hemogram parameters and DVT. In their study, Zorlu et al. [9] emphasized that increased red cell distribution width (RDW) poses an increased risk of mortality in acute pulmonary embolism patients. In another study, Cay et al. found a significant correlation especially between high RDW level and the presence and severity of non-chronic proximal DVT in patients with DVT [10]. In another study on Turkish patients, the mean platelet volume (MPV), an indicator of platelet activation, was found to be higher in patients with DVT than in the control group [11]. When we evaluate the hemogram results of our patients in the light of the data obtained, we found a significant difference between the female and male patients depending on gender in eosinophil ($p = 0.025$), erythrocyte ($p = 0.003$), hematocrit ($p = 0.000$), hemoglobin ($p = 0.000$), MCH ($p = 0.010$), MCHC ($p = 0.014$) and PCT ($p = 0.009$) values. VDU findings have an important role in the diagnosis of DVT and even provide great convenience to the physician in the diagnosis of patients whose diagnosis has been missed on the initial examination. Of venous thrombosis, 85% occur in the tibial vein, 9% in the popliteal fossa, and 6% occur in the thigh veins. Of venous thrombosis, 35% is bilateral. It occurs more commonly in the left lower extremity [12]. In a retrospective study evaluating VDU results of 1,328 Turkish patients, 26.7% of the patients were found to have deep venous insufficiency in at least one vein [13]. According to the VDU results evaluated in another study, 62.4% of patients were found to have thrombosis in the left lower extremity and 37.6% were found to have thrombosis in the right lower extremity. Of these patients, 41.6% had DVT in the femoral vein, 31.8% in the iliac vein and 26.6% had DVT in the popliteal vein and its distal [14]. According to VDU results evaluated in the present study, 80.3% of our patients (122 patients) were found to have venous insufficiency. In light of these results, we see that VDU findings have an important place in the diagnosis of DVT and provide great convenience to the physician in the diagnosis, although hemogram findings do not show any variability.

Table 1. Demographic data of the patients

	Women	Men	Total	p
Participants (n)	85	67	152	
Risk factors and biological data				
Age (years)	46.9 ± 11.8	50.8 ± 12.4	48.6 ± 12.2	0.146
Gender (%)	55.9	44.1	100	
WBC ($10^3/mm^3$)	8.1 ± 1.8	8.4 ± 2.2	8.2 ± 1.9	0.534
Neutrophil ($10^3/mm^3$)	5.0 ± 1.7	5.2 ± 1.8	5.1 ± 1.7	0.524
Lymphocytes ($10^3/mm^3$)	2.3 ± 0.5	2.4 ± 0.9	2.3 ± 0.7	0.778
Monocytes ($10^3/mm^3$)	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.1	0.057
Eosinophil ($10^3/mm^3$)	0.1 ± 0.07	0.2 ± 0.1	0.2 ± 0.1	0.025*
Basophil ($10^3/mm^3$)	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.197
Neutrophil (%)	60.5 ± 7.7	60.5 ± 9.9	60.5 ± 8.7	0.803
Lymphocytes (%)	29.9 ± 6.8	29.3 ± 9.3	29.6 ± 7.9	0.415
Monocytes (%)	7.1 ± 1.2	7.3 ± 1.3	7.2 ± 1.2	0.089
Eosinophil (%)	1.8 ± 0.8	2.2 ± 1.4	1.9 ± 1.1	0.144
Basophil (%)	0.6 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.110
Erythrocyte ($10^3/mm^3$)	4.8 ± 0.4	5.1 ± 0.5	4.9 ± 0.4	0.003*
Hemoglobin (g/dL)	13.4 ± 1.3	14.4 ± 1.7	13.8 ± 1.5	0.000*
Hematocrit (%)	41.2 ± 3.7	43.6 ± 4.2	42.2 ± 4.1	0.000*
MCV (fl)	84.9 ± 4.5	86.0 ± 3.5	85.4 ± 4.1	0.279
MCH (fl)	27.6 ± 1.8	28.3 ± 1.8	27.9 ± 1.8	0.010*
MCHC (g/dL)	32.6 ± 1.1	32.8 ± 1.4	32.6 ± 1.2	0.014*
RDW-CV (%)	13.9 ± 1.2	13.9 ± 1.5	13.9 ± 1.4	0.646
Platelets ($10^3/mm^3$)	284.8 ± 52.2	266.1 ± 53.8	276.6 ± 53.5	0.162
PCT (%)	0.3 ± 0.05	0.3 ± 0.05	0.3 ± 0.05	0.009*
MPV (fL)	10.5 ± 0.7	10.3 ± 0.7	10.4 ± 0.7	0.205
PDW	12.3 ± 1.7	11.9 ± 1.6	12.2 ± 1.6	0.502
NRBC (fL)	0.002 ± 0.005	0.004 ± 0.02	0.003 ± 0.01	0.510
NRBC (%)	0.02 ± 0.04	0.03 ± 0.14	0.02 ± 0.09	0.411
IG	0.05 ± 0.07	0.07 ± 0.12	0.06 ± 0.1	0.105
IG (%)	0.5 ± 0.5	0.8 ± 1.5	0.6 ± 1.06	0.066
PLCR (%)	28.2 ± 5.3	27.3 ± 5.2	27.8 ± 5.3	0.551
Genetics data				
FII G20210A				0.187
WT	77 (%90.6)	65 (%97)	142 (%93.4)	
HT	8 (%9.4)	2 (%3)	10 (%6.6)	
FVL G1691A				0.003*
WT	69 (%81.2)	37 (%55.2)	106 (%69.7)	
HT	14 (%16.5)	26 (%38.8)	40 (%26.3)	
HM	2 (%2.4)	4 (%6)	6 (%3.9)	
MTHFR C677T				0.612
WT	48 (%56.5)	34 (%50.7)	82 (%53.9)	
HT	29 (%34.1)	28 (%41.8)	57 (%37.5)	
HM	8 (%9.4)	5 (%7.5)	13 (%8.6)	
MTHFR A1298C				0.500
WT	30 (%35.3)	21 (%31.3)	51 (%33.6)	
HT	42 (%49.4)	39 (%58.2)	81 (%53.3)	
HM	13 (%15.3)	7 (%10.4)	20 (%13.2)	

Values presented as mean ± standard deviation; *$p < 0.05$; IG: immature granulocyte; MCV: mean corpuscular volume; MCH: mean cell hemoglobin; MCHC: mean corpuscular hemoglobin concentration; MPV: mean platelet volume; NRBC: erythroblasts; PCT: procalcitonin; PDW: platelet distribution width; PLCR: platelet large cell ratio; RDW-CV: red cell distribution width %; WBC: leucocytes

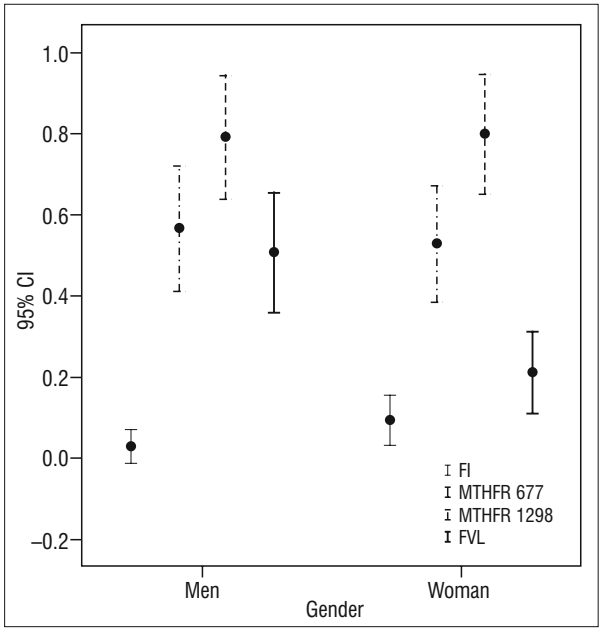

Figure 1. In the diagram, genetic changes according to gender are presented in 95% confidence interval

In the literature, there are many studies analyzing the genetic risk factors in the etiology of DVT in different ethnic populations [15, 16]. In various studies, FII G20210A, FVL G1691A, MTHFR C677T and MTHFR A1298C polymorphism regions especially have been reported to be risk factors in venous thrombosis [5, 7, 17]. In a study on the Croatian population, the FVL G1691A mutation was identified to be the most common and the FII gene G20210A was identified to be the second most common mutation [18]. In a similar study on Iranian DVT patients, FVL G1691A was found to be the most common mutation. In addition, it was emphasized that it is more common in female patients [19]. In another study on the Iranian population, FVL G1691A, MTHFR C677T and MTHFR A1298C mutations were associated with increased risk of DVT. However, it was indicated that G20210A mutation in the FII gene was not a risk factor for DVT [20]. In a meta-analysis study with a large sample size, FVL G1691A and FII G20210A mutations were identified to be moderate risk factors for vascular thromboembolism. The risk was found to increase in FVL G1691A and FII G20210A heterozygous carriers but not associated with MTHFR C677T mutation [21]. There are also other studies showing no correlation between MTHFR C677T polymorphism and venous thrombosis [22]. In a similar study on the Turkish population, it was stated that there was a correlation between MTHFR C677T polymorphism and DVT, but the same results were not found for MTHFR A1298C polymorphism [23]. When we evaluated our results based on these data, we could not identify a homozygous mutation for the FII G20210A polymorphism region. We identified heterozygous mutations in 6.6% of the patients. In terms of the FVL G1691A polymorphism region, we found that of the patients, 26.3% had heterozygous mutation and 3.9% had homozygous mutation. In contrast to the results of the study conducted on the Iranian population, we found it to be higher in male patients than in female patients ($p = 0.003$) [19]. We found that of the patients, 37.5% had the heterozygous mutation, 8.6% had the homozygous mutation for the MTHFR C677T polymorphism region, 53.3% had the heterozygous mutation and 13.2% had the homozygous mutation for the A1298C polymorphism.

In conclusion, there were many factors affecting the etiology of DVT. In this study, hemogram, clinical and genetic parameters of DVT patients were evaluated. In light of the data, the hemogram parameters were found not to be sufficient markers. VDU was found to be a clinically necessary marker in diagnosis, together with genetic outcomes, in initiating appropriate early-phase treatment. Whether thrombotic events occur due to genetic predispositions that differ according to gender requires studies with larger populations. Subsequent studies will be needed to further our knowledge on these issues.

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Conflict of interest

None.

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Impact of risk factors on serum levels of vasoactive substances in patients with peripheral arterial occlusive disease at different Fontaine's stages

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Abstract

Introduction: *There is a bulk of literature data on the physiological roles of nitric oxide (NO) and endothelin-1 (ET-1), but many facts remain unknown, especially in certain diseases such as peripheral arterial disease.*

Material and methods: *This is an observational cross-sectional study. The subjects were patients diagnosed with PAD. Serum levels of NO and ET-1 were determined for all patients, and statistical data processing was performed according to the set goals.*

Results: *The study included 64 patients with mean age 60.2 ± 12.7 years, mostly in stage II PAD according to Fontain (46.9%). Statistical analysis failed to determine a significant difference in serum NO or ET-1 values with respect to disease stage, sex, and body mass index (BMI). Certain oscillations were found in the mean values of NO related to smoking and diabetes but without statistical significance. There were also oscillations in the values of ET-1, with higher levels found in women, smokers and non-diabetics in whom this difference reached statistical significance ($p = 0.041$).*

Conclusion: *Serum levels of NO and ET-1 in this study show some causal relationship with certain risk factors for PAD such as diabetes and smoking, but additional research is needed to fully understand their effects and interactions.*

Key words: local circulation, peripheral arterial disease, endothelin-1, nitric oxide

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Introduction

The endothelium of blood vessels synthesizes a lot of different mediators that are involved in vasodilatation, vasoconstriction, adhesion, growth, differentiation, proliferation and other aspects of endothelial function that are responsible for cardiovascular homeostasis and health [1–3]. Endothelial dysfunction is characterized by imbalanced vasodilatation and vasoconstriction, elevated levels of reactive oxygen species and proinflammatory factors, as well as deficiency of nitric oxide bioavailability [3]. Endothelium dysfunction may result

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in the loss of important homeostatic functions, which, in turn, leads to various pathologies. For example, endothelial dysfunction is the initial stage in the pathogenesis of peripheral artery disease, cardiovascular disease, chronic venous disease, chronic kidney failure, cancer, infectious diseases, and obesity [4].

Endothelial cell in the walls of arterioles and small arteries synthetize a number of vasoactive substances, some of which are potent vasodilators, such as nitric oxide (NO), or potent vasoconstrictors, such as endothelin-1 (ET-1) [5].

Nitric oxide is also considered to be responsible for platelet aggregation, non-adrenergic-non-cholinergic neurotransmission and cytotoxic reactions [6], while ET-1 has a 100 times more potent vasoconstrictor effect than adrenaline, and can also have effects on muscle contractility, secretory activity, cell transport management, growth and proliferation as well as modulation of the immune response [7–10].

ET-1 is inextricably linked to a number of vascular mediators, primarily NO, to which it acts as a natural counterpart. However, there is evidence that they do not have a simple opposite effect on the vascular system, but rather there is a complex system of coordinated cascade of reactions [11, 12].

The dominant vasoconstrictor effect of ET-1 is manifested through ET-A receptors, while the initial short-term vasodilatating effect is explained by the autocrine feedback of ET-1 through ET-B receptors on endothelial cell membranes, which promotes transient production of prostacyclin and NO which cause vasodilatation [13, 14]. In pathophysiological conditions where the availability of NO is compromised, vasoconstrictor and other effects of ET-1 such as proliferation and migration become disinhibited. This can lead to the development of a certain pathological process or worsening of the existing one. All the evidence suggests that balance among these mediators is crucial for preserving vascular function and that loss of balance between them leads to disruption or exacerbation of pre-existing vascular disease [1].

Homeostasis of endothelial vasoactive substances is disturbed in many diseases, and one of them is peripheral arterial disease (PAD). Most patients with PAD have impaired vascular function and vascular regulation, and the studies of homeostatic mechanisms involving these substances and their receptors are very actual.

Material and methods

Study design and location

The study was designed as an observational cross-sectional study and it was conducted in the period from January to July 2016, during multimodal hospital treatment of patients diagnosed with PAD at the Department of Physical Medicine and Rehabilitation "Dr Miroslav Zotovic" Banja Luka — at the Center for Hyperbaric Medicine and Rehabilitation and Treatment of Chronic Wounds.

The study was approved by the Ethics Committee of the Institute for the Physical Medicine and Rehabilitation "Dr Miroslav Zotovic" Banja Luka (the number of agreement: 6947/13). The informed consent was signed by the patient or authorized representative of the patient. Measurements were performed at two separate certified laboratories.

Patients

Inclusion criteria for this study were age over 20 years, signed consent to participate in the study (informed consent), documented findings with a diagnosis of PAD, and data on the stage of Fontaine's disease. In relation to the classification of PAD according to Fontaine, the study included patients with stages II (intermittent claudication as the dominant symptom), III (pain at rest as the dominant symptom) and IV (the dominant finding is irreversible ischemia with necrosis and gangrene). Exclusion criteria were age less than 20 years, pregnancy and patients without established diagnosis of PAD. Patients were interviewed, and after signing informed consent, demographic data (gender and age), anthropometric data (height and weight from which BMI was calculated), and data on smoking status and a history of diabetes were recorded for each patient.

Blood sampling

Blood for analysis was obtained by venipuncture of the cubital vein. A total sample of 2 milliliters of blood was taken before initiating any therapeutic measures prescribed by hospital treatment at the same time each day (between 8 and 10 AM) before the start of any therapeutic procedures.

Serum NO level measurement

NO levels were determined by spectrophotometry using the Griess reagent (Griess method). Immediately after sampling, the samples were treated with 30% ZnSO4 to deproteinize the blood and release hemoglobin-bounded $NO3^{2-}$. It was then centrifuged for 10 minutes, and separated supernatants were stored in the freezer at –80°C. NO concentration was measured using the classical colorimetric Griess reaction, the conversion of $NO3^{2-}$ to $NO2^{2-}$ by elemental zinc followed by measuring $NO2^{2-}$ concentration. The concentration of NO (in mmol/L) was determined from a standard curve with known concentrations of NaNO2 (from 1.56–100 mmol/L). Purified water with Griess reagent was used as a blank probe. The mean value of three

consecutive measurement performed on the same sample was taken as the definitive level of NO. Intra and inter assay CV were 6.3 and 5.9%, respectively.

Serum ET-1 level measurement

Serum was separated from the whole blood using water bath at 37°C, after which it was frozen at –80°C until analysis. Determination of serum ET-1 levels was performed with EIA methodology based on an immunometric assay, the so-called "Sandwich technique" using the Endothelin-1 ELISA kit, IBL Hamburg, Germany. The concentration of ET-1 is determined measuring the change in color intensity. This measurement was performed electronically using an ELISA reader (Elx 800 Universal Microplate Reader Biotek Instruments, INC) at a wavelength of 405 nm. An automatic ELISA washer from the same manufacturer was used to rinse the plate. The standard curve was obtained from known ET-1 activities within the kit. The values obtained are expressed in pg/ml. Since 8 standard probes were used to obtain the standard curve, with decreasing concentrations in the S1–S8 standards, the S1 sample chamber contained a concentration of about 250 pg/ /mL, the next one 125 pg/mL, and further 62.5 pg/mL, 26.3 pg/mL and in each of the following two times less than in the previous one, while the S8 sample contained only diluted human plasma from the kit itself, with the manufacturer's guarantee that the concentration of ET-1 was less than 1.5 pg/mL. Thus, for the adsorbents obtained below the absorbance values for standard S8, we interpreted the serum ET-1 levels of subjects below 1.5 pg/mL as expected (normal) values in potentially healthy subjects. For these adsorption findings, level of 1.5 pg/mL was noted in the table. The mean value of three consecutive measurement performed on the same sample was taken as the definitive level of ET-1. Intra- and inter-assay CVs were 5.9 and 6.2 %, respectively.

Statistical analysis

The obtained results were stored in table (MS Excel 2013), and the SPSS software (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was used for all statistical analysis. Data were processed by standard statistical methods, both from the domain of descriptive statistics (basic descriptive measures: mean, mode, median, standard deviation) and from the area of statistical inference (Student's t test for small independent samples, Student's t test for small dependent samples, χ^2 test, ANOVA test). Values of $p < 0.05$ were considered statistically significant.

Table 1. Descriptive parameters of studied patients

	n (%)
Males	36 (56.3)
Age 20–29 years	2 (3.1)
Age 30–39 years	4 (6.3)
Age 40–49 years	3 (4.7)
Age 50–59 years	19 (29.7)
Age 60–69 years	25 (39.0)
Age > 70 years	11 (17.2)
Stage II PAD	30 (46.9)
Stage III PAD	21 (32.8)
Stage IV PAD	13 (28.3)
Smokers	15 (23)
Diabetes	52 (81)
Body mass index > 24.9	44 (68.7)

Results

During the study period, a total of 64 patients (36 men and 28 women) treated at the Center for Hyperbaric Medicine and Rehabilitation and Treatment of Chronic Wounds were included using previously defined criteria. Patients' characteristics are shown in Table 1.

Table 2 presents comparison of NO (mean values ± SD) (mmol/L) and ET-1 (mean values ± SD) (pg/ /mL) serum levels between different groups of studied patients. Patients were divided in groups according to presence of risk factors (stage of PAD, gender, smoking and diabetes).

Significantly higher NO concentrations were found in the age group 20–29 years compared to almost all other age groups using post hoc Tukey analysis [group $_{20–29\ (\text{mean} \pm \text{SD})}$ 41.45 ± 16.7 vs. group $_{30–39\ (\text{mean} \pm \text{SD})}$ 16 ± 3.3 ($p = 0.01$); group $_{20–29\ (\text{mean} \pm \text{SD})}$ 41.45 ± 16.7 vs. group $_{50–59\ (\text{mean} \pm \text{SD})}$ 19.07 ± 7.82 ($p = 0.09$); group $_{20–29\ (\text{mean} \pm \text{SD})}$ 41.45 ± 16.7 vs. group $_{60–69\ (\text{mean} \pm \text{SD})}$ 23.58 ± 9.52 ($p = 0.05$); group $_{20–29\ (\text{mean} \pm \text{SD})}$ 41.45 ± 16.7 vs. group $_{>70\ (\text{mean} \pm \text{SD})}$ 19.19 ± 6.74 ($p = 0.01$)].

The highest ET-1 concentrations were detected in the age group 50–59 years but the difference between different age groups did not reach statistical significance.

Discussion

We showed in this study that patients with PAD have impaired homeostasis of the observed vasoactive substances. In addition to PAD, levels of vasoactives were also affected by smoking status and presence of diabetes.

Table 2. Serum levels of NO and ET-1 (mean value ± SD) in regards to presence of risk factors

	NO (mean value ± SD) (mmol/L)	p
Stage II/III/IV PAD	21.9 ± 9.6/20.0 ± 6.9/23.5 ± 11.5	0.554*
Males/Females	22.3 ± 8.9/21.1 ± 9.5	0.604#
BMI ≤ 24.9/BMI > 24.9	23.9 ± 12.4/20.6 ± 7.3	0.182#
Smoker/Non-smoker	19.6 ± 7.1/22.3 ± 9.8	0.341#
Diabetes/No diabetes	20.9 ± 8.0/24.8 ± 13.2	0.182#
	ET-1 (mean value ± SD) (pg/mL)	**p**
Stage II/III/IV PAD	6.3 ± 15.9/1.5 ± 0.000/3.8 ± 8.2	0.343*
Males/Females	1.50 ± 0.000/6.48 ± 15.37	0.080#
BMI ≤ 24.9/BMI > 24.9	4.2 ± 8.4/4.2 ± 12.8	0.998#
Smoker/Non-smoker	8.9 ± 21.7/2.8 ± 5.4	0.075#
Diabetes/No diabetes	2.8 ± 5.6/10.3 ± 23.8	0.041#

*ANOVA; #Student's t test

NO levels differed between patients in different stages of PAD but without statistical significance. The fact that PAD is basically an inflammatory process and that stimulation of endothelial procoagulant tissue factors, leukocyte adhesion molecules and chemotactic substances as well as endothelial NO synthase inhibitors leads to a decrease in NO production, our finding was contradictory [15].

We showed higher oscillations of ET-1 concentrations between patients in different stages of PAD but without statistical significance. The predominance of ET-1vasoconstrictor effects was expected, but lower values were detected in more severe stages of the disease. The reason for this may be found in the chronic nature of the condition, as well as the involvement of other mechanisms and mediators [16–18].

When looking at demographic data we haven't found significant difference in NO levels between patients' groups, while ET-1 levels were lower in male patients but without statistical significance. Data from the works of other authors on impact of gender on ET-1 levels are very scarce.

Obesity as one of the risk factors for the development of metabolic syndrome was expressed through BMI and patients with BMI > 24.9 were classified as obese. The effect of hyperglycemia on blocking the function of endothelial NO synthetase and increasing the production of free radicals that interfere with vasodilatatory homeostasis has been known for some time [19], and in the light of these findings lower NO levels could be attributed to higher BMI. Elevated ET-1 values were initially observed in obese patients, which was confirmed in experimental studies where higher expression of ET-A was demonstrated relative to ET-B receptors in adipose tissue [20, 21]. Our findings related to ET-1 do not support these findings.

A very significant finding of this study is that ET-1 levels are higher and NO levels are lower in smokers, which is unequivocal evidence of the predominance of vasoconstrictor effects in smokers. Other authors state that cigarette smoking is a risk factor for vascular diseases, such as hypertension, atherosclerosis and aneurysm [22, 23]. A crucial feature of smoking-induced vascular injury is endothelial dysfunction, defined as decreased nitric oxide bioavailability in the vascular wall [24]. Among studies that tested the effect of nicotine on NO-dependent endothelial relaxation, one stands out [25] which showed that cigarettes containing nicotine extract more severely damage the integrity of the endothelium compared to those that are nicotine free. Subsequent analyzes have shown that other components of tobacco smoke also damage NO homeostasis, although to a much greater extent if combined with nicotine [26–28].

A strong relationship between ET-1 levels and smoking status (current smoker) was demonstrated in one

large cohort study that monitored ET-1 levels in healthy young adults where a link with global cardiovascular risks including smoking, blood pressure, and inflammation was demonstrated [29]. Additionally, smoking is thought to affect vascular tone via the endothelin system, moreover it is activated via ET-receptor pivotal protein kinases, such as mitogen-activated (MAP) kinases involved in important cell cycle and inflammatory cascades [30, 31].

There is evidence that diabetes leads to the activation of alternative signaling pathways that stimulate cell growth and proliferation, thus stimulating the release of ET-1 [32]. This has been confirmed by observations in animal models [33].

Limitations of this study are the inability to accurately determine the serum concentration of ET-1 for serum values less than 1.5 pg/mL due to the limitations of the kit that was used. Also, one of the shortcomings is the lack of classification of smokers into categories according to the smoking pack years, and classification of diabetes according to the present complications. We also did not use ankle brachial index for determination of correlation between PAD and CAD like certain studies [34–36]. Additionally, we did not have control group of healthy subject.

Conclusions

Finally, the results of this study showed that NO and ET-1 represent important factors in modeling the pathological process in PAD. The fact that the basic values of the examined vasoactive substances show oscillations depending on the presence of certain risk factors and comorbidities, gives us the right to consider them in one context as an etiological cause, and in another as a driver of the pathophysiological path for PAD.

Conflict of interest

None.

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CASE REPORT

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Neurogenic muscle hypertrophy as an uncommon case of the calf enlargement — a case report and literature review

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Abstract

Unilateral limb edema is a common diagnostic problem. Apart from the most common pathologies, i.e. chronic venous insufficiency and lymphedema, differential diagnosis should include unusual causes of asymmetric extremity edema. We presented a case of 41-year-old man with suspicion of lymphedema of the left calf, who reported to a lymphology clinic. We discuss subsequent steps of the diagnostic procedure in case of the calf edema. In our patient, neurogenic muscle hypertrophy was found to be the cause of the calf enlargement. The diagnosis was confirmed by results of ultrasound, magnetic resonance, computed tomography, lymphoscintigraphy and electromyography examinations. Neurogenic muscle hypertrophy is a very rare and unusual cause of the calf enlargement. Nevertheless, it should be taken into account in the differential diagnosis.

Key words: lymphedema, neurogenic muscle hypertrophy, radiculopathy

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Introduction

Unilateral limb edema is a common diagnostic problem, usually consulted by vascular disease specialists. It is usually defined as a palpable thickening of the limb circumference caused by increased amounts of the interstitial fluid [1]; however, this definition does not include less common causes of an increase in limb circumference, e.g. as a result of the overgrowth of anatomical structures (e.g. muscles).

Undoubtedly, one of the most common causes of unilateral edema of the lower extremities is chronic venous insufficiency, which affects almost 50% of women and 40% of men in Poland. A frequently observed cause in everyday practice is also lymphedema, defined as an abnormal accumulation of the interstitial fluid and fibrous-lipid tissue mainly in the subcutaneous tissue due to oncological treatment, trauma, infection or congenital lymphatic system abnormalities. Apart from the two most common causes, the differential diagnosis should take into account other numerous causes of unilateral edema of the lower limb [2], listed in Table 1.

Apart from meticulous physical examination, ultrasound is very helpful in establishing the diagnosis, as it allows visualization of a vast majority of the venous and arterial systems disorders and obtaining information about the location of the edema (suprafascial or subfascial). The choice of further imaging methods (computed tomography, magnetic resonance imaging, lymphoscintigraphy, angiography) in clinical practice depends on the result of physical and ultrasound examinations.

We present a case of a 41-year-old man with neurogenic calf muscle hypertrophy who was originally referred to an angiologist with the suspicion of lymphedema. We discuss subsequent steps of the

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Table 1. Causes of unilateral lower extremity edema, based on [2], modified

Venous	Primary venous disease Post-thrombotic syndrome Iliac vein compression (May-Thurner syndrome) Deep vein thrombosis Superficial thrombophlebitis Klippel-Trénaunay syndrome Venous adventitial cystic disease
Musculosceletal	Ruptured Baker's cyst Ruptured leg muscle Sprain/strain Static foot disorders Fracture
Lymphatic	Lymphedema
Arterial	Intramuscular hematoma Muscular infarction (particularly in diabetes) Vascular malformation Critical limb ischemia (consequence of leg position) Compartment syndrome
Miscellaneous	Infection (bacterial, parasitic) Radiation Mass/tumor Insect/animal bites Complex regional pain syndrome type I Atrophy/hypertrophy Overgrowth syndromes Granulomatous myositis Amyloidosis

diagnostic procedure that enabled the final diagnosis of a rare cause of limb enlargement.

Case report

A 41-year-old man after surgical treatment of the left iliac bone fracture more than a decade earlier, with suspected left leg edema reported to a lymphology clinic. Since the fracture, the patient observed edema and an increase of the calf circumference. He also complained of alternating numbness, particularly of the left foot, for a few years. On physical examination, circumferences of the left thigh and calf were 3–4 cm larger than in contralateral extremities, numerous telangiectasias and small varicose veins were observed in the ankle area. On doppler ultrasound normal arterial flow was confirmed and venous thrombosis was excluded, there were signs of the great saphenous vein insufficiency and its varicose veins, the popliteal vein did not collapse completely in compression test, the subcutaneous tissue hypertrophy were not present in the thighs and calves. Computed tomography (CT) did not reveal any filling defects in the common femoral veins, external iliac veins, internal iliac veins, common iliac veins and in the visible portion of the inferior vena cava.

Post-thrombotic syndrome and lymphovenous edema of the left lower extremity was suspected. Further diagnostic investigations, i.a. lymphoscintigraphy, were scheduled, and the patient was instructed to continue compression therapy and reduce his body weight.

Lymphoscintigraphy revealed normal iliac and inguinal lymph nodes and an increased number of the superficial lymphatic vessels on the thighs and calves bilaterally (Fig. 1). Magnetic resonance imaging (MRI) revealed signs of left calf hypertrophy, particularly of the medial and lateral heads of the gastrocnemius (Fig. 2). No focal abnormalities or post-contrast enhancement were demonstrated. The signal from bone and muscle structures was unchanged. A discrete zone of edematous lesions of the adipose tissue was disclosed just below the inferior pole of the lateral head of the gastrocnemius. In addition, moderate varicose veins of the left calf were observed, particularly those originating from the great saphenous vein. The right calf was unaffected and had normal muscle and bone structures, with no focal lesions and no areas of abnormal post-contrast enhancement. Based on clinical presentation and the diagnostic procedures performed, hypertrophy of the left calf muscle was diagnosed and the patient was referred for further tests.

Electromyography (EMG) revealed reduced amplitudes of motor potentials in the left peroneal and tibial nerves and prolonged F wave latencies. Sensory conduction of the sural nerve and motor conduction of the femoral nerve were within normal limits. The results supported the diagnosis of axonal sciatic nerve injury.

CT of the lumbosacral spine showed slight midline protrusion of the L5/S1 intervertebral disc with no significant compression of nerves, the L4/L5 intervertebral disc protruding towards the back moderately asymmetrically, more on the left side, with thecal sac and left nerve root indentation, and moderate hypertrophic changes of L4/L5 and L5/S1 facet joints. No signs of central or foraminal stenosis were observed.

In our patient, neurogenic muscle hypertrophy was found to be the cause of the calf enlargement. The patient started physiotherapy.

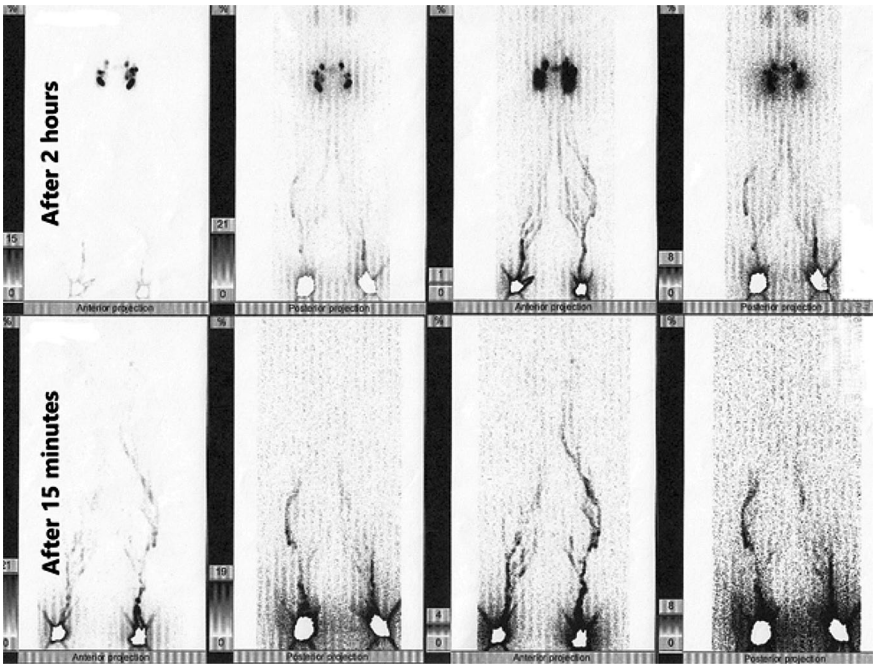

Figure 1. Lymphoscintigraphy. Normal visualization of the iliac and inguinal lymph nodes, increased number of superficial lymph vessels of the thighs and calves bilaterally

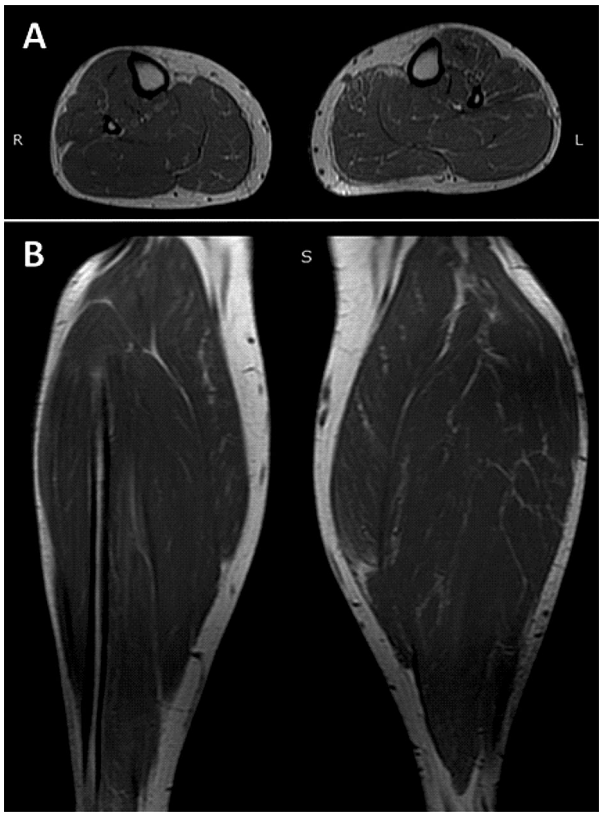

Figure 2. Magnetic resonance imaging (MRI) in transverse (A) and coronal (B) plane. Muscle hypertrophy of the left calf, particularly the medial and lateral head of the gastrocnemius muscle; the skin, subcutaneous tissue, and bones of similar size bilaterally, normal

Discussion

In a patient with unilateral edema of the lower limb, the angiologist first of all would search for pathologies of the venous, arterial and lymphatic system, probably taking into account also the most important traumatic causes. By means of ultrasound examination it is possible to rule out the majority of venous and arterial disorders. Moreover, the thickness of the subcutaneous tissue and its echogeneity may be premises of possible lymphedema, but the results of lymphoscintigraphy is important for establishment of the latter diagnosis (normal lymphoscintigraphy does not exclude lymphedema). In the presented patient, no clinically significant vascular pathologies were found, and the thickness of the skin and subcutaneous tissue was similar on both sides. Therefore, the patient was referred for an MRI, which showed the hypertrophy of the muscles of the calf.

Muscle hypertrophy in neurogenic disorders is unusual finding, as a rule in these conditions is muscle atrophy [3]. Neurogenic muscle hypertrophy, referred to as "denervation hypertrophy", was first described in the 19th century, and is caused mainly by compression of the nerve root, thus most commonly affects the calf muscles (78% of all reported cases) [4], including the gastrocnemius muscle or all muscles of the posterolateral lower leg compartment [5]. In a review of the available literature, comprising less than 30 cases, Zabel et al. [6] showed that neurogenic muscle hypertrophy is usually manifested by painful calf enlargement in males

at the age between 32 and 60, with a previous history of low back pain and sciatica, and that the average time from the onset of the trigger factor to the development of the hypertrophy was 7.3 years. In the vast majority of cases, the cause was a damage to the S1 root [5, 7–10], single cases also reported involvement of the L5 [4] or L4 [11] root or even the cervical spine [12]. In the presented case, at the L4/L5 and L5/S1 levels discopathic lesions were visualized, and the EMG examination confirmed axonal damage to the left sciatic nerve.

The pathomechanism of muscle hypertrophy is not completely clear and is certainly multifactorial. In radiculopathies may occur complex repetitive discharges (CRDs, observed as denervation signs in EMG [7]), which explains some, but not all, cases of calf hypertrophy secondary to L5 or S1 radiculopathy. When carbamazepine or botulinum toxin are administered, positive motor activity is not only reduced or stopped, but also the accompanying muscle hypertrophy is reduced, that additionally supports this hypothesis [13]. In addition to above mechanism, other possible explanations for the pathogenesis of neurogenic hypertrophy have also been proposed: work load — the workload of healthy muscle fibers remaining after damage and stretching and release of growth factors [8]. In addition, attempts have been made to explain the pathomechanism of focal myositis as a continuum of changes induced by chronic stimulation [14]. If chronic stimulation is the trigger for hypertrophy, it is conceivable that as the size of the fiber increases, splitting and then necrosis occur, either because the overgrown fiber exceeds its nutrient supply, or simply because of overstimulation; necrosis is a stimulus that attracts inflammatory cells, especially macrophages [14].

The treatment of neurogenic muscle hypertrophy is not well-established. It can be surgical (microdiscectomy) or conservative, including described as an effective injection of botulinum toxin [4, 8]. Physiotherapy plays an important role as well.

Conclusions

Detailed physical examination remains an essential part of our routine practice, because slowly changing muscle size may go unnoticed by the patient and may be omitted in the medical history [13]. The clinician should be aware that isolated muscle hypertrophy may be a sign of partial damage to peripheral nerves or nerve roots and is likely to occur in any skeletal muscle [11].

Unilateral calf edema should be considered as a rare symptom of lumbosacral radiculopathy. When suspected — history and physical examination, lumbar spine imaging should be performed in conjunction with EMG. Upon confirmation of radiculopathy that correlates with anatomical compression, this rare association should be suspected. However, the remaining non-invasive tests for other most common causes of unilateral calf enlargement, including doppler ultrasound, should not be delayed [15].

Neurogenic muscle hypertrophy is an uncommon, but well-documented phenomenon that physiotherapists should also be aware of, because patients with chronic muscle imbalance due to neuropathic disorders are often referred for a physiological evaluation [16].

Conflict of interest

None.

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CASE REPORT

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Infectious arteritis of the femoral artery as a cause of recurrent hemorrhage after endovascular treatment of thrombosed popliteal artery aneurysm

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Abstract

Due to a great number of endovascular interventions, conducted over the last decades, a huge percentage of obese patients and administration of double antiplatelet therapy, manual compression (MC) is more and more frequently replaced by a vascular closure device of which the most common is Angio-Seal. The study presents a case of a 63-year-old female patient with recurrent hemorrhages of the common femoral artery (CFA), originally closed after an endovascular intervention of a popliteal artery aneurysm with the use of Angio-Seal VIP. After around 3 weeks following the original surgery, the patient at first developed a pseudoaneurysm and then septic inflammation of the femoral artery, manifested with recurrent hemorrhages from the groin. The patient was operated on many times and an implantation of an ilio-femoral bypass from the femoral vein turned out to be an effective solution. Staphylococcus epidermidis MRSA was cultured in the femoral artery wall and a histopathological examination confirmed infectious arteritis. The presented case of a rare septic complication after the application of a closure device shows that it is essential to carefully monitor surgical approach areas in patients who are quickly discharged from hospital after surgical interventions, to select treatment methods tailored for individual patients as well as implement particular surgical management.

Key words: Angio-Seal, infectious complications, pseudoaneurysm, vascular closure device, popliteal artery aneurysm

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Introduction

Manual compression (MC) is a basic hemostatic method after an arterial puncture in endovascular surgery. However, a rapid increase in the number of endovascular interventions over the last few decades, a huge percentage of obese patients, administration of double antiplatelet therapy as well as the fact MC is a disadvantage for both medical personnel and patients, led to a more frequent replacement of MC with a vascular closure device (VCDs). VCDs, introduced at the beginning of the 1990s, considerably shorten the time needed to stop bleeding after performing an arterial puncture [1]. The percentage of complications after application of VCDs in comparison to MC is different. Some previous studies revealed that these devices significantly decrease the number of complications [2]. However, authors of recently published analyses claim that the number of VCDs- and MC-related complications is similar [1]. Besides, Angio-Seal VIP device was introduced into practice more than 20 years ago [3]. It allows closing arteriotomy between a polymer anchor and a plug of collagen sponge which are self-tightening. The anchor is placed in the arterial lumen and the collagen plug – outside. Hemostasis is achieved by a mechanical pressure and stimulation of coagulation

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with collagen. All elements of the Angio-Seal VIP set are absorbable [4]. Potential complications related to application of Angio-Seal VIP can be divided into three main groups: ischemic (stenosis/occlusion of the access artery, distal embolization), hemorrhagic (bleeding, hematoma, pseudoaneurysm) and infectious (foreign body reaction, infection) [5].

The article presents a case of a female patient with recurrent hemorrhages of the common femoral artery (CFA), originally closed after an endovascular intervention with the use of Angio-Seal VIP device.

Case report

A 63-year-old patient, hypertensive, with insulin-dependent diabetes, chronic renal failure (on admission serum creatinine was 1.69 mg%), after cerebral stroke, was admitted to the clinic due to critical ischemia of the left lower limb. Imaging examinations (DUS – duplex doppler ultrasound, CTA — computed tomography angiography) revealed on the left side a thrombosed aneurysm of the popliteal artery (PA), up to 24 mm in diameter and occluded anterior and posterior tibial arteries. The patient was qualified for endovascular treatment. Until the surgery, the patient had been undergoing antithrombotic treatment, involving administration of low molecular weight heparin (Clexane 80 mg every 12h). Through a puncture in the common femoral artery (CFA) on the right side, conducted with the cross-over technique, 5F PIG Impress catheter was inserted to the external iliac artery (EIA) on Lake Region Medical Starter Guidewire 0.035. Besides, arteriography was performed. It confirmed a thrombosed aneurysm of the left PA, with a narrow fibular artery (anterior and posterior tibial arteries — occluded). After getting through the occluded PA with the use of the mentioned catheter and 5F VERT Impress catheter, 4F Cragg-Mcnamara Infusion Catheter 100 × 20 was inserted. Its end was placed in the fibular artery. Intra-arterial administration of (50 mg/50 mL) with an infusion pump (1 mL/h) was continued for 48 h, regularly every 6 h, monitoring fibrinogen. Simultaneously, the vascular sheath was rinsed with solution of unfractioned heparin (25 mg/50 mL), also administered from an infusion pump (500 U/h). After 24 h, the thrombolytic process was controlled in angiography which confirmed that a huge amount of embolic material is still observed. Only next angiography, conducted after 48 hours, revealed a patent canal of PA aneurysm and a patent tibial artery. A covered stent Gore Viabahn Endoprosthesis 8 × 150 mm was implanted into PA, modeling it with Dorado balloon 7 × 150 mm, Bard. After achieving a good morphological and hemodynamic effect, the approach area in the right CFA (after sheath 8F) was secured with Angio-Seal VIP device. An antibiotic (Biofazolin 1 g every 12 h) was administered during the thrombolytic treatment. The course of the surgery was uncomplicated; on day 3 following the surgery, the patient' pulse on both PAs was regular and she did not demonstrate any hematoma or aneurysm in the right groin so she was discharged home and ordered a double antiplatelet therapy (ASA 75 mg daily and clopidogrel 75 mg daily). Five days following the discharge, the patient was routinely examined in the outpatient clinic. The pulse on both PAs was regular and no hematoma or clinical manifestations of pseudoaneurysm were observed.

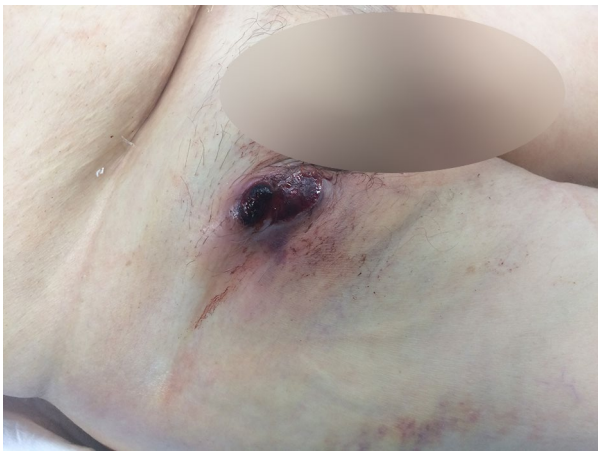

Figure 1. Ruptured common femoral artery pseudoaneurysm in the right groin

On day 19 following the surgery, the patient was readmitted to the clinic with symptoms of ruptured aneurysm in the right groin, accompanied by skin necrosis above the aneurysm and elevated inflammatory parameters (leukocytosis 25.500/mm^3, RCPR 173.7 mg%) (Fig. 1). The patient underwent an emergency operation. The visualized anterior wall of CFA appeared to be damaged. There was a hole 3 mm in diameter, which was closed with two single sutures (Prolene 5/0). Next, the aneurysm was removed and some considerably inflamed subcutaneous and dermal tissue was resected (culture was also collected). After the operation, the patient was initiated empirical intravenous antibiotic therapy (Biotraxon 2 g every 12 h and Metronidazole 0.5 g every 8 h). After obtaining culture results (*Proteus vulgaris*, *Staphylococcus aureus MSSA*), this therapy was replaced with a targeted antibiotic therapy (Ciprinol 0.2 g every 12 h).

On day 27, an inguinal hemorrhage occurred. During a reoperation, the doctors observed suture dehiscence and a hole on the anterior wall of CFA, 8 mm in diameter. The damaged fragment of the CFA anterior wall was resected and the defect was closed with a saphenous

vein patch. The sample of the damaged wall was sent to a bacteriological analysis (negative culture).

On day 33, the patient suffered another hemorrhage from the groin. During a reoperation, the doctors observed suture dehiscence between the medial edge of the patch and the CFA wall. The damaged fragment of the CFA was resected and a 4-cm saphenous vein graft, collected from the left groin, was implanted. The lower anastomosis of the graft was performed about 3 mm from the ostium of the deep femoral artery (PFA). The removed fragment of CFA was sent to a bacteriological analysis but the culture appeared to be negative. A control CTA revealed huge inflamed infiltration, modeling the course of CFA and the implanted graft and no manifestations of aneurysm. The patient with a post-operative wound, partially healing by granulation, was discharged home (culture from the wound collected a week prior to the discharge, turned out to be negative).

On day 75, the patient was readmitted due to another inguinal hemorrhage. CTA visualized aneurysm 76 mm in diameter, adjacent to the right CFA (Fig. 2). Another operation revealed that CFA wall, the implanted graft and the PFA ostium are completely damaged. Since PFA walls were inflamed and fragile, the artery was punctured. Next, a 15-cm ilio-femoral bypass, made of the femoral vein (collected from the right lower limb), was implanted. An upper anastomosis was performed around 5 cm above the inguinal fold, whereas a lower anastomosis was performed in the upper 1/3 of the thigh (the surgeons attempted to make both the anastomoses within uninflamed tissue). The resected fragment of CFA was again sent to a microbiological and histopathological analysis. This time, the culture appeared to contain *Staphylococcus epidermidis* MRSA (MLSB), which, according to an antibiogram, was managed with Vancomycin 0.5 g, administered every 6h. A histopathological examination confirmed arteritis (Fig. 3). The post-operative course was uncomplicated. The patient's pulse on both PAs was regular and the wound healed so she was discharged home. Follow-up examinations on days 7 and 30 showed that the wound was healed and the pulse was present on both PAs.

On day 166, the patient was readmitted to the clinic due to rest pains of the right limb which had lasted for almost 24 h. CTA (performed two weeks before) confirmed a critical stenosis of the right EIA (at the level of the upper anastomosis). The patient was qualified for endovascular treatment. By puncturing the left CFA with the cross-over technique, the surgeon inserted 5F PIG Impress catheter to the right common iliac artery (CIA) on Lake Region Medical Starter Guidewire 0.035". Arteriography visualized occluded EIA, the ilio-femoral bypass and the initial segment of the superficial femoral artery (SFA) (Fig. 4A). With the use of the mentioned guidewire and 5F VERT Impress 125 cm 0.035" catheter, the author managed to get through the occlusion and placed the end of the guidewire in PA. Next percutaneous transluminal angioplasty (PTA) with the application of Dorado balloon 5 × 150 mm, Bard of

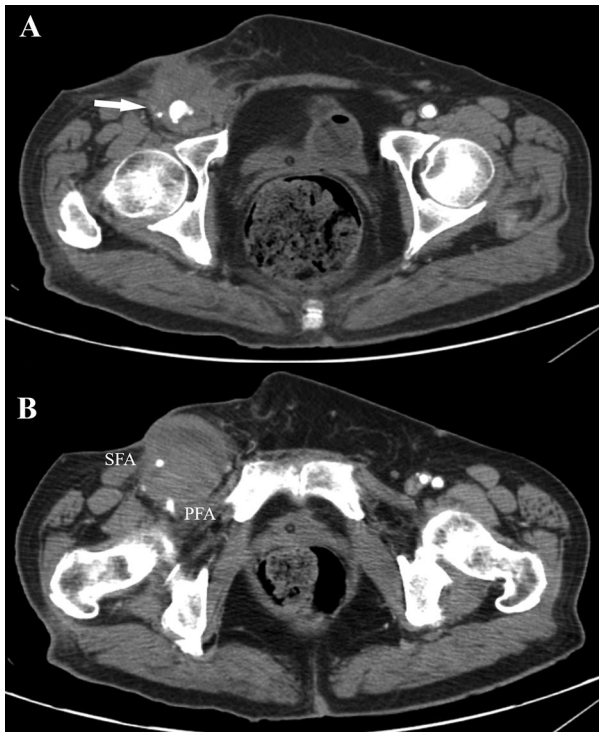

Figure 2. CT shows large common artery pseudoaneurysm. **A** — CT scan at the level of CFA (arrow shows CFA with visible leakage of contrast); **B** — CT scan at level of 1–2 cm below the division of CFA into SFA and PFA (massive aneurysm shifts both femoral arteries to the side)

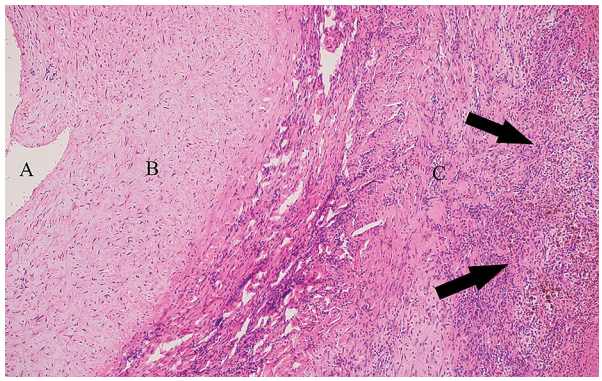

Figure 3. Histopathological examination of the common femoral artery wall: **A** — artery lumen; **B** — Tunica media; **C** — Tunica adventitia. Arrows show inflammatory infiltrate from lymphocytes and granulocytes.

the bypass and SFA was performed. The balloon angioplasty was accompanied by implantation of two stents Everflex, going from top 7 × 120 mm and 6 × 150 mm. A follow-up angiography showed stenosis above the upper stent. Hence, the stent was extended upwards with the third stent — Boston Express LD Vascular 7 × 27 mm. After achieving a good hemodynamic effect, the approach in the right CFA was closed with MC (Fig. 4B). The post-operative course was uncomplicated; on day 3 following the intervention, the patient did not demonstrate clinical signs of hematoma or aneurysm in the left groin and her pulse on both PAs was regular. She was discharged home with recommendation of double antiplatelet therapy. Follow-up examinations were conducted in an outpatient clinic on days 7 and 30 and next, every month for half a year. During this time, no lower limb ischemia or suspicion of aneurysm were observed. CTA (2 months after the last operation) did not show significant stenosis or any other abnormalities, requiring an intervention (Fig. 5).

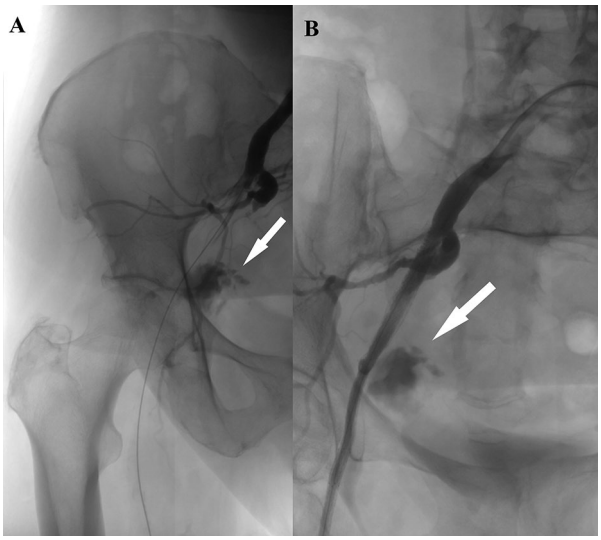

Figure 4. Arteriography 3 month after implantation of ilio-femoral bypass. **A** — occlusion of external iliac artery, ilio-femoral bypass and common femoral artery; **B** — final arteriography after angioplasty and stenting (arrows show extravasation after lesion collateral artery)

Discussion

Angio-Seal studies report a rate of hemorrhagic complications of 3.8–7.7% and hematomas resulting from these complications may potentially contribute to infectious complications [6, 7]. The risk of infection of the approach definitely increases in the event of a foreign body. Elements of VCD are such a foreign body. Despite this, the risk of infectious complications after application of Angio-Seal is still low. If such complications occur, they are usually mild and rare (0.3%) [8]. An infection of the arterial wall in the approach area poses a far more serious threat. It subsequently leads to a septic form of arteritis which is particularly resistant to pharmacotherapy. Frazee and Flaherty [9] presented 10 cases of septic arteritis which occurred after repeat PTCA and were related to recatheterization of the original approach area. In all patients, *Staphylococcus aureus* was the etiological factor. In six cases, aneurysm occurred. It was surgically treated by resection of a fragment of FA with implantation of a saphenous vein bypass. In our patient, culture of aneurysm appeared to contain *Staphylococcus aureus* MSSA, whereas in the culture of the FA wall contained *Staphylococcus epidermidis* MRSE/MLSB. What is interesting, a positive result of the culture of the arterial wall was obtained only in the third test, during the fourth surgical intervention. Despite initiating targeted therapy after the first two operations, no clinical improvement was observed. Minor surgical techniques (a vascular patch, a short saphenous vein graft) appeared to be ineffective, either. Only a reconstructive surgery, consisting in a complete resection of CFA and replacing it with a femoral vein bypass, implanted from

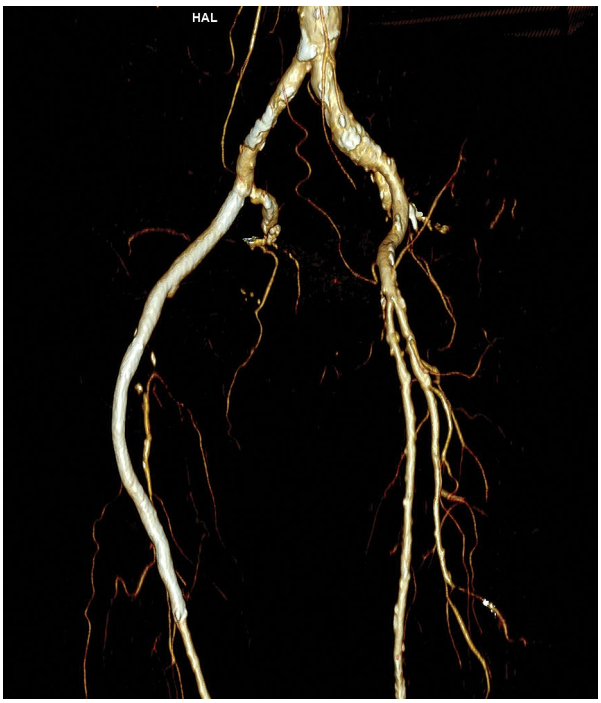

Figure 5. CTA 2 month after angioplasty shows good hemodynamic effect

EIA to SFA, where both anastomoses were performed in health tissue, brought expected clinical effect. Severe infections after administration of Angio-Seal occur rarely. The Medline database reports single cases [10].

Copper and Miller [11] presented one case of confirmed endartertis in a patient after application of Angio-Seal, where *Staphylococcus aureus* was the etiological factor. The patient was operated on, had CFA and its bifurcation resected. He was also implanted a femoro-femoral suprapubic bypass (from the left CFA to the right SFA) from a Dacron prosthesis. Such solution seems to be risky because implantation of an artificial prosthesis in infected environment might contribute to development of another infection. However, distant prognosis of that patient was not given. On the other hand, the adopted solution decreased the risk of restenosis in the place of anastomosis, an incident which was observed in our patient (stenosis of anastomosis of the femoral vein and the iliac artery). An important question arises: How to avoid such complications in the future? If, according to the instruction, the sheath is placed for longer than 8h, antibiotics should be applied, which, by the way, was done. The manufacturer of Angio-Seal recommends taking precautions and safety measures in patients who have undergone thrombolytic treatment, which was done as the patient was much more often monitored than other patients. However, she got many infections. The presented case of a rare septic complication after an application of a closure device shows that it is essential to carefully monitor surgical approaches in patients who are quickly discharged from hospital after surgical interventions, to select treatment methods tailored for individual patients as well as implement particular surgical management.

Conflict of interest

None.

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