We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

4,800
Open access books available

122,000
International authors and editors

135M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

Is Restenosis/Reocclusion after Femoropopliteal Percutaneous Transluminal Angioplasty (PTA) the Consequence of Reduced Blood Flow, Inflammation, and/or Hemostasis Disturbances?

Mojca Božič-Mijovski, Vinko Boc, Vladka Salapura, Aleš Blinc and Mojca Stegnar

Additional information is available at the end of the chapter

Abstract

Percutaneous transluminal angioplasty (PTA) is an established method for treatment of peripheral artery disease (PAD) of the femoropopliteal artery. However, in up to 50% of patients restenosis and/or reocclusion remain a frequent complication occurring in the first year after the procedure. In this study, we focused on the influence of compromised postprocedural infrapopliteal runoff of the affected limb, on the hypercoagulability as detected by a global hemostasis assay and on genetic predisposition to hypercoagulability and on the regulation of the inflammation through the nuclear receptor related 1 protein (NuRR1). Consecutive PAD patients treated by femoropopliteal PTA because of disabling claudication or critical limb ischemia were followed up by vascular ultrasound imaging at 1, 6, and 12 months after the procedure. Venous blood samples for hemostasis, inflammation, and gene analysis were obtained before and 24 h after PTA. One month after femoropopliteal PTA, 23% of patients with compromised runoff developed the combined end point restenosis/reocclusion in comparison to 11% with good runoff (p = 0.03). After 6 months, the differences were no longer significant. It was concluded that compromised postprocedural infrapopliteal runoff predisposes to early restenosis/reocclusion after femoropopliteal PTA and that the deterioration of infrapopliteal runoff in the year after femoropopliteal PTA is accompanied by worsening of long-term femoropopliteal patency. Patients were genotyped for the prothrombotic gene polymorphisms: platelet receptor glycoprotein IIIa T1565C, coagulation factor V G1691A, coagulation factor II G20210A, coagulation factor XII C(-4)T, and plasminogen activator inhibitor-1 4G5G. We were not able to show any association between these polymorphisms and the restenosis/reocclusion rate in patients treated with femoropopliteal PTA. Furthermore, no association between thrombin generation and reste-

nosis/reocclusion rate was established. NuRR1 haplotypes significantly increased the restenosis/reocclusion rate after PTA (adjusted relative risks were 1.6, 95% CI 1.1–2.3 for haplotype 2 and 2.0, 95% CI 1.3–2.8 for haplotype 3). To conclude, this study suggested a significantly higher restenosis/reocclusion rate in patients with compromised runoff compared to patients with a good runoff 1 month after the procedure. Hypercoagulability was not associated with the restenosis/reocclusion rate, and the prothrombotic polymorphisms were equally distributed among patient with and without restenosis/reocclusion, suggesting minor or no role in restenosis/reocclusion. Haplotypes 2 and 3 in the NuRR1 gene significantly increased the restenosis/reocclusion rate, suggesting significant role of inflammation. In this ongoing study, further analysis on a larger group of patients is warranted.

Keywords: Percutaneous transluminal angioplasty, peripheral artery disease, inflammation, hemostasis

---

1. Introduction

Peripheral artery disease (PAD) is a prevalent circulatory problem in which narrowed or occluded arteries reduce blood flow to the limbs. PAD is most often a manifestation of generalized atherosclerosis that reduces the patients' quality of life by reducing their walking ability and also confers an increased risk for cardiac death, acute coronary syndrome, and ischemic stroke. Patients with PAD die more than 3 times more often than peers of the same age [1]. Early diagnosis is important for improving the patient's quality of life and for reducing the risk of serious secondary vascular events. PAD, defined by decreased ankle brachial pressure index, is found in 15–20% of population aged 55 years or more [2]. All patients with PAD require preventive treatment against vascular events by lifestyle modification and protective medication, but only a fraction ever requires a revascularization procedure [3, 4]. This procedure is needed in order to establish suitable blood flow to the affected limb if PAD severely hampers walking ability or in cases of critical limb ischemia [3, 4]. Percutaneous transluminal angioplasty (PTA) is an established revascularization method for treatment of PAD and is associated with low morbidity and mortality rates [3, 4]. However, in up to 60% of patients, restenosis and/or reocclusion remain a frequent complication occurring in the first year after the procedure [5]. Our understanding of the mechanisms of restenosis/reocclusion of the femoral artery after PTA is incomplete. The comprehension of the factors that contribute to the pathophysiology of restenosis/reocclusion is the foundation to develop effective strategies for improvement of patients' post-PTA outcome. Once identified, reliable predictors of the restenosis/reocclusion risk could facilitate the use of preventive measures, help to save healthcare resources, and assist in new drug development. In this study, we focused (i) on the influence of compromised postprocedural infrapopliteal runoff of the affected limb, (ii) on the hypercoagulability as detected by a global hemostasis assay and on genetic predisposition to hypercoagulability due to altered hemostatic factors that could support thrombus formation in the arterial segment injured by PTA, and (iii) on the regulation of the inflammation through

the nuclear receptor related 1 protein (NuRR1) that could presumable favor increased restenosis/reocclusion rate after successful PTA.

2. Problem statement

PAD causes inadequate blood flow to the limbs, mostly lower limbs. Femoropopliteal artery is the most commonly affected arterial segment. The patency of femoropopliteal artery after PTA is affected by several factors [37], including clinical severity of PAD, patient comorbidities, such as diabetes or renal failure, morphological characteristics of the arterial lesions, i.e., occlusion vs. stenosis, length of the lesion and their number, calcification of plaques, functional characteristics of the affected artery, i.e., the extent of vascular inflammation, and hemodynamic conditions that are to a large extent defined by the arterial runoff.

The STAR registry and several older studies listed poor tibial runoff as strongly predictive of bad long-term patency [6–10], but some authors found no association of femoral artery patency with tibial runoff 1 year after recanalization [11]. There is not much data on the role of concomitant infrapopliteal PTA in maintaining the long-term patency of the femoropopliteal segment after PTA. This question is difficult to address directly since it is not ethically acceptable to deny PTA of accessible lesions in the calf arteries to any group of patients with clinically relevant limb ischemia who are already treated by femoropopliteal PTA.

The acute response to arterial injury induced by PTA involves the adhesion of platelets and leukocytes, which react with the damaged arterial wall in proportion to the degree of injury. In addition, arterial injury activates hemostasis presumably, resulting in thrombus formation on the injured vessel segment [12]. The laboratory recognition of activated hemostasis (hypercoagulability) is a very demanding task due to the complexity of the hemostatic system. Hypercoagulability can be detected by global tests, such as the thrombin generation assay that provide an overview of the entire hemostatic system, including enzymes, cofactors, and inhibitors. With this assay hypercoagulability was detected in patients with atherothrombosis [13]. Another approach to detect hypercoagulability is to measure specific substances (peptides, enzymes, and enzyme–inhibitor complexes) that are liberated with the activation of hemostasis, namely, specific hemostasis activation markers such as D-dimer [14]. A permanent prothrombotic state caused by gene polymorphisms that affect coagulation factors or platelets could supplement hypercoagulability and contribute to increased risk for restenosis/reocclusion. Such prothrombotic polymorphisms include glycoprotein IIIa T1565C polymorphism (GPIIIa T1565C), which increases platelet adhesion and aggregation, factor V G1691A, which causes resistance to activated protein C, factor II G20210A associated with elevated prothrombin levels, factor XII C46T associated with lower factor XII levels, and plasminogen activator inhibitor-1 (PAI-1) 4G5G associated with lower PAI-1 levels.

In the following months after PTA, the hyperplasia of smooth muscle cells (SMCs) in vascular wall that is regulated by proinflammatory mediators can lead to restenosis [12, 15]. The association between inflammation and PAD is well established, and the prognostic value of inflammation in restenosis has also been recognized [16]. Shear stress during balloon inflation

and vascular injury stimulates the production of proinflammatory molecules and the activation of circulating monocytes. The level of monocyte activation and adherence to the vascular wall, mediated by selectins and adhesion molecules, was suggested to promote late lumen loss [17]. The regulation of the inflammation through the nuclear receptor related 1 protein (NuRR1) has recently been associated with restenosis. NuRR1 (or NR4A2) together with NR4A1 and NR4A3 constitutes the nuclear receptor subfamily 4, group A (NR4A). This subfamily is also referred to as the nerve growth factor-induced protein-B subfamily of nuclear receptors because these receptors were first described as early response transcription factors expressed following stimulation by growth factors. All three subfamily members bind the same response element(s). They are referred to as orphan receptors because the ligands that may regulate their transcriptional activity have not yet been identified. These transcriptional factors have been described in the regulation of differentiation, proliferation, apoptosis, and survival of many different cell types [18]. Besides direct binding to the promoter of target genes, NuRR1 modulates gene transcription by the transrepression of other transcription factors. Its role in inflammatory responses has been recognized when the overexpression of NuRR1 in human atherosclerotic lesions compared to normal healthy human arteries has been observed [19]. An antiproliferative and anti-inflammatory function of NuRR1 in human SMCs and its protective role against arterial wall injury-induced SMC-rich lesion formation in mice has been shown [20]. The NuRR1 gene lies in one linkage disequilibrium block spanning approximately 36 kb of DNA on chromosome 2q22–2q23. Several gene polymorphisms were described in the NuRR1 gene; however, from the three tagging single-nucleotide polymorphisms (rs1466408, rs13428968, and rs12803), four haplotypes had been inferred with frequencies >1% that explained 96% of the variation in this linkage disequilibrium block [21]. In patients undergoing percutaneous coronary intervention, haplotypes 3 and 4 increased the risk of in-stent restenosis, target lesion revascularization, percutaneous coronary reinterventions, and the rate of major cardiac events (MACE) about 2- to 3-fold in the first year after the procedure [20]. A similar role in femoropopliteal restenosis after PTA was expected. To our knowledge, the role of NuRR1 haplotypes in femoropopliteal restenosis after PTA has not been investigated yet, although it could be expected.

3. Patients and methods

In our study on the effect of tibial runoff on femoropopliteal patency after PTA [22], consecutive consenting patients with claudication or critical limb ischemia admitted for femoropopliteal PTA to the Department of Vascular Diseases of the University Medical Centre Ljubljana have been enrolled and prospectively followed up. In addition to femoropopliteal PTA, infrapopliteal PTA has been performed in all cases when lesions of the calf arteries have been judged suitable for intervention. At enrolment, risk factors for PAD and clinical stage of PAD by the Fontaine classification have been determined for each patient [3]. The morphological changes of femoropopliteal lesions have been evaluated according to the TASC II classification [3]. Ankle brachial pressure index has been measured routinely before and after PTA [23]. The PTA procedures have been performed in a catheter laboratory by interventional radiologists.

The ipsilateral anterograde approach via the common femoral artery has been used except in cases of ostial lesions of the femoral artery, where the contralateral or transpopliteal approach has been used, introducing 5 Fr sheaths for vascular access. All patients, already treated with low-dose aspirin, have received local anesthesia and 3,000 IU heparin i.v. at the beginning of the procedure. Most stenotic lesions have been crossed by a soft 0.035-inch J-wire (Terumo Medical Corporation, USA) and in the majority of occlusions have been crossed by the direct recanalization technique. Alternatively, the subintimal approach has been used in cases of unsuccessful direct recanalization. Noncompliant balloons of 5 or 6 mm diameter from different manufacturers have been used, depending on the vessel diameter in the adjacent nondiseased parts. The balloons have been inflated for at least 1 min to 8 atm pressure. Stents (nitinol self-expanding stents) have been implanted only in cases of flow-limiting dissections or residual stenosis of >50% even after repeated balloon inflation. In patients with accessible concomitant infrapopliteal lesions, the PTA of the calf arteries has also been performed. For infrapopliteal lesions, 0.025-inch J-Terumo wire or 0.014/0.018-inch (Pointer, Denmark or Invatec, Italy) wires have been used for intraluminal crossing, with balloon diameters from 2 to 3.5 mm (different manufacturers). All angiographies have been performed by the standard digital subtraction technique. The technical success of PTA and the infrapopliteal runoff has been evaluated by periprocedural angiography. Immediate technical success has been defined as ≤ 50% residual angiographic stenosis [24]. Infrapopliteal runoff has been scored by a modification of the Society for Vascular Surgery criteria, originally intended for quantifying bypass runoff, where a higher score implies worse runoff [25]. This scoring system ascribes 3 points for occlusion throughout the vessel, 2.5 points for occlusion of less than half of the arterial length, 2 points for maximal stenosis of 50–99%, 1 point for maximal stenosis of 20–49%, and 0 points for less than 20% maximal stenosis. Each of the calf arteries has been ascribed a weight, i.e., multiplication factor, of 1, and the distal popliteal artery has been ascribed a weight of 3, with one point always added to the total score [26]. Thus, the cumulative score for the distal popliteal artery (a maximum of 9 + 1) and for the tibial vessels (a maximum of 3 × 3) gives a maximum score of 19 [23]. We have divided the patients' limbs into two categories: good runoff (<5 points) and compromised runoff (≥ 5 points). In the good runoff group, a limb has to have a patent popliteal artery and at least two patent calf arteries with less than 50% maximal stenosis. An occlusion of one calf artery (3 points) and more than 50% stenosis in another calf artery (2.5 points) already implies compromised runoff. Bad runoff with a score of 11 or more points after femoropopliteal PTA implies complete occlusion of all 3 calf arteries plus at least 20–49% residual stenosis of the popliteal artery. Some typical examples of infrapopliteal runoff scoring are shown in Figure 1.

All subjects have examined by vascular ultrasonography (US) at 1 month (range 29–60 days), 6 months (range 6–8 months), and 12 months (range 12–16 months) after PTA to evaluate the development of restenosis/reocclusion of the femoropopliteal arterial segment on a Vivid 3 ultrasound machine (GE Medical Systems, USA) with a linear vascular probe (Vascular 10L). An adverse outcome of PTA has defined as identification of femoropopliteal stenosis of ≥50%, confirmed by at least doubling of the maximal systolic velocity in comparison to a proximal nondiseased arterial segment, or by identifying a reocclusion confirmed by the absence of a Doppler flow signal [25]. The patency of calf arteries has been assessed by US at the third
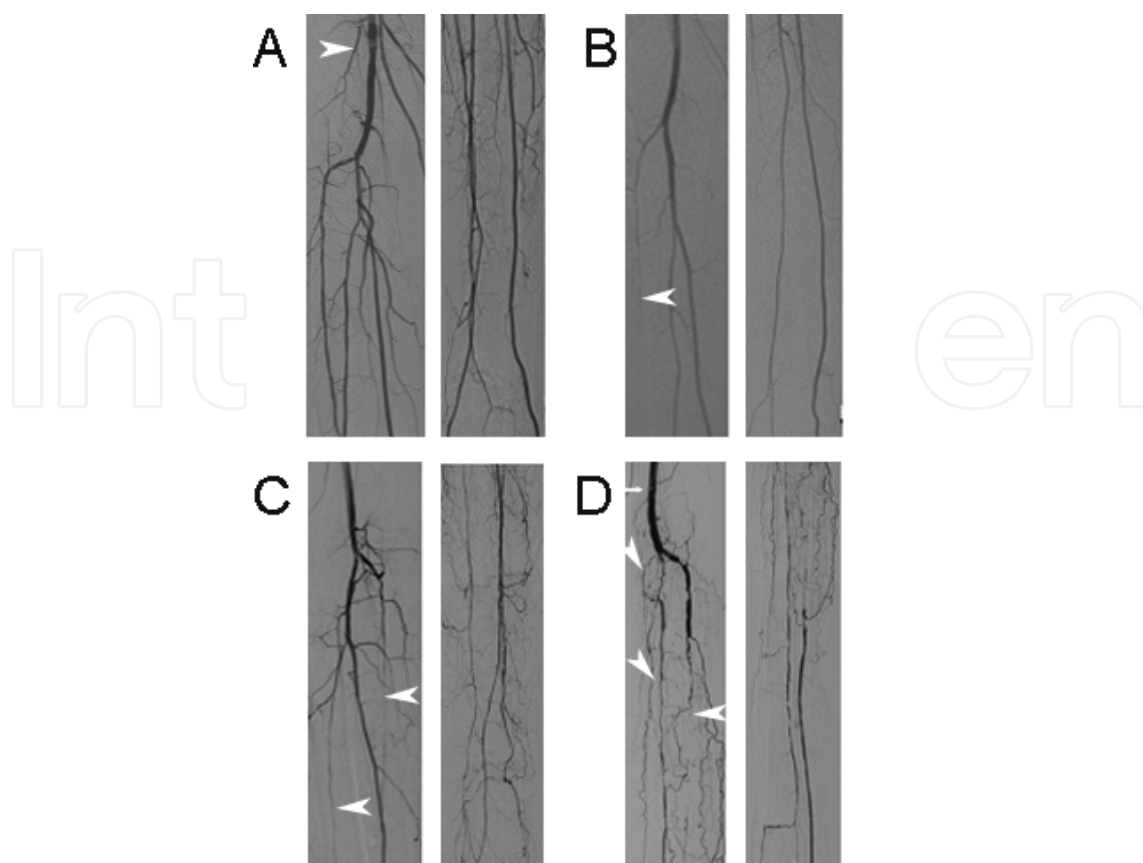
Figure 1. Examples of infrapopliteal scoring according to a modification of the Society for Vascular Surgery criteria [25]. In each set of angiograms, the left image represents the popliteal artery and the upper calf, while the right image represents the lower calf. (A) Good runoff with a score of 1: 3 × 0 + 1 point for a patent popliteal artery with <20% popliteal stenosis (arrowhead) plus 3 × 0 points for patent calf arteries. (B) Good runoff with a score of 4: 3 × 0 + 1 for the good patency of popliteal artery and 3 points for anterior tibial artery occlusion (arrowhead). (C) Compromised runoff with a score 5.5: 3 × 0 + 1 for good popliteal patency and 2 points for >50% posterior tibial artery stenosis (lower arrowhead) plus 2.5 points for anterior tibial artery occlusion spanning less than half of the arterial length (upper arrowhead). (D) Bad runoff with a score of 12: 3 × 1 + 1 points for 20–49% popliteal stenosis (small arrow) plus 2.5 points for occlusion of less than half of the length of the anterior tibial artery (lower horizontal arrowhead), plus 2.5 points for occlusion of less than half of the length of the peroneal artery with collateral filling (upper skewed arrowhead) plus 3 points for total occlusion of the posterior tibial artery with a collateral artery running along its path (lower skewed arrowhead). Reproduced with permission from [22].

follow-up examination 12 months after femoropopliteal PTA and compared to the periprocedural angiographic result. During US of the calf arteries, attempts have been made at visualizing as much as possible of the whole length of the two tibial arteries and the peroneal artery, i.e., interosseal artery. As in the femoropopliteal arterial segment, a stenosis of ≥50% has been diagnosed by at least doubling of the maximal systolic velocity in comparison to a proximal nondiseased arterial segment, whereas an occlusion has been documented in the absence of Doppler flow signal. In addition, the Doppler waveform at the level of the ankle in each of the three calf arteries has been compared to the waveform in the tibioperoneal trunk. A change from triphasic to monophasic signal with a marked reduction in peak systolic velocity and a decrease in the slope of systolic upstroke or absence of distal flow have been taken as additional evidence of hemodynamically significant compromise of the investigated calf artery [25].

Patients' blood has been collected 1 day before PTA (preprocedural sample) and on the day of PTA after the procedure (postprocedural sample). Blood has been drawn into 4.5 mL Vacutainer® tubes (Becton Dickinson, Plymouth, UK) containing 0.11 mol/L sodium citrate. From whole blood, DNA has been extracted either manually utilizing the silica-membrane-based DNA purification (QIAamp DNA Blood Mini Kit, Qiagen, Germany) or with magnetic beads on an automated nucleic acid purification instrument with the iPrep™ PureLink® gDNA Blood Kit (Life Technologies, USA). The remaining blood has been centrifuged at 2,000g and 4°C for 30 min to obtain platelet-poor plasma. Plasma has been transferred to small plastic vials, frozen in liquid nitrogen, and stored at –70°C until analyzed.

Genotyping of prothrombotic polymorphisms (GPIIIa T1565C, factor V G1691A, factor II G20210A, factor XII C46T and PAI-1 4G5G) and NuRR1 has been performed with real-time PCR on an ABI PRISM 7000 system (Applied Biosystems), using TaqMan® chemistry. In plasma, thrombin generation and D-dimer levels have been measured. Thrombin generation has been determined using a commercial kit (Technothrombin® TGA, Technoclone, Austria), which is based on monitoring the fluorescence generated by thrombin cleavage of a fluorogenic substrate over time on the activation of the coagulation cascade with 5 pmol/L tissue factor. The following parameters have been registered: lag phase, time to peak thrombin concentration, peak thrombin concentration and area under the curve–endogenous thrombin potential (ETP). The amount of microparticle-induced thrombin generation has also been determined by measuring thrombin generation in microparticle-free (filtered using 0.2 μm vacuum filtration device Ceveron® MFU-500, Technoclone, Austria) plasma versus thrombin generation in nonfiltered plasma containing microparticles. The amount of thrombin (peak thrombin concentration) induced by microparticles has been calculated (in per cent). D-dimer concentration has been measured with TriniLIA Auto-Dimer reagent (Trinity Biotech, Ireland) on an automated coagulation analyzer CS2100i (Siemens Healthcare Diagnostics, Germany) [27].

4. Results and discussion

4.1. Infrapopliteal runoff

Data on the infrapopliteal runoff have been analyzed for 176 patients [22]. We found a significantly higher restenosis/reocclusion rate in patients with compromised runoff (23%) compared to patients with a good runoff (11%) 1 month after femoropopliteal PTA ($p = 0.03$, Figure 2) [22]. The statistical significance was lost later on (after 6 months 49% in the compromised runoff group vs. 43% in the good runoff group, $p = 0.49$ and 57% vs. 52% after 12 months, respectively, $p = 0.51$). However, in patients' limbs with good periprocedural runoff that deteriorated into compromised runoff in the year after PTA, femoropopliteal restenosis/ reocclusion occurred more often than in limbs which retained good runoff: 10/14 (71%) vs. 18/51 (35%), $p = 0.02$ [22]. The results were similar if only patients with Fontaine stages III and IV, i.e., critical limb ischemia were regarded. These results suggest that mechanisms of intermediate and long-term restenosis/reocclusion act simultaneously in the calf and the femoropopliteal arterial segment. The higher rate of early femoropopliteal restenosis/reocclu-

sion after PTA in limbs with compromised infrapopliteal runoff could at least in part be the consequence of a diminished arterial blood flow predisposing to thrombosis. We recorded four early femoropopliteal reocclusions among limbs with compromised infrapopliteal runoff and one early reocclusion among patients' limbs with good runoff, but due to small number, the difference was not statistically significant. Our results at 6 and 12 months suggest that the postprocedural infrapopliteal runoff is not a prognostic indicator of intermediate and late restenosis/reocclusion, which are mainly caused by neointimal hyperplasia and advancing atherosclerosis.

In interpreting these results, we must keep in mind that 40% of the subjects had their infra-popliteal runoff improved by PTA, and that our study tested the effects of postprocedural not preprocedural runoff of diseased arterial segments [22]. In this respect, our work differs from previous studies that found poor runoff strongly predictive of a bad long-term outcome of femoropopliteal PTA [57] and agrees with the finding of no effect of tibial runoff on the rate of the 1-year patency of recanalized superficial femoral artery occlusions in patients with at least 1 patent tibial artery in the affected limb [11]. When we calculated the outcomes with respect to preprocedural runoff, we found no association between the rate of restenosis/reocclusion and the infrapopliteal runoff before it was improved by PTA [22]. This finding in combination with our results according to postprocedural runoff strongly suggests that improving the infrapopliteal runoff by PTA delays the time to femoropopliteal restenosis/reocclusion, which may be especially beneficial in cases of critical limb ischemia. Patients' limbs that experienced deterioration of good postprocedural infrapopliteal runoff in the first year after PTA were affected by an approximately doubled rate of restenosis/reocclusion of the femoropopliteal artery in comparison with limbs that retained good runoff [22]. This means that worsening of infrapopliteal runoff was accompanied not only by early but also by intermediate and late femoropopliteal restenosis or reocclusion, probably due to neointimal hyperplasia and progression of atherosclerotic disease.

The combined complication rate of the PTA in our patients was 7%: 3 minor heamatomas, 2 pseudoaneurysms (managed by conservative treatment), and 1 periprocedural thrombosis. The average ABI improved from 0.60 ± 0.41 before PTA to 0.82 ± 0.25 after PTA ($p < 0.001$). Among the 176 treated patients, 3 had minor limb amputations within 1 month after PTA (2 transmetatarsal and 1 toe amputation), 4 additional patients had limb amputations within 6 months (2 above the knee and 2 below the knee), and 5 additional patients within 12 months (2 above the knee, 2 below the knee, and 1 with an undisclosed level of amputation). Overall, the amputation rate was 12/176 patients (7%) after 1 year. Two patients died within 6 months after PTA and a total of 8/176 patients (5%) died within the first year [22]. Overall, our results with a combined femoropopliteal restenosis/reocclusion rate of 55% and a reocclusion rate of 21% 1 year after PTA [22] were comparable to the published data for patients without implantation of self-expanding femoral stents [24, 28].This was expected since we used femoral stents only for bailout indications, i.e., in 3 out of 176 treated patients' limbs. The clinical success of PTA among our series of patients was demonstrated by the low 1-year amputation rate despite the advanced stages of PAD among our patients (36% in Fontaine stage III with rest pain and 40% in Fontaine stage IV with skin ulcerations). This result is at the upper level of the reported limb salvage rate with traditional recanalization techniques [29].

Among our patients, no significant differences in postprocedural runoff were found with respect to the presence of diabetes or renal failure, but there were more smokers in the group with compromised postprocedural runoff in comparison to the group with good runoff and more patients with hypercholesterolemia [22]. While smoking might have decreased the feasibility of infrapopliteal PTA, the greater prevalence of hypercholesterolemia among patients with compromised postprocedural runoff could be just a chance finding, although hypercholesterolemia has been associated with restenosis of TASC B and C femoropopliteal lesions after PTA [30].
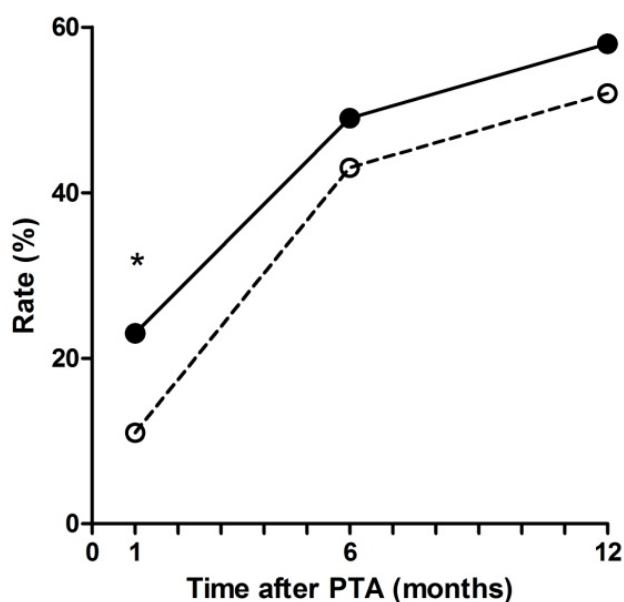

Figure 2. Time course of restenosis/reocclusion rates (%) in compromised runoff (full circle) and restenosis/reocclusion in good runoff (empty circle). Reproduced with permission from [22]. *$p < 0.03$

4.2. Hemostasis

To detect a possible prothrombotic state in patients referred for femoropopliteal PTA, thrombin generation and D-dimer concentration were measured before and after PTA in 88 patients. Thrombin generation assay indicates the potential of plasma to generate thrombin following the in vitro activation of coagulation with tissue factor or another trigger. The resulting thrombin generation curve reflects all pro- and anticoagulant reactions that regulate the formation and inhibition of thrombin [31]. D-dimer is a specific degradation product of cross-linked fibrin and is thus a marker of both activated coagulation and fibrinolysis. D-dimer is best known today as the biochemical gold standard for initial assessment of hypercoagulability in suspected venous thrombosis [32].

We detected a hemostatic shift toward hypercoagulability induced by PTA by a significantly higher postprocedurel thrombin generation expressed by increased ETP and higher D-dimer concentration compared to preprocedural values (Table 1). However, we found association

neither between thrombin generation nor D-dimer (either before or after PTA) and restenosis/reocclusion rate. No association between preprocedural thrombin generation and restenosis rate has also been observed in another study [33]. On the other hand, preprocedural hypercoagulability detected as shortening of the thromboelastometry-derived coagulation time (<444.5 s) reliably identified patients with high-degree in-stent restenosis in the superficial femoral artery [34]. Higher levels of preprocedural fibrinogen were also documented in patients with restenosis compared to patients with patent arteries [35].

	Before PTA	After PTA	p
Thrombin generation			
Lag phase (min)	10.8 ± 2.5	10.8 ± 2.2	NS
Peak thrombin (nM)	385 ± 96	393 ± 80	NS
Time to peak (min)	13.6 ± 3.1	13.8 ± 2.6	NS
Velocity	157 ± 83	154 ± 81	NS
ETP	3559 ± 542	3739 ± 490	<0.001
Microparticles (%)	26.8 ± 11.1	26.0 ± 10.2	NS
D-dimer (µg/L)	168 (99–479)	242 (138–584)	<0.001

ETP—endogenous thrombin potential, NS—not significant.

Table 1. Thrombin generation and D-dimer before and after PTA (mean ± standard deviation with Student's paired *t*-test *p* or median, 1st–3rd quartile with Wilcoxon signed-rank test *p*).

Genotyping of the prothrombotic polymorphisms was performed in 128 patients. All the tested polymorphisms were equally distributed among patients with or without restenosis/reocclusion in the first year after PTA (Table 2), suggesting that these polymorphisms have probably no major role in restenosis/reocclusion [36]. However, in order to detect possible weak association between these polymorphisms and femoropopliteal restenosis/reocclusion rate after PTA, a larger study population would be required.

With the exception of the study showing association of factor V G1691A with failed vascular reconstructions in patients with PTA [37], associations between prothrombotic gene polymorphisms and the risk of restenosis have been studied predominantly after percutaneous transluminal coronary angioplasty (PTCA). GPIIIa T1565C polymorphism was associated with higher risk of stent thrombosis after revascularization [38, 39] and with restenosis after PTCA in some [40], but not other studies [41]. Among other prothrombotic polymorphisms, factor V G1691A and PAI-1 4G5G may also play a role in the process of restenosis after PTCA. The PAI-1 4G variant was associated with an increased risk of restenosis after this procedure in contrast to factor V G1691A, which decreased the risk [42]. As far as we know, there has been no studies on the association of GPIIIa T1565C, factor II G20210A, factor XII C46T, and PAI-1 4G5G polymorphism with restenosis/reocclusion after PTA.

Polymorphism		Restenosis/reocclusion (N = 74)	Patent arteries (N = 54)	p
GP IIIa T1565C				
Genotype	TT	45 (61)	35 (65)	
	TC	27 (36)	17 (31)	NS
	CC	2 (3)	2 (4)	
1565C allele frequency		0.20	0.21	
FVL G1691A				
Genotype	GG	71 (96)	51 (94)	
	AG	3 (4)	3 (6)	NS
	AA	0 (0)	0 (0)	
1691A allele frequency		0.02	0.03	NS
Factor II G20210A				
Genotype	GG	70 (95)	53 (98)	
	AG	4 (5)	1 (2)	NS
	AA	0 (0)	0 (0)	
20210A allele frequency		0.01	0.03	NS
PAI-1 4G5G				
Genotype	5G5G	21 (28)	6 (11)	
	4G5G	22 (30)	29 (54)	NS
	4G4G	31 (42)	19 (35)	
4G allele frequency		0.62	0.57	
FXII C46T				
Genotype	CC	46 (62)	33 (61)	
	CT	23 (31)	18 (33)	NS
	TT	5 (7)	3 (6)	
FXII 46T allele frequency		0.77	0.72	NS

NS—not significant.

Table 2. Genotype in allele distribution in patients with restenosis/reocclusion or patients with patent arteries in the first year after PTA.

4.3. Inflammation

Genotyping of the three tagging single-nucleotide polymorphisms (rs1466408, rs13428968, and rs12803) in the NuRR1 gene was performed in 142 patients with femoropopliteal PTA who finished a 12-month follow-up. From these three polymorphisms, four haplotypes were

inferred as described earlier, and their frequencies were similar to that earlier observed in Caucasian population [20]. Haplotype 1 was the most frequent and served as the reference haplotype. Haplotypes 2 and 3 significantly increased the restenosis/reocclusion rate as shown by the relative risks adjusted for sex, age, and Fontaine classification calculated by Cox regression (Table 3) [43].

Haplotype	rs1466408	rs13428968	rs12803	Frequency (%)	Relative risk (95% CI)
Haplotype 1	T	T	G	49.4	-
Haplotype 2	T	T	T	23.3	1.6 (1.1–2.3)
Haplotype 3	T	C	T	19.7	2.0 (1.3–2.8)
Haplotype 4	A	T	T	6.6	NS

CI—confidence interval, NS—not significant.

Table 3. Composition and frequencies of the 4 NuRR1 haplotypes with frequencies >1% and adjusted relative risk associated with each haplotype in our study population.

Similar to our study, haplotype 3 increased the risk of in-stent restenosis, target lesion revascularization, percutaneous coronary interventions, and the rate of MACE about 2-fold in the first year after the procedure [20]. This study reported no association of haplotype 2, while haplotype 4 increased the risk of in-stent restenosis, target lesion revascularization, percutaneous coronary interventions, and the rate of MACE about 2- to 3-fold in the first year after the procedure [20]. We were not able to confirm an increased risk of restenosis/reocclusion in patients with haplotype 4 probably due to the small number of patients with this haplotype, and further analysis on a larger group of patients is warranted.

Despite a well-recognized role of inflammation in restenosis and known polymorphisms in inflammation marker genes that influence their level or function, the influence of these polymorphisms on restenosis rate has not yet been extensively studied. In addition, most studies to date focused on patients with PTCA rather than PTA. Among the most extensively studied polymorphisms is the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene. Meta-analysis of 33 cohort studies involving 11,099 subjects confirmed that carriers of the ACE DD genotype are subjected to a significantly increased risk (odds ratio 1.61, 95% CI 1.27–2.04, $p < 0.001$) for post-PTCA restenosis [44].

In patients with femoropopliteal PTA, two studies were reported that involved gene polymorphisms in interleukins. In the first study, a combined effect of the interleukin-1B C(-511)T single-nucleotide polymorphism and a variable number tandem repeat polymorphism in intron 2 of the interleukin-1 receptor antagonist gene (IL-1RN VNTR) were associated with a higher restenosis risk [45]. In the second study, a 2.4-fold increased adjusted risk for restenosis was observed in carriers of the interleukin-6 (-174)CC genotype compared to carriers of the (-174)GG genotype [46].

5. Conclusion

Our understanding of the mechanisms of restenosis/reocclusion of the femoral artery after PTA is deficient, and this study provided some additional evidence on the subject. The study suggested a significantly higher restenosis/reocclusion rate in patients with compromised runoff compared to patients with a good runoff 1 month after the procedure. In all patients, hypercoagulability as assessed by a thrombin generation assay and D-dimer was observed after PTA but was not associated with the restenosis/reocclusion rate. Prothrombotic poly-morphisms were equally distributed among patient with and without restenosis/reocclusion suggesting minor or no role of these polymorphisms in the risk of restenosis/reocclusion. On the other hand, haplotypes 2 and 3 in the NuRR1 gene significantly increased the restenosis/reocclusion rate, suggesting significant role of inflammation. In this ongoing study, further analysis on a larger group of patients is warranted, and possible consideration of combinations of genetic markers rather than isolated polymorphisms in the analysis of this multifactorial vascular disease might provide further evidence on the risk of restenosis/reocclusion after PTA.

Acknowledgements

Author details

Mojca Božič-Mijovski[1*], Vinko Boc[1], Vladka Salapura[2], Aleš Blinc[1,2] and Mojca Stegnar[1]

*Address all correspondence to: mojca.bozic@kclj.si

1 Department of Vascular Diseases, University Medical Centre Ljubljana, Ljubljana, Slovenia

2 Institute of Radiology, University Medical Centre Ljubljana and Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia

References

[1] Criqui MH, Langer RD, Fronek A, Feigelson HS, Klauber MR, McCann TJ, Browner D. Mortality over a period of 10 years in patients with peripheral arterial disease. New England Journal of Medicine 1992;326: 381–6.

[2] Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JW, Krook SH, Hunninghake DB, Comerota AJ, Walsh ME, McDermott MM, Hiatt WR.

Peripheral arterial disease detection, awareness, and treatment in primary care. JAMA 2001;286: 1317–24.

[3] European Stroke O, Tendera M, Aboyans V, Bartelink ML, Baumgartner I, Clement D, Collet JP, Cremonesi A, De Carlo M, Erbel R, Fowkes FG, Heras M, Kownator S, Minar E, Ostergren J, Poldermans D, Riambau V, Roffi M, Rother J, Sievert H, van Sambeek M, Zeller T, Guidelines ESCCfP. ESC guidelines on the diagnosis and treatment of peripheral artery diseases: document covering atherosclerotic disease of extracranial carotid and vertebral, mesenteric, renal, upper and lower extremity arteries: the Task Force on the Diagnosis and Treatment of Peripheral Artery Diseases of the European Society of Cardiology (ESC). European Heart Journal 2011;32: 2851–906.

[4] Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG, Group TIW. Inter-society consensus for the management of peripheral arterial disease (TASC II). Journal of Vascular Surgery 2007;45(Suppl S): S5–67.

[5] Matsi PJ, Manninen HI, Vanninen RL, Suhonen MT, Oksala I, Laakso M, Hakkarainen T, Soimakallio S. Femoropopliteal angioplasty in patients with claudication: primary and secondary patency in 140 limbs with 1–3-year follow-up. Radiology 1994;191: 727–33.

[6] Clark TW, Groffsky JL, Soulen MC. Predictors of long-term patency after femoropopliteal angioplasty: results from the STAR registry. Journal of Vascular Interventional Radiology 2001;12: 923–33.

[7] Johnston KW. Femoral and popliteal arteries: reanalysis of results of balloon angioplasty. Radiology 1992;183: 767–71.

[8] Krepel VM, van Andel GJ, van Erp WF, Breslau PJ. Percutaneous transluminal angioplasty of the femoropopliteal artery: initial and long-term results. Radiology 1985;156: 325–8.

[9] Matsi PJ, Manninen HI, Suhonen MT, Pirinen AE, Soimakallio S. Chronic critical lower-limb ischemia: prospective trial of angioplasty with 1–36 months follow-up. Radiology 1993;188: 381–7.

[10] Stokes KR, Strunk HM, Campbell DR, Gibbons GW, Wheeler HG, Clouse ME. Five-year results of iliac and femoropopliteal angioplasty in diabetic patients. Radiology 1990;174: 977–82.

[11] Gordon IL, Conroy RM, Tobis JM, Kohl C, Wilson SE. Determinants of patency after percutaneous angioplasty and atherectomy of occluded superficial femoral arteries. American Journal of Surgery 1994;168: 115–9.

[12] Fuster V, Falk E, Fallon JT, Badimon L, Chesebro JH, Badimon JJ. The three processes leading to Post PTCA restenosis: dependence on the lesion substrate. Thrombosis and Haemostasis 1995;74: 552–9.

[13] Ten Cate H. Thrombin generation in clinical conditions. Thrombosis Research 2012;129: 367–70.

[14] Vižintin Cuderman T, Božič M, Peternel P, Stegnar M. Hemostasis activation in thrombophilic subjects with or without a history of venous thrombosis. Clinical and Applied Thrombosis and Hemostasis 2008;14: 55–62.

[15] Chistiakov DA, Orekhov AN, Bobryshev YV. Vascular smooth muscle cell in atherosclerosis. Acta Physiologica (Oxf) 2015.

[16] Schillinger M, Minar E. Restenosis after percutaneous angioplasty: the role of vascular inflammation. Vascular Health Risk Management 2005;1: 73–8.

[17] Mickelson JK, Lakkis NM, Villarreal-Levy G, Hughes BJ, Smith CW. Leukocyte activation with platelet adhesion after coronary angioplasty: a mechanism for recurrent disease? Journal of the American College of Cardiology 1996;28: 345–53.

[18] Bonta PI, Pols TW, de Vries CJ. Nr4a nuclear receptors in atherosclerosis and vein-graft disease. Trends in Cardiovascular Medicine 2007;17: 105–11.

[19] Bonta PI, van Tiel CM, Vos M, Pols TW, van Thienen JV, Ferreira V, Arkenbout EK, Seppen J, Spek CA, van der Poll T, Pannekoek H, de Vries CJ. Nuclear receptors Nur77, Nurr1, and nor-1 expressed in atherosclerotic lesion macrophages reduce lipid loading and inflammatory responses. Arteriosclerosis, Thrombosis, and Vascular Biology 2006;26: 2288–94.

[20] Bonta PI, Pols TW, van Tiel CM, Vos M, Arkenbout EK, Rohlena J, Koch KT, de Maat MP, Tanck MW, de Winter RJ, Pannekoek H, Biessen EA, Bot I, de Vries CJ. Nuclear receptor Nurr1 is expressed in and is associated with human restenosis and inhibits vascular lesion formation in mice involving inhibition of smooth muscle cell proliferation and inflammation. Circulation 2010;121: 2023–32.

[21] Kardys I, van Tiel CM, de Vries CJ, Pannekoek H, Uitterlinden AG, Hofman A, Witteman JC, de Maat MP. Haplotypes of the Nr4a2/Nurr1 gene and cardiovascular disease: the Rotterdam Study. Human Mutation 2009;30: 417–23.

[22] Salapura V, Blinc A, Kozak M, Ježovnik MK, Pohar Perme M, Berden P, Kuhelj D, Ključevšek T, Popovič P, Stankovič M, Vrtovec M, Šurlan M. Infrapopliteal run-off and the outcome of femoropopliteal percutaneous transluminal angioplasty. Vasa 2010;39: 159–68.

[23] Orchard TJ, Strandness DE, Jr. Assessment of peripheral vascular disease in diabetes. Report and recommendations of an international workshop sponsored by the American Diabetes Association and the American Heart Association September 18–20, 1992 New Orleans, Louisiana. Circulation 1993;88: 819–28.

[24] Schillinger M, Sabeti S, Loewe C, Dick P, Amighi J, Mlekusch W, Schlager O, Cejna M, Lammer J, Minar E. Balloon angioplasty versus implantation of nitinol stents in the superficial femoral artery. New England Journal of Medicine 2006;354: 1879–88.

[25] MacAllister, L. Peripheral Vascular Sonography. Philadelphia: Lippincott Williams and Wilkins; 2004.

[26] Rutherford RB, Baker JD, Ernst C, Johnston KW, Porter JM, Ahn S, Jones DN. Recommended standards for reports dealing with lower extremity ischemia: revised version. Journal of Vascular Surgery 1997;26: 517–38.

[27] Božič M, Stegnar M. Validation of an automated immunoturbidimetric assay for measurement of plasma D-dimer. Clinical Chemistry and Laboratory Medicine 2003;41: 958–62.

[28] Schillinger M, Minar E. Endovascular stent implantation for treatment of peripheral artery disease. European Journal of Clinical Investigation 2007;37: 165–70.

[29] Zeller T, Sixt S, Rastan A. New techniques for endovascular treatment of peripheral artery disease with focus on chronic critical limb ischemia. Vasa 2009;38: 3–12.

[30] Baril DT, Marone LK, Kim J, Go MR, Chaer RA, Rhee RY. Outcomes of endovascular interventions for TASC II B and C femoropopliteal lesions. Journal of Vascular Surgery 2008;48: 627–33.

[31] Hemker HC, Giesen P, AlDieri R, Regnault V, de Smed E, Wagenvoord R, Lecompte T, Beguin S. The calibrated automated thrombogram (CAT): a universal routine test for hyper- and hypocoagulability. Pathophysiology of Haemostasis and Thrombosis 2002;32: 249–53.

[32] Righini M, Perrier A, De Moerloose P, Bounameaux H. D-Dimer for venous thromboembolism diagnosis: 20 years later. Journal of Thrombosis and Haemostasis 2008;6: 1059–71.

[33] Gremmel T, Koppensteiner R, Ay C, Panzer S. Residual thrombin generation potential is inversely linked to the occurrence of atherothrombotic events in patients with peripheral arterial disease. European Journal of Clinical Investigation 2014;44: 319–324.

[34] Cvirn G, Hoerl G, Schlagenhauf A, Tafeit E, Brodmann M, Juergens G, Koestenberger M, Gary T. Stent implantation in the superficial femoral artery: short thrombelastometry-derived coagulation times identify patients with late in-stent restenosis. Thrombosis Research 2012;130: 485–90.

[35] Tschopl M, Tsakiris DA, Marbet GA, Labs KH, Jager K. Role of hemostatic risk factors for restenosis in peripheral arterial occlusive disease after transluminal angioplasty. Arteriosclerosis, Thrombosis, and Vascular Biology 1997;17: 3208–14.

[36] Bedenčič M, Božič M, Salapura V, Blinc A, Stegnar M. Genetic predisposition to the risk of restenosis after femoropopliteal percutaneous transluminal angioplasty. Pathophysiology of Haemostasis and Thrombosis 2008;36:A41.

[37] Sampram ES, Lindblad B, Dahlback B. Activated protein C resistance in patients with peripheral vascular disease. Journal of Vascular Surgery 1998;28: 624–9.

[38] Di Castelnuovo A, de Gaetano G, Donati MB, Iacoviello L. Platelet glycoprotein receptor IIIA polymorphism Pla1/Pla2 and coronary risk: a meta-analysis. Thrombosis and Haemostasis 2001;85: 626–33.

[39] Williams MS, Bray PF. Genetics of arterial prothrombotic risk states. Experimental Biology and Medicine (Maywood) 2001;226: 409–19.

[40] Abbate R, Marcucci R, Camacho-Vanegas O, Pepe G, Gori AM, Capanni M, Simonetti I, Prisco D, Gensini GF. Role of platelet glycoprotein Pl(A1/A2) polymorphism in restenosis after percutaneous transluminal coronary angioplasty. American Journal of Cardiology 1998;82: 524–5.

[41] Mamotte CD, van Bockxmeer FM, Taylor RR. Pia1/A2 polymorphism of glycoprotein IIIA and risk of coronary artery disease and restenosis following coronary angioplasty. American Journal of Cardiology 1998;82: 13–6.

[42] Pons D, Monraats PS, de Maat MP, Pires NM, Quax PH, van Vlijmen BJ, Rosendaal FR, Zwinderman AH, Doevendans PA, Waltenberger J, de Winter RJ, Tio RA, Frants RR, van der Laarse A, van der Wall EE, Jukema JW. The influence of established genetic variation in the haemostatic system on clinical restenosis after percutaneous coronary interventions. Thrombosis and Haemostasis 2007;98: 1323–8.

[43] Božič-Mijovski M, Bedenčič M, Stegnar M, Salapura V, Ježovnik MK, Kozak M, Blinc A. Nurr1 haplotypes are associated with femoropopliteal restenosis/re-occlusion after percutaneous transluminal angioplasty. European Journal of Vascular and Endovascular Surgery 2012;43: 337–8.

[44] Wang S, Dai Y, Chen L, Dong Z, Chen Y, Li C, Zhong X, Lin W, Zhang J. Genetic polymorphism of angiotensin converting enzyme and risk of coronary restenosis after percutaneous transluminal coronary angioplasties: evidence from 33 cohort studies. PloS One 2013;8: e75285.

[45] Marculescu R, Mlekusch W, Exner M, Sabeti S, Michor S, Rumpold H, Mannhalter C, Minar E, Wagner O, Schillinger M. Interleukin-1 cluster combined genotype and restenosis after balloon angioplasty. Thrombosis and Haemostasis 2003;90: 491–500.

[46] Exner M, Schillinger M, Minar E, Mlekusch W, Sabeti S, Endler G, Raith M, Mannhalter C, Wagner O. Interleukin-6 promoter genotype and restenosis after femoropopliteal balloon angioplasty: initial observations. Radiology 2004;231: 839–44.