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Stent implantation in the superficial femoral artery: Short thrombelastometry-derived coagulation times identify patients with late in-stent restenosis

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ABSTRACT

Introduction: The mechanisms of restenosis, the recurrence of luminal narrowing, are complex and incompletely understood to date. Thrombin, the pivotal enzyme in haemostasis, presumably contributes to the formation of in-stent restenosis (ISR). It was therefore the aim of our study to investigate whether blood coagulation/thrombin generation plays a critical role in the formation of ISR in peripheral artery disease patients with stent angioplasty in the superficial femoral artery.
Materials and Methods: We aimed to examine in this retrospective study whether patients with high-degree restenosis (50-75% lumen diameter reduction, n = 20) are in a hypercoaguable state implying enhanced readiness to generate thrombin compared to patients with low-degree restenosis (<50% lumen diameter reduction, n = 14).
Results: The coagulation tests calibrated automated thrombography, activated partial thromboplastin time, platelet aggregation, platelet adhesion, fibrinogen, and microparticles' procoagulant activity did not indicate a different coagulation status in the two patient groups. However, the thrombelastometry-derived value Coagulation Time (CT) was significantly shorter in the high-degree restenosis group ($p = 0.012$), indicating a hypercoagulable state of patients with high-degree restenosis. Under our experimental conditions, CTs shorter than 444.5 s identify patients at high risk (sensitivity = 95%) for luminal narrowing.
Conclusions: Our study supports the assumption that blood coagulation/thrombin generation plays a critical role in the development of ISR in peripheral arteries after stent insertion and that the thrombelastometry-derived CT might be a suitable value to identify peripheral artery disease patients at risk for development of high-degree in-stent restenosis in the superficial femoral artery.

Introduction

Treatment of superficial femoral artery (SFA) disease by stent implantation has demonstrated superiority to percutaneous transluminal angioplasty (PTA) alone. However, in-stent restenosis (ISR) remains a frequent complication [1]. The mechanisms of restenosis are complex and incompletely understood to date. Thrombin, the pivotal enzyme in haemostasis, has recently been recognized as an important factor in the development of restenosis [2–5]. Thrombin supports restenosis via activation of thrombin receptors on endothelial cells, smooth muscle cells, macrophages, and fibroblasts [6,7].

Thus, a critical role of blood coagulation implying thrombin generation has to be expected in the development of ISR in the SFA in patients with peripheral arterial disease (PAD). However, Wahlgren et al. did not observe an influence of thrombin generation on stent restenosis in thirty-four patients with PAD undergoing angioplasty of the iliac and superficial femoral arteries [8]. In this study, plasma levels of prothrombin fragment 1 + 2 (F 1 + 2) served as an indicator of thrombin generation. There were no significant changes in the plasma levels of F 1 + 2 after PTA. Moreover, F 1 + 2 level was no statistically significant predictor of luminal narrowing or restenosis.

The aim of our study was therefore to further scrutinize whether development of ISR after stent implantation in the SFA in patients with PAD is promoted by a hypercoagulable state and enhanced

Abbreviations: APTT, activated partial thromboplastin time; ETP, endogenous thrombin potential; CAT, calibrated automated thrombography; CFT, clot formation time; CRP, C-reactive protein; CT, coagulation time; F 1 + 2, prothrombin fragment 1 + 2; GPRP, fibrin polymerization inhibitor H-Gly-Pro-Arg-Pro-OH; HDL, high-density lipoprotein; ISR, in-stent restenosis; LDL, low-density lipoprotein; MCF, maximum clot firmness; PAD, peripheral arterial disease; PCI, percutaneous coronary intervention; PPP, platelet poor plasma; PT, prothrombin time; PTA, percutaneous transluminal angioplasty; PTCA, percutaneous transluminal coronary angioplasty; SD, standard deviation; SFA, superficial femoral artery; TEM, thrombelastometry; TF, lipidated tissue factor; WB, whole blood.

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thrombin generation, respectively. For the present study, thirty-four patients were admitted to the department of angiology of the university hospital Graz/Austria approximately three years after SFA-stent insertion. Twenty out of thirty-four patients presented with high-degree restenosis, defined as 50–75 % lumen diameter reduction at the site of PTA. We examined whether patients with high-degree ISR are hypercoagulable/generate higher amounts of thrombin when compared to patients with low-degree ISR. Patients' coagulable state was assessed particularly by measuring thrombin generation curves and by monitoring whole blood clot development courses.

Thrombin generation curves were detected in platelet poor plasma (PPP) samples by means of calibrated automated thrombography (CAT) [9,10]. Whole blood clot development courses were monitored by means of tissue factor (TF) triggered thrombelastometry (TEM). This method has been shown to allow a sensitive and a close to the in-vivo situation estimation of the patients' coagulable state [11].

In order to extend the haemostatic profiling in our patients with low vs. high-degree restenosis, we additionally determined activated partial thromboplastin times (APTTs) as well as platelet adhesion and aggregation. Moreover, a possible platelet-activating effect of the implanted stent might result in shedding of procoagulant microparticles. Therefore, we also determined microparticles' procoagulant activities in both patient groups.

Materials and Methods

Patients

The diagnosis of PAD was assigned in our outpatient clinic by means of clinical evaluation, ankle-brachial index, and duplex scan. The current study is based on 34 patients scheduled for stent implantation throughout the years 2007 and 2008. The results presented herein are derived from blood collected from the same patients between February and April 2011. Stent implantation was applied as secondary stent implantation for unsatisfactory results of percutaneous transluminal angioplasty (PTA) alone, which means residual stenosis of >50%, flow-limiting dissection, elastic recoil, or acute thrombotic occlusion in the area of balloon angioplasty. A standardized protocol was used for stent implantation. All our patients were treated with self-expandable nitinol stents (Absolute stent from Abbot Vascular, Des Plaines, IL). During the intervention, 3000 IU of unfractionated heparin was administered to avoid stent occlusion. After the procedure, patients were treated with low-molecular-weight heparin (enoxaparin 40 mg twice daily) for 48 h. All patients received antithrombotic medication with 100 mg aspirin before intervention and 100 mg aspirin and 75 mg clopidogrel after stent implantation for 3 month. After 3 month, the patients were treated with 100 mg aspirin indefinitely. Our patients were divided into two groups: patients with low-degree restenosis (<50% lumen diameter reduction, n = 14) and patients with high-degree restenosis (50-75% lumen diameter reduction, n = 20). ISR was assessed with duplex scan. An obstruction was considered as hemodynamically relevant when the grade of stenosis was above 50%, defined as at least doubling of the peak velocity in the obstruction area. The exact degree of stenosis was calculated by means of an area stenosis degree in the cross-sectional view.

Reagents and Devices

Blood cell counts were determined on a Sysmex KX-21 N Automated Hematology Analyzer from Sysmex (Illinois, USA). Thrombin generation curves were monitored by means of CAT purchased from Thrombinoscope BV, Maastricht, the Netherlands. The TEM coagulation analyser (ROTEM®05) was purchased from Matel Medizintechnik, Graz, Austria. Activated partial thromboplastin time and prothrombin time were measured on the optomechanical coagulation analyzer Behring Fibrintimer from Behring Diagnostics GmbH, Marburg, Germany. Plasma levels of fibrinogen were determined on a Schnitger-Gros coagulometer according to the Clauss-method. Whole-blood aggregation experiments were performed on the Chrono-Log Whole Blood Aggregometer Model 590 from Probe & Go (Endingen, Germany). Platelet adhesion was measured on a Cone and Platelet Analyzer (CPA) from DiaMed (Linz, Austria). Microparticles' procoagulant activity was determined by the functional assay ZYMUPHEN MP-Activity from HYPHEN BioMed (Neuville, France). Microplate Scanning Spectrometer was purchased from Bio-Tek Instruments, Inc., Winooski, Vermont, USA.

Collection of WB and Preparation of Plasma

Venous blood samples were obtained from 34 PAD patients with SFA-stent implantation in. The study was approved by the appropriate Institutional Review Board. Informed consent was obtained. Blood (2.7 ml) was collected into precitrated S-Monovette premarked tubes (three from each individual) from Sarstedt (Nümbrecht, Germany), containing 300 μl of 0.106 mol/l sodium citrate. The first tube aspirated was discarded. The whole blood from the two remaining tubes was pooled, and subsequently used for determination of blood cell counts as well as for thrombelastometry and platelet function measurements. The remaining whole blood was centrifuged at room temperature for 15 min at 1200 ×*g* to obtain PPP for subsequent determination of standard coagulation times, fibrinogen plasma concentrations, thrombin generation curves, and microparticles' procoagulant activity.

Automated Fluorogenic Measurement of the Thrombin Generation

Measurement of the thrombin generation was performed using CAT [9]. The ability of a given plasma sample to generate thrombin was assessed with respect to lag time preceding the thrombin burst (Lag Time), time to peak (ttPeak), peak height (Peak), and endogenous thrombin potential (ETP), and the time point at which free thrombin has disappeared (StartTail). Measurements were carried out in the presence of 5 pmol/l of tissue factor (TF) (final concentration).

WB Tissue Factor Triggered TEM Assay

We obtained the following values: Coagulation Time (CT), the period of time from initiation of the test to the initial fibrin formation; Clot Formation Time (CFT), time of beginning of clot formation until the amplitude of thrombelastogram reaches 20 mm; Maximum Clot Firmness (MCF), expressing the maximum strength in millimeters of the final clot; and Alpha, the angle between the line in the middle of the TEM tracing and the line tangential to the developing "body" of the TEM tracing. The alpha angle represents the acceleration (kinetics) of fibrin build up and cross-linking. This method has been described in detail recently (11).

Whole Blood Platelet Aggregation Assay

WB aggregation was assayed with WB aggregometer by the impedance method [12,13]. Impedance aggregometry results are expressed as "amplitude (or maximum aggregation) [ohm]" at 6 minutes after reagent addition and as "lag time (or aggregation time) [seconds]", the time interval until the onset of platelet aggregation. The rate of platelet aggregation is expressed as "slope [ohm/min]". Collagen and endogenously generated thrombin were used as platelet agonists, respectively, described in our previous study [11].

Whole Blood Platelet Adhesion Assay

The method has been described in detail previously [14]. Briefly, 130 μl of citrated WB were placed in polystyrene tubes and subjected to flow ($1300\ s^{-1}$) for 2 minutes using a rotating Teflon cone. The wells were washed with phosphate buffered saline, stained with