Thrombin Generation Profile in Patients With Steady State Peripheral Arterial Disease

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Residual thrombin generation potential is linked to the occurrence of atherothrombotic events in patients with peripheral arterial disease (PAD).[1] The assessment of thrombin generation reflects the global balance of blood coagulation process in patients with vascular diseases.[2] In this present hypothesis-generating study, we evaluated thrombin generation as a potential tool for the diagnosis of blood coagulation alterations in patients with steady state PAD.

The study protocol was approved by the local institutional ethics committee, and the study was performed in compliance with the principles of good clinical practice and in accordance to the Declaration of Helsinki. Forty-two patients with PAD (mean age: 68.0 $\pm$ 8.8 years; 76.2% male) and 17 age- and gender-matched participants with no PAD (control group) were prospectively enrolled in this multicenter study, following informed consent. The patients in the PAD cohort had a mean duration of symptomatic PAD of 7.8 $\pm$ 4.2 years. Their mean body mass index and blood pressure were 27.8 $\pm$ 4.2 kg/m^2 and 140.3 $\pm$ 16.0/78.8 $\pm$ 7.5 mm Hg, respectively. All patients with PAD were in a stable clinical condition and received antiplatelet therapy with either aspirin or clopidogrel. All participants in the control group have no personal history of thrombotic or hemorrhagic episodes and did not have any other evident disease. They also had normal hematological parameters. None of the recruited participants had received blood transfusion for at least 12 weeks prior to inclusion into the current study. They also did not receive any anticoagulant or antiplatelet treatment (heparins, vitamin K antagonists, aspirin, clopidogrel, or prasugrel) during the 2 months prior to inclusion into the study. Moreover, participants with liver or renal impairment were excluded from the study. All participants received best medical treatment and optimal pharmacological management for treatment of their dyslipidemia and hypertension.

Samples from all participants obtained for the study were acquired in the setting of routine outpatient consultation for routine laboratory and clinical follow-up of their disease. Venous blood samples were drawn into siliconized vacutainer tubes containing 3.2% trisodium citrate (Becton Dickinson, Le Pont-de-Claix, France) as anticoagulant in a ratio of 9 parts of blood to 1 part of citrate. Platelet-poor plasma (PPP), used for the assessment of thrombin generation, was prepared using double centrifugation at 2000*g* for 20 minutes in room temperature. All samples were aliquoted and frozen at -80°C. Thrombin generation was assessed using the calibrated automated thrombogram (CAT) assay (Diagnostica Stago, Asnieres, France) in accordance with the manufacturers' instructions, as previously described.[3] Briefly, 80 µL of PPP was added to 20 µL of PPP reagent 5 pM (Thrombinoscope

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B.V., Maastricht, Netherlands), which consisted of a mixture of tissue factor (TF; 5 pM final concentration in plasma) and phospholipids (4 μM final concentration in plasma). Plasma from each participant was analyzed in duplicate. In a third well, PPP reagent was replaced with the same volume of thrombin calibrator (Thrombinoscope B.V., Maastricht, Netherlands) to correct the thrombin generation curves for substrate consumption and inner filter fluorescence effects. Thrombin generation was triggered with 20 μL solution containing $CaCl_2$ (16.7 mM final concentration) and the fluorogenic substrate Z-Gly-Gly-Arg-AMC (417 μM final concentration). All measurements were done using thawed plasma samples. Fluorescence was measured with fluorometer (Fluoroskan Ascent; Thermo Labsystems, Helsinki, Finland). Acquisition of thrombin generation parameters was performed using the Thrombinoscope software (Diagnostica Stago, Gennevilliers, France) (CAT, Maastricht, the Netherlands). The following parameters of thrombogram were studied—the lag time of thrombin generation, the time to reach the peak of thrombin (ttPeak), the thrombin peak (Peak), the endogenous thrombin potential (ETP) that reflects the total amount of thrombin activity, and the mean rate index (MRI) that reflects the velocity of the propagation phase of thrombin generation (The MRI was calculated using the formula MRI = Peak/(ttPeak − lag time). The levels of factor V and antithrombin were measured using the conventional assays. The comparisons between continuous variables were performed using Student *t* test. Upper normal limit for each parameter of thrombin generation was defined as mean ± standard deviation (SD) of the values obtained by assessing thrombogram in the group of healthy volunteers. A 2-sided *P* value < .05 was considered significant. Statistical analysis was performed using the STATA software for Windows (STATA Inc, release 14, Chicago, Illinois).

This is the first study that provides direct comparison of the thrombin generation profile in patients with steady state PAD versus healthy age- and gender-matched controls. Our results showed that thrombin generation was significantly decreased in patients with steady state PAD as compared to the control group. The MRI was significantly lower in patients with steady state PAD as compared to the control group (6.5 [3.9-12.5] nM/min vs 143.7 [121.7-189.8] nM/min; $P < .001$). The thrombin peak was also significantly lower in patients with steady state PAD as compared to the control group (41.6 [30.5-69.0] nM vs 379.6 [329.5-405.2] nM; $P < .001$). Furthermore, the time to reach the peak of thrombin (12.8 [10.0-14.7] minutes vs 5.2 [5.0-5.5] minutes; $P < .001$) and the lag time (5.2 [4.5-6.4] minutes vs 3.0 [2.7-3.3] minutes; $P < .001$) were significantly increased in our cohort with PAD as compared to the control group. On the other hand, the ETP was significantly decreased in patients with steady state PAD in comparison with the control group (546 [431-807] nM/min vs 1751.5 [1603.5-1967.5] nM/min; $P < .001$). The levels for factor V and antithrombin in our cohort with PAD were within the normal range and were not significantly different as compared to the control group, thus excluding the presence of compensated disseminated intravascular coagulation.

Since all patients were maintained on antiplatelet treatment and were also routinely treated with statins, the reduced thrombin generation at steady state conditions could reflect a favorable alteration in the global hemostatic balance by these treatments. This hypothesis is supported from the data presented by Mobarrez et al who showed that atorvastatin treatment in participants with PAD induces a reduction in the number of platelet-derived microparticles, expression of TF, and reduction in plasma prothrombin fragment 1+2.[4] Furthermore, Tripodi et al have, in fact, shown that statin was associated with a significant reduction in median ETP in patients with hypercholesterolaemia.[5] Thus, the potential beneficial effect of statin treatment on thrombin generation process in patients with PAD merits to be studied in a prospective trial. Furthermore, the potential additive or confounding effect of antiplatelet and other concomitant therapy warrants further clarification. The exploration of the relationship between thrombin generation profile and the atherosclerotic burden is currently a subject of an ongoing study.

In conclusion, the present pilot study demonstrates that thrombin generation is significantly downregulated in patients with steady state PAD who were maintained on antiplatelet therapy and lipid-lowering treatment with statin. The assessment of thrombin generation could be a potential tool for the evaluation of the effect of these treatments on the global balance of blood coagulation in patients with PAD. A prospective study is warranted to evaluate the clinical relevance of these findings, specifically, the potential identification of patients at risk of treatment failure to prevent acute thrombotic episodes or aggravation of PAD.

References

1. 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. *Eur J Clin Invest*. 2014;44(3):319-324. doi:10.1111/eci.12236.
2. Tournois C, Pignon B, Sevestre MA, et al. Critical limb ischemia: thrombogenic evaluation of two autologous cell therapy products and biologic profile in treated patients. *Transfusion*. 2015;55(11): 2692-2701. doi:10.1111/trf.13203.
3. Chaari M, Ayadi I, Rousseau A, et al. Impact of breast cancer stage, time from diagnosis and chemotherapy on plasma and cellular biomarkers of hypercoagulability. *BMC Cancer*. 2014;14:991. doi:10.1186/1471-2407-14-991.
4. Mobarrez F, He S, Broijersen A, et al. Atorvastatin reduces thrombin generation and expression of tissue factor, P-selectin and GPIIIa on platelet-derived microparticles in patients with peripheral arterial occlusive disease. *Thromb Haemost*. 2011;106(2): 344-352. doi:10.1160/TH10-12-0810.
5. Tripodi A, Pellegatta F, Chantarangkul V, et al. Statins decrease thrombin generation in patients with hypercholesterolemia. *Eur J Intern Med*. 2014;25(5):449-451. doi:10.1016/j.ejim.2014.03.016.