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Full Length Article

Clot structure and fibrinolytic potential in patients with post thrombotic syndrome☆

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ABSTRACT

Introduction: Post-thrombotic syndrome (PTS) is a chronic sequel of deep vein thrombosis (DVT). The clot structure and fibrinolytic potential in PTS is currently unknown.
Objective: To assess the fibrinolytic potential and clot structure in patients with PTS.
Materials and methods: Patients with a history of DVT were included in a case–control study: patients with PTS (cases n = 30) and without PTS (controls n = 30), and 30 apparently healthy individuals (HI) without venous thromboembolism (VTE) or venous insufficiency were enrolled. Fibrinolysis and clot structure were assessed by turbidimetric assays, permeation, and confocal microscopy. Fibrinogen was measured by Clauss and fibrinogen γ′ by ELISA.
Results: We observed a significant trend of decreasing maximum turbidity from HI (median 0.52 [IQR 0.46–0.62]), to controls (0.49 [IQR 0.41–0.55]), to cases (0.46 [IQR 0.39–0.49]) p = 0.020. Fibrinogen was lower in patients (cases and controls) (3.69 g/L [IQR 3.31–4.26]) compared to HI (4.17 [IQR 3.69–4.65]) p = 0.041. Patients with recurrent VTE had lower maximum turbidity and lower permeation than patients with one episode of VTE; (0.31 [IQR 0.25–0.39] versus 0.38 [IQR 0.34–0.44] p = 0.008) and (6.0×10^{-9}/cm^2 [IQR 5.1–7.9] versus 7.7×10^{-9}/cm^2 [IQR 6.0–10.0] p = 0.047) respectively, at equal fibrinogen levels. There were no differences in lysis time, confocal microscopy, or fibrinogen γ′.
Conclusions: Lower maximum turbidity, indicating a tendency towards thinner fibres and denser clots, was found in patients with PTS as well as in patients with recurrent VTE. Fibrinogen levels did not explain these differences in clot structure. The abnormal clot structure may contribute to the increased thrombotic risk profile in patients with PTS.

1. Introduction

Post thrombotic syndrome (PTS) is a long-term complication of deep vein thrombosis (DVT), characterized by chronic complaints of the leg affected by DVT. Patients experience pain, heaviness, cramps, tingling, itching, and/or skin changes, and in severe cases ulceration of the leg can occur [1]. PTS is a syndrome and is diagnosed based on a clinical score [2]. PTS significantly reduces quality of life, and associated costs are substantial [3,4].

According to current consensus, the signs and symptoms of PTS are the end organ manifestation of venous hypertension. Venous hypertension is caused by a combination of reflux of blood, due to destruction of the venous valves; and stiff fibrotic vein walls, due to processes of tissue remodelling; and venous outflow resistance, due to impaired thrombus resolution [5,6].

Differences in the processes of thrombus formation and thrombus resolution between patients may therefore be associated with the occurrence of PTS. In a recent case–control study patients with PTS were found to have a decreased activated protein C (APC) ratio and decreased levels of zymogen thrombin activatable fibrinolysis inhibitor (proTAFI), as compared to controls [7]. Based on these findings, we hypothesised that patients with PTS might have an impaired fibrinolysis, due to a decreased inhibition of thrombin generation by APC, which results in increased conversion of proTAFI to active TAFI and increased activation of Factor (F) XIII [7,8]. Apart from impaired fibrinolysis, also fibrin clot structure might contribute to the development of PTS. Recent studies

Abbreviations: A/C, oral anticoagulants; APC, activated protein C; BMI, Body Mass Index; CRP, C-reactive protein; DVT, deep vein thrombosis; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; FXIII, Factor XIII; HI, healthy individuals; IQR, interquartile range; n/a, not applicable; proTAFI, pro thrombin activatable fibrinolysis inhibitor; PTS, post thrombotic syndrome; TAT, thrombin-antithrombin complex; TMB, 3,3′,5,5′-tetramethylbenzidine; tPA, tissue plasminogen activator; VTE, venous thromboembolism.

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Table 1
Baseline characteristics.

	Cases (n = 24)	Controls (n = 28)	HI (n = 22)
Gender			
Male	12 (50%)	12 (43%)	11 (50%)
Female	12 (50%)	16 (57%)	11 (50%)
Age			
Median (IQR)	66 (49–76)	69 (58–78)	63 (54–69)
BMI			
Median (IQR)	28 (25–33)	25 (24–31)	26 (25–30)
Average Villalta[a]			
Median (IQR)	7 (6–8)	2 (1–3)	n/a
Severity PTS[b]			
Mild	12 (50%)	n/a	n/a
Moderate	8 (33%)	n/a	n/a
Severe	4 (17%)	n/a	n/a
Characteristics most recent DVT			
Median time between DVT and blood withdrawal for study (IQR)	55 months (35–78)	64 months (41–91)	n/a
Highest extent			
Iliac or femoral	13 (54%)	14 (50%)	n/a
Popliteal	11 (46%)	13 (46%)	n/a
Isolated calf	0 (0%)	1 (4%)	n/a
Side			
Left	13 (54%)	15 (54%)	n/a
Right	11 (46%)	13 (46%)	n/a
Provoked/unprovoked			
Provoked	6 (25%)	6 (21%)	n/a
Unprovoked	18 (75%)	22 (79%)	n/a
Concurrent symptomatic PE	0 (0%)	2 (7%)	n/a
Previous DVT	10 (42%)	8 (29%)	n/a
Previous ipsilateral DVT	7 (29%)	6 (21%)	n/a
Previous PE	0 (0%)	2 (7%)	n/a
Distal DVT only[c]	0 (0%)	1 (4%)	n/a
A/C			
Yes	11 (46%)	7 (25%)	2 (9%)
No	13 (54%)	21 (75%)	20 (91%)

A/C, oral anticoagulants; DVT, deep vein thrombosis, PE, pulmonary embolism.
[a] Average of 1–4 measurements of Villalta score.
[b] Based on highest Villalta score during follow-up.
[c] Only episodes of distal DVT during follow-up.

have shown denser fibrin clot structures with smaller pores and increased resistance to fibrinolysis in patients with DVT and other thrombotic disorders [9–13]. The role of fibrin structure in PTS is currently unknown. Therefore, the aim of the current study is to assess the fibrinolytic potential and the clot structure of patients with PTS, by means of functional fibrinolysis tests and confocal microscopy.

2. Materials and methods

2.1. Patients and study design

A case–control study was performed as previously described [7]. In short, 60 patients with a history of objectively confirmed DVT, 30 patients with PTS and 30 patients without PTS, were recruited from the outpatient clinic of the Maastricht University Medical Centre and the Flevohospital Almere, The Netherlands. Patients who had developed PTS were defined as cases. Patients that had not developed PTS, after a minimal follow-up of 2 years after DVT, were defined as controls. PTS was defined as a Villalta score of ≥5 on two consecutive visits that were at least three months apart. Most patients were followed after DVT and Villalta scores were recorded during follow-up. In case Villalta scores during follow-up were lacking (2 patients), diagnosis was based upon the Villalta score taken at the time of inclusion in this study, and in that case a single score of ≥5 was defined as PTS.

As a reference to the normal population, apparently healthy individuals (HI) without a history of venous thromboembolism (VTE) were included. For the current analyses subjects with known venous insufficiency were excluded. Cases, controls, and HI were similar in gender, age, and Body Mass Index (BMI).

This study was approved by the Medical Ethical committee of the Maastricht University Medical Centre, and all participants gave written informed consent.

2.2. Blood collection and plasma preparation

Venous blood was collected in citrate (3,2% *w*/*v*) and ethylenediaminetetraacetic acid (EDTA) polypropylene tubes for plasma (BD Vacutainer), and in clot activator (coating of micronized silica particles) containing polypropylene tubes for serum (BD Vacutainer). Citrate tubes were centrifuged for 5 min at 2500 *g* (room temperature) and for 10 min at 10,000 *g* (18 °C). EDTA tubes were centrifuged for 5 min at 2500 *g* (room temperature). Serum tubes were left for 30 min at 37 °C and consequently centrifuged for 5 min at 2500 *g* (room temperature). Plasma and serum samples were stored at −80 °C until analysis.

2.3. Laboratory analyses

2.3.1. Reagents

Human thrombin (Calbiochem, Nottingham, UK) was reconstituted in Milli-Q water and stored in aliquots at −80 °C until use. Recombinant tissue plasminogen activator (tPA) (Pathway Diagnostics, Dorking, UK) was dissolved in Milli-Q water and stored in aliquots at −80 °C.

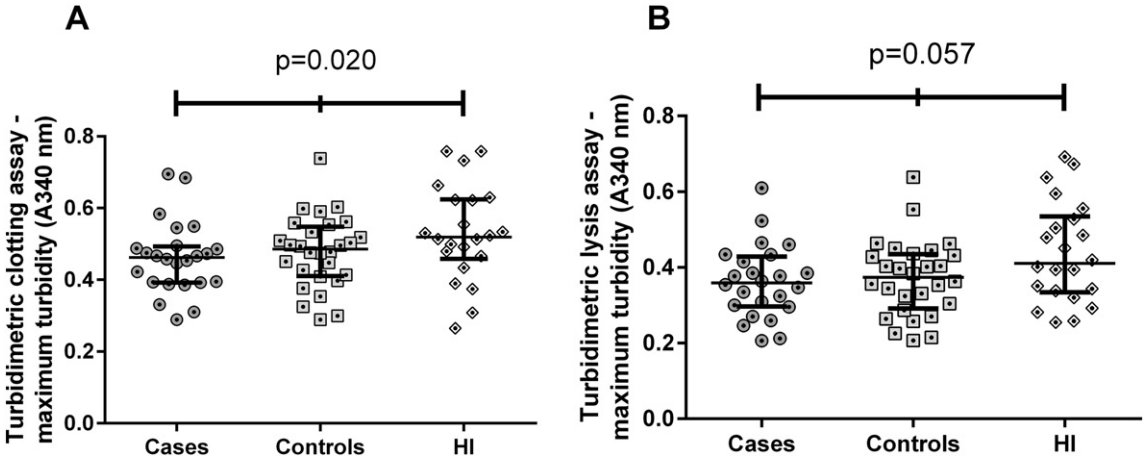

Fig. 1. Maximum turbidity: cases, controls, HI. A) Turbidimetric clotting assay. Cases, controls, HI. p-Value of Jonckheere–Terpstra test. B) Turbidimetric lysis assay. Cases, controls, HI. p-Value of Jonckheere–Terpstra test.