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Full Length Article

Circulating levels of tissue factor and the risk of thrombosis associated with antiphospholipid syndrome

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ABSTRACT

The mechanisms behind the severe hypercoagulable state in antiphospholipid syndrome (APS) have not yet been fully elucidated. Knowledge on the etiology of thrombosis in APS is needed to improve treatment. We performed a case control study to evaluate the association of the levels of circulating tissue factor (TF) with thrombotic APS and unprovoked venous thromboembolism (VTE), as compared with controls without a history of thrombosis. Study participants were selected in the same geographic area. Linear regression was used to evaluate possible determinants of TF levels among controls and logistic regression was used to evaluate the association between TF, unprovoked VTE and t-APS. TF levels were grouped into three categories based on: below 50th percentile [reference], between 50-75th percentiles [second category] and 75th percentile [third category]. Two hundred and eighty participants were included in the study; 51 patients with unprovoked VTE, 111 patients with t-APS and 118 control individuals. The levels of TF were not associated with an increased risk of unprovoked VTE, as compared with controls. The adjusted odds ratio for t-APS was 2.62 (95%CI 1.03 to 6.62) with TF levels between 50-75th percentiles and 8.62 (95%CI 3.76 to 19.80) with TF levels above the 75th percentile, as compared with the reference category (below the 50th percentile). In the subgroup analysis, higher levels of TF were associated with both arterial and venous thrombosis in APS and with both primary and secondary APS. Circulating TF is associated with thrombotic complications related to APS, but not with the risk of unprovoked VTE.

1. Introduction

Antiphospholipid syndrome (APS) is an rare disorder characterized by the occurrence of thrombosis or gestational complications associated with the presence of at least one antiphospholipid antibody (aPL): lupus anticoagulant (LAC), anticardiolipin (aCL) or anti-beta2glycoprotein I (aβ2GP1) [1,2]. APS can lead to a wide spectrum of thrombotic complications, such as venous thromboembolism (VTE), venous thrombosis in unusual sites, arterial and capillary thrombosis, which are highly susceptible to recurrence [3,4]. The mechanisms that are at the basis of the severe hypercoagulable state and thrombosis in APS have not yet been fully elucidated, but seem to differ from those observed in unprovoked VTE [5].

A mechanism that could explain the occurrence of thrombosis in APS is the overexpression of tissue factor (TF) [6]. TF is a coagulation factor encountered in endothelial cells, monocytes and in blood circulation [7,8]. Circulating TF comprise either soluble forms of TF or TF-bearing microparticles released from TF-producing cells [7,8]. These forms of TF encountered in blood can participate in thrombus growth and extension [9,10]. High levels of circulating TF have been associated with the risk of thrombosis in several diseases, as sepsis, diabetes, cardiovascular disease, sickle cell disease, stroke and cancer [11–14]. Despite basic research studies having demonstrated that aPL may increase TF expression in endothelial cells and monocytes [15–18], clinical evidence on the association between circulating TF and APS-related thrombosis (t-APS) are scarce [19,20].

The evaluation of circulating TF can provide clinical information on risk factors associated with thrombotic complications in APS. Identifying these risk factors is a critical step towards the identification of potential alternative treatments for the prevention of t-APS.

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Therefore, the aim of this study was to investigate the association of the levels of circulating TF with t-APS and unprovoked VTE.

2. Materials and methods

2.1. Participant selection

This study enrolled patients with t-APS or unprovoked VTE treated at the Hematology and Hemostasis Center at the University of Campinas, Brazil, between November 2013 and February 2016. During the same period, individuals with no history of thrombosis and who tested negative for aPL antibodies were enrolled as healthy controls. Inclusion criteria for the APS group comprised a confirmed diagnosis of APS with a history of at least one thrombotic event. Inclusion criteria for the group of unprovoked VTE comprised a history of deep venous thrombosis or pulmonary embolism with no clear transient risk factor (immobilization, air flight, surgery, contraceptives, pregnancy). Active neoplasia, pregnancy and lack of clinical information were reasons for exclusion. Clinical information was obtained at the day of the enrollment for the study by reviewing the medical records or interviewing patients. All patients with t-APS received prolonged anticoagulant treatment with warfarin; patients with arterial thrombosis also received antiplatelet agents. Patients with VTE were enrolled after the end of the anticoagulant treatment.

The total period of enrollment for the study was 28 months, in which 455 patients with a history of thrombotic disease were treated at the outpatient unit in the Hematology and Hemostasis Center at the University of Campinas, Brazil. Consecutive patients with unprovoked VTE or APS were enrolled for the study. Healthy individuals from the same geographic area were selected among individuals who were accompanying a patient to the medical appointment, employees at the hospital or their relatives or friends. Fig. 1 illustrates the study enrollment, selection and reasons for exclusion. The study was conducted in compliance with the Helsinki Declaration. The local Ethical Committee on Human Research approved this study and written informed consent was obtained from patients or their attending relatives.

2.2. Outcomes

The information regarding the diagnosis of VTE and the presence of risk factors for VTE, such as immobilization, air flight, surgery, oral contraceptives or pregnancy was obtained from medical records. The diagnosis of deep vein thrombosis was confirmed by Doppler ultrasonography and the diagnosis of pulmonary embolism was confirmed by ventilation-perfusion lung scan or computer tomography of the chest. All VTE patients tested negative for aPL antibodies.

Information on APS diagnosis and classification were assessed in the medical records.

APS was diagnosed in patients with persistent positive aPL antibody plus a history of thrombosis (confirmed by imaging examinations) or obstetric complications. Persistent positive aPL was defined as persistent positive LAC; persistent positive IgG or IgM aCL at moderate to high titers (> 40 GPL or MPL) or persistent positive (> the 99th percentile) IgG/IgM anti-beta2 glycoprotein 1, at two distinct times, with an interval of at least 12 weeks [2].

The detection of aPL was performed at diagnosis following the international guidelines from the International Society of Thrombosis and Haemostasis (ISTH) and Clinical and Laboratory Standard Institute (CLSI). Blood was collected in 0.109 M sodium citrate at a proportion of 9:1 and in serum separating tubes, prior to the initiation of any anticoagulant drug regimen or after a sufficient period of drug discontinuation.

For LAC, plasma samples were used and two assays based on different principles were applied: Dilute Russell's viper venom time (dRVVT) and Silica Clotting Time (SCT). Results of screening tests were potentially suggestive of LAC when their clotting times were longer than the local cut-off value (percentile 99th) and the results were confirmed for LAC at correction percentage of above the local cut-off value (99th percentile). The antiphospholipid antibodies with solid phase were tested in patient serum by “in house” ELISA immunological assays, with cardiolipin or β2GP1 as antigen (Sigma-Aldrich, USA), as previously described [21,22] A calibration curve and commercial controls were used, positive patient samples were also used as positive controls, and samples were tested in duplicate. The local cut-off value for aβ2GP1 was determined by the 99th percentile.

APS was classified into primary (PAPS) or secondary (SAPS) depending on the diagnosis of an underlying autoimmune disease, such as systemic lupus erythematosus (SLE). All patients with APS were screened for SLE and the diagnosis of SLE was confirmed according to established criteria [23].

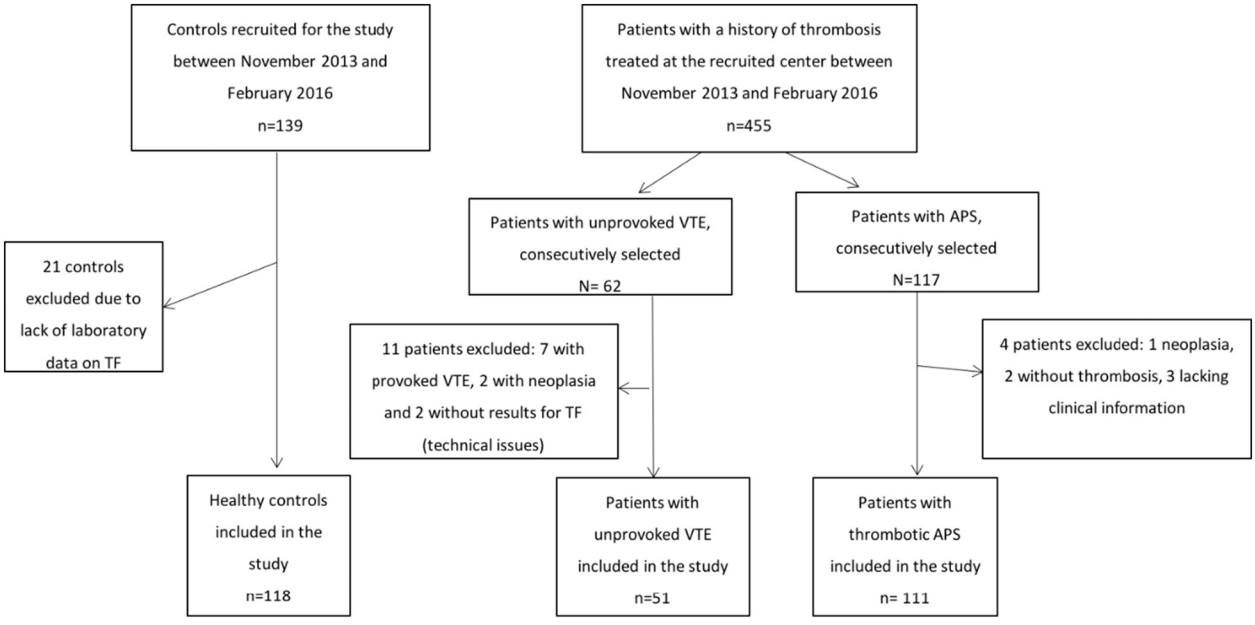

Fig. 1. Flow chart of participants' selection and reasons for exclusion. VTE = venous thromboembolism; APS = antiphospholipid syndrome; TF = tissue factor.