Journal of
Vascular Surgery
Venous and Lymphatic Disorders™

REVIEW ARTICLE

Richard P. Cambria, MD, Section Editor

Biomarkers for post-thrombotic syndrome

Annemieke C. Bouman, MD,[a] Selma Atalay, MD,[b] Hugo ten Cate, MD, PhD,[a] Marije ten Wolde, MD, PhD,[b] and **Arina J. ten Cate-Hoek, MD, PhD,[a]** *Maastricht and Almere, The Netherlands*

Background: **Post-thrombotic syndrome (PTS) is a serious condition that occurs in 20%-50% of patients following deep venous thrombosis (DVT). Biomarkers can be of use in further exploring the etiology as well as in developing risk stratification tools for PTS. The relationship between PTS and specific biomarkers may help guide prevention and therapy based on a patient's individual risk profile. This review gives an overview of the current knowledge on biomarkers in relation to PTS.**

Methods: **A systematic search was executed in three databases (Pubmed, Embase/Medline, Cochrane) to identify all publications on biomarkers in relation to PTS. Where possible, results of studies were pooled and a meta-analysis was performed using Review Manager 5.1 (The Cochrane Collaboration).**

Results: **Twenty-four papers were included in this review. In patients after DVT, increased D-dimer appeared to be associated with the development of PTS (odds ratio [OR], 2.04; 95% confidence interval [CI], 1.02-4.08; $P = .04$). Neither prothrombin G20210A (OR, 0.95; 95% CI, 0.53-1.69; $P = .86$, nor increased factor VIII (OR, 1.78; 95% CI, 0.88-3.57; $P = .11$) were associated with PTS. For factor V Leiden (FVL), conflicting results were found. FVL was not associated with PTS within a population of patients with a history of DVT (OR, 0.98; 95% CI, 0.74-1.29; $P = .88$), but FVL was positively associated with post-thrombotic ulceration in severe PTS, in patients compared with healthy individuals without a history of DVT (OR, 11.42; 95% CI, 6.37-20.48; $P < .00001$). A meta-analysis could not be performed for markers of inflammation and tissue remodelling in relation to PTS.**

Conclusions: **Increased D-dimer levels are associated with a twofold increased risk for PTS. Inherited hypercoagulability, including FVL is not associated with PTS in general. In contrast, FVL is strongly associated with post-thrombotic ulceration in severe PTS. The role of inflammation in the etiology of PTS still has to be elucidated. (J Vasc Surg: Venous and Lym Dis 2014;2:79-88.)**

Post-thrombotic syndrome (PTS) is a chronic complication of deep venous thrombosis (DVT) of the leg, which arises in 20%-50% of patients, usually within 1-2 years after acute DVT.[1,2] The symptoms and signs of PTS consist of heaviness, pain, cramps, itching, tingling, edema, venous ectasia, hyperpigmentation, and in severe cases even venous ulceration. These symptoms and signs are thought to be caused by venous hypertension. Since PTS is a syndrome, no golden standard diagnostic test exists. It is recommended to establish the diagnosis of PTS using the Villalta scale.[3] The ruling theory is that venous hypertension, precipitated by venous thrombosis, leads to venous stasis and disturbs the microcirculation and blood supply to the muscles of the lower limb. Consequently, this chain of events gives rise to the signs and symptoms of PTS.[4-6]

This review focuses on biomarkers in relation to PTS. The reason for studying biomarkers in PTS is twofold. (1) Identification of biomarkers is a clinically feasible start for unravelling the pathogenesis of PTS. Knowledge on the exact etiology of PTS is worthwhile to pursue, since it may contribute to the development of new therapeutic tools, which are most certainly needed for this burdensome and expensive condition.[7-11] (2) Biomarkers might be helpful in risk stratification for PTS. A combination of biomarkers and risk factors could be used to assess the individual risk of PTS in a patient who experienced DVT. Potentially, this may increase the options for optimizing preventive or therapeutic measures according to the individual risk profile.

The objective of this systematic review is to give an overview of current knowledge on biomarkers in relation to PTS. The primary endpoint was PTS in all its different manifestations, ranging from mild PTS to post-thrombotic ulceration.

METHODS

Identification of studies. Our aim was to identify all relevant original studies on biomarkers in relation to PTS

From the Laboratory for Thrombosis and Hemostasis, Department of Internal Medicine, Maastricht University Medical Centre, Maastricht[a]; and the Department of Internal Medicine, Flevohospital, Almere.[b]

Author conflict of interest: none.

Additional material for this article may be found online at www.jvsvenous.org.

Reprint requests: Annemieke C. Bouman, MD, Laboratory for Clinical Thrombosis and Hemostasis, Department of Internal Medicine, Maastricht University Medical Center, Universiteitssingel 50, PO Box 616, 6200 MD Maastricht, The Netherlands (e-mail: annemieke.bouman@maastrichtuniversity.nl).

The editors and reviewers of this article have no relevant financial relationships to disclose per the Journal policy that requires reviewers to decline review of any manuscript for which they may have a conflict of interest.

2213-333X/$36.00

http://dx.doi.org/10.1016/j.jvsv.2013.07.001

in human adults. A systematic search of the literature was performed using the following academic search strategy: *biomarkers* OR *fibrin fragment D* OR *D-dimer* OR *biological markers* OR *inflammation mediators* OR *intercellular signaling peptides and proteins* OR *intracellular signaling peptides and proteins* OR *lipoproteins* OR *blood coagulation factors* AND *postthrombotic syndrome* OR *post thrombotic syndrome* OR *post-thrombotic syndrome* OR *postphlebitic syndrome* OR *post-phlebitic syndrome* OR *post phlebitic syndrome*. This combination of search terms was entered in Pubmed central database (January 1, 1950 to October 2012), Embase/Medline database (January 1, 1974 to October 2012), and Cochrane Central Register of Controlled Trials (January 1980 to August 2012). A manual search of references was also performed.

Selection and data extraction. Studies were considered eligible if (1) it concerned an original study on biomarkers in relation to PTS, performed in human adults; (2) information on PTS incidence was available; (3) biomarkers were measured and related to the development of PTS, and results of these analyses could be extracted; and (4) articles were written in the English, Dutch, German or French language. Two authors performed selection of articles independently (A.B., S.A.). Disagreements were solved, by consulting 2 other authors (M.W., A.C.). Two authors read full text of all eligible articles independently (A.B., S.A.). Relevant information was recorded in a predefined data extraction sheet, which included data on study design, recruitment of patients, treatment of patients, strategy of PTS diagnosis, biomarker assays, characteristics of study population, and results.

Quality assessment of studies. Methodological quality of studies was assessed using the Newcastle-Ottawa quality assessment scale for cohort studies and case-control studies. To achieve comparability, the Newcastle-Ottawa quality assessment scale for case-control studies was also used for a cross-sectional study and a family study.[12,13]

Statistical analyses. Associations between different biomarkers and PTS were examined by pooling the results of the separate studies. Odds ratios (ORs) of the variable PTS (yes/no) vs dichotomized biomarker levels (>cut-off value/<cut-off value) or thrombophilia factors (present/absent), were pooled using the generic inverse variance method. Since in the majority of studies results were reported as ORs only, it was inevitable to use the generic inverse variance method. Random-effects models were incorporated in the generic inverse variance method of data pooling in Review Manager 5.1 (The Cochrane Collaboration).

Heterogeneity was assessed using I^2 statistics, $I^2 \geq 50\%$ was defined as substantial heterogeneity. To determine sources of heterogeneity, sensitivity analyses were performed in case of substantial heterogeneity.

RESULTS

The search of the three databases yielded a total of 354 publications; 321 of the 354 publications were excluded based on title and/or abstract for the following reasons: it concerned a review (132 articles), protocol (10 articles), or guideline (one article); it was written in a language other than English, Dutch, German, or French (six articles); the study was not performed in humans (eight articles); the study was performed in children (15); and it did not concern biomarkers (32 articles) or PTS (117 articles).

The full text of the remaining 33 articles was evaluated and a total of 16 articles were included. Through manual search of references another nine eligible articles were found. Two articles appeared to be identical (double publication), which led to a final number of 24 publications included in this review (Fig 1).

The methodological quality of the included studies on the Newcastle-Ottawa quality assessment scale is represented in Supplementary Table I (online only) and Supplementary Table II (online only). The cohort studies all scored fairly well. The average quality was 75% (five out of seven points); all studies but one had ≥five points on the seven-point scale (Supplementary Table I, online only). Methodological quality of the case-control studies was inferior, with an average quality of 57%. Seven of the 17 studies had a quality of ≤50% (Supplementary Table II, online only).

The studies were performed in two distinctive patient populations, therefore we separated the description of the results in two parts: (1) studies that compare PTS patients to patients without PTS, within a population of patients who all experienced a DVT (12 studies); and (2) studies that compare PTS patients to healthy individuals who never had a DVT (12 studies).

PTS vs no PTS, within a population after DVT. In 12 of the 24 studies, biomarkers were measured in a cohort of patients who all experienced a DVT.[14-25] Characteristics and results of these studies are summarized in Table I. The mean incidence of PTS in these studies was 39.6% (18.9%- 56.4%). Except for three studies, the Villalta scale was used to diagnose PTS in all studies.[15-21,23,24] The other three studies used the CEAP classification to make the diagnosis.[14,22,25] Five studies defined PTS as a Villalta score of ≥5 at two visits (either or not consecutive).[15,18,20,23,24] In three studies, one Villalta score of ≥5 was sufficient to make the diagnosis PTS.[16,17,21] In one study, it was not described whether one or two Villalta scores were used.[19] The studies that applied the CEAP classification considered a patient with signs and symptoms according to either class 1-6[22,25] or class 3-6[14] to have PTS. We were able to pool data for a limited number of biomarkers only; data on D-dimer, factor V Leiden (FVL), prothrombin G20210A, and factor VIII (FVIII) were pooled. None of the analyses showed an I^2 of ≥50%.

Levels of D-dimer. We found increased D-dimer (>500 μg/mL) to be significantly associated with PTS (OR, 2.04; 95% confidence interval [CI], 1.02-4.08; $P = .04$; Fig 2). Levels of D-dimer in relation to the occurrence of PTS were assessed in four studies.[15,18,19,25] Data of three studies were eligible to be included in the meta-analysis.[15,18,25] The fourth study did not find an association between increased D-dimer and PTS. This study could not be included in the meta-analysis because the data necessary to pool the results were not available nor provided by the