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Biomarkers for the prediction of venous thromboembolism in the community

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ABSTRACT

Background &objectives: Venous and arterial thrombosis share common pathophysiology. Multiple biomarkers reflecting various biological pathways can predict arterial thrombosis. We studied whether this approach could identify persons at risk of first venous thromboembolism (VTE).

Patients and methods

Framingham Heart Study Offspring participants ($N = 3120$) attending the sixth examination cycle (1995–1998) were eligible. Twelve biomarkers were tested: aldosterone-to-renin ratio, B-type natriuretic peptide, high-sensitivity C-reactive protein, cystatin-C, D-dimer, estimated glomerular filtration rate, fibrinogen, growth differentiation factor-15 (GDF-15), homocysteine, plasminogen activator inhibitor-1, soluble ST-2, and high-sensitivity cardiac troponin I. Cox proportional hazards regression models were used. Biomarker score was calculated using biomarkers significant in the stepwise procedure.

Results

During a median follow-up of 16.2 years, 139 participants developed VTE. Individually, six of the tested biomarkers were associated with VTE. In stepwise procedure, only ln-GDF-15 [hazards ratio (HR) per 1 SD increment 1.34 (95%CI 1.11–1.61); $p = 0.0024$)] and ln-D-dimer [HR per 1 SD increment 1.51 (95%CI 1.21, 1.89); $p = 0.0003$)] remained significant. Those in the highest tertile of the biomarker score had 3.6-times higher risk of VTE than those in the lowest tertile [HR 3.62 (95% CI 1.89–6.91), $p < 0.0001$]. C-statistic for the clinical model (including age, sex, BMI, prevalent CVD, smoking, cancer and hormone replacement therapy) was 0.720 (95%CI 0.678–0.762), and increased to 0.760 (95%CI 0.722–0.798) (upon adding D-dimer and GDF-15).

Conclusions

Higher circulating levels of GDF-15 and D-dimer are associated with increased risk of incident VTE in the community. A biomarker score could help identify individuals at increased risk for VTE.

Abbreviations: AUC, Area under the curve; BMI, Body mass index; BNP, B-type natriuretic peptide; CRP, C-reactive protein; CVD, Cardiovascular disease; DVT, Deep venous thrombosis; eGFR, Estimated glomerular filtration rate; FHS, Framingham Heart Study; GDF-15, Growth differentiation factor 15; hsTnI, High-sensitivity cardiac troponin I; IDI, Integrated Discrimination Improvement; PAI-1, Plasminogen activator inhibitor 1; PE, Pulmonary embolism; VTE, Venous thromboembolism.

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1. Introduction

Venous thromboembolism (VTE), consisting of pulmonary embolism (PE) and deep venous thrombosis (DVT), is a significant cause of cardiovascular morbidity and mortality [1,2]. VTE can be idiopathic (labeled as 'unprovoked'), or it may be associated with clinical predisposing factors (referred to as 'provoked') such as recent trauma, surgery, hospitalization, prolonged immobilization, pregnancy, use of oral contraceptives or hormone replacement therapy, or cancer. VTE has a high risk of recurrence [3]. The first manifestation of VTE may be an episode of fatal PE in an apparently healthy individual without any known predisposing factors, thereby underscoring the importance of identifying 'at risk' individuals and considering preventive measures. Yet, prediction models for the occurrence of a first episode of VTE in the community setting are scarce [4,5]. Prediction models for recurrent VTE do exist, even though their clinical utility is still limited [6]. These prediction algorithms incorporate clinical characteristics and select biomarkers. For instance, circulating levels of D-dimer measured after cessation of anticoagulant therapy have been associated with a higher risk of a recurrent VTE [7–9].

Arterial and venous thrombosis have traditionally been considered separate entities. However, this notion has been challenged recently. Both diseases share some common pathophysiologic bases, e.g., endothelial dysfunction, inflammation, and hypercoagulability [10,11]. Furthermore, several studies have shown an increase in VTE in patients with clinical or subclinical atherosclerosis [12,13]. We have previously shown that a combination of multiple biomarkers reflecting various biological pathways may be used in predicting first major cardiovascular events and death in the general population, conditions where the underlying pathology is often arterial thrombosis [14,15]. Therefore, we hypothesized that a panel of multiple biomarkers reflecting various biological pathways can predict first VTE in the community. We tested this hypothesis using data from the community-based Framingham Heart Study (FHS) cohort.

2. Materials and methods

2.1. Study sample

The FHS is a longitudinal community-based cohort of white individuals almost exclusively of European Ancestry. The design, selection criteria, and enrollment of the FHS participants have been detailed previously [16,17]. Participants in the Framingham Offspring cohort were eligible for the present investigation if they attended the sixth examination cycle (1995–1998), during which multiple biomarkers were assayed. A total of 3532 persons attended the sixth examination cycle of the Offspring cohort. Of these, 412 were excluded for the following reasons: history of VTE ($n = 30$), serum creatinine levels ≥2 mg/dL ($n = 15$) indicative of possible renal dysfunction, missing biomarker concentrations ($n = 323$), and missing covariates at exam 6 ($n = 44$). The Institutional Review Board of Boston University Medical Center approved the study protocol, and all participants provided written informed consent.

2.2. Biomarker selection and measurement

Twelve clinical biomarkers associated with various biological pathways were selected based on biological plausibility of an association with thrombotic events, and availability at the sixth FHS Offspring cohort examination cycle. Biomarker measurements from the sixth examination cycle included: high-sensitivity C-reactive protein (CRP, a marker of inflammation); fibrinogen (a marker of inflammation and thrombosis); plasminogen activator inhibitor-1 (PAI-1, a marker of fibrinolytic potential and endothelial function); D-dimer (a marker of hemostasis activation); homocysteine (a marker of endothelial function and oxidative stress); B-type natriuretic peptide (BNP), and aldosterone-to-renin ratio (markers of neurohormonal activity); high-sensitivity cardiac troponin I (hsTnI) (a marker of cardiac injury); growth differentiation factor-15 (GDF-15; a marker of oxidative stress and inflammation); soluble ST-2 (a marker of inflammation); cystatin-C and estimated glomerular filtration rate (eGFR; markers of kidney function).

2.3. Outcomes

The primary outcome for this study was the development of first symptomatic VTE. At each Framingham Heart Study clinic examination, participants underwent a medical history and physical examination. If a participant saw a physician or was hospitalized between Framingham examinations for any conditions, including symptoms that could be related to VTE, the records and imaging reports from the clinical encounter were sought. An end-points review panel consisting of three experienced physicians reviewed all medical records and adjudicated the occurrence of VTE events. Briefly, the diagnostic criteria for VTE included clinical symptoms and signs of VTE and objective evidence of venous thrombosis on imaging studies or based on findings at autopsy. PE was diagnosed using either ventilation perfusion (V/Q) scans, CT angiography, or at autopsy. DVT was diagnosed using venograms, Doppler ultrasound studies of culprit veins, impedance plethysmography (IPG), ^{125}I fibrinogen leg scan, or a combination of the IPG and leg radionuclide scans. Thrombosis in the superficial veins, restricted to the calf, or intracranial veins were not counted as VTE.

Any of the following factors were considered predisposing factors if present within 3 months prior to VTE: Surgery defined as any surgical operation; any fracture/trauma requiring medical attention; hospitalization defined as any admission to a hospital ward; immobilization defined as bed rest ≥2 days; self-reported use of hormone replacement therapy or oral contraceptives. Self-reported travel >4 h within 1 month prior to VTE was counted as a predisposing factor. Pregnancy was defined as a predisposing factor if VTE occurred any time during pregnancy or within 3 months after delivery (puerperium) or miscarriage. A VTE event was considered provoked if at least one predisposing factor (surgery, fracture, hospitalization, immobilization, HRT/OC use, travel >4 h, pregnancy/puerperium) other than cancer was present; a VTE event was considered cancer-related if there was a diagnosis of active cancer (excluding only non-melanoma skin cancer). Active cancer was defined as new cancer diagnosis within six months prior to event or three months after VTE, any treatment for cancer within the six months prior to event, recurrent cancer, or the presence of metastasis. In the absence of a documented provoking factor or cancer, a VTE event was considered unprovoked. These three VTE groups were mutually exclusive.

2.4. Statistical analysis

All biomarkers were natural-logarithmically transformed to normalize their skewed distributions (in the text we refer to the ln-values without the prefix in order to simplify reading).

First, we modeled incident VTE with each individual biomarker using Cox proportional hazards regression (after confirming the assumption of proportionality of hazards was met). We adjusted for age, sex, body mass index (BMI), history of cardiovascular disease (CVD), history of cancer (excluding non-melanoma skin cancer), smoking status, and use of hormone replacement therapy at baseline; these covariates are known to be associated with VTE. Biomarkers associated with VTE ($p < 0.20$ for entry) were then included in a stepwise procedure using a Cox model, where we set $\alpha = 0.0042$ (Bonferroni corrected 0.05/12) as the statistical significance threshold to account for multiple statistical testing. We also estimated the incremental contribution of biomarkers by comparing the C statistics from two models: (i) standard VTE risk factors (age, sex, body mass index (BMI), history of cardiovascular disease (CVD), history of cancer, smoking status, and use of