

Association between atopic diseases and venous thromboembolism: a case-control study in patients aged 45 years or less

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Activation of the blood coagulation pathways occurs in patients with allergic diseases such as allergic asthma and nasal allergy [1]. Platelet activation also plays a role in allergic inflammation [2]. Mast cells produce plasminogen activator inhibitor-1 (PAI-1), a major inhibitor of plasminogen activation [3]. Mast cell chymase-mediated proteolysis of tissue factor pathway inhibitor contributes to reduced anticoagulant activity at the site of inflammation [4]. It is unknown whether allergic diseases, including common atopic disorders such as allergic rhinitis (AR), allergic asthma and atopic dermatitis (AD), are associated with thrombosis. Recently, we have reported that denser fibrin clot formation associated with reduced plasmin-mediated lysis can be detected in AD patients [5]. Given the data showing that venous thromboembolism (VTE) [6] is associated with the formation of dense fibrin clots resistant to lysis, unfavorably altered fibrin clot properties might constitute a prothrombotic mechanism that operates in atopy. The aim of this case-control study was to compare the incidence of atopic diseases among VTE patients and controls with no history of thrombosis. Of the 654 VTE patients screened from 2006 to 2009, we identified 129 consecutive white patients aged 20–45 years who experienced the first-ever VTE. The diagnosis of deep vein thrombosis (DVT) was established by a positive finding on color duplex sonography. The diagnosis of pulmonary embolism (PE) was based on the presence of typical symptoms and positive results of high-resolution spiral computed tomography. VTE was defined as secondary if it was preceded within 90 days by major trauma, surgery, immobilization of 3 days or more, distant travel (> 8 h), or childbirth, or if it was associated with oral contraceptives or pregnancy.

The presence of atopic diseases was recorded on the basis of history, physical examination, and medical records. AD was

defined according to the Hanifin and Rajka diagnostic criteria. Allergic asthma was diagnosed on the basis of typical symptoms and spirometry criteria based on the Global Initiative for Asthma 2008 guidelines. AR was defined as the patient being atopic and reporting nasal allergy. Skin reactivity tests were performed in all symptomatic patients with mixtures of grass, tree and weed pollens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, *Alternaria*, and *Cladosporium*. All patients classified as having atopic diseases had at least one positive skin-prick test. The diagnoses of atopic disease were determined at the time of the VTE event, and laboratory allergic markers were assessed at the time of study enrollment.

The control group comprised 144 individuals matched for age, sex, and smoking habit, without previous VTE, recruited from the hospital staff and students.

The Jagiellonian University Ethical Committee approved the study, and patients provided written, informed consent.

Fibrinogen was determined with the Clauss method. Fibrin D-dimer levels were measured by immunoturbidimetry (Dade Behring, Marburg, Germany). PAI-1 antigen was determined with an ELISA (American Diagnostica, Greenwich, CT, USA). Fibrin clot lysis time was measured with a plasma-based assay [7,8]. Intra-assay and interassay coefficients of variation for all variables were < 7%.

Total serum IgE and IgE specific to house dust mite, timothy grass, cat and *Cladosporium* were measured with the Pharmacia CAP system (Pharmacia, Uppsala, Sweden). Specific IgE values of 0.35 kIU L⁻¹ or more were considered to be positive.

Thrombophilia screening performed in VTE patients involved deficiency of antithrombin, protein C, or protein S, factor V Leiden, prothrombin 20210A mutation, lupus anticoagulant, and significant levels of anti-cardiolipin or anti-β₂-glycoprotein I IgG and IgM antibodies.

Continuous variables were checked for normal distribution by the Shapiro–Wilk statistic and compared with Student's *t*-test, the Mann–Whitney test or the Wilcoxon test as appropriate. Categorical variables were compared by χ²-test or Fisher's exact test as appropriate. The odds ratios (ORs) and 95% confidence intervals (CIs) were computed by logistic regression. A *P*-value < 0.05 was considered to be statistically significant.

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Of the 129 VTE patients, 87 (67.4%) had DVT without symptomatic PE (proximal DVT, $n = 42$ [32.6%]; distal DVT, $n = 45$ [34.9%]), and 42 (32.6%) had symptomatic PE (DVT combined with PE, $n = 25$ [19.4%]; PE alone, $n = 17$ [13.2%]). The median time from the event was 10 months (range, 4–26 months). The mean duration of anticoagulation was 7 months (range, 3–24 months). A positive family VTE history was noted in 49 (38.0%) VTE patients and in five controls (3.5%; $P < 0.001$). The most common VTE risk factors were: oral contraceptives ($n = 20$), pregnancy/postpartum ($n = 19$), surgery ($n = 18$), prolonged travel ($n = 11$), and immobilization ($n = 5$). Unprovoked VTE was diagnosed in 56 (43.4%) patients.

The VTE and control groups differed with respect to total serum IgE (Table 1). Atopic diseases occurred more commonly in VTE patients than in controls (49 [38.0%] vs. 33 [22.9%]; OR 2.06, 95% CI 1.21–3.50; $P = 0.007$). There was an increased prevalence of AR among VTE patients ($P = 0.04$), with no between-group differences in AD and allergic asthma (Table 1). Atopy defined as at least one positive specific IgE test result was more common among VTE patients (Table 1). Allergy to timothy grass pollen was overrepresented in the VTE group (OR 3.08, 95% CI 1.36–7.00; $P = 0.007$).

In VTE patients, we observed 44.1% higher PAI-1 ($P = 0.006$) and 35.9% longer clot lysis time ($P < 0.001$) than in controls (Table 1). Clot lysis time showed an association with fibrinogen in the VTE patients (Spearman coefficient, $r = 0.59$, $P < 0.0001$).

VTE patients with atopic diseases were younger than the remaining subjects (Table 2). The two subgroups did not differ with regard to the medication used, including vitamin K antagonists ($P = 0.49$). A positive family history

for VTE was observed more commonly in atopic VTE patients (31 [63.3%] vs. 18 [22.5%]; OR 5.93, 95% CI 2.69–13.08; $P < 0.001$).

Distal DVT alone, defined as involving any vein segment distal to the knee joint, was more common among patients with atopic diseases, whereas proximal DVT, defined as thrombosis involving the popliteal or more proximal deep vein segments, occurred less commonly in atopic patients (Table 2). In the patients with diagnosed atopic diseases as compared with the remaining VTE patients, most of the distal DVT cases occurred between March and September (20 of the 28 events [69%] vs. four of the 16 events [25%]; OR 7.5, 95% CI 1.80–31.62; $P = 0.005$), and were associated with immobilization, travel, surgery, or trauma (21 of the 28 events [72.4%] vs. five of the 16 events [33.3%]; OR 6.6, 95% CI 1.63–26.74; $P = 0.007$). Four incidents of proximal DVT (66.6%) were also provoked by surgery or immobilization in the patients with atopic diseases, whereas in the remainder there were 11 episodes (27.5%) (OR 6.6, 95% CI 0.84–37.41; $P = 0.067$).

In VTE patients with atopic diseases, we observed 32.0% higher PAI-1 ($P = 0.005$) and 21.4% longer clot lysis time ($P = 0.003$) than in the remaining VTE patients (Table 2). Weak correlations were noted between total IgE and both PAI-1 and clot lysis time in VTE patients with atopic diseases (Spearman coefficient, $r = 0.39$, $P = 0.04$, and $r = 0.43$, $P = 0.02$, respectively).

This study shows an increased incidence of atopic diseases among VTE patients aged 20–45 years, indicating that atopy *per se* might have prothrombotic effects of clinical relevance. We observed that, as compared with the controls, patients with signs of AR had a predisposition to DVT, but not PE, that developed mainly in high-risk situations. Interestingly, atopic diseases were overrepresented among patients with distal DVT, accompanied by a higher relative frequency of individuals

Table 1 Characteristics of patients with venous thromboembolism (VTE) and controls

	VTE patients ($n = 129$)	Controls ($n = 144$)	OR (95% CI)
Age (years)	36.2 $\pm$ 4.2	36.9 $\pm$ 6.1	0.98 (0.81–1.46)
Male sex, n (%)	75 (58.1)	79 (74.5)	1.14 (0.71–1.85)
BMI (kg m^{-2})	25.2 (23.0–28.1)	25.6 (23.6–28.1)	0.97 (0.76–1.42)
Current smoking, n (%)	48 (37.2)	60 (41.7)	0.83 (0.51–1.35)
Allergic rhinitis, n (%)	39 (30.2)	27 (18.8)	1.88 (1.07–3.30)
Asthma, n (%)	7 (5.4)	5 (3.5)	1.60 (0.49–5.18)
Atopic dermatitis, n (%)	3 (2.3)	1 (0.7)	3.41 (0.35–33.5)
Atopy with positive specific IgE, n (%)	56 (43.4)	36 (25.0)	2.30 (1.37–3.85)
Medications			
Aspirin, n (%)	10 (6.9)	5 (3.5)	2.06 (1.21–3.50)
Oral corticosteroids, n (%)	5 (3.9)	3 (2.1)	2.34 (0.77–7.06)
Antihistamines, n (%)	25 (19.4)	24 (16.7)	1.89 (0.44–8.15)
Vitamin K antagonists, n (%)	55 (42.6)	0	–
Laboratory parameters			
Fibrinogen (g L^{-1})	2.57 $\pm$ 0.63	2.51 $\pm$ 0.56	1.06 (0.95–1.14)
D-dimer (ng mL^{-1})	186.0 $\pm$ 81.5	167.4 $\pm$ 56.7	1.11 (0.84–1.25)
Total IgE (IU L^{-1})	97.5 (37.2–124.9)	48.6 (20.8–91.3)	2.04 (1.44–4.12)
PAI-1 antigen (ng mL^{-1})	20.9 (13.8–29.2)	14.5 (9.4–23.6)	1.49 (1.21–1.72)
Clot lysis time (min)	87 (68–110)	64 (50–86)	1.42 (1.23–1.66)

BMI, body mass index; CI, confidence interval; OR, odds ratio; PAI-1, plasminogen activator inhibitor-1. Quantitative values are given as mean $\pm$ standard deviation (SD) or median (interquartile range). ORs for continuous variables were computed for one SD increment.

Table 2 Comparison between patients with venous thromboembolism (VTE) and atopic diseases and the remaining VTE patients

Variable	Atopic patients (n = 49)	Non-atopic patients (n = 80)	OR (95% CI)
Age (years)	32.3 ± 5.4	39.1 ± 4.6	1.22 (1.11–1.45)
Male sex, n (%)	33 (67.3)	42 (52.5)	1.87 (0.88–3.94)
BMI (kg m ⁻²)	24.5 (22.9–26.1)	25.0 (23.3–26.8)	0.95 (0.84–1.19)
Current smoking, n (%)	16 (32.6)	29 (36.3)	0.85 (0.40–1.82)
DVT alone, n (%)	34 (69.4)	52 (65.0)	1.22 (0.57–2.63)
DVT with PE, n (%)	10 (20.4)	15 (18.8)	1.11 (0.45–2.74)
PE alone, n (%)	8 (16.3)	9 (11.3)	1.54 (0.55–4.34)
Distal DVT, n (%)	28 (57.1)	16 (20.0)	5.33 (2.41–11.8)
Proximal DVT, n (%)	6 (12.2)	36 (45.0)	0.17 (0.07–0.45)
Unprovoked VTE, n (%)	21 (42.9)	35 (43.8)	0.96 (0.47–2.00)
Thrombophilia			
FV Leiden, n (%)	12 (21.4)	19 (21.6)	1.04 (0.45–2.41)
FII 20210A, n (%)	5 (8.9)	7 (8.0)	1.19 (0.35–4.01)
APS, n (%)	5 (8.9)	8 (9.1)	1.02 (0.31–3.35)
Other factors*, n (%)	1 (1.8)	2 (2.3)	0.81 (0.07–9.42)
Laboratory parameters			
Fibrinogen (g L ⁻¹)	2.52 ± 0.65	2.65 ± 0.64	0.89 (0.78–1.85)
D-dimer (ng mL ⁻¹)	200.8 ± 68.9	171.7 ± 88.6	1.39 (0.84–2.13)
Total IgE (IU L ⁻¹)	159.3 (98.5–429.8)	55.7 (22.1–121.9)	2.97 (1.44–6.35)
PAI-1 antigen (ng mL ⁻¹)	28.4 (20.2–34.1)	19.3 (14.0–24.8)	1.59 (1.12–2.06)
Clot lysis time (min)	103 (88–115)	81 (69–97)	1.28 (1.10–1.74)

APS, antiphospholipid syndrome; BMI, body mass index; CI, confidence interval; DVT, deep vein thrombosis; OR, odds ratio; PAI-1, plasminogen activator inhibitor-1; PE, pulmonary embolism. Quantitative values are given as mean ± standard deviation (SD) or median (interquartile range). ORs for continuous variables were computed for one SD increment. *Deficiency of antithrombin, protein C, or protein S.

allergic to timothy grass, but not other allergens tested. It appears that patients with AR during the pollen spring–summer season had a higher risk of distal DVT. Unexpectedly, atopic diseases seem to protect against proximal DVT. Given the small size of these subgroups, however, the findings regarding proximal DVT should be interpreted with caution.

In adults, atopic diseases (apart from severe asthma, which was absent in the present study) did not affect occupational activity or life-expectancy. The healthy worker effect in our control group appears to be unlikely.

Mechanisms underlying the intriguing association between atopic diseases and VTE remain obscure. Thrombophilia cannot explain the association. Given reports on increased PAI-1 levels in allergic diseases [9] and a positive association between clot lysis and PAI-1 [8], we hypothesized that a PAI-1-mediated impairment of fibrinolysis could, in part, account for the increased prevalence of atopy among VTE patients. Our current findings confirmed this concept. Moreover, we found a positive correlation between PAI-1 and total IgE. Lisman *et al.* [8] showed that prolonged clot lysis time predisposes to VTE, which is in line with the findings of the current study. Apart from fibrinogen, elevated PAI-1 was the only parameter that correlated with clot lysis, providing a potential mechanism underlying impaired fibrin clot lysis. Other prothrombotic mechanisms, including those related to platelet activation [2], might also contribute to VTE in atopy.

The current study has limitations. The number of study participants was limited, particularly in the subgroups. Our findings cannot be extrapolated to a whole VTE patient population, as atopic diseases become less frequent with

increasing age, and acquired thrombosis risk factors are more prevalent in subjects aged 50 years or more, who represent a majority of VTE patients. The link between atopy, a genetic predisposition to elevated IgE levels and prothrombotic genetic mutations other than FV Leiden or prothrombin 20210A [10] could be of importance, given frequent positive family histories in atopic VTE patients.

In conclusion, we have demonstrated that atopic diseases occur more commonly among VTE patients aged 45 years or less, which leads to the intriguing hypothesis that atopy might favor thrombosis, mostly provoked distal DVT not complicated with PE, in subjects at risk of thrombosis. This preliminary report sheds new light on the complex links between allergic inflammation/atopic diseases and blood coagulation.

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Disclosure of Conflict of Interests

The authors state that they have no conflict of interest.

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Increased tissue factor expression in diabetes mellitus type 2 monocytes caused by insulin resistance

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Diabetes mellitus type 2 (DM2) is associated with accelerated atherosclerosis [1] and a two-fold to eight-fold increased risk of cardiovascular and thrombotic complications [2]. Plasma from DM2 patients shows signs of a hypercoagulable state with elevated coagulation factors and activated coagulation [3]. Tissue factor (TF) is the prime initiator of blood coagulation, and precise regulation of its production and activation is vital for normal hemostasis. DM2 patients have elevated plasma TF antigen [4] and TF procoagulant activity [5]. There is increased TF expression in circulating monocytes [6] and microparticles [7]. In DM2, levels of soluble TF correlate with microvascular complications, making soluble TF a predictive marker of thrombotic complications [8]. Interestingly, improvement of glycemic control reduces circulating TF by 1.5-fold [9].

In healthy individuals, stimulation of monocytes with lipopolysaccharide (LPS) induces TF surface expression and

procoagulant activity, and insulin interferes with these processes [10]. Insulin induces phosphorylation of the insulin receptor (INS-R). The activated receptor binds the G-protein $G_{i\alpha_2}$, forming an INS-R- $G_{i\alpha_2}$ complex. This association inactivates $G_{i\alpha_2}$, disturbing the suppression of cAMP, an inhibitor of TF synthesis. This property makes insulin a suppressor of TF synthesis in monocytes. Suppression by insulin also reduces TF expression on monocyte-derived microparticles without changing microparticle shedding [10].

A main feature of DM2 is the loss of insulin sensitivity of many cell types, including platelets. We investigated whether DM2 monocytes lose responsiveness to insulin and thereby the suppression of TF synthesis. This would result in higher TF production than in normal individuals, and might explain the hypercoagulable state seen in DM2.

In healthy subjects, LPS induced a rise in monocyte TF procoagulant activity from 76 ± 56 to 128 ± 38 ng per 10^7 cells (means $\pm$ standard errors of the mean [SEMs], $n = 9$). A concentration of $1 \mu\text{g mL}^{-1}$ LPS was used to create a condition of high TF levels for the optimal study of insulin inhibition [11,12]. Insulin at concentrations between 0.1 and 100 nM suppresses TF expression, confirming its negative role in the regulation of TF synthesis (Fig. 1A) [10]. In DM2 monocytes, LPS raised TF expression from 42 ± 3 to 192 ± 48 ng per 10^7 cells (means $\pm$ SEMs, $n = 9$). The increase in TF expression was unchanged in the presence of 100 nM insulin. Microparticles shed from activated monocytes

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