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Rosendaal, F.R.

British Journal of Haematology 2002 116 173 177

Interleukin 8 and venous thrombosis: evidence for a role of inflammation in thrombosis

BENJAMIN E VAN AKEN [1,2] PIETER H REITSMA [1] AND FRITS R ROSENDAAL [3,4] [1]*Laboratory for Experimental Internal Medicine and* [2]*Department of Vascular Medicine Academic Medical Center Amsterdam and* [3]*Department of Hematology and* [4]*Department of Clinical Epidemiology Leiden University Medical Center Leiden The Netherlands*

Received 19 July 2001 accepted for publication 31 August 2001

Summary. Elevated plasma levels of interleukin 8 (IL-8) were previously shown to be associated with recurrent venous thrombosis To assess the risk of venous thrombosis IL-8 plasma concentrations were measured in patients and control subjects of the Leiden Thrombophilia Study (LETS) This population based case–control study included 474 patients with a first deep-vein thrombosis and 474 age- and sex-matched controls The risk of venous thrombosis for subjects with elevated IL-8 levels (above 90th percentile of controls) compared with subjects with IL-8 levels below the 90th percentile was increased 1 8-fold (95%CI 1 2–2 8) Adjusted for age and sex the odds ratio was 1 9 (95% CI 1 3–2 8) IL 8 concentrations were weakly correlated with age male sex and concentrations of C-reactive protein factor VIII coagulation activity and homocysteine but adjustment for these factors did not substantially affect the association between IL-8 and venous thrombosis Our results suggest that IL-8 is a risk factor for venous thrombosis

Keywords venous thrombosis epidemiology interleukin 8 inflammation risk factors

Classic risk factors for venous thrombosis include acquired factors such as immobilization surgery and malignancies and genetic risk factors i e activated protein C resistance (factor V Leiden) and deficiencies of protein C (PC) protein S (PS) or antithrombin (AT) Recently elevated plasma levels of factor VIII hyperhomocysteinaemia and prothrombin 20210A have been added to the series of risk factors (reviewed by Rosendaal 1999) A previous study in patients with recurrent venous thrombosis suggested interleukin 8 (IL-8) to be a risk factor for venous thrombosis (Van Aken *et al* 2000)

Interleukin 8 a C-X-C chemokine is produced by several cell types including endothelial cells peripheral blood monocytes neutrophils epithelial cells and fibroblasts (reviewed by Hoch *et al* 1996 and Luster 1998) The main function of IL-8 is the activation of integrin-mediated adhesion of neutrophils (Luster 1998) and it was recently shown that IL-8 is a powerful trigger for adhesion of monocytes to vascular endothelium (Gerszten *et al* 1999) Several studies have identified IL–8 in association with various acute and chronic inflammatory conditions including sepsis psoriasis rheumatoid arthritis and asthma (reviewed by Hoch *et al* 1996) Proinflammatory cytokines IL 1β and tumour necrosis factor α (TNF α) and viral and bacterial stimuli can induce IL 8 production

The importance of inflammatory responses in venous thrombosis has been shown *in in vitro* studies (Johnson *et al* 1996 Senden *et al* 1998) and in animal models (Wakefield *et al* 1993 1995 1997) but little is known about the association of inflammatory mediators and venous thrombosis in humans Studies using a human experimental endotoxaemia model have suggested an interaction between inflammatory mediators and blood coagulation (Van Deventer *et al* 1990) Infusion of endotoxin in healthy volunteers resulted in increased plasma levels of IL-1β IL-6 IL 8 and TNF α as well as of coagulation activation markers Recently elevated concentrations of C reactive protein (CRP) have been detected in patients with a first episode of venous thrombosis suggesting an association with systemic inflammation (Kamphuisen *et al* 1999)

Based on these considerations we theorized that IL 8 levels may play a causative role in venous thrombosis In order to obtain evidence for this supposition the association between elevated plasma levels of IL 8 and venous thrombosis was analysed in a case–control study of patients with a first episode of venous thrombosis

Correspondence Dr P H Reitsma Laboratory for Experimental Internal Medicine Academic Medical Center G2–129 Meibergdreef 9 1105 AZ Amsterdam The Netherlands E mail P H Reitsma@amc uva nl

PATIENTS AND METHODS

Study population The Leiden Thrombophilia Study (LETS) has been described previously (Koster *et al* 1993). Briefly 474 patients with a first episode of objectively demonstrated deep venous thrombosis and 474 age and sex-matched healthy controls were included. Patients with known malignant disorders were excluded and patients were seen only after anticoagulant treatment had been discontinued for at least 3 months. This was always > 6 months after the event because oral anticoagulant (OAC) treatment was routinely given for 3 months. Forty-eight (10%) of the patients were on long-term coumarin treatment and were not allowed to interrupt their medication for various reasons (Koster *et al* 1995). The healthy control subjects were acquaintances of patients or partners of other patients and were selected according to the following criteria: same sex, same age (± 5 years), no biological relationship, no history of venous thromboembolism, no use of coumarin-derivatives for at least 3 months and no known malignancies.

Laboratory studies Blood was collected from the antecubital vein into tubes containing 0·106 mol/l trisodium citrate. Plasma was prepared by centrifugation for 10 min at 2000 *g* at room temperature and stored at −70°C. IL-8 concentrations were measured using an enzyme linked immunosorbent assay (ELISA) with a detection limit of 2·0 pg IL-8/ml (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service CLB, Amsterdam, The Netherlands). Plasma samples were assayed in a blind manner. If the difference between the mean of the duplicate measurements and the measurements separately exceeded 10% of the mean value, the measurement was not repeated and the sample was excluded from further analysis. This resulted in a case/control ratio of 466/462. Samples below the detection limit were designated as 2 pg/ml.

Statistical analysis We calculated odds ratios as an estimate of the relative risk of venous thrombosis in subjects with elevated IL-8 concentrations using the 90th percentile of IL-8 as a cut-off value based on the distribution in the control population and under the assumption that levels measured after the event fairly represent those of before the event. The odds ratios in the group with elevated IL-8 concentrations were calculated using logistic regression analysis with the group below the 90th percentile as the reference category. All odds ratio calculations were adjusted for age and sex except when stratification for sex or age was performed. The 90th percentile of the IL-8 concentrations of the control study population was 7·1 pg/ml for men it was 7·5 pg/ml and for women it was 6·8 pg/ml. The possible confounding effect of age on the association between IL-8 and venous thrombosis was analysed using equally distributed age-tertiles designated young (lowest tertile), middle (second tertile) and old (upper tertile). For the total study population the age tertiles were < 40, 40–51 and > 51 years.

RESULTS

The mean age of the whole population was 45 years (range 15–72). 57% were women. Thirty-six per cent of both patients and control subjects were current smokers while the mean body mass index (BMI) was 26 (range 14–46) kg/m^2. The mean IL-8 concentration was higher in the patients than the controls [8·0 pg/ml (95%CI 6·0–10·0) versus 4·4 pg/ml (95%CI 4·0–4·8)]. Eleven per cent of the controls and 9% of the patients had plasma levels below the detection limit (2·0 pg/ml). The IL-8 plasma concentrations of individual patients and controls are shown in Fig 1.

To evaluate the association between IL-8 plasma concentrations and venous thrombosis a cut-off value of 7·1 pg/ml (90th percentile of the control population) was used. Seventy-seven (17%) patients were detected with elevated IL-8 concentrations (above 90th percentile) compared with 45 (10% by definition) of the controls [odds ratio 1·9 (95%CI 1·2–2·8)]. Using the cut-off of 16·2 pg/ml (99th percentile) 23 (5%) patients with elevated IL-8 were detected in comparison with four (1% by definition) controls [odds ratio 6·0 (95%CI 2·0–17)]. Odds ratios using other cut-off values are shown in Table I. By dividing the IL-8 concentrations into quintiles and comparing each quintile with the lowest we found a concentration-dependent association pointing towards a threshold effect. The risk was not increased in the second [odds ratio 1·0 (95%CI 0·7–1·6)] and third quintile [odds ratio 1·0 (95%CI 0·6–1·5)] while in the fourth quintile [odds ratio 1·2 (95%CI 0·8–1·8)] and in the fifth quintile [odds ratio 1·8 (95%CI 1·2–2·8)] the odds ratio was increased.

In control subjects we examined which factors influenced the IL-8 concentration. The mean IL-8 concentration was higher in men [5·3 pg/ml (95%CI 4·4–6·1)] than in women [3·8 pg/ml (95%CI 3·4–4·1)]. IL-8 concentrations increased by 0·2 pg/ml (95%CI −0·5–1·0) per 10 years of age. Distribution of the control subjects in age groups showed that 30 (10%) of the subjects between 40 and 51 years and 70 (23%) above the age of 51 years had an elevated IL-8 concentration (> 7·1 pg/ml 90th percentile) compared with 22 (7%) of the subjects under the age of 40 years. The mean IL-8 concentration was higher in subjects with elevated (> 5·1 μg/ml 90th percentile) CRP plasma levels [5·5 pg/ml (95%CI 3·0–8·0)] than in other subjects [4·3 pg/ml (95%CI 3·9–4·7)].

Risk factors for venous thrombosis include high levels of factor VIII coagulant activity (FVIII:C) and homocysteine. Among healthy subjects with elevated FVIII:C concentrations (> 100 IU/dl) the IL-8 concentration was slightly increased [n = 344, 4·6 pg/ml (95% CI 4·0–5·1)] compared with subjects with low FVIII:C (< 100 IU/dl)[n = 130, 4·0 pg/ml (95%CI 3·6–4·4)]. Homocysteine was analysed in the subjects recruited in the Leiden area only (control subjects n = 309). The IL-8 concentration was increased in subjects with elevated homocysteine concentrations (> 90th percentile 16·7 μmol/l) [n = 30, 4·8 pg/ml (95%CI 3·9–5·7) versus n = 279, 4·2 pg/ml (95%CI 4·0–4·5)]. Analysis of life-style factors BMI and smoking showed that among overweight subjects (BMI > 90th percentile 31 kg/m^2) the mean IL-8 was higher [4·6 mg/l (95%CI 2·8–6·3)] than among other subjects [4·4 mg/l (95%CI 4·0–4·8)]. In smokers the IL-8 concentration was not different from

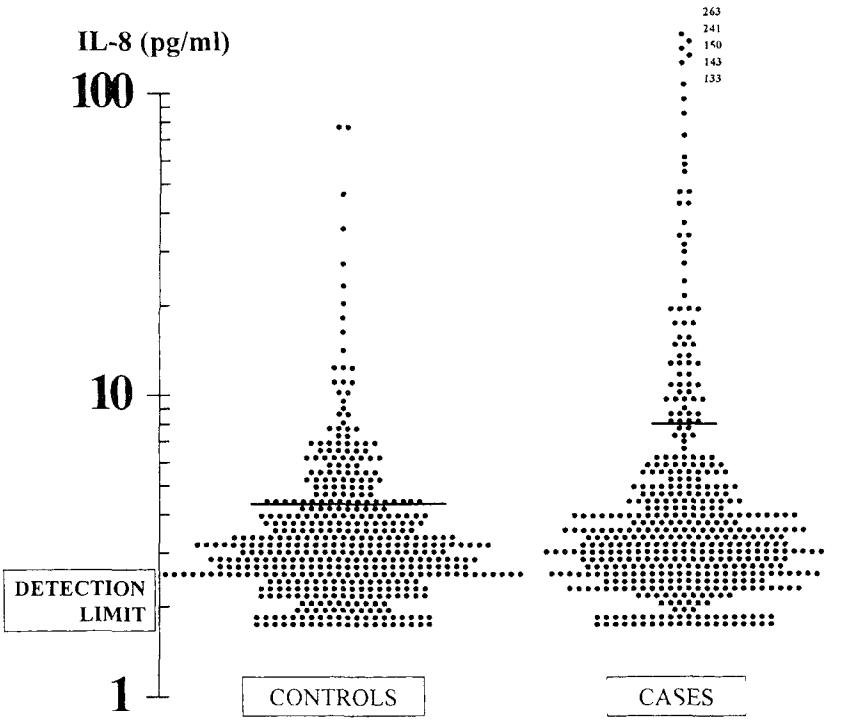

Fig 1 Plasma levels of IL-8 are elevated in patients with venous thrombosis compared with control subjects The solid lines represent the mean the dotted lines the detection limit *The numbers at the top of the cases column* indicate the IL-8 levels that were above 100 pg/ml in five patients

Table I Thrombosis risk for different cut off points of IL 8 concentrations

IL-8 plasma concentrations	Patients (466)	Controls (462)	OR (95 CI)
< 80th percentile (5 6 pg/ml)	336	373	
> 80th percentile	130	89	1 7 (1 2–2 3)
< 90th percentile (7 1 pg/ml)	389	417	
> 90th percentile	77	45	1 9 (1 2–2 8)
< 95th percentile (8 2 pg/ml)	401	440	
> 95th percentile	65	22	3 3 (2 0 5 5)
< 99th percentile (16 2 pg/ml)	443	458	
> 99th percentile	23	4	6 0 (2 0 17)

Odds ratio adjusted for age and sex

non smokers [respectively 4 2 mg/l (95%CI 3 8–4 7) versus 4 5 (95%CI 3 9–5 1)]

Next we investigated the role of the above-mentioned factors on the association between elevated IL-8 (> 90th percentile) and venous thrombosis Adjustment for FVIII C [odds ratio 1 7 (95%CI 1 1–2 6)] homocysteine [odds ratio 1 7 (95%CI 1 1–2 6)] CRP [odds ratio 1 8 (95%CI 1 2–2 6)] or BMI [odds ratio 1 7 (95%CI 1 1–2 6)] only marginally affected the association between venous thrombosis and elevated IL-8 plasma concentrations

The presence of other thrombosis risk factors (factor V Leiden prothrombin G20210A factor VIII factor IX factor XI and antithrombin protein C or protein S deficiencies) did not affect the association between elevated IL 8 (> 90th percentile) and venous thrombosis [odds ratio 1 7 (95%CI 1 1–2 7)] and neither did exclusion of these patients [odds ratio 1 9 (95%CI 1 2–2 9)]

Patients were included in the study at different time intervals after the thrombotic event [mean 21 (range 6–68) months] IL 8 concentration was not related to this time interval Patients who were seen more than 28 months after the thrombotic event had a similar frequency (16%) of elevated IL 8 concentration as those seen within 12 months after the thrombosis (18%) Additionally analysis after exclusion of the subjects with an elevated CRP concentration (> 5 1 μg/ml 90th percentile) did not affect the association between IL 8 and venous thrombosis although 51 (13%) patients with elevated IL-8 concentrations were present compared with 37 (9/) of the controls [odds ratio 1 6 (95%CI 1 0–2 5)]

IL-8 concentrations were higher among oral anticoagulant (OAC) users than among non users The reasons for continuing OAC treatment were as follows 15 of these 48 patients had a recurrence six had a history of myocardial

infarction (MI) and seven were diagnosed with PC/PS/AT deficiency. Thus 28 out of 48 individuals had a clear medical reason for continuing OAC treatment. These conditions (recurrence MI, second thrombophilic defect) were present in only 19 of the other 399 patients.

Twelve out of 46 (26%) patients still using OAC had elevated IL-8 concentrations in contrast to 65 (15%) of the 420 patients without OAC. Exclusion of OAC users resulted in 6 (16%) patients with elevated IL-8 concentrations (> 90th percentile) versus 45 (10%) controls [1·7 (95%CI 1·1–2·5)]. Using the cut-off of 7·1 pg/ml (90th percentile) the adjusted odds ratio was 2·9 (95%CI 2·1–8–4·9) and using the cut-off of 16·2 pg/ml (99th percentile) the adjusted odds ratio was 6·0 (95%CI 2·0–17·7).

Separate analysis showed similar odds ratios for men and women with elevated IL-8 concentrations [for men 1·5 (95%CI 0·8–2·6) and for women 2·2 (95%CI 1·3–3·9)]. Stratification by age showed that the effect of IL-8 was limited to middle-aged subjects [age 30–51 years odds ratio 3·4 (95%CI 1·6–7·2)] while in younger [age < 30 odds ratio 2·9 (95%CI 0·6–14·7)] or elderly subjects [age > 51 years odds ratio 1·3 (95%CI 0·8–2·2)] an increased risk of venous thrombosis was less obvious.

DISCUSSION

Elevated concentrations of IL-8 are associated with venous thrombosis. Plasma concentrations of IL-8 (above the 90th percentile) lead to a 1·9-fold (adjusted for age and sex) increased risk of venous thrombosis. The association between venous thrombosis and IL-8 is most pronounced between the age of 40 and 51 years and is not affected by levels of FVIII:C or homocysteine.

Several mechanisms may explain the association between elevated IL-8 concentrations and venous thrombosis. First, IL-8 has been shown to induce tissue factor, an important inducer of blood coagulation, on monocytes (Neumann *et al* 1997). Second, leucocyte recruitment is induced by IL-8 (Luster 1998). Leucocytes are the first cells to adhere to venous endothelium in a model of stasis-induced deep venous thrombosis and these leucocytes then stimulate thrombus formation (Schaub *et al* 1984). Furthermore, IL-8 could contribute to the induction of a procoagulant surface by triggering the adhesion of monocytes to the endothelium (Gerszten *et al* 1999).

The main stimuli for IL-8 production are proinflammatory cytokines such as IL-1 and TNF-α, bacterial products and viral infection (Baggiolini *et al* 1994). It can only be speculated which stimuli are responsible for the increased IL-8 concentrations associated with venous thrombosis. The potential role of infectious agents in the pathogenesis of vascular disease has been studied in relation to atherosclerosis. These studies have suggested that infections with, for example, *Chlamydia pneumoniae*, *Helicobacter pylori* and cytomegalovirus are associated with atherosclerosis (Ross 1999). These infectious agents may induce the production of IL-8 in the endothelium and monocytes (Kragsbjerg *et al* 1995, Bliss *et al* 1998, Murayama *et al* 1998). Therefore it is worthwhile to explore the potential role of these infections in the induction of IL-8 in relation to venous thrombosis.

Studies in baboons have shown elevations in IL-8 levels after induction of a thrombus, suggesting that the thrombotic event could contribute to an increased IL-8 concentration (Wakefield *et al* 1993). Nevertheless, it is unlikely that the thrombosis itself was responsible for the increased IL-8 concentrations, as the subjects were included at least 6 months after the thrombotic event. IL-8 concentration was not related to the time interval between thrombotic event and measurement of IL-8 and no decrease in IL-8 level was detected in patients who were included more than 28 months after their thrombotic event, compared with the patients included within 12 months. Although plasma levels of CRP, a marker for inflammation, are high in patients, adjustment for CRP did not affect the thrombosis risk of elevated acute phase proteins FVIII:C and fibrinogen (Kamphuisen *et al* 1999) or IL-8, suggesting that these associations are not owing to a post-thrombotic acute phase response. However, we cannot exclude the possibility that post-thrombotic syndrome or underlying malignancies contributed to the elevated IL-8 concentration in some patients, which may in part or completely be responsible for the observed association.

Another possibility is that the increased concentrations of IL-8 reflect occult cancer because many cancers produce IL-8. It is also well-known that patients with idiopathic venous thrombosis have a higher frequency of underlying cancer (Monreal *et al* 1997). In fact, venous thrombosis is often the first sign of malignancy in most patients with prostatic and pancreatic carcinoma. The design of the LETS study excludes patients with known cancer and overt cancer does not therefore explain the results. The LETS protocol, however, did not include an exhaustive search for occult cancer and non-symptomatic cancer may therefore explain all or part of the increased IL-8 concentrations. Only large prospective studies focusing on cancer during follow-up will provide a definite answer.

In conclusion, the association between elevated IL-8 concentrations and a first thrombotic event provides evidence for the notion that IL-8 increases the risk of venous thrombosis.

ACKNOWLEDGMENTS

We thank Dr T. Koster for collecting blood samples of patients and controls, Dr F. J. M. van der Meer (head Thrombosis Centre Leiden), Dr L. P. Colly (head Thrombosis Centre Amsterdam) and Dr P. H. Trienekens (head Thrombosis Centre Rotterdam) for their kind cooperation and Mrs T. Vissel and Ms A. P. Groot for skilful technical assistance.

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