Inflammatory response in the acute phase of deep vein thrombosis

Edith M. Roumen-Klappe, MD,[a] Martin den Heijer, MD, PhD,[b,c] Stan H. M. van Uum, MD,[c] Johanna van der Ven-Jongekrijg, MS,[d] Fedde van der Graaf, PhD,[e] and **Hub Wollersheim, MD, PhD,[a]**
Nijmegen, The Netherlands

Objective: **Deep vein thrombosis (DVT) is a multifactorial disease. Recently, inflammation has been suggested as a risk factor for DVT. The question is whether inflammation is a cause of venous thrombosis or rather a result of the thrombotic process.**
Methods: **We studied the inflammatory response in the acute phase of DVT with interleukin-6, interleukin-8, and C-reactive protein (CRP) as inflammatory markers. Plasma concentrations were measured on the day of admission (day 0) in 40 patients with acute DVT confirmed with phlebography and in 33 patients with clinical suspicion of DVT but negative phlebography results (controls). In patients with DVT, inflammatory markers were also examined on five subsequent days.**
***Results*: On day 0, the median concentrations in plasma of interleukin-6, interleukin-8, and CRP were 15.0 pg/mL (range, <3 to 70 pg/mL), 7.0 pg/mL (range, <3 to 76 pg/mL), 37.5 mg/L (range, <7 to 164 mg/L), respectively, in the patient group and less than 3 pg/mL (range, <3 to 11 pg/mL; *P* < .001), 6.0 pg/mL (range, <3 to 52 pg/mL; *P* = .08), and 5.0 pg/L (range, <7 to 66 pg/L; *P* < .001), respectively, in the controls. During the next days, interleukin-6 concentration showed a gradual decline in patients with DVT from 15.0 to 5.5 pg/mL (*P* < .001), interleukin-8 concentration was relatively constant in time, and CRP concentration declined from 37.5 to 21.5 mg/L (*P* = .01).**
Conclusion: **Our data show an apparent inflammatory response with highest measured concentrations of inflammatory markers on the day of admission and a subsequent decrease during the next days. This response supports the hypothesis that elevated inflammatory markers are a result rather than a cause of venous thrombosis. (J Vasc Surg 2002;35:701-6.)**

Deep vein thrombosis (DVT) occurs with an incidence rate of 1 to 2 per 1000. Known risk factors are deficiencies of protein C, protein S, antithrombin III, factor V Leiden and a mutation of prothrombin (factor II), immobilization, surgery, trauma, pregnancy, malignant disease, use of oral contraceptives, hyperhomocysteinemia, and elevated concentrations of factor VIII.[1-3] In the last few years, several new risk factors have been detected, such as factor IX,[4] factor XI,[5] and thrombin activatable fibrinolysis inhibitor.[6]

Chronic systemic inflammation is a risk factor for atherosclerosis, and it has been shown that markers of low-grade systemic inflammation are associated with a future risk of coronary heart disease.[7] The relationship between inflammation and venous thrombosis remains less well studied. An increase of the concentration of inflammatory markers interleukin-6, interleukin-8, and monocyte chemoattractive protein–1 was found in patients with recurrent DVT,[8] and elevated interleukin-8 plasma concentrations were measured in patients after a first thrombotic event.[9] Because these markers were measured at least 6 months after the thrombotic event, it was unlikely that the thrombosis itself was responsible for the increased plasma levels. Therefore, it was suggested that inflammation was a risk factor for DVT. In the latter study, the concentration of another inflammatory parameter, C-reactive protein (CRP), was also increased in patients with thrombosis.[10] However, in a prospective study, higher baseline CRP concentration was associated with a higher risk of future myocardial infarction and stroke but not with DVT.[11] In contrast to the suggestion that low-grade inflammation is a risk factor, it can also be hypothesized that acute DVT causes an inflammatory response, which may persist for a longer period.

To test this hypothesis, we studied the question of whether an immediate systemic inflammatory response occurs in DVT. To answer this question, we determined interleukin-6, interleukin-8, and CRP concentrations as inflammatory markers in patients with DVT, both at diagnosis and during the follow-up period, and compared these with control subjects on the day of presentation. Furthermore, we analyzed the diagnostic value of the inflammatory markers on the day of admission.

METHODS

Patients. The study population consisted of patients who participated in a previous study that dealt with the exclusion of DVT with D dimer testing.[12] In short, 112 consecutive outpatients were referred to the emergency

From the Departments of General Internal Medicine,[a] Epidemiology and Biostatistics,[b] and Endocrinology[c] and the Laboratory of General Internal Medicine[d] of the University Medical Center Nijmegen and the Clinical Laboratories of the Sint Joseph Hospital.[e]
Competition of interest: nil.
Reprint requests: E.M. Roumen-Klappe, Department of General Internal Medicine, University Medical Center Nijmegen, Postbox 9101, 6500 HB Nijmegen, The Netherlands (e-mail: E.Roumen@aig.azn.nl).

0741-5214/2002/$35.00 + 0 **24/1/121746**
doi:10.1067/mva.2002.121746

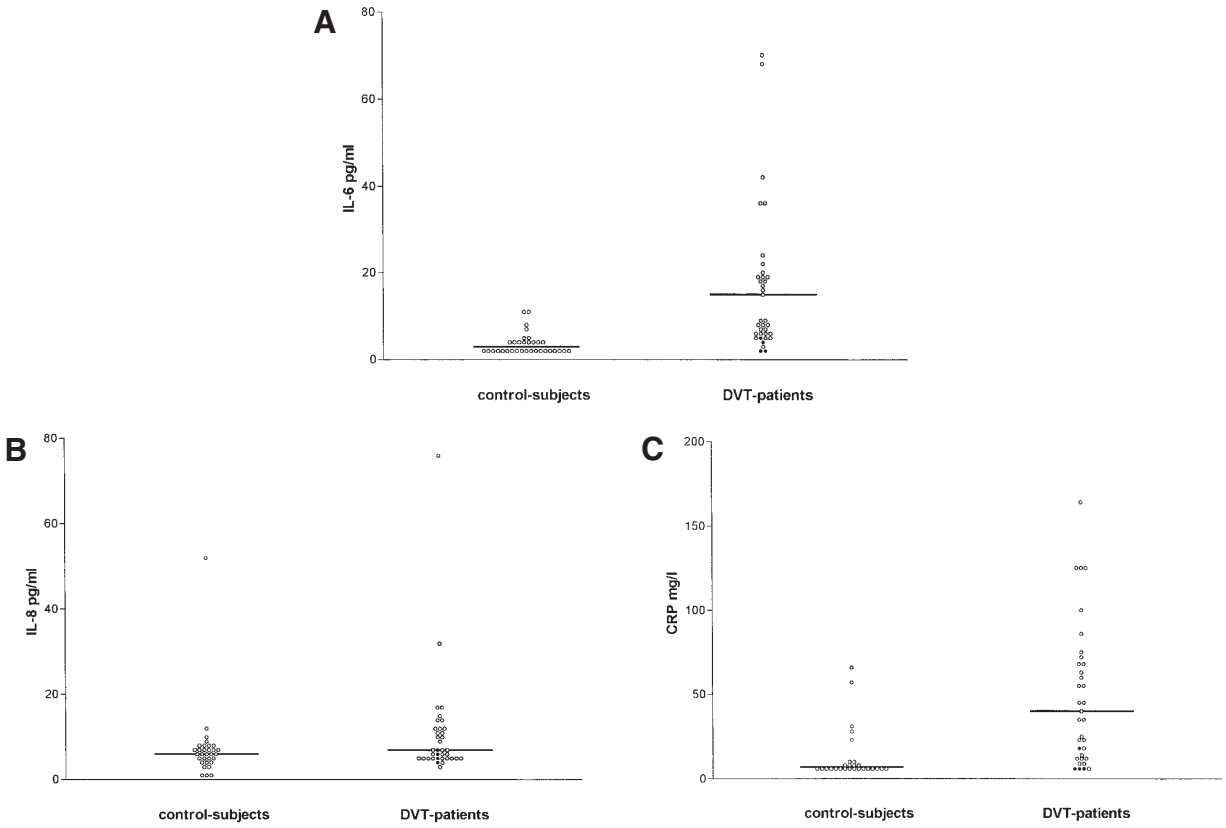

Fig 1. Plasma levels in control subjects and patients with deep vein thrombosis *(DVT)* of **(A)** interleukin-6 *(IL-6)*, **(B)** interleukin-8 *(IL-8)*, and **(C)** C-reactive protein *(CRP)*. *Straight lines* represent median levels, and *black dots* represent patients with isolated distal DVT.

department because of clinical suspicion of DVT. The patients were excluded with one or more of the following criteria: treatment with anticoagulant therapy (n = 1), recent surgery less than 8 days previously (n = 0), refusal or inability to give informed consent (n = 5), and inability for ascending contrast venography or inadequate results of the venographic examination (n = 7). Ninety-nine patients were included and evaluated with ascending contrast venography for the diagnosis of DVT, defined as a persistent intraluminal filling defect in at least two projections. When DVT was diagnosed, the patients were admitted to the hospital and treated with intravenous heparin therapy for a minimum of 5 days. Warfarin therapy was started concurrently for 3 months. When DVT was excluded, the patients were discharged and no further follow-up examination was performed (these patients are further named control subjects). The study was restricted to patients with a complete set of sera for the day of admission and the 5 subsequent days because, in some patients, after measurement of D dimers, no sera remained. The study protocol was approved by the Institutional Review Board of the Sint Joseph Hospital Veldhoven, and informed consent was obtained from all the study participants.

Blood collection. Blood was collected within 2 hours of presentation in the emergency department and before administration of heparin therapy if DVT was diagnosed. After a positive diagnosis of DVT and admission to the hospital, blood was drawn daily between 8:00 and 10:00 AM for the next 5 days. Blood was collected into 4.5-mL siliconized glass vacutainer tubes that contained 0.45 mL of 0.109 mol/L sodium citrate. Platelet poor plasma was prepared with centrifugation at 3000*g* for 15 minutes at 20°C, mixed, aliquoted, frozen, and stored at –80°C until analysis. At the time of the assay, the samples were thawed in a waterbath at 37°C for 10 minutes and, after mixing, were allowed to stand at room temperature for at least 15 minutes before use.

Analysis of plasma concentrations of interleukin-6, interleukin-8, C-reactive protein, and D dimer. Interleukin-6 and interleukin-8 concentrations were determined with a commercially available enzyme-linked immunosorbent assay (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands). These enzyme-linked immunosorbent assay kits are calibrated according to the standards of the National Institute for Biological Standards and Control (Potters Bar, United Kingdom).

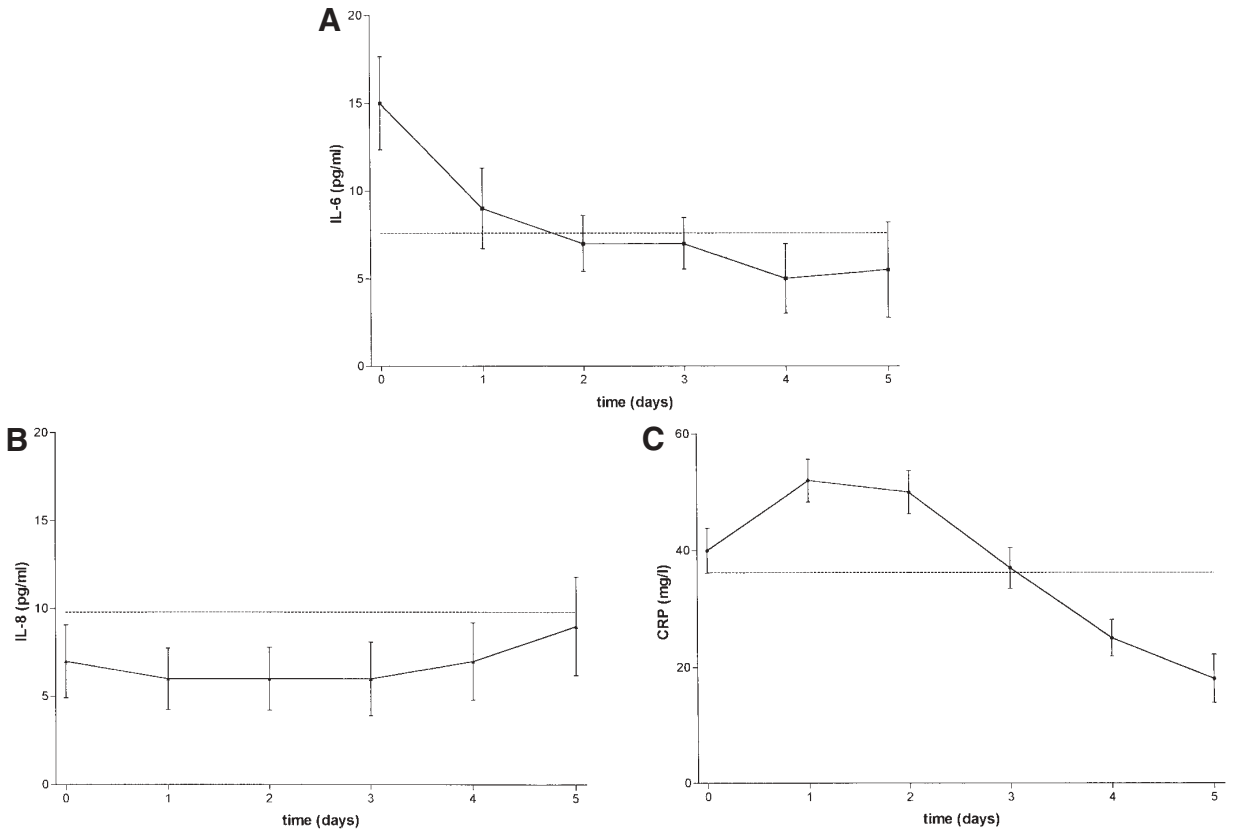

Fig 2. Median levels of inflammatory parameters in patients with deep vein thrombosis on day of admission (day 0) and during 5 subsequent days. **A,** Interleukin-6 *(IL-6)*. **B,** Interleukin-8 *(IL-8)*. **C,** C-reactive protein *(CRP)*. *Dotted lines* represent 90th percentile of distribution of control subjects on day of presentation.

The detection limit is 3.0 pg/mL for both interleukin-6 and interleukin-8. CRP was assayed with an immunoassay on a Vitros 250 (Ortho Clinical Diagnostics, Rochester, NY), with a detection limit of 7 □g/mL.

In the previous study, 13 D dimer methods were tested.[12] The Tinaquant D dimer assay (Roche Diagnostics, Mannheim, Germany) was one of the two assays with the highest sensitivity, so the results of the Tinaquant assay were used for this study.

Statistics. The significance of differences in concentrations between the patients and the control subjects was tested with the Mann-Whitney test. *P* values of less than .05 were considered significant. Furthermore, we compared the presence of elevated plasma concentrations in control subjects and patients with DVT with the □^{2} test. Elevated levels of interleukin-6, interleukin-8, and CRP were defined as concentrations of more than the 90th percentile of the distribution in the control subjects. The significance of the difference in concentration during the time course was evaluated with the Mann-Whitney test for paired samples.

The correlation coefficient was used for the assessment of the relation between inflammatory parameters and D dimer levels. The correlation was defined as good if more than 0.75, as moderate if between 0.50 and 0.75, and as poor if less than 0.50. With the Pearson correlation test, the significance of the correlation coefficient was calculated and defined as significant at the .01 level (two-tailed). The diagnostic values of the inflammatory parameters were evaluated with receiver operating curves, which were constructed for interleukin-6, interleukin-8, CRP, and D dimer by plotting sensitivity (true positive fraction) towards one specificity (false-positive fraction). Areas under the curves (AUCs) were derived from these curves as an estimate of the overall diagnostic performance.

RESULTS

In 50 of the 99 included patients, the clinical suspicion of DVT was confirmed with venography. In the remaining 49 patients, the diagnosis was excluded. Plasma was available in 40 of the patients with DVT (17 male and 23 female) and in 33 control subjects (12 male and 21 female). In the other patients, after measurement of D dimers, not enough sera remained for all the days. There are no differences in the clinical characteristics of the patients included in this study when compared with all the patients. The median age of the patients with DVT was 55.7 years (range, 27 to 87 years) and of the control subjects was 52.0 years (range, 23 to 79 years). The median

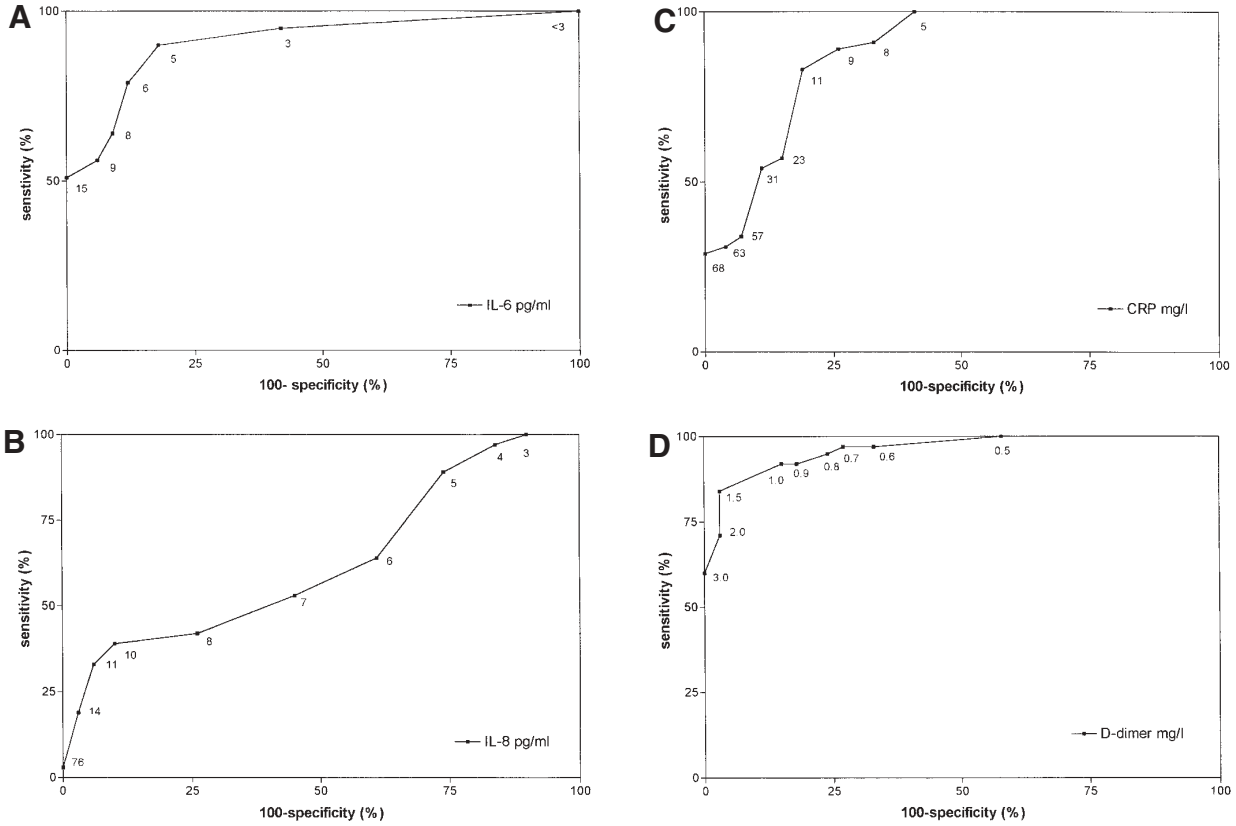

Fig 3. Receiver operating curve analysis of accuracy of **(A)** interleukin-6 *(IL-6)*, **(B)** interleukin-8 *(IL-8)*, **(C)** C-reactive protein *(CRP)*, and **(D)** D dimer levels at day of presentation for diagnosis of deep vein thrombosis.

duration of symptoms was 8 days (range, 1 to 42 days). Five patients (7%) had symptoms for longer than 10 days. The following risk factors for DVT were present in 18 patients: malignant disease (n = 5), immobilization (n = 5), recent trauma (n = 3), oral contraceptive use (n = 2), and previous operation (n = 3; <8 days were excluded). Of the DVT group, four patients had distal thrombosis and 36 had proximal thrombosis.

At the day of admission, the median plasma concentrations of interleukin-6, interleukin-8, and CRP were higher in the patients with DVT as compared with the control subjects (Fig 1). In the patients with DVT, the median plasma levels of interleukin-6, interleukin-8, and CRP were 15.0 pg/mL (range, <3 to 70 pg/mL), 7.0 pg/mL (range, <3 to 76 pg/mL), and 37.5 mg/L (range, <7 to 164 mg/L), respectively, and in the control subjects were less than 3 pg/mL (range, <3 to 11 pg/mL; $P < .001$), 6.0 pg/mL (range, <3 to 52 pg/mL; $P = .08$), and 5.0 pg/L (range, <7 to 66 pg/L; $P < .001$), respectively. The 90th percentile of control subjects was, respectively, 7.6 pg/mL, 9.8 pg/mL, and 36.2 mg/L. In patients with DVT, elevated plasma levels of interleukin-6 were found in 64%, of interleukin-8 in 39%, and of CRP in 51%, as compared with 10% in the control group ($P < .01$). Although the number of patients with distal thrombosis is small (n = 4), these patients had considerably lower levels of inflammatory markers (median plasma levels: interleukin-6, <3 pg/mL; interleukin-8, 5.5 pg/mL; and CRP, 6.0 mg/L) than did patients with proximal DVT (median plasma levels: interleukin-6, 19.4 pg/mL; interleukin-8, 11.3 mg/mL; and CRP, 53.2 mg/L). There were no differences in inflammatory markers between patients with known risk factors, such as malignant disease or recent trauma, and patients with idiopathic thrombosis.

During the follow-up period, levels of interleukin-6 declined to 5.5 pg/mL after 5 days of treatment (Fig 2). Median levels of interleukin-8 did not change over time. The median CRP level showed an initial rise on the 2nd and 3rd day from 37.5 mg/L to 50.0 and 51.0 mg/L and then declined to 21.5 mg/L. Despite the decline in interleukin-6 and CRP levels, the concentrations after 5 days were still increased as compared with the controls at the day of presentation (interleukin-6, 5.5 pg/mL versus <3.0 pg/mL; $P = .003$; and CRP, 21.5 mg/L versus 5.0 mg/L; $P = .001$). At day 5, elevated levels (>90th percentile of controls) of interleukin-6, interleukin-8, and CRP were still found in 35%, 41%, and 39%, respectively, of patients with DVT (all $P < .01$).

At day of presentation, the median D dimer levels were 4.50 mg/L in patients with DVT versus 0.54 mg/L

in controls. The correlation with interleukin-6, interleukin-8, and CRP was only moderate (interleukin-6: $r = 0.62$; n = 70; $P < .01$; interleukin-8: $r = 0.46$; n = 65; $P < .01$; CRP: $r = 0.72$; n = 62; $P < .01$)). The receiver operating curves for interleukin-6, interleukin-8, CRP, and D dimer levels are shown in Fig 3. AUCs of the inflammatory parameters were 0.91, 0.63, and 0.88, respectively, as compared with the AUCs of D dimers (0.96).

DISCUSSION

This study shows an apparent systemic inflammatory response with elevated plasma levels of interleukin-6, interleukin-8, and CRP in patients with DVT. Interleukin-6 and CRP levels showed a gradual decline during treatment, whereas interleukin-8 levels remained elevated in time. These results support the hypothesis that inflammation is a result of the thrombotic process rather than a cause of DVT.

It has been suggested that low-grade inflammation is a cause of DVT because elevated interleukin-6, interleukin-8, and monocyte chemoattractive protein–1 concentrations were found in patients after a thrombotic event.[8,9] We cannot exclude that higher levels of inflammatory markers can be present because of risk factors in some of the patients with DVT (like malignant disease or recent trauma). However, there was no difference in markers between patients with idiopathic thrombosis or patients with a risk factor. It could be expected that, in case of persistent systemic inflammation as a cause of DVT, concentrations of inflammatory markers are steady instead of declining. Although this alternative hypothesis of low-grade persistent inflammation as a cause of DVT is not finally excluded, the course of the inflammatory markers is in our opinion strongly suggestive for the hypothesis that DVT is followed by an inflammatory response.

Except for elevated CRP levels at the diagnosis of DVT,[13,14] no other studies have been published on inflammatory markers in the acute phase of DVT in humans. In an experimental baboon model of DVT, interleukin-6 and interleukin-8 concentrations peaked on day 2 after stasis-induced DVT. Unlike other cytokines, only interleukin-6 showed a significant relation with the extent of thrombosis on fibrinogen scanning. DVT was associated with a venous wall inflammatory response that consisted of an early neutrophil infiltration into the vein wall, followed by extravasation of monocytes, macrophages, and lymphocytes.[15] The inflammatory response in the vein wall could be visualized with gadolinium-enhanced magnetic resonance venography that showed enhancement around the thrombosed veins, which diminished in approximately 2 weeks.[16] In our study, during treatment in the subsequent days after the diagnosis of DVT, interleukin-6 and CRP levels declined and interleukin-8 levels were relatively constant. CRP concentration peaked during 2 days, similar to another study in DVT.[13] Because the inflammatory response is initiated and propagated by further thrombosis,[15] the decline of markers is most probably the effect of treatment with anticoagulants. Although antiinflammatory agents may prevent DVT[17] and heparin possesses antiinflammatory properties distinct from its anticoagulant properties and inhibited vein wall neutrophils in an animal thrombosis model, the mechanism is unknown and the antiinflammatory effects may not be cytokine-mediated.[18] For this reason, we think that the course of the inflammatory markers is not caused by the antiinflammatory effects of the treatment with heparin but is the result of decrease of the thrombotic process.

The control subjects in this study were patients with a clinical suspicion of DVT with negative phlebography results. Because the main priority was exclusion of DVT, an alternative diagnosis was not always clear and the control subjects were referred to their general practitioner. The differential diagnosis of DVT includes, for example, lymphedema, muscle trauma, hemorrhage, arthritis, tendinitis, and erysipelas. Some of these disorders may also cause elevations of inflammatory parameters. Although in most patients no substantial elevation of inflammatory parameters was found, it can be expected that in comparison with healthy controls the inflammatory response in DVT is even more apparent.

Several earlier studies showed elevated CRP levels at the diagnosis of DVT. Although CRP concentration was not a reliable marker for exclusion of DVT, the sensitivity of an elevated CRP level at time of diagnosis was 72% to 100%.[13,14] As in our study, lower CRP levels were found in patients with distal thrombosis.[13] When elevation of inflammatory markers are supposed to be the result of the DVT, they might also be used as diagnostic markers for DVT. Therefore, we looked at the diagnostic value of these markers as compared with D dimer. Although in our study, D dimer levels showed the highest sensitivity and specificity, the AUCs of interleukin-6 and CRP compared with D dimer can also be considered as good. The correlation of interleukin-6, interleukin-8, and CRP levels with D dimer levels at diagnosis was only moderate. Although it has been suggested that initial D dimer levels may be related to the extent of the thrombosis, this correlation is relatively weak. This may be because of differences in fibrinolytic activity between individuals.[19] It appears that the processes of fibrinolysis and inflammation may interact in DVT, but the extent of the inflammatory process can not be estimated with determination of D dimer levels.

In conclusion, in the acute phase of DVT, we found an apparent systemic inflammatory response with highest measured concentrations of inflammatory markers on day of admission and a subsequent decrease during the next days. This supports the hypothesis that elevated cytokines in patients with DVT are more likely to be a result of the thrombotic process rather than a cause of DVT.

REFERENCES

1. Rosendaal FR. Venous thrombosis: a multicausal disease. Lancet 1999;353:1167-73.
2. den Heijer M, Rosendaal FR, Blom HJ, Gerrits WB, Bos GM. Hyperhomocysteinemia and venous thrombosis: a meta-analysis. Thromb Haemost 1998;80:874-7.

3. Koster T, Blann AD, Briet E, Vandenbroucke JP, Rosendaal FR. Role of clotting factor VIII in effect of von Willebrand factor on occurrence of deep-vein thrombosis. Lancet 1995;345:152-5.
4. van Hylckama V, van der Linden I, Bertina RM, Rosendaal FR. High levels of factor IX increase the risk of venous thrombosis. Blood 2000;95:3678-82.
5. Meijers JC, Tekelenburg WL, Bouma BN, Bertina RM, Rosendaal FR. High levels of coagulation factor XI as a risk factor for venous thrombosis. N Engl J Med 2000;342:696-701.
6. van Tilburg NH, Rosendaal FR, Bertina RM. Thrombin activatable fibrinolysis inhibitor and the risk for deep vein thrombosis. Blood 2000;95:2855-9.
7. Danesh J, Whincup P, Walker M, et al. Low grade inflammation and coronary heart disease: prospective study and updated meta-analyses. BMJ 2000;321:199-204.
8. van Aken BE, den Heijer M, Bos GM, van Deventer SJ, Reitsma PH. Recurrent venous thrombosis and markers of inflammation. Thromb Haemost 2000;83:536-9.
9. van Aken BE, Reitsma PH, Rosendaal FR. Interleukin 8 and venous thrombosis: evidence for a role of inflammation in the development of thrombosis. Brit J Haem 2002;116:1-5.
10. Kamphuisen PW, Eikenboom JC, Vos HL, et al. Increased levels of factor VIII and fibrinogen in patients with venous thrombosis are not caused by acute phase reactions. Thromb Haemost 1999;81:680-3.
11. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973-9.
12. van der Graaf F, van den Borne H, van der Kolk M, de Wild P, Janssen G, Van Uum S. Exclusion of deep venous thrombosis with D-dimer testing—comparison of 13 D-dimer methods in 99 outpatients suspected of deep venous thrombosis using venography as reference standard. Thromb Haemost 2000;83:191-8.
13. Syrjala H, Haukipuro K, Kiviniemi H. Acute phase response and deep lower limb venous thrombosis. J Clin Pathol 1990;43:519-20.
14. Wong NA, Laitt RD, Goddard PR, Virjee J. Serum C reactive protein does not reliably exclude lower limb deep venous thrombosis. Thromb Haemost 1996;76:816-7.
15. Wakefield TW, Greenfield LJ, Rolfe MW, et al. Inflammatory and procoagulant mediator interactions in an experimental baboon model of venous thrombosis. Thromb Haemost 1993;69:164-72.
16. Froehlich JB, Prince MR, Greenfield LJ, Downing LJ, Shah NL, Wakefield TW. "Bull's-eye" sign on gadolinium-enhanced magnetic resonance venography determines thrombus presence and age: a preliminary study. J Vasc Surg 1997;26:809-16.
17. Wakefield TW, Strieter RM, Schaub R, et al. Venous thrombosis prophylaxis by inflammatory inhibition without anticoagulation therapy. J Vasc Surg 2000;31:309-24.
18. Downing LJ, Strieter RM, Kadell AM, Wilke CA, Greenfield LJ, Wakefield TW. Low-dose low-molecular-weight heparin is anti-inflammatory during venous thrombosis. J Vasc Surg 1998;28:848-54.
19. Meissner MH, Zierler BK, Bergelin RO, Chandler WC, Manzo RA, Strandness DE. Markers of plasma coagulation and fibrinolysis after acute deep venous thrombosis. J Vasc Surg 2000;32:870-80.

Submitted Jul 18, 2001; accepted Oct 9, 2001.