The relationship between inflammation and venous thrombosis

A systematic review of clinical studies

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Summary

During the past decade, the role of inflammation in the pathophysiology of arterial thrombosis has been elucidated. However, comparatively little is known about the relationship between inflammation and venous thrombosis. The aim of this study was to perform a systematic review of clinical studies that have examined the association between inflammation and venous thrombosis, specifically: (1) the value of inflammatory markers in predicting the future development of venous thrombosis; (2) test characteristics of markers of inflammation in the diagnosis of acute venous thrombosis; and (3) effect of venous thrombosis on blood levels of inflammatory markers.

Using keywords venous thrombosis, venous thromboembolism, inflammation, acute phase markers, C-reactive protein (CRP), interleukin (IL)-6, IL-8, and monocyte chemotactic protein (MCP)-1, PubMed and Medline computerized databases were searched for English language articles published after 1980. Search results were restricted to clinical studies in humans that used study designs that were appropriate to address the above objectives. Results show that plasma CRP levels do not appear to predict risk of future venous thrombosis (two studies; N=41,308). Four studies (N=562) have examined the utility of plasma CRP in the diagnosis of venous thrombosis; pooled positive and negative predictive values were 53% (95% CI: 47%, 59%) and 85% (95% CI: 81%, 89%), respectively. A two- to six-fold increase in the risk of deep vein thrombosis (DVT) is associated with elevations in plasma levels of CRP, IL-6, IL-8, MCP-1 or TNF-α (three studies).

We can conclude that the nature of the relationship between inflammation and clinical venous thrombosis is not yet established. CRP does not appear to be useful in predicting future venous thrombosis or in the diagnosis of acute venous thrombosis. While several markers of inflammation are elevated in acute venous thrombosis, further research is needed to determine the precise relationship between these markers and venous thrombosis. The identification and elucidation of inflammatory markers relevant to venous thrombosis could provide targets for future therapy.

Keywords

Venous thromboembolism, markers of inflammation, prognosis, predictors, pathophysiology

Introduction

In recent years, a clear link has been established between inflammation and the development of arterial atherothrombosis. For example, increased plasma levels of C-reactive protein (CRP), a predominant acute phase reactant and marker of inflammation, are predictive of future myocardial infarction and stroke (1).

Inflammation may also play a role in venous thromboembolism (VTE), a common thrombotic vascular condition that affects about 1/1000 people per year (2, 3). Clinically, it is apparent that patients with deep vein thrombosis (DVT) manifest the four cardinal signs of inflammation, namely heat, redness, pain, and swelling. It is known that the procoagulant thrombin is capable of stimulating multiple inflammatory pathways, and, equally, inflammatory cytokines such as interleukin (IL)-6, IL-8, and monocyte chemotactic protein (MCP)-1 are capable of activating coagulation (4, 5). Lab studies have shown that peripheral blood monocytes, when incubated with highly purified (>90%) human CRP for 6 hours, exhibit a significant increase in procoagulant activity due to an increase in the expression of tissue factor, an initiator of the extrinsic pathway of coagulation (6). Animal studies have demonstrated that thrombosis directly elicits an inflammatory response in the vein wall, which involves neutrophil activation and expression of selectins, inflammatory cytokines, and cellular adhesion molecules (4, 7, 8). Additionally, human saphenous vein endothelial cells have been shown to
increase secretion of IL-6 and MCP-1 when incubated with human recombinant CRP (9).

In light of these observations, several clinical investigators have undertaken studies to attempt to elucidate the nature of the relationship between markers of inflammation and VTE. The objective of this paper is to critically review and synthesize published clinical studies that have examined the link between the inflammatory response and VTE. We discuss the role of markers of inflammation in predicting future VTE, their utility in the diagnosis of VTE, and how their levels vary in response to VTE.

**Methods**

Medline was searched in the English language for articles published after 1980 using the keywords venous thrombosis, venous thromboembolism, inflammation, acute phase markers, C-reactive protein, interleukin (IL)-6, IL-8, and monocyte chemotactic protein (MCP)-1. Search results were restricted to clinical human studies. Review articles and case reports were excluded. Bibliographies of retrieved articles were then searched for additional relevant articles.

Studies were too few in number and too heterogeneous to perform formal metaanalysis with generation of summary statistics. Hence, results are presented descriptively. Where possible, pooled weighted indices of accuracy (e.g. sensitivity, specificity, predictive values) are presented.

**Results**

**Markers of inflammation for predicting future VTE**

Two prospective studies have examined the predictive value of plasma CRP level for the development of VTE. The Physician’s Health Study (1) enrolled 22,071 U.S. male physicians between the ages of 40–84 who had no history of myocardial infarction, stroke, transient ischemic attack, or cancer. Study subjects were followed for up to 14 years. Mean plasma CRP levels in subjects who developed VTE during follow-up were 1.24 mg/l, compared with 1.10 mg/l in subjects who did not develop VTE (p=0.34). The lack of statistical significance of these differences might be explained, at least in part, by the small number of participants in the study who developed VTE (n=101). However, Tsai et al (10) also found that there was no association between baseline CRP levels and the subsequent development of VTE. For this analysis, those participants in the Cardiovascular Health Study (CHS) and the Atherosclerosis Risk In Communities (ARIC) study without prevalent VTE, a history of cancer or use of warfarin at baseline were studied. Participants in the CHS study were aged ≥65 years at entry, while those in the ARIC study were aged 45–64 years at entry. Of the 19,237 patients in the pooled population, 159 patients developed VTE. Patients from the CHS and ARIC studies who developed VTE had mean (SD) plasma CRP levels (mg/l) of 4.0 ± 6.7 and 2.8 ± 2.4 respectively, vs. 3.6 ± 6.0 and 3.2 ± 5.9 respectively in those who did not develop VTE. The measured differences in plasma CRP levels were not statistically significant in either study population (p=0.52 in CHS and p=0.48 in ARIC). Furthermore, there were no differences in age-, race- and sex-adjusted VTE incidence rates per 1000 person-years according to quartile of CRP levels. Overall, these results are consistent with those reported for the Physician’s Health Study (1) and taken together, indicate that plasma CRP levels do not appear to predict future VTE events.

Elevated plasma Factor VIII:C (FVIII:C) is a risk factor for VTE (11, 12). Several studies have investigated whether acute phase reactants cause this observed increase in FVIII:C (13–15) and could therefore be predictive of future VTE. O’Donnell et al (13) investigated 45 patients in whom the sole risk factor for VTE was elevated plasma FVIII:C (≥1.5 IU/ml). In these patients, there were no significant correlations between FVIII:C or FVIII:Ag levels and levels of the acute phase reactants CRP or fibrinogen. Subsequently, the same group serially investigated 35 patients who had objectively confirmed VTE 3 months – 12 years earlier (median 9 months) and whose only VTE risk factor was elevated plasma FVIII:C (≥1.5 IU/ml) (14). Throughout follow-up (median 8 months), 94% of patients continued to have elevated levels of plasma FVIII:C. Conversely, only 6 patients had elevated levels of acute phase reactants (CRP ≥ 10 mg/ml or fibrinogen ≥ 4.0 mg/ml) at the beginning of the study, these persisted throughout follow-up in only 3 patients, and there was no correlation between FVIII:C levels and acute phase reactant levels. Finally, Kamphuisen et al (15) conducted a case control study of 474 patients with a first episode of objectively confirmed DVT and 474 age and sex matched healthy controls. Blood was collected in cases a median of 18 months after the thrombotic event. Mean CRP levels were higher in thrombosis patients than in controls. After adjusting for CRP levels, however, high factor VIII:C levels still increased the thrombosis risk 6-fold. Hence, based on the results of the above studies, it appears that the risk for VTE conferred by increased levels of FVIII:C is not caused by acute phase reactants such as CRP.

**Markers of inflammation in the diagnosis of VTE**

The potential role of CRP in the diagnosis of VTE among patients with clinically suspected VTE has been evaluated in four studies (Table 1). All studies defined elevated plasma CRP levels as >10 mg/l. Using contrast venography as a gold standard, Thomas et al (16) tested whether plasma CRP levels were useful to diagnose deep venous thrombosis (DVT) in 47 patients with suspected DVT, 18 (38%) of whom were confirmed to have DVT on venography. Elevated CRP levels had a sensitivity of 100% (95% confidence interval [CI] 78%-100%) and a specificity of 52% (95% CI 34%-70%) for the diagnosis of DVT. The positive predictive value (PPV) and negative predictive value (NPV) were 56% and 100%, respectively, as calculated from the data. These results suggested that non-elevated CRP levels might be useful to exclude DVT in patients with clinically suspected DVT. However, several larger subsequent studies by Wong et al (17), Maskell et al (18), and Bucek et al (19) have refuted that plasma CRP levels are of value in the diagnosis of VTE (Table 1). Combining the data from the four studies, all of which had a similar prevalence of DVT in their study populations, yielded a pooled weighted sensitivity of 77%, specificity of 66%, PPV of 53% and NPV of 85%. These results indicate that plasma CRP level, in itself, does not have utility for ruling in or ruling out DVT in patients with clinically suspected DVT. The potential role of other markers of inflammation in the diagnosis of VTE has not been evaluated.
Levels of markers of inflammation in acute VTE

A number of clinical studies have measured levels of markers of inflammation in patients with DVT compared with controls without DVT (Table 2). In a case-control study, van Aken et al (20) investigated whether elevated plasma markers (defined as >90% control values) of the inflammatory cytokines IL-6, IL-8, and MCP-1 were associated with VTE. Plasma levels of these markers were measured in subjects who had experienced at least 2 VTE episodes between one and thirty years previously, compared with apparently healthy volunteers. In patients with previous VTE, the odds ratio (OR) for elevated plasma IL-6 was 2.4 (95% CI 1.5–3.8), elevated IL-8, 2.0 (95% CI 1.2–3.5), and elevated MCP-1, 1.9 (95% CI 1.2–3.2). Elevated plasma IL-8 was also shown to be associated with VTE in a second case-control study, also by van Aken, of 474 subjects with a first episode of objectively confirmed DVT (21) who were compared with 474 age and sex-matched controls who had no history of VTE and were not biologically related to the subjects (Leiden Thrombophilia Study). IL-8 levels >90% control values were associated with an OR of 1.9 (95% CI 1.2–2.8) and IL-8 levels >99% control values were associated with an OR of 6.0 (95% CI 2.0–17). The authors postulated that because levels of inflammatory markers did not differ in patients with recent VTE (less than 2 years previously (20), or less than 12 months previously (21)) compared to those with more remote VTE (more than 9 years previously (20), or more than 28 months previously (21)), it is possible that the inflammatory state preceded the VTE, although this was not directly established. Reitsma and Rosendaal recently reported further on levels of inflammatory mediators in patients and controls from the Leiden Thrombophilia Study (22). Tumor necrosis factor (TNF)-α, IL-6 and IL-8 levels were found to be risk determinants for venous thrombosis, such that individuals with detectable levels of these markers had OR that ranged from 2–3 and those with levels >95% of control levels had OR ranging from 2–5. Interestingly, high levels of the anti-inflammatory cytokine IL-10 were found to be protective against venous thrombosis. Roumen-Klappe et al (23) measured levels of IL-6, IL-8 and CRP at the time of diagnosis of acute DVT and 5 days later. Consistent with van Aken’s and Reitsma’s results, plasma IL-8 levels were elevated at both time points, however plasma IL-6 and CRP levels decreased from Day 0 to Day 5. This may be an indication that inflammation is a consequence rather than a cause of DVT (23). The observed decrease in plasma IL-6 and CRP may have been partly attributable to the treatment regimen, as blood samples were taken during the first 5 days of the study while the participants were being treated with heparin, which has anti-inflammatory effects in rats (24).

Prospective studies to confirm these findings and help clarify the sequence of events would be desirable.

Discussion

Of late, there has been a great deal of research interest in the relation between mediators of the inflammatory reaction and cardiovascular disease. Elevated levels of CRP have been shown to significantly increase the risk of clinical manifestations of atherosclerosis (1). In contrast, however, our review did not find direct evidence to support that markers of inflammation are predictive of future development of clinical venous thrombosis. Two large prospective studies (1, 10) compared plasma CRP levels in people who developed VTE to those in people who did not develop VTE, and both concluded that plasma CRP levels were not predictive of future development of VTE. Additionally, the risk for VTE conferred by elevated plasma FVIII:C does not appear to be related to acute phase reactants (13–15). Hence, a temporal association between markers of inflammation and the development of venous thrombosis has not yet been demonstrated.

We found indirect evidence, however, to support the view that markers of inflammation such as IL-6, IL-8, and MCP-1 are involved in the pathogenesis of VTE. Three separate studies showed that patients with VTE were more likely to have elevated

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### Table 1: Studies examining C-reactive protein in the diagnosis of venous thromboembolism

<table>
<thead>
<tr>
<th>Authors</th>
<th>Number of Patients</th>
<th>Number of Patients with DVT</th>
<th>Gold Standard</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>PPV (95% CI)</th>
<th>NPV (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas (1989)</td>
<td>47</td>
<td>18</td>
<td>Phlebography</td>
<td>100% (78–100)</td>
<td>52% (74–70)</td>
<td>56% (39–72)</td>
<td>100% (80–100)</td>
</tr>
<tr>
<td>Wong (1996)</td>
<td>150</td>
<td>56</td>
<td>Venography</td>
<td>84% (72–92)</td>
<td>62% (51–72)</td>
<td>57% (46–67)</td>
<td>87% (77–93)</td>
</tr>
<tr>
<td>Maskell (2001)</td>
<td>132</td>
<td>40</td>
<td>Venography</td>
<td>60% (45–74)</td>
<td>70% (60–78)</td>
<td>46% (33–59)</td>
<td>80% (70–87)</td>
</tr>
<tr>
<td>Bucik (2002)</td>
<td>233</td>
<td>73</td>
<td>Venography or Colour Duplex Sonography</td>
<td>75% (64–85)</td>
<td>69% (61–76)</td>
<td>52% (42–62)</td>
<td>86% (79–91)</td>
</tr>
<tr>
<td>Pooled weighted results</td>
<td>562</td>
<td>187</td>
<td></td>
<td>77% (70–82)</td>
<td>66% (61–71)</td>
<td>53% (47–59)</td>
<td>85% (80–89)</td>
</tr>
</tbody>
</table>

### Table 2: Odds ratios associated with levels of inflammatory markers in patients with venous thromboembolism vs. controls.

<table>
<thead>
<tr>
<th>Authors</th>
<th>Marker of Inflammation (cut-off for abnormal)</th>
<th>Number of Patients</th>
<th>Number of Controls</th>
<th>Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>van Aken (2000)</td>
<td>IL-6 (&gt;90% ctrl)</td>
<td>182</td>
<td>350</td>
<td>2.4 (1.5–3.8)</td>
</tr>
<tr>
<td></td>
<td>IL-8 (&gt;90% ctrl)</td>
<td>167</td>
<td>316</td>
<td>2.0 (1.2–3.5)</td>
</tr>
<tr>
<td></td>
<td>MCP-1 (&gt;99% ctrl)</td>
<td>182</td>
<td>347</td>
<td>1.9 (1.2–3.2)</td>
</tr>
<tr>
<td>van Aken (2002)</td>
<td>IL-8 (&gt;90% ctrl)</td>
<td>466</td>
<td>462</td>
<td>1.9 (1.2–2.8)</td>
</tr>
<tr>
<td></td>
<td>IL-8 (&gt;99% ctrl)</td>
<td>466</td>
<td>462</td>
<td>6.0 (2.0–17)</td>
</tr>
<tr>
<td>Reitsma (2004)</td>
<td>TNFα (&gt;95% ctrl)</td>
<td>470</td>
<td>470</td>
<td>2.3 (1.1–5.1)</td>
</tr>
<tr>
<td></td>
<td>IL-6 (&gt;95% ctrl)</td>
<td>470</td>
<td>470</td>
<td>1.5 (0.7–3.4)</td>
</tr>
<tr>
<td></td>
<td>IL-8 (&gt;95% ctrl)</td>
<td>470</td>
<td>470</td>
<td>2.2 (0.9–5.4)</td>
</tr>
<tr>
<td></td>
<td>II-10 (&gt;95% ctrl)</td>
<td>470</td>
<td>470</td>
<td>4.9 (1.7–16.4)</td>
</tr>
<tr>
<td></td>
<td>II-12 (&gt;95% ctrl)</td>
<td>470</td>
<td>470</td>
<td>0.4 (0.2–1.3)</td>
</tr>
<tr>
<td></td>
<td>II-12 (&gt;95% ctrl)</td>
<td>470</td>
<td>470</td>
<td>1.0 (0.0–2.2)</td>
</tr>
</tbody>
</table>
plasma IL-8 levels (20–22) than those without VTE. Elevated plasma IL-6, MCP-1 and TNF-α levels have also been documented in patients with VTE (20, 22), and plasma IL-6 and CRP levels appear to decrease during treatment with heparin (23), which has anti-inflammatory properties. Taken together, these findings suggest that inflammation plays a role in the pathogenesis of VTE, and that IL-6 and IL-8 in particular may be of interest in future studies.

Based on the literature published to date, CRP, used alone, does not appear to be a good candidate for a diagnostic tool to detect or exclude VTE due to low positive and negative predictive values (17–19). The potentially lethal consequences of VTE require that the negative predictive value of any single or combination of diagnostic tests be near 100%. Further research is needed to determine whether other markers of inflammation could be of use as an adjunct to the currently available diagnostic tools in the detection of VTE.

In conclusion, the nature of the relationship between inflammation and clinical VTE has not yet been elucidated. Efforts to associate CRP with VTE have failed to demonstrate that it can either predict future VTE or is useful in the diagnosis of VTE. However, recent research demonstrates a probable association between VTE and several other markers of inflammation, notably IL-6, IL-8, MCP-1 and TNF-α being of particular interest. Overall, little has been published in this area and further research is needed to determine the precise relationship between these markers of inflammation and VTE. The identification and elucidation of inflammatory markers relevant to VTE could provide targets for future therapy. Whether these markers may be useful in the diagnosis of VTE is as yet uncertain.

References