C-reactive Protein is Elevated in Symptomatic Compared with Asymptomatic Patients with Carotid Artery Disease

K. Rerkasem¹, C. P. Shearman¹, J. A. Williams¹, G. E. Morris¹, M. J. Phillips¹, P. C. Calder² and R. F. Grimble²

¹Department of Vascular Surgery, Southampton General Hospital, U.K. and ²Institute of Human Nutrition, University of Southampton, U.K.

Objectives: to investigate the level of inflammatory markers between symptomatic and asymptomatic carotid stenosis patients.

Design: cross-sectional study

Materials and methods: a prospective study of 137 consecutive patients, admitted electively for carotid endarterectomy during 1997–2000, was conducted. 125 patients had cerebrovascular symptoms: either stroke (neurological deficit >24 h), transient ischaemic attack (neurological deficit <24 h) or amaurosis fugax. Twelve patients were asymptomatic. A medical history and a fasting venous blood sample were taken from each patient around 6 weeks before surgery. The plasma concentrations of cholesterol and of inflammatory markers; (high sensitivity C-reactive protein (hs-CRP), sICAM-1, sVCAM-1, sE-selectin) were determined.

Results: the concentration of hs-CRP in the symptomatic group (3.9 mg/L) was significantly higher than in the asymptomatic group (2.1 mg/L; p<0.04). These concentrations were within normal range (<10 mg/L). sICAM-1, sVCAM-1, sE-selectin and total cholesterol concentrations were not different between the two groups.

Conclusion: plasma hs-CRP was elevated in symptomatic compared to asymptomatic patients with carotid artery disease. High sensitivity C-reactive protein has been shown to be of prognostic value in a number of cardiovascular conditions and this study suggests it may be of value to identify patient at high risk of developing neurological deficits.

Key Words: C-reactive protein; Carotid endarterectomy; Atherosclerosis; Carotid stenosis.

Introduction

The Asymptomatic Carotid Artery Stenosis Trial (ACAS) has shown the benefit of carotid endarterectomy (CEA) in preventing future strokes.¹ The cost effectiveness of this procedure is limited however. As within 5 years only 1 stroke is prevented for approximately 17 CEAs undertaken. In particular, asymptomatic patients with diabetes mellitus and contralateral siphon stenosis have a significantly higher risk of perioperative stroke than other patients, so the benefit of CEA in preventing strokes in this group is marginal.² A blood test, which could predict the risk of a carotid atherosclerotic plaque leading to a stroke, might be useful in identifying patients with asymptomatic carotid stenosis, who have higher risk of atheroembolism or haemodynamic insufficiency and is beneficial the most from surgery. No such studies has been carried out before.

High sensitivity C-Reactive Protein (hs-CRP) has been shown to be a predictive factor for cardiovascular events. Ridker et al. studied a large prospective cohort study and found that high concentrations of hs-CRP were associated with increased risk of stroke, myocardial infarction and of developing symptomatic peripheral vascular disease by 2, 3 and 4 fold, respectively.³,⁴ In addition to hs-CRP, many other mediators have been identified that can predict a cardiovascular event, but from the head-to-head comparison study, high hs-CRP concentration had a stronger relationship with stroke and myocardial infarction than serum amyloid A, soluble intercellular adhesion molecule-1 (sICAM-1), interleukin 6, total cholesterol, low density lipoprotein (LDL) cholesterol, apolipoprotein A1, Lp(a) lipoprotein and homocysteine.⁵ Interestingly, few studies have explored the level of hs-CRP compared to other inflammatory markers in patients with symptomatic and asymptomatic carotid stenosis. We
therefore hypothesised that inflammatory markers, especially hs-CRP, in symptomatic patients with carotid stenosis are elevated compared to asymptomatic patients. If that is the case, it might lead to future cohort studies to identify the mediators, which can be used to select asymptomatic carotid stenosis patients at high risk of developing neurological deficits for surgery. The aims of this paper are to compare the level of plasma inflammatory markers and total cholesterol concentration between symptomatic and asymptomatic carotid stenosis.

**Methods**

We prospectively included 137 consecutive patients who were due for CEA. This was carried out in the Department of Vascular Surgery, Southampton General Hospital, during 1997–2000. The indication for surgery included symptomatic severe carotid stenosis (>70%) and asymptomatic bilateral severe carotid stenosis (>90%). The degree of stenosis of all patients was assessed by duplex scan. There were 137 patients: 85 males and 52 females. The median age was 73 years (range 45–85 years). Twelve patients were in the asymptomatic group and 125 patients in the symptomatic group. Patients were defined as asymptomatic if they had never had any experience of neurological deficit, either a transient ischaemic attack (TIA) or stroke related to the affected vessel. Baseline clinical data and cardiovascular events were evenly matched in the two groups (Tables 1 and 2). In the symptomatic group, the median duration between the last reported symptom and venous blood sampling was 3 months.

A medical history was recorded and a physical examination was undertaken. At the initial visit, fasting venous blood was collected into vacutainer tubes containing 0.12 ml of 15% EDTA. Plasma was prepared by centrifugation at 1500×g for 10 min and stored at −80°C. Plasma hs-CRP was measured by an immunoturbidimetric technique using a commercially available kit, produced by Wako Laboratories and available from Alpha Laboratories (Eastleigh, U.K.). The technique had inter- and intra-assay coefficients of variation of 6.4%, and 3.4%, respectively. Plasma sICAM-1 and soluble vascular cell adhesion molecule-1 (sVCAM-1) concentrations were measured using Cytoscreen ELISA kits from BioSource (Nivelles, Belgium). Limits of detection were 0.04 ng/mL (sICAM-1) and 0.5 ng/mL (sVCAM-1). Inter- and intra-assay coefficients of variation were <5% for both assays. Plasma soluble E-selectin (sE-selectin) concentrations were measured using Quantikine ELISA kits from R & D Systems Europe (Abingdon, U.K.). The limit of detection was 0.1 ng/mL, and inter- and intra-assay coefficients of variation were <10% and <5%, respectively. Plasma total cholesterol concentrations were measured using a commercially available, enzyme-based diagnostic kit (Sigma Chemical Co., Poole, U.K.).

**Statistics**

Since the data were not normally distributed, univariate analysis was performed by the Mann–Whitney U-test and Spearman’s rank correlation test. Hs-CRP concentration was logarithmically (ln) transformed prior to multivariate analysis to obtain a normal distribution. Statistical analyses were performed using
Results

The median concentration of hs-CRP among all patients was 3.5 mg/L (range 0.2–84.9 mg/L). In univariate analysis, the concentration of hs-CRP in the symptomatic group was significantly higher than in the asymptomatic group (Table 3). However, there were no differences between these groups in terms of the concentrations of sICAM-1, sVCAM-1, sE-selectin, and total cholesterol. The median concentration of hs-CRP in stroke patients (4.7 mg/L, range 0.2–84.9 mg/L) was not significantly higher than in the transient neurological deficit group (3.5 mg/L, range 0.2–36.9 mg/L) ($p = 0.36$). No correlation was found between hs-CRP concentration and the time delay between onset of last symptoms and blood withdrawal ($r = 0.08 ; p = 0.33$). The median concentration of hs-CRP in males (3.7 mg/L, range 0.2–84.9) was not significantly different compared with females (3.5 mg/L, range 0.3–29.1) ($p = 0.81$).

In multivariate analysis, the significant association between hs-CRP concentration and symptoms remained unaffected when adjusted for the duration from the last event and became even more pronounced when possible confounders were taken into account (Table 4).

Discussion

This study shows that symptomatic patients with severe carotid stenosis have elevated hs-CRP compared with asymptomatic patients. This is the first study to show the difference of the level of hs-CRP between symptomatic and asymptomatic carotid stenosis. However this study cannot conclude that high CRP level is the predictor of the progression of carotid disease because it is a cross-sectional study. This issue can only resolve with a large-scale cohort study. If that is the case, hs-CRP may have a benefit in identifying asymptomatic carotid stenosis patients at a higher risk of neurological deficit. To confirm this benefit, a randomised controlled trial should be carried out between surgical and medical treatment in the asymptomatic patients with high level of hs-CRP.

The upper limit of normal of hs-CRP is 10 mg/L. The median level of hs-CRP in the carotid stenosis patients both symptomatic and asymptomatic groups, was within this (3.9 and 2.1 mg/L, respectively). However, hs-CRP did not correlate with the severity of the clinical condition (stroke, TIA) or with the duration between the onset of the last neurological symptom and sampling. This supports the findings of Canova et al. who showed that CRP levels in 138 patients with acute neurological deficit was not different between 5 clinical categories: transient ischaemic attack, reversible neurological deficit, complete stroke and recovery, stroke without recovery and cerebral haemorrhage. The concentration of hs-CRP increases in response to acute ischaemia, like

<table>
<thead>
<tr>
<th>Median concentration of markers and range</th>
<th>Symptomatic group</th>
<th>Asymptomatic group</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mg/L)</td>
<td>3.9 (0.2–84.9)</td>
<td>2.1 (0.2–9.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>sICAM-1 (ng/mL)</td>
<td>323 (130–719)</td>
<td>337 (235–597)</td>
<td>0.66</td>
</tr>
<tr>
<td>sVCAM-1 (ng/mL)</td>
<td>616 (319–2192)</td>
<td>582 (396–860)</td>
<td>0.44</td>
</tr>
<tr>
<td>sE-selection (ng/mL)</td>
<td>43 (14–109)</td>
<td>44 (22–87)</td>
<td>0.44</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.6 (3.0–11.0)</td>
<td>5.3 (3.4–7.2)</td>
<td>0.08</td>
</tr>
</tbody>
</table>

Table 4. Hs-CRP regression coefficients from multivariate regression analysis of the presence of neurological deficit on in hs-CRP in various degrees of adjustment.

<table>
<thead>
<tr>
<th>Adjustment</th>
<th>Regression coefficient</th>
<th>Standard Errors</th>
<th>Lower 95% confidence limit</th>
<th>Upper 95% confidence limit</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.73</td>
<td>0.32</td>
<td>0.10</td>
<td>1.36</td>
<td>0.02</td>
</tr>
<tr>
<td>Duration since last event (months)</td>
<td>0.75</td>
<td>0.32</td>
<td>0.12</td>
<td>1.38</td>
<td>0.02</td>
</tr>
<tr>
<td>Possible confounders*</td>
<td>0.84</td>
<td>0.33</td>
<td>0.18</td>
<td>1.50</td>
<td>0.01</td>
</tr>
</tbody>
</table>

* Possible confounders = duration since last neurological event, age, smoking status, history of angina pectoralis or myocardial infarction, history of intermittent claudication or critical limb ischaemia, body-mass index, gender, hypertension history, hypercholesterolaemia (plasma cholesterol < 5 mmol/L), history of diabetes mellitus.
most other acute phase proteins. Furthermore, raised hs-CRP concentrations have been reported in many conditions, such as cigarette consumption. Therefore, cerebral ischaemic events and other factors (e.g. smoking) are likely to be confounding variables in the current study. However, the association between symptoms and the concentration of hs-CRP remained significant after adjusting for the duration since the most recent neurological deficit (Table 4). Furthermore, the difference was still significant after adjusting for the many possible confounding variables including smoking status and the ischaemia of the heart and the lower extremities.

A number of prospective studies have identified peptide mediators such as other acute-phase proteins, cytokines and soluble adhesion molecules to be markers of cardiovascular disease. However, hs-CRP appears superior than other markers: the results of the current study and also from the large cohort study in women show hs-CRP to be more closely associated with cardiovascular events than other markers. CRP is a very sensitive marker. The plasma concentration of CRP can increase several hundred fold following stimulation and is not effected by hormonal changes or anti-inflammatory drugs. Furthermore, in contrast to many other inflammatory markers, the assay techniques for hs-CRP are highly sensitive and can detect even mild elevation of CRP within the normal range. On the whole, hs-CRP assay provides a simple and reliable measurement for the assessment of systemic inflammation.

Although CEA has proved to be beneficial in severe symptomatic carotid stenosis in two large trials (ECST and NASCET), 8–10 CEAs are needed to prevent one stroke over three years. Rothwell et al. tried to increase the efficacy of surgical intervention by developing a model that targeted patients, who have a high chance of developing further neurological events. Hs-CRP concentration after stroke has been found to be a marker of increased 1 year risk in ischaemic stroke. If hs-CRP is able to predict future neurological events in patients with severe carotid stenosis in future cohort study, this might improve targeting of surgery.

The mechanism of the association between raised hs-CRP and cardiovascular symptoms is still uncertain. The raised level of this inflammatory marker may reflect an epi-phenomenon associated with the atherosclerotic plaque, as some experimental studies could identify CRP binding in the foamy macrophages of atherosclerotic lesions. It has been hypothesised that CRP might encourage lipid intake and cytolysis of the atherosclerotic lesion, which will enlarge the necrotic area in the lesion. An alternative hypothesis is that raised hs-CRP concentrations might be related to cardiovascular events via a causal pathway such as increased thrombus formation, lipid oxidation, and cell activation and proliferation. Tohgi et al. found the activation of the coagulation/fibrinolysis system and platelet function in patients with acute thrombotic stroke with raised CRP.

### Limitations

As CRP level is usually elevated with injury or inflammation including ischaemia or infection. Our study took blood sampling after some of the patients had symptoms, so the result might be confounded by the effect of cerebral ischaemia. Thus a longitudinal study should be carried out. Although we did not sample when patients were unwell from any kind of obvious infection such as cold. This cannot exclude infection. Erythrocyte sedimentation rate (ESR) may be used to identify patients with inflammatory processes. Also our study potentially contains selection bias. For example, there was a high proportion of symptomatic cases (12 asymptomatic patients/125 symptomatic patients), so our conclusions are based on only 12 patients, resulting in a high chance of a type 1 error. There was a high proportion of bilateral severe asymptomatic carotid stenosis in these 12 patients, because unilateral severe carotid stenosis in asymptomatic patients is not a routine indication of surgery in our unit.

### Conclusion

Patients with symptomatic carotid stenosis have elevated hs-CRP compared with asymptomatic patients. This elevation of hs-CRP concentration is associated with the presence of neurological events. The hs-CRP level might be useful in selecting asymptomatic carotid stenosis patients who will gain benefit from CEA. A large prospective cohort study is necessary to confirm and judge the clinical application of this measurement.

### Acknowledgements

The authors would like to acknowledge the Ministry of Agriculture, Fisheries and Food for financial support for this study (Grant no. ANO238). We would like to thank Dr Peters and Mr Farey for statistical support and Jennifer Garry (Institute of Human Nutrition, University of Southampton) and Drs Jonathan Powell and Tina Hurst (Unilever Research Colworth Laboratory, Sharnbrook) for...
technical expertise. Dr Rerkasem was supported by the Faculty of Medicine, Chiang Mai University, Thailand and The Royal Thai Government.

References


14 European Carotid Surgery Trialists' Collaborative Group. MRC European Carotid Surgery Trial: interim results for symptomatic patient with severe (70–99%) or with mild (0–29%) carotid stenosis. Lancet 1991; 337: 1235–1243.


Accepted 4 March 2002