Clinical Research

Inflammatory markers and elderly patients with stroke

Antonello Silvestri, Cristina Vitale, Pasquale Galetta,
Daniela Onorati, Massimo Fini, Giuseppe MC Rosano

Research Unit, Department of Medical Sciences, San Raffaele - Roma, TOSINVEST SANITA', Roma, Italy

Objective C-reactive protein (CRP) is associated with unfavorable outcome in patients with acute ischemic syndromes and in patients with chronic stable angina. Elevated CRP levels suggestive of heightened inflammatory state in vascular conditions are often associated with elevated interleukin-6 (IL-6) levels. The aim of our study was to show the predictive importance of CRP and IL-6 levels in patients with ischemic stroke that has not been fully elucidated. Design We studied 647 consecutive elderly patients (>65 years) with stroke who were documented with ischemic stroke, presence of significant carotid athrombosis and absence of atrial fibrillation. The study population included 150 patients (74 men, 76 women, mean age 74 ± 2). Patients underwent evaluation of high sensitive CRP and IL-6 levels at baseline, during hospitalization and at discharge. Results In-hospital mortality was 6%, 1 year mortality was 15% and a second cerebrovascular event occurred in 12% of patients. Those with inhospital events had significantly higher baseline CRP and IL-6 levels than patients without events (3.8 ± 1.1 vs 1.9 ± 0.9 mg/L, P < 0.01 and 13.8 ± 3.4 vs 6.3 ± 2.1 pg/ml, P < 0.01, respectively). Also CRP and IL-6 levels were significantly higher in those patients with an event within 3 months of discharge compared to patients without an event (3.6 ± 1.3 vs 1.1 ± 0.7 mg/L, P < 0.01 and 14.2 ± 3.7 vs 5.4 ± 1.6 pg/ml, P < 0.01, respectively). Both baseline CRP levels and IL-6 were predictive of events both in-hospital and after 3 months while CRP and IL-6 levels at baseline were not associated with a poor 1 year prognosis. Elevated CRP levels were associated with an unfavorable outcome only when IL-6 levels were also elevated. In a stepwise multivariate analysis IL-6 level was a stronger predictor of outcome than CRP. Conclusions In conclusion, elevated CRP and IL-6 levels may identify elderly patients at increased medium term risk, but do not predict one year events in this subset of patients. CRP levels predict events only when they are coupled with IL-6 levels. (J Geriatr Cardiol 2004;1:44-48.)

Key Words c-reactive protein; interleukin-6; inflammatory markers; stroke

Introduction

Atherosclerosis is associated with chronic vascular inflammation and the adhesion between vascular endothelial cells and circulating leukocytes plays a key role in the inflammatory process.1,2 Several studies have found that the acute inflammatory response to tissue injury, which is marked by elevated levels of acute-phase proteins such as fibrinogen and C-reactive protein (CRP) and by leukocytosis, may have an important causal role in stroke.3 In longitudinal studies of cardiovascular health, baseline CRP has been found to be higher not only in subjects who develop ischemic heart disease or peripheral vascular disease, but also in subjects who will later develop a stroke.4,5 Moreover, in a large population-based cross-sectional study, Ford ES et al found a significant positive association between C-reactive protein and self-reported past history of stroke among men, women and among whites, African Americans and Mexican Americans.6 CRP has been reported to be a prognostic marker for cerebrovascular and cardiovascular ischemic events,7 and elevated serum C-reactive protein levels have been reported to be associated with a worse prognosis in patients with acute myocardial ischemia, myocardial infarction, unstable angina and chronic stable angina.8 In these patients high plasma levels of C-reactive protein reflecting the increased inflammatory activity, predict a rapid progression of the disease.9,10 Specific cytokines, particularly interleukin-1β, interleukin-6 (IL-6) and tumor necrosis factor α, are largely responsible for the increased expression of inflammatory markers by the liver. IL-6 is elevated in acute stroke, with levels in
cerebrospinal fluid of acute-stroke patients. Serum levels of IL-6 are predictive of cardiovascular risk, and are correlated with the levels of C-reactive protein. Recently, it has been suggested that elevated CRP levels independently predict the risk of future stroke and transient ischemic attack in the elderly. The importance in determining future cardiovascular and cerebrovascular events from elevated serum levels of CRP in the elderly is still a matter of speculation as these patients often have elevated plasma levels of CRP because of comorbidities without being at increased cardiovascular risk.

After acute stroke, a single CRP concentration measured within 72 hours or at discharge was found to be an independent predictor of a poor outcome. However, the predictive relative importance of CRP and IL-6 levels during the early phase of hospitalization in elderly patients with ischemic stroke has never been fully elucidated. The aim of the present study was to evaluate the predictive role of CRP and IL-6 levels obtained during hospitalization in elderly patients with ischemic stroke.

Subjects and methods

We studied between January 2000 and December 2000 a population of 647 consecutive elderly patients (>65 years, Table 1) admitted to our unit who were documented with ischemic stroke by CT, presence of significant carotid atherosclerosis (>50% stenosis of CCA or ICA by ultrasound), absence of atrial fibrillation and refusal to undergo thromb-endoarterectomy. Patients receiving thrombolitics, those with chronic or acute inflammatory or infectious disease, and those with conditions requiring oral anticoagulation (dilated cardiomyopathy), were excluded.

Study protocol and blood sampling

We measured CRP and IL-6 plasma levels within 12 hours from admission, on day 3 during hospitalization and at discharge. Venous blood samples were taken in a supine position after 20 minutes of rest with a Vacutainer system (Becton Dickinson, Meylan, France). Baseline blood samples were collected in tubes containing EDTA or trisodium citrate (1:9 vol/vol). The blood samples were immediately placed on ice and centrifuged within 1 hour from collection. Plasma was divided into aliquots and stored at -80 °C until laboratory analysis. All serum samples were assessed in duplicate.

Plasma samples obtained were thawed and assayed for CRP by use of a high-sensitivity assay with a coefficient of variation below 5% (hs-CRP, Dade Behring). An ELISA method was used to measure IL-6 plasma concentration (R & D Systems Inc, Minneapolis, Minnesota, USA). The lower limit for the detection of IL-6 was 0.2 ng/L.

Follow-up

Patients were evaluated at 3-month intervals during a mean follow-up duration of 16 ± 5 months. At each visit a full medical examination was performed and occurrence of any event was noted. For those patients that were not seen at the last follow-up visit, vital statistics were obtained from the health status registry and hospital charts.

Unfavorable outcome was defined as occurrence of cardiac death, death related to a cerebral ischemic event, new cerebrovascular event, or myocardial infarction (MI).

Statistical analysis

Results are presented as means ± 1 SD or percentages where appropriate. Differences between and amongst groups in the continuous variables were tested using either the two tailed Student’s t test or the Mann-Whitney U test. Chi-Squared or Fisher’s exact test were used to compare categorical variables. Results were considered significant if P < 0.05. Univariate Cox regression analysis was performed with cardiovascular events as the outcomes variable. The following univariate predictors were tested: age, diabetes, smoking status, BMI, blood pressure, total cholesterol, HDL cholesterol, HbA1c, fibrinogen, left ventricular hypertrophy, episodes of angina, CRP, IL-6, calculated cardiovascular risk, presence of carotid atherosclerosis, or coronary artery disease. Univariate predictors with P < 0.10 were entered into multivariate Cox regression analysis. All calculations were performed using the GBSTAT statistical package.

Results

Clinical features of study patients are reported in Table 1. A high incidence of hyperlipidemia, arterial hypertension and diabetes were found while an even male/female distribution was noted. The in-hospital mortality was 6%, during follow up overall mortality was 15% and another cerebrovascular event occurred in 12% of patients. During follow up 13 patients had an ischemic stroke, 5 had a transient ischemic attack, 12 suffered an acute myocardial infarction and 16 were hospitalized because of unstable angina. No significant difference in plasma levels of IL-6 and CRP were detected between on-admission and 3 days later. Patients with in-hospital events had significantly higher CRP and IL-6 levels at admission than patients without events (3.8 ± 1.1 vs 1.9
± 0.9 mg/L, \( P < 0.01 \) and 13.8 ± 3.4 vs 6.3 ± 2.1 pg/ml, \( P < 0.01 \), respectively (Fig. 1). Also CRP and IL-6 levels at admission were significantly higher in those patients with a new event within 3 months of discharge compared to those patients without an event (3.6 ± 1.3 vs 1.1 ± 0.7 mg/L, \( P < 0.01 \) and 14.2 ± 3.7 vs 5.4 ± 1.6 pg/ml, \( P < 0.01 \), respectively). In a multivariate analysis both baseline CRP levels and IL-6 were predictive of future in-hospital and 3 months future cerebrovascular events while high CRP and IL-6 levels at baseline were not associated with a poor 1-year prognosis (Table 2). Elevated CRP levels were associated with an unfavorable outcome only when IL-6 levels were also elevated. CRP and IL-6 levels at discharge were significantly lower than admission levels and did not correlate with future cardiovascular events. In a stepwise multivariate analysis IL-6 level was a stronger predictor of outcome than CRP.

Table 1. General information of patients

<table>
<thead>
<tr>
<th>Predictor factors</th>
<th>After 3 months</th>
<th>After 12 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP</td>
<td>( P = 0.046 )</td>
<td>( P = 0.078 )</td>
</tr>
<tr>
<td>IL-6</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.054 )</td>
</tr>
<tr>
<td>Unstable carotid plaque</td>
<td>( P = 0.032 )</td>
<td>( P = 0.018 )</td>
</tr>
<tr>
<td>Angina/CAD</td>
<td>( P = 0.048 )</td>
<td>( P = 0.046 )</td>
</tr>
<tr>
<td>Hypertension</td>
<td>( P = 0.029 )</td>
<td>( P = 0.037 )</td>
</tr>
</tbody>
</table>

Discussion

The results of our study show that both CRP and IL-6 measured at admission in patients with an ischemic stroke are predictive of short and medium term future cardiovascular events in patients with carotid atherosclerosis, but they are not predictors of long term events.

Various cytokines,\(^{14-16}\) chemokines,\(^{17-18}\) and adhesion molecules\(^ {19-20}\) have been shown to be expressed in the ischemic brain of experimental animals. A few studies have also revealed increased plasma levels of certain cytokines in patients with ischemic stroke.\(^ {22}\) Inflammation in atherosclerotic plaque is thought to be a significant contributory factor to the plaque rupture that precedes unstable vascular syndromes. CRP and IL-6 are now believed to be clinically useful risk markers of unstable atherosclerotic disease, as well as predictors of future cardiovascular morbidity and mortality.\(^ {23}\) C-reactive protein in particular is an independent marker for cardiovascular disease risk in men with and without coronary artery disease and in postmenopausal women without clinically evident coronary artery disease.\(^ {13}\) IL-6, one of the few cytokines measurable in human serum, is stimulated by proinflammatory cytokines TNF-alfa and IL-1, and it appears to reflect the proinflammatory status of patients with acute vascular syndromes.\(^ {22}\) Recent demonstrations of higher levels of IL-6 in the cerebrospinal fluid than in the serum of patients with stroke\(^ {24}\) or head injury\(^ {25}\) suggested that the cellular origin of serum cytokines in these patients may be the central nervous system. Brain cells, including astrocytes, microglia, and neurons, were shown to produce IL-6\(^ {26-29}\) and TGF-\(\beta\) under certain pathological conditions.\(^ {30-31}\)

Although the underlying mechanisms triggering the inflammatory response in atherosclerosis are unknown, elevation of C-reactive protein levels in the context of cardiovascular disease is involved in the pathophysiology of progression of atherosclerosis and in its complications. These experimental and human data suggest that cytokines and adhesion molecules are involved in the pathogenesis of stroke and related diseases.\(^ {7}\) In our study, patients with in-hospital events had significantly higher CRP and IL-6 levels than patients without events. These findings support the observations of Beamer et al that found significantly elevated IL-6 in patients after stroke; they
also support the fact that IL-6 was significantly correlated with levels of CRP and that elevated IL-6 and CRP concentrations were present in patients with large established infarcts on CT. We also found that CRP and IL-6 levels at admission were significantly higher in those patients with a new event within 3 months after discharge compared to those patients without an event. There is no clear data in literature about the possibility to predict the outcome in elderly patients with stroke. We found that both baseline CRP levels and IL-6 were predictor of future in-hospital events. This finding may be explained by the fact that CRP and IL-6 concentrations may reflect the degree of stroke severity, which correlate with the degree of inflammation directly related to cerebral infarction; it may also be related to the degree of carotid vascular inflammation.

Our data show that plasma levels of IL-6 were a more significant predictor of future events than CRP; this is because elevated CRP plasma levels were associated with an unfavorable outcome only when IL-6 levels were also elevated. In a stepwise multivariate analysis IL-6 levels were a stronger predictor of outcome than CRP. CRP levels were predictors of future events only after disregarding IL-6 levels and lack of presence of cardiovascular symptoms from the analysis. We found that both baseline CRP levels and IL-6 were predictive of 3 months future cerebrovascular events, but were not associated with a poor 1 year prognosis. These findings are consistent with a role for inflammation in acute ischemic stroke, as well as with the hypothesis that elevated CRP and IL-6 may predict future cardiovascular mortality. If increased CRP and IL-6 shortly after stroke is confirmed as an index of subsequent cardiovascular risk, these patients could be targeted for more aggressive conventional treatment or novel therapies intended to stabilize atherosclerotic plaque.

In conclusion elevated IL-6 and CRP plasma levels in patients with acute ischemic stroke and significant carotid atherosclerosis are predictors of in-hospital and short term cardiovascular events; this is probably because they reflect both stroke severity and unstability of atherosclerotic plaque. If these data will be confirmed by further studies, then IL-6 and CRP levels may be used as markers for a more aggressive management of such patients.

References


