Original Article

Can C-Reactive Protein and Fibrinogen Predict Major Adverse Cardiac Events in Cardiovascular and Cerebrovascular Patients?

Jafar Golshahi, M.D.1, Somayeh Khalesi, M.D.2, Masoumeh Sadeghi, M.D.2*, Nizal Sarrafzadegan, M.D.1, Mojgan Gharipour, Ph.D.3, Minoo Dianatkhah, M.D.4, Asma Salehi, M.D.5

ABSTRACT

Background: We aimed to examine the value of C-reactive protein (CRP) and fibrinogen levels to predict cardiovascular events and compare their predicting power between patients with a history of the acute coronary syndrome, patients with a history of stroke (ischemic type), and healthy individuals.

Methods: This case-control study assessed 79 patients with a history of the acute coronary syndrome and 88 patients with a history of stroke (cerebral ischemia) occurring at least 3 months previously. The patients were selected and followed up from September 2013 to September 2014 for 3 and 6 months after initial assessment to determine 6-month major adverse cardiac events (MACE). The serum levels of CRP and fibrinogen were measured using ELISA kits.

Results: The serum CRP level was significantly higher in the group with the acute coronary syndrome than in the group with a history of stroke and in the healthy group (P=0.045). The Cox regression model showed increased levels of CRP (HR=1.29 [1.01-1.66]; P=0.038) and fibrinogen (HR=1.01 [1.01-1.02]; P<0.001) in the group with a history of the acute coronary syndrome. It also demonstrated increased levels of CRP (HR=1.61 [0.97-2.67]; P=0.065) and fibrinogen (HR=1.02 [1.01-1.04]; P=0.010) in the stroke group and increased levels of CRP (HR=2.06 [0.71-5.99]; P=0.183) and fibrinogen (HR=1.01 [0.99-1.04]; P=0.294) in the normal group. Consequently, the groups with a history of the acute coronary syndrome and a history of stroke effectively predicted 6-month MACE in the crude and age- and sex-adjusted models.

Conclusions: Our study achieved 2 important findings. First, our results showed that higher values of these biomarkers were able to predict MACE, even after the inclusion of baseline covariates. Increased levels of CRP and fibrinogen, measured after evaluating the acute phase and their related outcome, were able to predict recurrent cardiovascular events in the patients with a history of cerebrovascular ischemia and the acute coronary syndrome. In addition, there were higher levels of both CRP and fibrinogen markers in the patients with a history of the acute coronary syndrome and stroke than in the healthy individuals. (Iranian Heart Journal 2015; 16(4): 19-27)

Keywords: CRP, Fibrinogen, Cerebrovascular, Cardiovascular, MACE
Atherosclerosis has been fundamentally understood as the chronic inflammatory disease of the cardiovascular system. In this regard, serum and plasma markers of inflammation provide an avenue of insight into the pathophysiology of atherosclerosis and its complications. The presence of inflammatory reactions in atherosclerotic plaques plays a certain role in both plaque rupture and platelet aggregation, leading to acute atherothrombotic events and serious complications. Increasing evidence shows that inflammatory mediators play a major role in determining the degree of plaque inflammation and in contributing to its evaluation from uncomplicated to complex atheroma, which leads to coronary artery disease and stroke. It has been proven that inflammatory reactions in coronary plaques play an important role in the pathogenesis of acute atherothrombotic events; inflammation elsewhere is also associated with both atherogenesis generally and its thrombotic complications. Moreover, with respect to the role of inflammation in cerebrovascular events, it has been shown that increased levels of inflammatory markers such as intracellular and muscular cell adhesion molecules may be related to the development of white matter lesions and lacunar infarcts. In addition, increased levels of inflammatory markers in damaged ischemic neurological tissues may be evidence of the role of inflammation in the development of cerebrovascular ischemic events. Along with the role of C-reactive protein (CRP) as a sensitive marker in inflammatory pathways, the role of fibrinogen as a risk factor for coronary artery disease has also been suggested. It has even been introduced as a risk component for scoring cardiovascular risk. This marker can trigger the coagulation process, which is a major component for the development of atherosclerotic plaques, leading to both cardiovascular and cerebrovascular ischemic events. In this regard, its valuable role in the prediction of further serious morbidities and even mortality has also been suggested. Despite some evidence regarding the role of these 2 inflammatory and coagulative biomarkers in the progression of ischemic events and their valuable role in the prediction of adverse consequences, several recent longitudinal studies have reported that CRP and fibrinogen are not associated with the future risk of ischemic stroke. Nonetheless, others have demonstrated that high-sensitivity CRP (hsCRP) is not associated with ischemic stroke, although it is modestly associated with myocardial infarction and mortality. Currently, there is not sufficient evidence to recommend the measurement of these 2 markers in primary prevention to predict cerebrovascular disease risk because there is insufficient evidence as to whether early detection or intervention based on detection improves health outcomes. Nevertheless, the shared risk of cardiovascular disease indicates that this may be of value. Still, the results of epidemiological studies have demonstrated an association between low-grade inflammation and vascular risk. The application of CRP and fibrinogen testing in clinical practice requires the estimation of risk across a spectrum of
CRP and fibrinogen levels.\textsuperscript{14,15} To the best of our knowledge, there are limited data regarding the role of new biomarkers in stroke, especially in ischemic stroke, as well as in coronary events. Therefore, we designed this study to compare the values of CRP and fibrinogen levels between patients with a history of the acute coronary syndrome, patients with a history of stroke, and healthy individuals. Subsequently, we examined the value of these markers to predict the 6-month outcome of these cardiovascular events.

**METHODS**

**Study Population**
This case-control cohort study recruited 79 patients with a history of the acute coronary syndrome, 88 patients with a history of stroke (ischemic) occurring at least 3 months previously, and also 77 sex- and age-matched healthy individuals (with normal exercise test and neurological assessments), who were referred to general, cardiovascular, or physical medicine clinics in the Iranian cities of Isfahan and Shahrekord from 2013 to 2014. The main inclusion criteria were age >35 years and the occurrence of cardiovascular or cerebrovascular events occurring at least 3 months previously. In this context, those with a previous history of trauma, inflammatory disorders, chronic rheumatismal disorders, acute febrile or infectious disorders (in the previous 3 months), incomplete clinical or laboratory data, and pregnancy were not included. Also excluded were patients who did not use low-dose statin drugs (anti-inflammatory drugs) and those who could not be followed up. Additionally, patients with CRP levels >10 mg/L were excluded since this may reflect acute inflammation. The diagnosis of the acute coronary syndrome was based on the diagnostic criteria of the American College of Cardiology. Data on sex, age, and history of coronary artery disease were obtained by trained nurses. The data collected were either physical and laboratory examinations (anthropometric testing, blood sample laboratory analysis, and blood pressure measurements) or questionnaires. Weight and height were measured with calibrated instruments under the standard protocol. Waist circumference and hip circumference were measured and recorded in centimeters, using the standard methods.\textsuperscript{16} Blood pressure was measured twice from the right hand.\textsuperscript{17} The study protocol was approved by the Research and Ethics Committee of Isfahan University of Medical Sciences. All the baseline information—including demographic characteristics, anthropometric parameters, systolic or diastolic blood pressure values, oral medications, and laboratory parameters—was collected by reviewing the recorded files. All blood and inflammatory indices were tested after a 12-hour fasting period. The serum levels of fasting blood glucose (FBS) and lipid profile were examined using the enzymatic method. The serum levels of CRP and fibrinogen were also measured using ELISA kits.\textsuperscript{13}

**Study End points**
The primary end point was major adverse cardiac events (MACE), comprising fatal and non-fatal Q wave and non-Q wave myocardial infarction, unstable angina, stroke (ischemic), and re-hospitalization due to cardiovascular causes. Cardiovascular death was considered as any death with a cardiovascular cause such as sudden cardiac death. Myocardial infarction was defined by symptoms suggestive of infarction, electrocardiographic changes, and positive cardiac enzymes. Unstable angina was defined as angina pectoris characterized by at least 1 of the following: occurs at rest or minimal exertion and usually lasts <20 minutes (if nitroglycerin is not administered), is severe and is described as flank pain and of new onset (i.e., within 1 month), and occurs with a crescendo pattern (more severe, prolonged, or with increased frequency than previously).\textsuperscript{18,19}
Stroke was defined as the relative sudden occurrence of a focal neurological deficit. Ischemic stroke was classified by the underlying cause of the vascular occlusion and atherosclerosis with superimposed thrombosis affecting large cerebral or extracerebral blood vessels. The patients were assessed 6 months after initial assessment by telephone follow-up or by periodical visiting to determine the occurrence of fatal or non-fatal myocardial infarction, fatal and non-fatal stroke, sudden cardiac death, unstable angina, need to hospitalization, or any changes in medication.

Statistical Analysis
The data were analyzed using the statistical software SPSS, version 20, for Windows (SPSS Inc., Chicago IL). The quantitative variables are presented as means ± standard deviations, and the categorical variables are presented by absolute frequencies and percentages. The continuous variables were compared using the ANOVA, and the Tukey test was used as post hoc. The categorical variables were compared using the chi-square test. The Cox regression model was used to determine the value of CRP and fibrinogen increased levels for the prediction of MACE in the group with a history of the acute coronary syndrome and the group with a history of stroke. P values ≤0.05 were considered statistically significant.

RESULTS
In total, 244 consecutive patients were assessed: 79 patients with a history of the acute coronary syndrome, 88 patients with a history of stroke occurring at least 3 months previously, and 77 healthy cases as the control. After 5.84±0.62 months of follow-up, 16 subjects had events: 4 patients in the group with a history of stroke, 11 in the group with a history of the acute coronary syndrome, and 1 in the normal group. The 3 groups were similar in terms of sex and age distribution as well as mean body mass index and traditional risk factors for cardiovascular events (Table 1). Regarding laboratory parameters, the mean levels of FBS and LDL were significantly higher in those with a history of the acute coronary syndrome and the healthy individuals, while the former group had lower serum HDL levels than did the other 2 groups. Using the Tukey post-hoc for ANOVA analysis, the serum CRP level was significantly higher in the group with a history of the acute coronary syndrome than in the group with a history of stroke (P=0.045) and in the healthy group (P<0.001). The mean CRP level was also higher in the patients with a history of stroke than in the normal subjects (P=0.012). Also, the level of serum fibrinogen was significantly higher in the group with a history of stroke than in the normal group (P=0.049), while no difference was observed in the fibrinogen level between the groups with a history of the acute coronary syndrome and stroke (P=0.918). Regarding 6-month MACE, the rates of the occurrence of the acute coronary syndrome in the healthy group, the group with previous cardiovascular ischemic events, and those with previous stroke were 1.3%, 12.6%, and 1.1%—with a significant difference (P=0.012). Also, the occurrence of stroke was shown in 3.41% of the patients with previous stroke, while it was not observed in the other 2 groups. Also, sudden cardiac death occurred in 1 patient in the cardiovascular disease group, while none of the patients in the other groups had this acute event.
Can C-Reactive Protein and Fibrinogen Predict Major Adverse Cardiac Events in Cardiovascular Patients? Golshahi J, et al.

Table 1. Baseline characteristics of the study groups

<table>
<thead>
<tr>
<th>Item</th>
<th>Normal (n=77)</th>
<th>Acute Coronary Syndrome (n=76)</th>
<th>Stroke (Cerebral Ischemia) (n=88)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>43 (55.8)</td>
<td>47 (53.4)</td>
<td>38 (45.6)</td>
<td>0.503</td>
</tr>
<tr>
<td>Age(y)</td>
<td>67.82 ± 10.20</td>
<td>64.70 ± 9.34</td>
<td>66.23 ± 12.40</td>
<td>0.444</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.98 ± 4.11</td>
<td>25.44 ± 3.94</td>
<td>26.56 ± 4.63</td>
<td>0.070</td>
</tr>
<tr>
<td>Hypertension</td>
<td>17 (22.1)</td>
<td>30 (34.1)</td>
<td>24 (30.4)</td>
<td>0.322</td>
</tr>
<tr>
<td>Diabetes</td>
<td>6 (7.8)</td>
<td>14 (15.9)</td>
<td>9 (11.4)</td>
<td>0.240</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>19 (24.7)</td>
<td>24 (27.3)</td>
<td>15 (19.0)</td>
<td>0.338</td>
</tr>
<tr>
<td>Smoking</td>
<td>5 (6.5)</td>
<td>12 (13.6)</td>
<td>10 (12.7)</td>
<td>0.458</td>
</tr>
<tr>
<td>History of CAD</td>
<td>3 (3.9)</td>
<td>4 (4.5)</td>
<td>5 (5.1)</td>
<td>0.931</td>
</tr>
<tr>
<td>FBS</td>
<td>106.99 ± 28.67</td>
<td>118.49 ± 32.13</td>
<td>104.43 ± 33.62</td>
<td>0.014</td>
</tr>
<tr>
<td>TC</td>
<td>156.60 ± 40.04</td>
<td>191.37 ± 47.76</td>
<td>185.20 ± 63.00</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>TG</td>
<td>134.15 ± 47.05</td>
<td>155.04 ± 50.61</td>
<td>146.57 ± 66.14</td>
<td>0.072</td>
</tr>
<tr>
<td>LDL</td>
<td>124.38 ± 30.62</td>
<td>131.95 ± 35.79</td>
<td>114.31 ± 29.62</td>
<td>0.004</td>
</tr>
<tr>
<td>HDL</td>
<td>45.76 ± 10.72</td>
<td>41.22 ± 11.14</td>
<td>42.61 ± 9.18</td>
<td>0.027</td>
</tr>
<tr>
<td>CRP</td>
<td>2.92 ± 2.45</td>
<td>5.02 ± 2.45</td>
<td>4.01 ± 2.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>276.56 ± 64.41</td>
<td>302.14 ± 93.52</td>
<td>315.07 ± 76.43</td>
<td>0.030</td>
</tr>
</tbody>
</table>

Abbreviations: CAD, Coronary artery disease; FBS, Fasting blood sugar; TC, Total cholesterol; TG, Triglyceride; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; CRP, C-reactive protein

a: Chi-square test for the categorical variables  
b: ANOVA for the continues variables  
c: Turkey test as a post hoc

Results of the Cox Regression Analysis

The Cox regression model (Table 2) showed increased levels of CRP (HR=1.29 [1.01-1.66]; P=0.038) and fibrinogen (HR=1.01 [1.01-1.02]; P<0.001) in the group with a history of the acute coronary syndrome. It also demonstrated increased levels of CRP (HR=1.61 [0.97-2.67]; P=0.065) and fibrinogen (HR=1.02 [1.01-1.04]; P=0.010) in the stroke group and increased levels of CRP (HR=2.06 [0.71-5.99]; P=0.183) and fibrinogen (HR=1.01 [0.99-1.04]; P=0.294) in the normal group. Consequently, the groups with a history of the acute coronary syndrome and a history of stroke effectively predicted 6-month MACE in the crude and age- and sex-adjusted models.

Table 2. Hazard ratio for the assessment of the amount of CRP and fibrinogen with cardiovascular events in the normal, stroke (cerebral ischemia), and acute coronary crude and age- and sex-adjusted models

<table>
<thead>
<tr>
<th></th>
<th>CRP</th>
<th>Fibrinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR</td>
<td>2.06</td>
<td>2.05</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.71 – 5.99</td>
<td>0.15 – 7.50</td>
</tr>
<tr>
<td>P Value</td>
<td>0.183</td>
<td>0.589</td>
</tr>
<tr>
<td></td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>Past history ACS group</td>
<td>1.30</td>
<td>1.30</td>
</tr>
<tr>
<td>95% CI</td>
<td>1.00 – 2.02</td>
<td>1.00 – 2.02</td>
</tr>
<tr>
<td>P Value</td>
<td>0.046</td>
<td>0.046</td>
</tr>
</tbody>
</table>

DISCUSSION

It is now debated whether increased serum levels of CRP and fibrinogen are considered as prognostic markers of vascular events. A few studies have addressed the relationship between increased levels of these biomarkers and atherosclerosis predisposing patients to vascular events.21,23 In the present study, we first showed high levels of both CRP and fibrinogen markers in the patients with a history of the acute coronary syndrome and the patients with a history of stroke compared with the healthy individuals. Notably, in a multiple regression model including baseline fibrinogen and CRP, we showed higher values of both biomarkers to predict the 6-month outcome, after adjusting for sex and age. In
Fibrinogen as a coagulation factor and acute-phase reactant can activate hemostasis, blood rheology, and platelet aggregation and it, thus, appears to be a mediator in arterial vessel damage.\textsuperscript{24} Furthermore, elevated CRP levels are associated with a profound impairment in systemic endothelial vascular reactivity in patients with coronary artery disease. The blunted systemic endothelial vasodilator function related to elevated plasma CRP levels is independent of classic risk factors for coronary artery disease.\textsuperscript{25} CRP as an acute-phase reactant can enhance and stimulate the production of tissue factor and of interleukin-1 (IL-1) and tumor necrosis factor-a (TNF) by monocytes and macrophages.\textsuperscript{26} It is well-identified that inflammation has a central role in the beginning and progression of atherosclerotic vascular disease.\textsuperscript{27} There is now general agreement that vessel-wall inflammation constitutes a major factor in the development of atherosclerosis, atheroma instability, and plaque disruption followed by local thrombosis.\textsuperscript{28,29} In this regard, we hypothesized that not only could these 2 inflammatory biomarkers predict the presence of ischemic vascular disorders, but also they could strongly predict the adverse outcome of vascular disorders in suspected patients. This predictive role for the 2 biomarkers was successfully confirmed in our survey even after adjusting major risk profiles. Similarly, Coppola et al.\textsuperscript{30} reported that the variables independently associated with non-fatal events included fibrinogen and plasma levels of hs-CRP, while fibrinogen independently associated with fatal events. Also, Grau et al.\textsuperscript{31} showed that patients with a history of cerebrovascular, cardiovascular, or peripheral arterial disease had higher fibrinogen and CRP than did subjects without vascular risk factors. They also found that subjects under the age of 65 with vascular risk factors but without ischemic diseases had higher fibrinogen and CRP and subjects older than 65 with risk factors had higher CRP than subjects without risk factors or ischemic diseases in the same age group.\textsuperscript{32} The treatment of ischemic cardiovascular and cerebrovascular events during the past decade has been improved; however, a substantial risk of death or new vascular events during the first year after the acute episode of these disorders has remained constant.\textsuperscript{33} In this regard, identifying new risk markers can facilitate the risk stratification and selection of individuals who might benefit from intensified therapy as well as facilitate the understanding of pathophysiological mechanisms of the diseases and their adverse outcomes.\textsuperscript{34} It seems that increased levels of acute-phase proteins, including fibrinogen and CRP, have a major role in predicting adverse clinical consequences of these vascular events.\textsuperscript{35,36} The present study revealed a relation between elevated CRP and fibrinogens levels and mid-term risk of death or new vascular events following ischemic stroke or ischemic cardiac disorders. Each biomarker seems to have specific pathophysiological pathways to trigger ischemic events. Elevated CRP levels can affect coagulation through the important role of tissue factor expression.\textsuperscript{37,38} High CRP values can also reflect the extent of the ischemic area.\textsuperscript{39} Obviously, necrosis triggers a rise in the circulating CRP. Fibrinogen has also 2 major roles in progressing ischemic vascular events as an acute-phase inflammatory and coagulative component.\textsuperscript{40} In total, because of the central role of these 2 markers in activating inflammation and coagulation pathways, their role in predicting vascular disorders and their related adverse outcome is expected. We hypothesized that the biomarkers of inflammation or coagulation might be
associated with the pathogenesis of the recurrence of cardiovascular events and sought to investigate whether these biomarkers could provide prognostic information on the risk of developing stroke and coronary artery disease. In fact, both biomarkers can be used to predict recurrent cardiovascular and cerebrovascular events in patients with a history of cardiovascular events or stroke (cerebral ischemia). We were interested in showing the predictive role of these 2 biomarkers or their ratio alongside traditional risk factors such as smoking, diabetes, hyperlipidemia, and hypertension. Thus, anti-inflammatory therapies can result in favorable outcomes in patients considered high-risk based on elevated levels of these markers.

Acknowledgments
This study was a residency thesis, written by S. Khalesi, M.D. and funded by the Research Deputyship of the School of Medicine, Isfahan University of Medical Sciences, Iran. The authors appreciate the mentioned deputyship and the Isfahan Cardiovascular Research Center for their kind cooperation in performing ultrasound and blood tests.

REFERENCES


24. Ernst E, Resch KL. Fibrinogen as a cardiovascular risk factor: a meta-analysis and
Can C-Reactive Protein and Fibrinogen Predict Major Adverse Cardiac Events in Cardiovascular Patients? Golshahi J, et al.


