Original article

Combination of high-sensitivity C-reactive protein and homocysteine may predict an increased risk of coronary artery disease in Korean population

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Keywords: C-reactive protein; homocysteine; fibrinogen; coronary artery disease

Background The association of emerging biomarkers such as high-sensitivity C-reactive protein (hs-CRP), homocysteine and fibrinogen with the risk of coronary artery disease (CAD) is still uncertain in Asian population including Koreans and little is known about the combined effect of biomarkers on the risk of CAD.

Methods A total of 10 650 subjects (6538 men and 4112 women) were enrolled in this study. A 10-year CAD risk was calculated using Framingham risk score modified by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) and levels of circulating hs-CRP, homocysteine and fibrinogen were measured using validated assays.

Results The 10-year CAD risk gradually augmented with increase in the circulating levels of hs-CRP, homocysteine and fibrinogen. For the highest quartile of hs-CRP, odds ratio (OR) of high-risk for CAD (10-year risk ≥ 20%) compared with the lowest quartile was 3.97 (95% CI: 2.51–6.29). For homocysteine and fibrinogen, ORs in the highest quartile compared to the lowest quartile were 5.10 (95% CI: 3.05–8.53, \( P < 0.001 \)) and 1.46 (95% CI: 0.69–3.11, \( P = 0.325 \)), respectively. OR of high-risk for CAD in both the highest quartile of hs-CRP and homocysteine was 9.05 (95% CI: 5.30–15.45) compared with the below median of hs-CRP and homocysteine.

Conclusions The present study demonstrated that hs-CRP and homocysteine are well associated with the 10-year CAD risk estimated using NCEP ATP III in Koreans and combination of hs-CRP and homocysteine can have strong synergy in predicting the development of CAD.

Coronary artery disease (CAD) is one of the leading causes of death in Asian population including Koreans as well as in western countries. Prevention of CAD has to be rooted on proper risk assessment reflecting ethnic variance. Current prevention of CAD by managing modifiable risk factors is based on the Framingham risk model. Complimentary markers may help getting a glimpse of the blind spot of Framingham risk model, for more accurate prediction.

Biomarkers such as high-sensitivity C-reactive protein (hs-CRP), homocysteine and fibrinogen have been emerged for identifying the population with high CAD risk from several cohort studies, independent of the other established risk factors. Recently, U.S. Preventive Services Task Force (USPSTF) have issued that the risk of CAD in the group with CRP >3 mg/L is 1.58-folds higher than that in the group with CRP <1 mg/L, and each 5 μmol/L increase in homocysteine level confers an approximately 9% increase in the risk for CAD events.

In spite of the clinical impact these emerging biomarkers have, few cohort studies or meta-analyses have validated the effect of biomarkers in the prediction of the CAD risk in Asian population. Only few cross-sectional and small sized cohort studies have been conducted to explore the association of individual biomarker and the risk of CAD in Japanese population. Little is known about the combined effect of biomarkers on the CAD risk prediction.

Inflammation, endothelial dysfunction, and platelet aggregation play different roles in pathogenesis of atherosclerosis. Indicators of these phenomena such as hs-CRP, homocysteine and fibrinogen would be associated with the risk of CAD. Therefore, it may be reasonable to investigate the synergy of these biomarkers reflecting different aspects of atherosclerosis progress in association with CAD risk.

The aim of the present study was to investigate the individual and combined association of emerging biomarkers with the risk of CAD, calculated using Framingham risk score (FRS) modified by the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) and levels of circulating hs-CRP, homocysteine and fibrinogen were measured using validated assays. The present study demonstrated that hs-CRP and homocysteine are well associated with the 10-year CAD risk estimated using NCEP ATP III in Koreans and combination of hs-CRP and homocysteine can have strong synergy in predicting the development of CAD.

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Panel III (ATP III) guideline in general population.

METHODS

Study subjects
This study was conducted as a part of periodic health examination program, total 35 617 individuals (20 982 men and 14 635 women) underwent health examination between January 2007 and December 2008 run by Ajou University Hospital. Subjects aged ranging from 20 to 79 years and with hs-CRP measurement, were identified as candidates for the study. Exclusion criteria were persons on statins or other lipid-lowering drugs, immunosuppressants, steroids, anti-inflammatory drugs, oral hormone replacement therapy after menopause, and those with a history or presence of malignancy, or acute or chronic inflammatory diseases. We also excluded any subjects with hs-CRP ≥10.0 mg/L to obviate any possible occult inflammatory or infectious disorder. Finally, 10 650 subjects (6538 men and 4112 women) participated in this study. This study protocol was approved by the Institutional Review Board (IRB) of Ajou University School of Medicine.

Subject’s data collection
A self-reported questionnaire was used to collect the subjects’ data including their past medical history, smoking status, family history of premature CAD, and the prescribed drugs. To calculate body mass index (BMI), height and weight measurements were taken on barefoot in light clothing. After subjects remained sitting for 10 minutes, blood pressure was measured more than two times with a 5-minute rest period between measurements.

Laboratory methods
Total cholesterol and triglyceride concentrations were determined enzymatically using a Beckman analyzer (Beckman Instruments, Brea, USA). High-density lipoprotein (HDL)-cholesterol levels were determined using a Sigma direct EZ-HDL assay. Low-density lipoprotein (LDL)-cholesterol was calculated from total cholesterol, triglycerides, and HDL cholesterol results, using the Friedewald equation. 15 hs-CRP was measured by the high-sensitivity nephelometric method (Dade Behring Marburg GMBH, Marburg, Germany). The lower detection limit of this method was 0.175 mg/L and the intra-assay coefficient of variation was <5%. Plasma total homocysteine concentration was measured by ion-exchange chromatography and fibrinogen level was determined using enzyme assay methods.

Calculation of 10-year CAD risk
A 10-year CAD risk was calculated using FRS modified by the NCEP ATP III guideline that used major independent risk factors including cigarette smoking, blood pressure, HDL-cholesterol, family history of premature CAD, age and type-2 diabetes. 2 Individual risk factor scores were assigned on the basis of age, total cholesterol, systolic blood pressure, HDL-cholesterol, and smoking status, and were summed to determine the 10-year absolute risk.

Statistical analysis
For continuous variables, values were presented as mean ± standard deviation (SD) or median (inter-quartile range), as appropriate. Categorical data were expressed as the number of subjects (percentage). To assess the relationship between biomarkers and the individual components of the FRS, Pearson correlation coefficients relating individual risk factor scores and the total FRS to the natural log of biomarkers levels were calculated. The study subjects were grouped into quartiles according to the circulating levels of hs-CRP, homocysteine and fibrinogen. Kruskal-Wallis test was used to test the difference in the 10-year CAD risk among quartiles of biomarkers, followed by the post hoc Bonferroni test for multiple comparisons (Bonferroni-adjusted $P < 0.017$ was considered statistically significant). Multiple Logistic regression analysis was performed to determine the odds ratio (OR) of high-risk for CAD (10-year risk ≥20%) in each quartile of biomarkers, before and after adjusting for LDL-cholesterol, triglyceride, BMI and diastolic blood pressure. The combined effect of biomarkers which were statistically significant in the individual analyses was also investigated by using of multiple Logistic regression analysis. All tests were 2-tailed. Statistical analyses were performed using the SPSS software version 12.0.1 (SPSS Inc., Chicago, IL, USA).

RESULTS

Compared with women, men had more CAD and CAD risk factors such as diabetes mellitus, hypertension, dyslipidemia and current smoking (Table 1). Therefore, total Framingham point score and 10-year CAD risk were also higher in men than in women.

The correlation coefficient between the 10-year CAD risk and log hs-CRP was $r=0.20$ ($P <0.001$) in men and $r=0.21$ ($P <0.001$) in women (Table 2). Log hs-CRP was well correlated with individual risk factors as well. The correlation coefficient between the 10-year CAD risk and log homocysteine was $r=0.09$ ($P <0.001$) in men and $r=0.22$ ($P <0.001$) in women and those between the 10-year CAD risk and log fibrinogen was $r=0.22$ ($P <0.001$) in men and $r=0.15$ ($P <0.001$) in women (data not shown).

The 10-year CAD risk augmented with an increase in the quartile of each biomarkers ($P <0.001$ for trend) (Figure 1). The 10-year CAD risk in the 4th quartile of hs-CRP and homocysteine was significantly higher than those in the other three quartiles ($P <0.001$). However fibrinogen level failed to demonstrate this association with 10-year CAD risk.

The OR for 10-year CAD risk ≥20% in 4th quartile of hs-CRP, homocysteine and fibrinogen compared to 1st
**Table 1.** Baseline characteristics and laboratory profiles of study subjects (n=10 650)

<table>
<thead>
<tr>
<th>Items</th>
<th>Men (n=6538)</th>
<th>Women (n=4112)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>46.7±10.5</td>
<td>47.3±11.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.5±2.9</td>
<td>23.0±3.1</td>
</tr>
<tr>
<td>CAD (n %)</td>
<td>113 (1.7)</td>
<td>38 (0.9)</td>
</tr>
<tr>
<td>Stroke (n %)</td>
<td>22 (0.3)</td>
<td>15 (0.4)</td>
</tr>
<tr>
<td>Diabetic (n %)</td>
<td>505 (7.7)</td>
<td>207 (5.0)</td>
</tr>
<tr>
<td>Hypertension (n %)</td>
<td>1220 (18.7)</td>
<td>611 (14.9)</td>
</tr>
<tr>
<td>Family history of premature CAD (n %)</td>
<td>49 (0.8)</td>
<td>28 (0.7)</td>
</tr>
<tr>
<td>Current smoker (n %)</td>
<td>2880 (44.1)</td>
<td>210 (5.1)</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>122.0±13.6</td>
<td>117.3±15.2</td>
</tr>
<tr>
<td>Diastolic</td>
<td>80.9±10.4</td>
<td>74.1±10.7</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>4.94±0.86</td>
<td>4.84±0.91</td>
</tr>
<tr>
<td>LDL</td>
<td>2.90±0.80</td>
<td>2.81±0.82</td>
</tr>
<tr>
<td>HDL</td>
<td>1.32±0.31</td>
<td>1.55±0.35</td>
</tr>
<tr>
<td>hs-CRP (mg/L, mean (interquartile range))</td>
<td>0.7 (0.4–1.5)</td>
<td>0.5 (0.2–0.9)</td>
</tr>
<tr>
<td>Homocysteine (μmol/L, mean (interquartile range))</td>
<td>12.3 (10.7–14.4)</td>
<td>9.4 (8.1–10.8)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In the present study, 10-year CAD risk gradually augmented with increasing in the circulating levels of hs-CRP, high-sensitivity C-reactive protein. HS-CRP: high-sensitivity C-reactive protein. LDL: low-density lipoprotein. BP: blood pressure. CAD: coronary artery disease.

The study subjects were also divided into nine groups (1st & 2nd quartile, 3rd quartile, 4th quartile) according to the hs-CRP and homocysteine levels which were statistically significant in the individual analyses (Figure 2). The reference category was having both 1st & 2nd quartile of hs-CRP and homocysteine. Taking hs-CRP and homocysteine into consideration together, strengthened their association with for 10-year CAD risk ≥20%. Adjusted OR of high-risk for CAD (10-year risk ≥20%) in both 4th quartile of hs-CRP and homocysteine was 9.05 (95% CI: 5.30–15.45) compared with the reference group.

**Table 2.** Pearson correlation coefficients relating individual components and the total Framingham point score to the natural log of hs-CRP levels

<table>
<thead>
<tr>
<th>Variables</th>
<th>Men (n=6424)</th>
<th>Women (n=4074)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age score</td>
<td>0.07</td>
<td>0.28</td>
</tr>
<tr>
<td>Total cholesterol score</td>
<td>0.08</td>
<td>0.03</td>
</tr>
<tr>
<td>HDL-C score</td>
<td>–0.20</td>
<td>–0.21</td>
</tr>
<tr>
<td>Smoking score</td>
<td>0.02</td>
<td>0.00</td>
</tr>
<tr>
<td>Systolic BP score</td>
<td>0.12</td>
<td>0.24</td>
</tr>
<tr>
<td>Total Framingham point score</td>
<td>0.18</td>
<td>0.32</td>
</tr>
<tr>
<td>10-year CAD risk</td>
<td>0.20</td>
<td>0.21</td>
</tr>
</tbody>
</table>

**Figure 1.** The 10-year CAD risks are different among quartiles of hs-CRP (A), homocysteine (B) and fibrinogen (C). CAD: coronary artery disease. hs-CRP: high-sensitivity C-reactive protein. NS: not significant. Q1: 1st quartile. Q2: 2nd quartile. Q3: 3rd quartile. Q4: 4th quartile.
hs-CRP, homocysteine and fibrinogen. Highest quartile of hs-CRP and homocysteine showed higher OR of high-risk for CAD (10-year risk ≥20%) significantly, but not with fibrinogen. These associations remained significant after adjustment for the LDL-cholesterol, triglyceride, BMI and diastolic blood pressure which were not used in the NECP ATP III but major traditional coronary risk factors. The use of a combination of hs-CRP and homocysteine showed strong synergy in predicting the development of CAD.

Individual correlations of circulating levels of hs-CRP and homocysteine, and FRS in the present study are in close agreement with those of earlier studies, which used NECP ATP II guideline.11,16 No significant OR of high-risk for CAD in the highest quartile of fibrinogen was found in this study, although the 10-year CAD risk was gradually increased with an increase in the fibrinogen. Fibrinogen levels have been consistently associated with several conventional CAD risk factors, and when these risk factors were included in multivariate analyses, the association between fibrinogen and CAD was attenuated but remained statistically significant.17,18 These disagreement with the previous reports about the association of fibrinogen and CAD risk may be due to the small sample size in the fibrinogen measured group in relation to the total study subjects.

Several studies have confirmed the close relationship between atherosclerosis and inflammatory condition.19,20 The interactions between leukocytes and endothelial cells in the process of atherosclerosis can promote the release of various cytokines, which stimulate hepatic synthesis of CRP and fibrinogen.21 Homocysteine can promote CAD by means of direct cytotoxic effects on the endothelium, increased adhesiveness of the platelets and effects on clotting cascade.22 Results of the present study are consistent with these proposed hypotheses by showing that circulating levels of hs-CRP and homocysteine were significantly higher in the high-risk group for CAD.

Our study also demonstrated that median hs-CRP levels were low in Korean population. Similar findings have been reported from several studies which included Asian population as subjects.11,23,24 The American Heart Association and the Centers for Disease Control and Prevention (AHA/CDC) have defined high CAD risk as CRP >3 mg/L and low risk as <1 mg/L, from the meta-analysis of prospective population-based studies, which mainly conducted in White and Black population.25 In view of the increasing evidence of ethnic difference in hs-CRP level, lower cut-off point for high-risk for future CAD should be developed for Asian population.

This study has several strengths. One of the strengths is large number of study subjects which covers both sex and all age group. Therefore, the results can be generalized into the entire Korean population. Second, we excluded any subjects with prescribed medications or diseases that could affect the circulating levels of biomarkers to control the possible confounding factors. Third, we used the categories of the estimated 10-year CAD risk calculated by using the NCEP ATP III, which is considered as a current standard for prediction of CAD risk. This study also has some limitations. We collected parts of subjects’ past medical history from self-reported questionnaires. Under-reporting should be considered in the smoking status, family history of premature CAD and use of over-the-counter drugs and vitamin supplements. In the correlation between hs-CRP and individual components of the FRS, negative correlation was observed in the smoking score in women. This finding could be attributed to the under-reporting of smoking status. Second, we used a cross-sectional design. Therefore the results could not provide sufficient information to the sub-group with low LDL-cholesterol (<100 mg/dl) which is a therapeutic goal in NCEP ATP III, but have elevated biomarkers.

In conclusion, the present study demonstrated that hs-CRP and homocysteine are associated with the estimated 10-year CAD risk calculated by using NCEP ATP III in Korean population and the use of a combination of hs-CRP and homocysteine appears to have additional benefit in the prediction of future development of CAD. Further longitudinal cohort studies are needed to evaluate the predictive value of biomarkers for the risk of CAD in Korean population.

REFERENCES


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