Biomarkers of Asymptomatic Carotid Stenosis in Patients Undergoing Coronary Artery Bypass Grafting

Suk Jae Kim, MD; Pamela Song, MD; Jae Hyun Park, MD; Young Tak Lee, MD; Wook Sung Kim, MD; Yun Gyoung Park, MD; Oh Young Bang, MD; Chin-Sang Chung, MD; Kwang Ho Lee, MD; Gyeong-Moon Kim, MD, PhD

Background and Purpose—Carotid artery stenosis is an important etiologic factor of stroke related to coronary artery bypass surgery. We evaluated clinical and laboratory factors to identify biomarkers for pre-existing carotid artery stenosis in patients undergoing coronary artery bypass surgery.

Methods—Between June 2006 and September 2008, 811 patients aged ≥50 years underwent preoperative carotid artery duplex scanning as part of a preoperative assessment for nonemergency cardiac procedures. Of these, 54 patients with previous stroke or transient ischemic attack were excluded. The association between various biomarkers and carotid artery stenosis was analyzed by multiple logistic regression analysis. The receiver operating characteristic curves were generated and analyzed to compare diagnostic performance and optimum diagnostic cutoff levels of biomarkers.

Results—A total of 757 patients was included in the study. The prevalence of asymptomatic carotid stenosis of ≥50% and ≥70% was 26.4% and 8.6%, respectively. In multivariate analysis, plasma levels of apolipoprotein B (apoB):apoA-I, lipoprotein(a), and homocysteine were independently associated with carotid stenosis of ≥50%: the OR (95% CI) for apoB/apoA-I, lipoprotein(a), and homocysteine in the highest versus lowest quartile was 2.07 (1.18 to 3.66), 2.17 (1.16 to 4.05), and 2.13 (1.20 to 3.79), respectively. Receiver operating characteristic curve analysis indicated area under the curve values of 0.708 (apoB:apoA-I), 0.678 (lipoprotein[a]), and 0.689 (homocysteine). The sensitivity, specificity, positive and negative predictive values (%) for diagnosis of carotid stenosis ≥50% were 80.0, 50.4, 38.0, and 86.9 for apoB:apoA-I; 47.0, 78.9, 46.1, and 79.5 for lipoprotein(a); and 69.3, 62.1, 41.2, and 84.1 for homocysteine, respectively.

Conclusion—Our findings indicated that plasma levels of apoB/apoA-I, lipoprotein(a), and homocysteine can predict asymptomatic carotid stenosis in patients undergoing coronary artery bypass surgery. (Stroke. 2011;42:00-00.)

Key Words: apoB:apoA-I ■ biomarker ■ CABG ■ carotid stenosis ■ homocysteine ■ lipoprotein(a)

Stroke remains a major cause of morbidity and mortality after coronary artery bypass graft (CABG). The incidence of cerebral infarction after CABG is estimated to be 2%.1 Cerebral embolization from a carotid plaque or intracardiac clot and a decrease in perfusion pressure to <60 mm Hg are the etiologic causes of stroke associated with CABG.2 Detecting carotid stenosis in patients undergoing CABG is important because it can be diagnosed preoperatively and managed concomitantly or sequentially.3-5

Several studies have been conducted to find risk factors of carotid stenosis using carotid duplex ultrasonography before CABG. These investigations have suggested that a history of stroke, peripheral arterial disease, a cervical bruit, and age >70 years are strong predictors of significant carotid disease.6-9

In this study, we analyzed patients with and without asymptomatic carotid artery stenosis detected by preoperative duplex ultrasonography who were scheduled for CABG to identify which biomarkers were associated with carotid artery stenosis.

Patients and Methods

Our analysis was performed using data collected from a prospective registry of patients admitted to Samsung Medical Center from June 2006 to September 2008. Patients who underwent scheduled CABG during this time period were included if they were aged ≥50 years. Before surgery, carotid screening for stenosis of the internal carotid artery was performed. Fifty-four patients with previous stroke or transient ischemic attack and 51 patients with age <50 years were excluded. Of the eligible 831 subjects, 757 (91.1%) patients agreed to participate in the study. Informed consent was obtained from all subjects and the local Institutional Review Board approved this study.

Carotid Screening

B-mode and duplex ultrasonography of the bilateral carotid arteries were performed for carotid screening by the same radiologist before CABG. Criteria for establishing carotid stenosis on duplex scanning were as follow:10: (1) <50% stenosis when internal carotid artery peak systolic velocity is <125 cm/s; (2) 50 to 69% stenosis when internal carotid artery peak systolic velocity is 125 to 230 cm/s and...
plaque is visible; (3) 70% to 99% stenosis when internal carotid artery peak systolic velocity is >230 cm/s and visible plaque and lumen narrowing are seen or there is a markedly narrowed lumen on color Doppler ultrasonography regardless of internal carotid artery peak systolic velocity; and (4) occlusion when there is no detectable patent lumen on gray-scale ultrasonography and no flow on spectral, power, and color Doppler ultrasonography.

**Plasma Lipid Profiles and Biomarker Assessment**

Blood samples were drawn after an overnight fast and centrifuged within 1 hour after collection. Plasma total cholesterol (T-chol) and triglyceride were analyzed by enzymatic procedures with diagnostic kits from Sigma Chemical Co (St Louis, MO). Total high-density lipoprotein cholesterol (HDL-C) was measured after precipitation of apolipoprotein (apo) B-containing lipoproteins, very low-density lipoprotein, and low-density lipoprotein with dextran-sulfate/Mg solution. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation (LDL-C = T-chol - HDL-C - 0.2 x triglyceride). In addition to T-chol and its subfractions, serum levels of low-density lipoprotein and high-density lipoprotein constituents, apoB and apoA-I, were also determined by nephelometry (Behring Nephelometer, Marburg, Germany). Plasma lipoprotein(a) was measured by an enzyme-linked immunosandwich assay method using a lipoprotein(a) kit (Immunozym Lp[a], Heidelberg, Germany). Plasma homocysteine levels were determined by the method of Vester and Rasmussen. The reductant, derivatizing agent, internal standard, and DL-homocysteine were obtained from Sigma.

**Statistical Analyses**

Statistical analyses were performed using a commercially available software package (SPSS Version 13.0; SPSS Inc, Chicago, IL). Pearson χ² or Fisher exact test was used to compare the demographic profiles and vascular risk factors between patients with <50% and ≥50% carotid stenosis. T-chol, triglyceride, HDL-C, LDL-C, non-HDL-C (T-chol - HDL-C), apoB, apoA-I, and lipoprotein(a) were assessed continuously. The following ratios were also calculated and assessed continuously: T-chol:HDL-C, LDL-C:HDL-C, and apoB:apoA-I. Mann-Whitney U test was conducted to compare these parameters between the 2 groups.

Multivariate logistic regression analysis was performed to identify the association between biomarkers and ≥50% carotid stenosis controlling for other pertinent lipid parameters, age, sex, vascular risk factors (hypertension, diabetes, hyperlipidemia, smoking habits), and premorbid medications (antiplatelet agents and statins). The
Results

A total of 757 patients (545 men and 212 women; aged 65.1±7.5 years; range, 50 to 87 years) were included in this study. The prevalence of asymptomatic carotid stenosis is summarized in Figure 1. Overall, 26.4% of the patients undergoing CABG had a 50% to 99% stenosis or occlusion (Figure 1A). Approximately 1 in 5 (18.5%) had a unilateral 50% to 99% stenosis, whereas 3.3% had carotid occlusion. The association between carotid stenosis and homocysteine was also found more frequently in patients with carotid stenosis (P=0.001). Other factors, including sex, diabetes, hyperlipidemia, smoking habits, and premorbid medications, including antiplatelet agents and statins were not significantly different between the 2 groups.

Table 2 shows the median (interquartile range) for each of the lipid parameters and biomarkers stratified by presence of carotid artery stenosis. Although lipid parameters were not different between the 2 groups, patients with carotid stenosis had higher lipoprotein(a) and homocysteine. The median apoB and apoA-I levels were not significantly different between the 2 groups; however, apoB:apoA-I ratios were higher in patients with carotid stenosis. The distribution of each biomarker is demonstrated in Figure 2. Patients with carotid stenosis had a higher quartile of apoB:apoA-I (P=0.034 for trends), lipoprotein(a) (P=0.004 for trends), and homocysteine (P<0.001 for trends).

Table 3 shows the OR and 95% CI for the relationship between each of the biomarkers and asymptomatic carotid stenosis in the multivariate-adjusted models. After adjusting for covariates, asymptomatic carotid stenosis ≥50% was associated with apoB:apoA-I (OR for highest quartile versus lowest quartile, 2.07; 95% CI, 1.18 to 3.66), lipoprotein(a) (OR for highest quartile versus lowest quartile, 2.17; 95% CI, 1.16 to 4.05) and homocysteine (OR for third quartile versus lowest quartile, 1.80; 95% CI, 1.10 to 3.21; OR for highest quartile versus lowest quartile, 2.13; 95% CI, 1.20 to 3.79), but not apoA-I only (OR for lowest quartile versus highest quartile, 1.10; 95% CI, 0.64 to 1.89). The trend tests suggest that the association between carotid stenosis and homocys-

<table>
<thead>
<tr>
<th>Table 1. Baseline Characteristics According to Carotid Artery Stenosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid Artery Stenosis &lt;50%</td>
</tr>
<tr>
<td>No. of patients</td>
</tr>
<tr>
<td>Age, years</td>
</tr>
<tr>
<td>Male sex</td>
</tr>
</tbody>
</table>

Values are median (interquartile range) or no. (percent).

Additionally, the receiver operating characteristic curves were generated and analyzed to compare diagnostic performance and optimum diagnostic cutoff levels of apoB:apoA-I, lipoprotein(a), and homocysteine. The area under the curve values were analyzed by pairwise comparison of receiver operating characteristic curves (MedCalc for Windows, Version 9.3; MedCalc Software, Mariakerke, Belgium). P<0.05 was considered statistically significant.

Table 2. Laboratory Findings According to Carotid Artery Stenosis

<table>
<thead>
<tr>
<th>Lipid parameters (N=722)</th>
<th>Carotid Artery Stenosis &lt;50%</th>
<th>Carotid Artery Stenosis ≥50%</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-chol, mg/dL</td>
<td>162 (135.5–192.5)</td>
<td>162 (138–192.5)</td>
<td>0.733</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>130 (96–182)</td>
<td>126 (91.5–170.5)</td>
<td>0.492</td>
</tr>
<tr>
<td>HDL-C, mg/dL</td>
<td>41 (35–48)</td>
<td>41 (33–48.5)</td>
<td>0.940</td>
</tr>
<tr>
<td>LDL-C, mg/dL</td>
<td>102 (80–126.5)</td>
<td>104 (80.5–127.5)</td>
<td>0.503</td>
</tr>
<tr>
<td>Non-HDL-C, mg/dL</td>
<td>122 (94.25–149)</td>
<td>122 (95.5–149.75)</td>
<td>0.849</td>
</tr>
<tr>
<td>T-chol:HDL-C</td>
<td>3.89 (3.17–4.87)</td>
<td>3.89 (3.20–5.05)</td>
<td>0.752</td>
</tr>
<tr>
<td>Low-density lipoprotein:high-density lipoprotein</td>
<td>2.47 (1.91–3.21)</td>
<td>2.55 (1.93–3.46)</td>
<td>0.349</td>
</tr>
</tbody>
</table>

Values are median (interquartile range).

Biomarkers from univariate analyses at P<0.1 were considered to represent possible explanatory variables and were entered into the multivariate analysis. Lipoprotein(a) was adjusted for HDL-C and triglyceride and apoB:apoA-I was adjusted for triglyceride. We also included interaction terms of statin use and apoB:apoA-I or lipoprotein(a) for exploring potential heterogeneity by statin therapy. For multivariate testing, apoB:apoA-I, lipoprotein(a), and homocysteine were categorized as quartiles. Additionally, the receiver operating characteristic curves were generated and analyzed to compare diagnostic performance and optimum diagnostic cutoff levels of apoB:apoA-I, lipoprotein(a), and homocysteine. The area under the curve values were analyzed by pairwise comparison of receiver operating characteristic curves (MedCalc for Windows, Version 9.3; MedCalc Software, Mariakerke, Belgium). P<0.05 was considered statistically significant.
tein may be dose-dependent, whereas the increased risk of stenosis appears limited to those in the fourth quartile for apoB:apoA-I and lipoprotein without an apparent dose–response relationship.

The comparative receiver operating characteristic curves for apoB:apoA-I, lipoprotein(a), homocysteine, and a combination of them are provided in Figure 3. A detailed figure for the area under the curve and other measurements of diagnostic performance (sensitivity, specificity, positive and negative predictive values) are also demonstrated in Supplemental Table I (available at http://stroke.ahajournals.org). The mean ± SE of the area under the curve for a combination of 3 biomarkers was 0.735 ± 0.025, significantly greater than that of apoB:apoA-I (0.708 ± 0.025; P = 0.039), lipoprotein(a) (0.678 ± 0.026; P = 0.004), and homocysteine alone (0.689 ± 0.026; P = 0.009), whereas no statically significant difference was found between each biomarker (P > 0.05).

Optimum diagnostic cutoff levels were identified from the receiver operating characteristic curves for apoB:apoA-I (> 0.99), lipoprotein(a) (> 60 mg/dL), and homocysteine (> 15 μg/mol). The sensitivity, specificity, positive predictive value, and negative predictive value (%) for diagnosis of carotid stenosis ≥ 50% at these levels were 80.0, 50.4, 38.0, and 86.9 for apoB:apoA-I; 47.0, 78.9, 46.1, and 79.5 for lipoprotein(a); and 69.3, 62.1, 41.2, and 84.1 for homocysteine, respectively.

Discussion

Coronary and carotid atherosclerosis tend to occur together.13 Previous studies have suggested that the prevalence of carotid stenosis ≥ 50% in patients undergoing CABG ranges from 2.2% to 22%.1,7 The prevalence of carotid stenosis increases with age,14 and we only included patients aged ≥ 50 years in this study, which may explain why the prevalence of carotid stenosis ≥ 50% was higher than in previous reports.

Many studies have demonstrated that patients with significant carotid stenosis have an increased risk of stroke after CABG.1 The accepted risk factors of significant carotid stenosis are old age, carotid bruit on physical examination, female sex, previous stroke or transient ischemic attack, peripheral vascular stenosis, hypertension, diabetes mellitus, history of smoking, hypercholesterolemia, and left main coronary disease.14–20 In this study, we found new risk markers of carotid stenosis ≥ 50% in patients undergoing CABG.

We demonstrated that apoB:apoA-I, but not conventional lipid parameters, can predict the risk of asymptomatic significant carotid stenosis in patients with coronary artery disease. Recent studies have shown that the risk of cardiovascular diseases is strongly related to the balance between the proatherogenic apoB lipoprotein particles and the antiatherogenic apoA-I particles.21–27 These studies have also suggested that this ratio is better than the conventionally used LDL-C and various other lipid ratios. In terms of carotid atherosclerosis, apoB:apoA-I level was related to the progression of carotid atherosclerosis in healthy subjects as well as carotid plaque progression in a population-based cohort.28,29

Lipoprotein(a) particles, which have a similar structure to low-density lipoprotein, are susceptible to oxidative modification and contain apo(a), a unique apolipoprotein that has structural homology with plasminogen; therefore, lipoprotein(a) could have proatherogenic and antifibrinolytic effects.30–32 Klein et al showed that lipoprotein(a) was a significant predictor of carotid stenosis and occlusion but not of carotid plaque.33 The antifibrinolytic effect of lipoprotein(a) may explain these results. Carotid stenosis and occlusion may not be attributable to plaque progression, but rather to plaque rupture and thrombosis. We think that our results provide further evidence of the association between lipoprotein(a) and carotid stenosis.

Homocysteine has been suggested as a risk factor for atherosclerosis.34 The proposed mechanisms responsible for its effects include endothelial dysfunction, increased oxidative stress, impairment of flow-mediated endothelium-derived relaxing factor with a subsequent reduction in arterial

Figure 2. Distribution of biomarkers according to carotid stenosis. Q indicates quartile.
vasodilation, proliferation of smooth muscle cells, and platelet activation.35–37 The association of plasma homocysteine concentration with carotid artery atherosclerosis is well known.38–40 The findings of our study suggest that plasma homocysteine levels are also a useful predictor of asymptomatic carotid stenosis in patients with a high risk of carotid atherosclerosis.

An important finding of receiver operating characteristic curve analysis is that of a high negative predictive value for asymptomatic carotid stenosis ≥50% in patients undergoing CABG for apoB:apoA-I, lipoprotein(a), and homocysteine. In a clinical setting, these biomarkers could be used for excluding high-risk patients of stroke after CABG. On the other hand, a low positive predictive value of the biomarkers raises concern about their effectiveness. Carotid ultrasonography is a noninvasive and sensitive method to evaluate carotid artery lesions.41 Hence, if carotid ultrasonography is readily available, the biomarkers study may be unnecessary at the time of presurgical evaluation. However, in terms of management for carotid disease, the biomarkers could be still useful because apoB:apoA-I, lipoprotein(a), and homocysteine are associated with progression of carotid atherosclerosis or occurrence of stroke.28,29,42,43

It is important to note that this study had several limitations. First, this single-center study was conducted on Korean patients only. Multicenter trials with diverse races/ethnicities are warranted to confirm the possible association of carotid stenosis with apoB:apoA-I, lipoprotein(a), and homocysteine in patients undergoing CABG. Second, we used a single measurement of the biomarkers rather than several measurements over time. However, most other studies that have been conducted to date used a similar approach. Lastly, patients in this study were not followed for outcome, limiting our ability to make broad recommendations.

In conclusion, apoB:apoA-I, lipoprotein(a), and homocysteine are useful markers for predicting asymptomatic carotid stenosis in patients undergoing CABG. Creation of a cost-effective algorithm with these new biomarkers is warranted to screen for carotid artery stenosis in this patient population.

**Table 3. Association Between Biomarkers and Carotid Artery Stenosis ≥50%, Adjusted for Demographics, Vascular Risk Factors, and Premorbid Medications**

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted OR (95% CI)</th>
<th>Adjusted for Age and Sex OR (95% CI)</th>
<th>Adjusted for Multiple Risk Factors* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoB:ApoA-I†</td>
<td>1.06 (1.17–2.04)</td>
<td>1.19 (1.08–2.10)</td>
<td>2.07 (1.18–3.66)</td>
</tr>
<tr>
<td>Lipoprotein(a), mg/dL‡</td>
<td>1.25 (1.30–2.26)</td>
<td>1.05 (1.54–2.05)</td>
<td>2.17 (1.16–4.05)</td>
</tr>
<tr>
<td>Homocysteine, μmol/L</td>
<td>2.14 (2.15–3.66)</td>
<td>2.17 (1.16–4.05)</td>
<td>2.17 (1.16–4.05)</td>
</tr>
</tbody>
</table>

*Age, sex, hypertension, diabetes, hyperlipidemia, smoking habits, and premorbid antiplatelet/statin use.
†Additionally adjusted for triglyceride.
‡Additionally adjusted for HDL-C and triglyceride.
§P for trends.

**Figure 3.** Predicting asymptomatic carotid stenosis ≥50% from apoB:apoA-I, lipoprotein(a), homocysteine, and their combination. area under the curve±SE was 0.735±0.025 for a combination of 3 biomarkers, which was greater than that of apoB:apoA-I (0.708±0.025; P=0.039), lipoprotein(a) (0.678±0.026; P=0.004), and homocysteine alone (0.689±0.026; P=0.008).
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13. Koschinsky ML. Lipoprotein(a) and the link between atherosclerosis and thrombosis. Can J Cardiol. 2004;20(suppl B):37B–43B.


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SUPPLEMENTAL METERIAL

Supplemental table. ROC curve analysis statistics for apoB:apoA-I, lipoprotein, homocysteine and their combination

<table>
<thead>
<tr>
<th></th>
<th>ApoB:ApoA-I (N=587)</th>
<th>Lipoprotein(a) (N=597)</th>
<th>Homocysteine (N=593)</th>
<th>Combination (N=570)</th>
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<tbody>
<tr>
<td>AUC</td>
<td>0.708</td>
<td>0.678</td>
<td>0.689</td>
<td>0.735</td>
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<tr>
<td>Optimum cut-off</td>
<td>0.99</td>
<td>60 mg/dL</td>
<td>15 μmol/L</td>
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<tr>
<td>Sensitivity</td>
<td>80.0%</td>
<td>47.0%</td>
<td>69.3%</td>
<td>68.6%</td>
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<tr>
<td>Specificity</td>
<td>50.4%</td>
<td>78.9%</td>
<td>62.1%</td>
<td>70.1%</td>
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<tr>
<td>Positive predictive value</td>
<td>38.0%</td>
<td>46.1%</td>
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<tr>
<td>Negative predictive value</td>
<td>86.9%</td>
<td>79.5%</td>
<td>84.1%</td>
<td>85.5%</td>
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</table>

See text for abbreviations