Remnant Lipoprotein Levels in Fasting Serum Predict Coronary Events in Patients With Coronary Artery Disease

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Background—Remnant lipoproteins are atherogenic, but assays of remnants have not been available in routine clinical laboratories because of the lack of practical and validated methods. A simple and reliable method for such an assay, using an immunochemical approach, has recently been developed. This study prospectively examined whether remnant lipoprotein levels in fasting serum, measured by our method, may have prognostic value in patients with coronary artery disease (CAD).

Methods and Results—Remnant lipoprotein levels in fasting serum were measured in 135 patients with CAD by an immunoaffinity mixed gel containing anti-apolipoprotein (apo) A-1 and anti–apoB-100 monoclonal antibodies. Patients were followed up for \( \geq 36 \) months until occurrence of 1 of the following clinical coronary events: recurrent or refractory angina pectoris requiring coronary revascularization, nonfatal myocardial infarction, or cardiac death. Kaplan-Meier analysis demonstrated a significantly higher probability of developing coronary events in patients with the highest tertile of remnant levels (>5.1 mg cholesterol/dL; 75th percentile of distribution of remnant levels) than in those with the lowest tertile of remnant levels (<3.3 mg cholesterol/dL; 50th percentile of the distribution). Higher levels of remnants were a significant and independent predictor of developing coronary events in multivariate Cox hazard analysis including the following covariates: extent of coronary artery stenosis, age, sex, smoking, hypertension, diabetes mellitus, hypercholesterolemia, low HDL cholesterol, and hypertriglyceridemia.

Conclusions—Higher levels of remnant lipoproteins in fasting serum predict future coronary events in patients with CAD independently of other risk factors. Thus, measurement of fasting remnant levels, assessed by the current immunoseparation method, may be helpful in assessment of CAD risk. (Circulation. 1999;99:2858-2860.)

Key Words: atherosclerosis □ coronary disease □ lipoproteins □ prognosis □ risk factors

R emnant lipoproteins, derived from VLDL and chylomicrons, are considered atherogenic. However, a simple and reliable method to isolate remnant lipoproteins has not been available because remnant lipoproteins have heterogeneous properties. Nakajima et al have recently developed a technique to isolate remnant-like lipoproteins using an immunoaffinity mixed gel containing anti-apolipoprotein (apo) A-1 and anti–apoB-100 monoclonal antibodies. This unique anti–apoB-100 monoclonal antibody has been shown to recognize apoB-100 in LDL and most VLDL but not in apoE-enriched VLDL. We and others have shown that this technique can isolate apoE-rich VLDL particles containing apoB-48, neither of which binds to the immunoaffinity gel. We further demonstrated that high levels of remnant lipoproteins, as assessed by this method, directly induced endothelial vasomotor dysfunction, an early sign of atherosclerosis, and were independently associated with the presence of myocardial infarction. In the present study, we prospectively examined whether remnant lipoprotein levels, measured by this method, may have predictive value for future coronary events in patients with coronary artery disease (CAD) and considered the possible validity of this assay in risk assessment of CAD.
disease, 40 patients; 3-vessel disease, 37 patients; and left main coronary artery disease, 12 patients). Written informed consent was obtained from all patients before the study. This study was conducted in agreement with guidelines approved by the ethics committee at our institution.

Measurement of Lipoproteins
At the beginning of the study, venous blood was taken in all patients after a 12-hour overnight fast. All patients ate a standard meal (1900 kcal/d; 25% fat, 59% carbohydrate, and 16% protein) the day before the sampling. Serum was stored at 4°C and was used for the assays within 3 days after sampling. Remnant lipoproteins were isolated by application of the fasting serum to the immunoaffinity mixed gel, which contained anti–apoA-1 and anti–apoB-100 monoclonal antibodies (Japan ImmunoResearch Laboratories), and the unbound fraction containing apoE-enriched lipoproteins was eluted with PBS, as described in our previous reports. This assay takes 2 hours to complete. Cholesterol concentrations in the unbound fraction were measured by the enzymatic method. Levels of HDL cholesterol, LDL cholesterol, and triglycerides in fasting serum were measured as described previously.

Follow-Up Study
After laboratory samples and angiographic data were obtained, the 147 patients with CAD were followed up every month in the hospital or with a clinic visit for ≤36 months until the occurrence of 1 of the following clinical coronary events: recurrent or refractory angina pectoris requiring coronary revascularization by PTCA or CABG, nonfatal myocardial infarction, or cardiac death. All patients received standardized medical therapy. Time to first coronary event was evaluated prospectively. Diagnosis of myocardial infarction was made by chest pain, appearance of new Q wave on the ECG, and elevation of creatinine kinase enzymes to more than twice the upper limit of normal. Cause of death was determined from hospital records.

Statistical Analysis
The Kaplan-Meier method (log-rank test) was applied in survival analysis according to the levels of remnant lipoproteins. The predictive value for coronary events during the follow-up period was assessed by Cox proportional hazard analysis with the following factors as categorical covariates: remnant levels, stenosis of the left main coronary artery, number of coronary arteries with stenosis, age (≥70 years), sex (male), smoking history (defined as smoking ≥10 cigarettes/d for ≥10 years), hypertension (>140/90 mm Hg or taking antihypertensive medication), diabetes mellitus (according to World Health Organization criteria), hypercholesterolemia (>220 mg/dL or use of cholesterol-lowering medications), low levels of HDL cholesterol (<35 mg/dL), and hypertriglyceridemia (>150 mg/dL). In these analyses, remnant levels were divided into tertiles that were based on the 75th and 50th percentiles (5.1 and 3.3 mg/dL, respectively) of the distribution of the fasting remnant levels in 250 consecutive patients hospitalized in the cardiology section of the hospital, as described in our previous report. When the number of coronary arteries with stenosis was scored, stenosis of the left main coronary artery was counted as 2-vessel disease. Statistical significance was defined as P<0.05.

Results
The distribution of fasting remnant levels in patients was skewed and shifted to lower levels, with a median level of 3.4 mg/dL (25th percentile, 2.0 mg/dL; 75th percentile, 5.4 mg/dL). The lipoproteins, isolated from fasting serum by the immunoaffinity mixed gel, consisted mainly of VLDL remnants and had only trace amounts of HDL, as shown in our previous analyses. Multivariate linear regression analysis (after log transformation) showed that remnant levels were independently correlated with triglyceride levels (partial regression coefficient, 0.480; P=0.001). Remnant levels were significantly higher in diabetic patients than in nondiabetic patients (n=44 and 103, respectively; P<0.01, Mann-Whitney U test).

Twelve patients were lost to follow-up. The remaining 135 patients were followed up for 26.8±13.9 (mean±SD) months. Patients with the highest tertile of remnant levels (39 patients) had 20 coronary events (5 PTCA, 6 CABG, 3 myocardial infarctions, and 6 coronary deaths) during the follow-up period, whereas patients in the lowest tertile (56 patients) had 11 events (3 PTCA, 5 CABG, 1 myocardial infarction, and 2 coronary deaths) (P<0.01 for frequency of coronary events between the 2 subgroups by χ² test). Kaplan-Meier analysis demonstrated a significantly higher probability of developing clinical coronary events in patients with higher remnant levels, as shown in the Figure. In univariate Cox proportional hazard model analysis, higher levels of remnants (OR, 5.91; 95% CI, 2.0 to 17.2; P<0.001, highest compared with lowest tertile), stenosis of left main coronary artery (OR, 3.55; 95% CI, 1.8 to 7.0; P<0.001), 3-vessel disease (OR, 2.96; 95% CI, 1.5 to 6.0; P=0.002 compared with 1-vessel disease), and diabetes mellitus (OR, 1.86; 95% CI, 1.1 to 3.4; P=0.04) were significant predictors of clinical coronary events. Multivariate Cox proportional hazard analysis showed that only higher levels of remnants were a significant and independent predictor of coronary events, as shown in the Table. Higher remnant levels remained a significant predictor of coronary events in multivariate Cox analysis after addition of high LDL-cholesterol levels (>130 mg/dL) into the covariates (OR, 6.12; 95% CI, 2.1 to 15.2; P=0.001, highest versus lowest tertile).

Discussion
The present study demonstrated that higher levels of remnant lipoproteins in fasting serum predicted the development of clinical coronary events in patients with CAD independently of other risk factors. Thus, measurement of remnant levels by the current method may be helpful for CAD risk assessment in clinical laboratories. Previous reports showed that postprandial levels of triglyceride-rich lipoproteins may be a better predictor of the presence of CAD than fasting levels,
Multivariate Cox Proportional Hazard Model Analysis of Risk of Developing Coronary Events According to Fasting Levels of Remnant Lipoproteins in Patients With CAD

<table>
<thead>
<tr>
<th>Remnant Levels, mg/dL</th>
<th>Patients With Coronary Events, n (% of Patients)</th>
<th>OR</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;5.1 (n=39)</td>
<td>20 (51)</td>
<td>6.38</td>
<td>2.3–17.6</td>
<td>0.0003</td>
</tr>
<tr>
<td>3.3 ≤ 5.1 (n=40)</td>
<td>14 (35)</td>
<td>2.43</td>
<td>1.1–5.8</td>
<td>0.04</td>
</tr>
<tr>
<td>≤3.3 (n=56)</td>
<td>11 (20)</td>
<td>1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fasting remnant levels were divided into tertiles that were based on 75th and 50th percentiles (5.1 and 3.3 mg/dL, respectively) of the distribution of fasting remnant levels in the 250 hospitalized patients. Remnant levels, stenosis of left main coronary artery, number of diseased coronary arteries, age (>70 y), sex (male), histories of smoking, diabetes mellitus, and hypertension, hypercholesterolemia, low levels of HDL cholesterol, and hypertriglyceridemia were included as categorical covariates in the multivariate analysis.

but the assessment of postprandial levels of remnant lipoproteins is likely to be difficult for routine clinical examination because of postprandial variability of remnant levels in individuals, as well as requirements that the patient eat a standard meal and that multiple blood samples be collected over a prolonged period afterward. On the basis of our previous results showing that fasting remnant levels were closely correlated with postprandial remnant levels, remnant lipoprotein levels could be measured in the fasting blood as a reflection of abnormal postprandial remnant lipoproteins.

A previous in vitro experiment showed that remnant lipoproteins were taken up by macrophages and caused foam cell formation. Furthermore, high levels of remnant lipoproteins caused endothelial vasomotor dysfunction in human coronary arteries, as shown in our previous report. Remnant lipoproteins induce proinflammatory and prothrombogenic genes in cultured endothelial cells (H. Doi, MD, unpublished data, 1999). This atherothrombogenic role of remnant lipoproteins may result in the association of higher remnant levels with the increasingly high prevalence of future coronary events in patients with CAD, as observed in the present study.

Lipid-lowering drugs (especially fibric acid derivatives), dietary interventions, aerobic exercise, and obesity reduction might decrease remnant lipoprotein levels in hypertriglyceridemic patients. The measurement of remnant levels may also be useful for monitoring the therapeutic effect on levels of this atherogenic lipoprotein in these patients.

In conclusion, fasting remnant levels predicted future coronary events in patients with CAD independently of other risk factors. Thus, measurement of fasting remnant levels, as assessed by the current immunoseparation method, may be helpful in the assessment of CAD risk.

References

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