High Remnant Lipoprotein Predicts Recurrent Cardiovascular Events on Statin Treatment After Acute Coronary Syndrome

Si Van Nguyen, MD; Takamitsu Nakamura, MD, PhD; Kiyotaka Kugiyama, MD, PhD

Background: After acute coronary syndrome (ACS), there is a high risk of recurrent cardiovascular events. Triglyceride-rich lipoproteins influence residual cardiovascular risk in patients taking statin. This study examined the predictive value of remnant lipoprotein level for secondary cardiovascular events in patients treated with statins after ACS.

Methods and Results: A total of 190 patients treated with statins after ACS were enrolled in the study. The serum level of remnant lipoproteins (remnant-like lipoprotein particle cholesterol; RLP-C) was measured using an immunoseparation method. All the patients were followed prospectively for a maximum period of 70 months or until the occurrence of one of the following events: cardiac death, non-fatal myocardial infarction, unstable angina requiring unplanned coronary revascularization, or ischemic stroke. During the follow-up period, 42 patients had a secondary event. Multivariate Cox analysis showed that a high level of RLP-C (≥5.4 mg/dl; determined on receiver operating characteristic curve analysis) was a significant risk factor for secondary events, independent of conventional risk factors (hazard ratio, 2.94; 95% confidence interval: 1.40–6.18; P<0.01). The addition of high RLP-C to traditional risk factors enhanced net reclassification improvement (NRI) and integrated discrimination improvement (IDI) (NRI, 0.66, P=0.0003; and IDI, 0.08, P=0.0002).

Conclusions: RLP-C is useful for risk assessment of secondary cardiovascular events in patients treated with statins after ACS. (Circ J 2014; 78: 2492–2500)

Key Words: Acute coronary syndrome; Prognosis; Remnant lipoprotein; Statin

In spite of controlling low-density lipoprotein cholesterol (LDL-C) with statins, patients may still have residual dyslipidemia, which can be attributed partially to triglyceride (TG)-rich lipoproteins, especially remnant lipoproteins. Patients with acute coronary syndrome (ACS) are considered to have high risk for recurrent cardiovascular events. The Pravastatin or Atorvastatin Evaluation and Infection Therapy-Thrombolysis In Myocardial Infarction 22 (PROVE IT-TIMI 22) Trial showed that TG level is an independent risk factor for recurrent cardiac events in patients treated with atorvastatin after ACS. It is not yet established, however, up to which specific lipoprotein fraction is responsible for this increased risk in these patients.

In the past, it has been difficult to assay remnant lipoprotein level due to their heterogeneous properties. A simple and reliable technique for measuring remnant-like lipoprotein particle cholesterol (RLP-C) using an immunoseparation method, however, has been developed. We had shown that the role of RLP-C may be more important in high-risk patient groups including postmenopausal women and those with coronary artery disease (CAD), metabolic syndrome, or type 2 diabetes mellitus (DM). We also confirmed that RLP-C was an important residual risk factor in patients with stable CAD and LDL-C <100 mg/dl. Currently, only a few studies have investigated the prognostic value of RLP-C in high-risk post-ACS patients treated with statins. The present study therefore investigated whether RLP-C predicted recurrent cardiovascular events in patients treated with statins after ACS.

Methods

Patients
This prospective study initially enrolled 358 patients with ACS who were admitted to University of Yamanashi Hospital between October 2004 and October 2013. All the patients with ACS had successful reperfusion therapy with percutaneous coronary intervention (PCI) immediately after admission. The inclusion criteria included statin treatment for at least 3 months prior to final enrollment. The diagnosis of ACS included unstable angina pectoris (uAP) and acute myocardial infarction (AMI). uAP was diagnosed on the presence of acute ischemic
symptoms lasting ≥20 min within 48 h prior to hospital admission, and consistent electrocardiogram changes without positive cardiac biomarkers. AMI was diagnosed on creatine kinase-MB increase ≥2-fold the upper limit of normal, or troponin T >0.1 ng/ml. Patients were excluded from analysis using the following criteria: (1) AMI with mechanical complications; (2) stroke, major surgery, trauma or serious infectious disease within the 4-week period prior to enrollment; (3) ne-
Statin type and dose depended on the attending doctors. Also, the patients were given lifestyle modification treatment. After 3 months treatment with statin, baseline lipid level and other clinical data were measured at University of Yamanashi Hospital. Then, 192 patients who met the inclusion and exclusion criteria were finally enrolled in the prospective follow-up study. During the follow-up study, all patients continued the same treatment with statin and other medications as taken at final enrollment. The primary physicians followed up the study patients monthly at the outpatient clinic for a maximum period of 70 months or until the occurrence of a major cardiovascular event including cardiac death, non-fatal myocardial infarction, refractory uAP requiring unplanned coronary revascularization, and ischemic stroke. Cardiac death was confirmed by hospital records. AMI and uAP were diagnosed as described in the previous section. The diagnosis of ischemic stroke was based on medical history, physical examination, and brain computed tomography or magnetic resonance imaging. The data were obtained every 3 months from the primary physicians and then collated by investigators who were blinded to patient status at enrollment. All endpoint data were checked for accuracy, consistency, and completeness by other investiga-

Flow chart of patient enrolment is shown in Figure 1. After ACS, all eligible patients received statin treatment and the standard medical treatment for ACS, as outlined in Table 1.

Figure 1. Flow chart of patient enrollment. AMI, acute myocardial infarction; RLP-C, remnant-like lipoprotein particle cholesterol.
Laboratory Tests

Venous blood was collected after 12-h fast. Serum was stored at 4°C and was used for the assays within 3 days after sampling.
Fasting serum total cholesterol (TC) and TG concentrations were measured enzymatically, while serum high-density lipoprotein cholesterol (HDL-C) concentration was measured on heparin-Ca$^{2+}$/Ni$^{2+}$ precipitation. Non-HDL-C was calculated as TC minus HDL-C. LDL-C was calculated using the Friedewald formula. When TG $\geq$400 mg/dl, LDL-C was measured by direct assay. RLP-C was measured using an immunoseparation assay as described previously.$^7,^8$ Intra- and inter-assay coefficients of variation of RLP-C were 2.5–3.7% and 2.5–6.1%, respectively.$^7$

**Statistical Analysis**
All descriptive data are expressed as either mean±SD, median, or frequency (%). Shapiro-Wilk test showed that age, hemoglobin A1c, estimated glomerular filtration rates, ejection fraction, brain natriuretic peptide, TG, HDL-C, and RLP-C were not distributed normally. These variables were therefore expressed as median and interquartile range (25th and 75th percentiles). Continuous variables were compared between the 2 groups using unpaired t-test or Mann-Whitney u-test, when appropriate. Frequencies were compared using Chi-squared test. Receiver operating characteristic (ROC) curve analysis was used to identify cut-offs for RLP-C and non-HDL-C as 5.4 mg/dl and 126 mg/dl, respectively. The cut-offs of LDL-C, HDL-C, and TG were 100 mg/dl, 40 mg/dl, and 150 mg/dl, respectively, and were chosen according to the Third Report of The National Cholesterol Education Program (NCEP), the Guidelines of the Japan Atherosclerosis Society (JAS 2012), and the Guidelines for Secondary Prevention of Myocardial Infarction (JCS 2011).$^2,^17,^18$ Predictive potential was assessed on univariate or multivariate Cox proportional hazards analyses. Multivariate Cox proportional hazards analysis was done using confounders that were significant in the univariate model. Univariate and multivariate Cox proportional hazards analysis examined 1-SD changes in continuous variables. The presence of dichotomous variables was coded as 1 and the absence as 0. Kaplan-Meier survival analysis was used to compare the 2 groups using the RLP-C cut-off.

C-statistics that incorporated ROC curve analysis was used.
Remnant Lipoproteins and ACS

Baseline Characteristics

Compared with patients without cardiovascular events, those with an event had a higher prevalence of multivessel CAD and increased TC, non-HDL-C, and RLP-C at baseline (Table 1). There was no significant difference in LDL-C between the 2 groups. The types of statin used were similar in patients with and without events (Table 1). RLP-C had a significant linear correlation with TG (r=0.73, P<0.01), TC (r=0.26, P<0.01), and non-HDL-C (r=0.34, P<0.01), but not LDL-C (r=–0.04, P=0.61). In Table 2, patients with higher RLP-C were younger and had higher TG compared with those with lower RLP-C.

Predictive Value of RLP-C

The RLP-C cut-off of 5.4 mg/dl determined on ROC curve analysis, provided a sensitivity of 61.9%, specificity of 72.0% and accuracy of 68.9% for prediction of future events (Figure 2A). The sensitivity and specificity of different RLP-C cut-offs are shown in Figure 2B.

When the patients were stratified into 2 groups using RLP-C cut-off, Kaplan-Meier analysis showed that high RLP-C (≥5.4 mg/dl) resulted in a higher probability of future cardiovascular events than a lower level (<5.4 mg/dl; P<0.01, log-rank test; Figure 3). According to Table 2, non-fatal MI and uAP occurred more frequently in patients with higher RLP than those with lower RLP. Univariate Cox proportional hazard analysis also showed that multivessel CAD (HR, 2.10; 95% CI: 1.00–4.41), non-HDL-C (≥126 mg/dl; HR, 2.62; 95% CI: 1.25–5.47) and RLP-C (≥5.4 mg/dl; HR, 2.99; 95% CI: 1.46–6.11) were significant predictors for cardiovascular events (Table 3).

As shown in Table 3, high RLP-C (HR, 2.94; 95% CI: 1.40–6.18) and non-HDL-C (HR, 2.44; 95% CI: 1.13–5.29) remained significant predictors of cardiovascular events in multivariate Cox proportional hazard analysis. Other cut-off values of RLP-C, which had been used in our previous reports (4.8 mg/dl to examine the additive effects of non-HDL-C and RLP-C on the predictive potential of the baseline model that consisted of conventional cardiovascular risk factors. The conventional risk factors included age ≥65 years, male gender, smoking, hypertension, DM, and LDL-C ≥100 mg/dl. We also performed category-free net reclassification improvement (NRI) and integrated discrimination improvement (IDI) to analyze the degree to which the addition of non-HDL-C and RLP-C to the baseline model of risk factors model improved predictive ability.

All probabilities were expressed as 2-tailed, with statistical significance inferred at P<0.05. All confidence intervals were computed at the 95% level. Statistical analysis was done using STATA 10.0 (StataCorp, College Station, TX, USA).

Sample Size

On the basis of our preliminary observations in patients taking statins after ACS, the composite endpoints occurred in approximately 40% of patients with higher RLP-C (≥5.1 mg/dl; this cut-off point was derived from our previous study) during 3 years of follow-up, and in 15% of patients with lower RLP-C (<5.1 mg/dl). To provide the 2-sided statistical analysis with sufficient statistical power of 0.90 (β=0.10 and α=0.05), a total of 146 patients taking statins after ACS were required for the follow-up study. A total of 192 patients provided this prospective study with sufficient statistical power.

Results

As shown in Figure 1, two patients were withdrawn from the study due to liver dysfunction. A total of 190 ACS patients completed the follow-up study. The duration of the follow-up period ranged from 2 to 70 months (mean, 30±19 months). During the follow-up period, 42 patients had a cardiovascular event that included 3 cardiac deaths, 10 AMI, 25 uAP requiring unplanned coronary revascularization, and 4 strokes.

Figure 3. Kaplan-Meier analysis according to remnant-like lipoprotein particle cholesterol (RLP-C). The cut-off value of 5.4 mg/dl was chosen based on receiver operating characteristic curve analysis of the entire patient study group.
NGUYEN SV et al.

less incremental effects on the predictive ability of the baseline model (ie, AUC, NRI and IDI indices) when compared to the cut-off 5.4 mg/dl (Tables S2, S3).

**Discussion**

The present study showed that high RLP-C was a significant predictor of secondary cardiovascular events in patients treated with statins after ACS. In addition, high RLP-C enhanced the prognostic value of traditional risk factors in these patients. Therefore, the addition of RLP-C to risk assessment algorithms may be useful for risk stratification in patients treated with statins after ACS. Our previous report showed that RLP impairs endothelial function and upregulates endothelial expression of intracellular adhesion molecule-1, vascular cell adhesion molecule-1, and tissue factor. These proatherothrombogenic effects of RLP-C may explain the association of high RLP-C with the increased incidence of future cardiovascular events observed in the present study.

High non-HDL-C is related closely to cardiovascular disease and is considered as a secondary target for treatment in dyslipidemic patients according to guidelines. In line with this, the present study showed that high non-HDL-C was a significant predictor of recurrent events in patients treated with statins after ACS. Moreover, the addition of high non-HDL-C to traditional risk factors enhanced category-free NRI and IDI (Table 5). Other cutpoints of RLP-C (4.8 mg/dl and 5.1 mg/dl) had significant but

| Table 3. Univariate and Multivariate Cox Proportional Hazard Analysis for Future CV Events |
|------------------|------------------|------------------|
|                  | Univariate       | Multivariate     |
|                  | HR | 95% CI | P-value | HR | 95% CI | P-value |
| Age ≥65          | 1.21 | 0.56–2.63 | 0.63 | – | – | – |
| Male             | 0.86 | 0.40–1.87 | 0.71 | – | – | – |
| Smoking          | 1.45 | 0.70–2.98 | 0.32 | – | – | – |
| BMI              | 2.12 | 0.52–8.60 | 0.30 | – | – | – |
| DM               | 1.13 | 0.54–2.35 | 0.75 | – | – | – |
| Hypertension     | 1.16 | 0.56–2.39 | 0.69 | – | – | – |
| Multivessel CAD  | 2.10 | 1.00–4.41 | 0.04 | 2.84 | 1.32–6.13 | <0.01 |
| hsCRP            | 0.79 | 0.68–1.51 | 0.57 | – | – | – |
| HbA1c            | 1.01 | 0.68–1.51 | 0.94 | – | – | – |
| TG ≥150 mg/dl    | 0.72 | 0.35–1.47 | 0.38 | – | – | – |
| HDL-C <40 mg/dl  | 1.00 | 0.49–2.06 | 0.41 | – | – | – |
| LDL-C ≥100 mg/dl | 1.56 | 0.77–3.16 | 0.22 | – | – | – |
| Non-HDL-C ≥126 mg/dl | 2.62 | 1.25–5.47 | 0.01 | 2.44 | 1.13–5.29 | 0.02 |
| RLP-C ≥5.4 mg/dl | 2.99 | 1.46–6.11 | <0.01 | 2.94 | 1.40–6.18 | <0.01 |

CI, confidence interval; CV, cardiovascular; HR, hazard ratio. Other abbreviations as in Table 1.

### Table 4. C-statistics for Discrimination Ability of non-HDL-C and RLP-C to Predict CV Events

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>95%CI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline model risk factors</td>
<td>0.57</td>
<td>0.47–0.67</td>
<td>–</td>
</tr>
<tr>
<td>+Non-HDL-C ≥126 mg/dl</td>
<td>0.63</td>
<td>0.53–0.74</td>
<td>0.27</td>
</tr>
<tr>
<td>+RLP-C ≥5.4 mg/dl</td>
<td>0.70</td>
<td>0.60–0.79</td>
<td>0.02</td>
</tr>
</tbody>
</table>

†Baseline risk factors: age ≥65 years, male gender, hypertension, DM, LDL-C ≥100 mg/dl and smoking. AUC, area under receiver operating characteristic curve. Other abbreviations as in Tables 1,3.

### Table 5. NRI and IDI of non-HDL-C and RLP-C to Predict CV Events

<table>
<thead>
<tr>
<th></th>
<th>NRI</th>
<th>P-value</th>
<th>IDI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline model risk factors</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>+Non-HDL-C ≥126 mg/dl</td>
<td>0.43</td>
<td>0.01</td>
<td>0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>+RLP-C ≥5.4 mg/dl</td>
<td>0.66</td>
<td>0.0003</td>
<td>0.08</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

†Baseline risk factors: age ≥65 years, male gender, smoking, hypertension, DM, and LDL-C ≥100 mg/dl. IDI, integrated discrimination improvement; NRI, net reclassification improvement. Other abbreviations as in Tables 1,3.
RCL-P is a useful residual risk factor for risk assessment of recurrent cardiovascular events in patients treated with statins after ACS.

Conclusions

RCL-P is a useful residual risk factor for risk assessment of recurrent cardiovascular events in patients treated with statins after ACS.

Acknowledgments

This work was supported by the Ministry of Education, Culture, Sports, Science, and Technology, Tokyo, Japan (grants-in-aid for B2-19390209 and B-22390158).

References


**Supplementary Files**

**Supplementary File 1**

Table S1. Strength of RLP-C cut-offs to predict cardiovascular events

Table S2. RLP-C cut-offs vs. AUC

Table S3. NRI and IDI vs. RLP-C cut-offs

Please find supplementary file(s);