Anticardiolipin Antibodies and Recurrent Coronary Events
A Prospective Study of 1150 Patients

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Background—The association of anticardiolipin (aCL) antibodies with coronary artery disease has been shown in several studies but remains controversial. We evaluated the association of aCL and anti-β2-glycoprotein I (aβ2GPI) antibodies with the risk of recurrent cardiac events in postinfarction patients.

Methods and Results—The study population consisted of 1150 patients with acute myocardial infarction. Levels of IgG and IgM aCL and aβ2GPI antibodies were determined on sera collected before hospital discharge. There were 131 recurrent cardiac events (nonfatal myocardial infarctions or cardiac deaths) over a mean follow-up period of 24.6 months. Patients with elevated IgG aCL antibodies had a higher event rate than patients with low levels (P=0.05). Multivariate Cox analysis after adjustment for relevant clinical covariates showed that elevated levels of IgG aCL (hazard ratio=1.63; P=0.01) and low levels of IgM aCL (hazard ratio of 1.76; P=0.02) antibodies contribute independent risks for recurrent cardiac events. Patients with elevated IgG aCL and low IgM aCL antibody levels had a 3-fold higher risk of recurrent cardiac events than patients with low IgG aCL and elevated IgM aCL antibody levels (P<0.001). There was no significant association of the aβ2GPI antibodies with recurrent cardiac events.

Conclusions—In postinfarction patients, elevated IgG aCL and low IgM aCL antibodies are independent risk factors for recurrent cardiac events. Patients with both elevated IgG aCL and low IgM aCL antibodies have the highest risk. These findings shed additional light on the mechanistic role of aCL antibodies in coronary artery disease in patients without autoimmune diseases. (Circulation. 2000;102:1258-1263.)

Key Words: antibodies • thrombosis • coronary disease • myocardial infarction

Anticardiolipin (aCL) antibodies and lupus anticoagulant are the main antibodies associated with the antiphospholipid syndrome, which manifests clinically as vascular thrombosis and pregnancy morbidity.1 aCL antibodies are strongly associated with venous and arterial thrombosis, both in patients with systemic lupus erythematosus (SLE) and in patients without any apparent autoimmune diseases.2,3 It is known that aCL antibodies require a serum cofactor, β2-glycoprotein I (β2GPI) or apolipoprotein H, for binding to cardiolipin in vitro.4 Antibodies to β2GPI can be measured directly and were shown to be more specific than aCL antibodies for the clinical manifestations of antiphospholipid syndrome.5-7 However, these are mostly retrospective studies and mainly involve patients with SLE.

An association of aCL antibodies with coronary artery disease has been shown in several8-13 but not all studies.14,15 In the present study, we evaluated the association of aCL and anti-β2GPI (aβ2GPI) antibodies with recurrent myocardial infarction (MI) or cardiac death in a large cohort of patients without autoimmune diseases. The patients were participants in the Thrombogenic Factors and Recurrent Coronary Events (Thrombo) study, a multicenter prospective cohort study that examined the risk of different thrombogenic factors for recurrent coronary events.16

Methods

Patients
The Thrombo study recruited 1161 patients hospitalized for acute MI (index MI). Serum samples were collected before hospital discharge and at 2 months after the acute event. We conducted our study on the predischarged sera (average time from index MI to discharge 10.9 days, with range of 1 to 31 days). The study included men or women ≥21 years of age with enzyme confirmation of the index MI with symptoms and/or ECG changes consistent with an acute MI. The index MI could be Q-wave or non-Q-wave in type and either a first or a recurrent infarction. Exclusion criteria were coronary artery bypass grafting surgery during the hospital phase of the index MI, malignancy, or severe hepatic, renal, or cerebral disease.16

Received January 4, 2000; revision received April 6, 2000; accepted April 7, 2000.

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*The Thrombo Investigators are listed in Reference 16.

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Circulation is available at http://www.circulationaha.org

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**Study Protocol**

Baseline demographic data regarding known risk factors for coronary artery disease and recurrent MI were collected in all patients. The patients were followed up by phone and with regular clinic visits. Eighteen patients were lost to follow-up, and they were included in the analysis up to the time of their last contact. The human investigation committees of all the participating hospitals approved the study protocol. Written informed consent was obtained from all study participants.

**Measurement of Antibodies**

All the tests were done in duplicate with sera stored at \(-70^\circ\)C, and the mean value was obtained. One of the authors performed all the tests in the same laboratory environment. Determination of aCL antibodies was performed by standardized ELISA for both IgG and IgM isotypes (REAADS Medical Products, Inc) with bovine calf serum in the sample diluent as the source of $\beta_2$ GPI. Results are expressed as GPL units for the IgG (positive \(\geq 23\) GPL) and MPL units for the IgM (positive \(\geq 11\) MPL) aCL antibodies, with 1 GPL or MPL unit being equivalent to 1 \(\mu g/mL\) of an affinity-purified standard IgG or IgM aCL antibody sample. Determination of IgM and IgG $\alpha\beta_2$GPI antibodies was performed by ELISA with irradiated and chemically activated plastic microwell plates containing purified human $\beta_2$GPI (INOVA, Inc). Results are expressed in standardized units, $SGU$ for the IgG (positive \(\geq 20\) SGU) and SMU for the IgM (positive \(\geq 20\) SMU) $\alpha\beta_2$GPI antibodies. All tests met the quality control standards as determined by the manufacturer.

**Statistical Analysis**

The end points were cardiac mortality and cardiac morbidity with rehospitalization as the result of recurrent nonfatal MI (cardiac

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All Patients (n=1150)</th>
<th>aCL Antibody IgG</th>
<th>aCL Antibody IgM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Q4 (n=288)</td>
<td>Q1–3 (n=862)</td>
<td>Q4 (n=257)</td>
</tr>
<tr>
<td>Age (\geq 60) y</td>
<td>48</td>
<td>53</td>
<td>46</td>
</tr>
<tr>
<td>Male sex</td>
<td>75</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>Nonwhite race</td>
<td>26</td>
<td>35*</td>
<td>23</td>
</tr>
<tr>
<td>History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prior MI</td>
<td>19</td>
<td>22</td>
<td>18</td>
</tr>
<tr>
<td>Treated hypertension</td>
<td>44</td>
<td>48</td>
<td>43</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>20</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Stroke</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Cigarette smoking status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoked</td>
<td>33</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>42</td>
<td>41</td>
<td>42</td>
</tr>
<tr>
<td>Current smoker</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Index MI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>New Q wave</td>
<td>51</td>
<td>48</td>
<td>52</td>
</tr>
<tr>
<td>Pulmonary congestion</td>
<td>20</td>
<td>23</td>
<td>19</td>
</tr>
<tr>
<td>Ejection fraction (&lt;0.30)</td>
<td>13</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Medications at enrollment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetylsalicylic acid</td>
<td>88</td>
<td>88</td>
<td>88</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>42</td>
<td>44</td>
<td>42</td>
</tr>
<tr>
<td>$\beta$-Blockers</td>
<td>78</td>
<td>78</td>
<td>78</td>
</tr>
<tr>
<td>Calcium-channel blockers</td>
<td>18</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Diuretics</td>
<td>14</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Oral anticoagulants†</td>
<td>21</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Lipid-lowering agents‡</td>
<td>27</td>
<td>26</td>
<td>28</td>
</tr>
</tbody>
</table>

\(Q_4\) indicates highest quartile; \(Q_{1–3}\), lower 3 quartiles. Values are percentage; plus-minus values are mean \(\pm\) SD.

*\$P<0.01\ between highest quartile and lower 3 quartiles.
†Dicumarol or warfarin.
‡Includes statin-type medications.
events). The same diagnostic criteria as for the index MI were used for the recurrent nonfatal MI. The first recurrent cardiac event (cardiac death or recurrent nonfatal MI) that occurred was used as the end point for each patient.

The baseline characteristics of patients with versus patients without cardiac events were compared by χ² test. Univariate analysis of the IgG and IgM aCL and aβ₂GPI antibodies in relation to the outcome was performed by Wilcoxon’s rank-sum test and χ² test where appropriate. For the survival analyses, the IgG and IgM aCL and IgM aβ₂GPI antibodies were dichotomized at a cutoff point closest to the 75th percentile, and comparisons of the highest quartile (Q₄) versus the lower 3 quartiles (Q₁–₃) were performed. This dichotomization parallels the analysis of the original Thrombo study and solves the problem of skewness. The cutoff values for the highest quartile were 12.5 GPL for IgG aCL, 4.1 MPL for IgM aCL, and 2.0 SMU for IgM aβ₂GPI antibodies. For IgG aβ₂GPI antibodies, values of the 95th percentile were used for analysis rather than the quartile approach because there were only 47 patients with any detectable level of these antibodies. Kaplan-Meier survival analysis was used for estimation of event rates in association with the IgG and IgM aCL and aβ₂GPI antibodies. The Cox proportional-hazards model was used to evaluate the independent contribution of risk variables to outcome events. In the construction of the Cox model, a clinical model was first obtained by including any clinical variable that entered the model at a level of significance <0.1 after a forward stepwise regression scheme. This approach was used to ensure inclusion of any added contribution to this model from important covariates with borderline levels of significance. Individual medications (as listed in Table 1) were tested in the model one at a time. The SAS statistical program (SAS Institute, Inc.) was used for data analysis.

Results

Of the 1161 patients recruited, serum samples were available for testing on 1150. The baseline characteristics for the entire study population and for patients in the highest and lower 3 IgG and IgM aCL antibody quartiles are shown in Table 1. Race was the only covariate with significantly different frequencies in the high and low quartile distributions of both IgG and IgM aCL antibodies.

During the mean follow-up period of 24.6 months (range 0 to 42 months), a total of 131 recurrent cardiac events (84 cardiac deaths and 47 nonfatal MI) occurred. The group with events was older, with a higher percentage having had a prior MI before the index MI and having hypertension, diabetes mellitus, pulmonary congestion, and ejection fraction ≤30%.

The mean levels and number of patients in the highest quartiles of IgG and IgM aCL and aβ₂GPI antibodies in those with and without coronary events are shown in Table 2. There is a trend for higher IgG aCL and lower IgM aCL antibody levels in patients with than without events (P = 0.06 for both). The highest quartiles of IgG aCL and IgM aCL antibodies show a similar trend (Table 2). The IgG and IgM aβ₂GPI antibodies did not show a significant association or a meaningful trend with recurrent cardiac events.

Kaplan-Meier survivorship analysis for the IgG and IgM aCL antibodies is shown in the Figure. Patients in the highest quartile of IgG aCL antibodies had a higher coronary event rate than patients in the lower 3 quartiles (P = 0.05). A reverse trend was observed for the IgM aCL antibodies (P = 0.06).

The risk contributed by aCL antibodies to the Cox model after adjustment for relevant clinical covariates is shown in Table 3. The variables that entered the clinical model at the 0.1 level of significance were age ≥ 60 years, prior MI before the index MI, diabetes mellitus, smoking, pulmonary congestion, and ejection fraction ≥ 30%. In this model, IgG aCL antibodies in the highest quartile of the distribution contributed an independent hazard ratio (HR) of 1.63 (95% CI 1.11 to 2.38; P = 0.01) for recurrent coronary events. In contrast, IgM aCL antibodies in the lower 3 quartiles of the distribution contributed an independent HR of 1.76 (95% CI 1.10 to 2.82; P = 0.02) for recurrent coronary events.

Because the IgG and IgM aCL antibodies contributed comparable opposite effects, we explored the joint risk for patients in the high- and low-risk partitions of both aCL antibody isotypes. The group of patients with IgM aCL antibodies in the highest quartile and IgG aCL antibodies in the lower 3 quartiles had the lowest risk and was chosen as the reference group. Patients with IgG aCL antibodies in the highest quartile and IgM aCL antibodies in the lower 3 quartiles had higher risks than any other aCL antibody group and 3-fold higher risk than the reference group (P = 0.001) (Table 4).

The cutoff values for the upper quartiles of the IgG (≥12.5 GPL) and IgM (≥4.1 MPL) aCL antibodies are lower than the values that are considered positive in the aCL antibody assay that was used in our study (≥23 GPL for IgG and ≥11 MPL for IgM aCL antibodies). When the data were analyzed according to the positive values, positive IgG and IgM aCL antibodies were associated with hazard ratios of 2.0 (P = 0.01) and 0.8 (P = 0.6), respectively. The low number of positive IgG and IgM aCL antibodies in the group of patients with events (Table 2) was the main obstacle to the interpretation of these results.
The correlation coefficients for aCL and aβ2GPI antibodies by isotype are shown in Table 5. A moderately strong correlation ($r=0.50$) was present only between IgM aCL and aβ2GPI IgM antibodies.

**Discussion**

This report represents the largest study to date evaluating the association between aCL and aβ2GPI antibodies and recurrent cardiac events in patients after MI. The data presented herein show that IgG aCL antibodies in the highest quartile represent an independent risk factor for recurrent MI and cardiac death, with an HR of 1.63 after adjusting for other relevant clinical variables. This risk is comparable to other known risk factors for recurrent coronary events in our study: prior MI (HR = 1.77), non–insulin-dependent diabetes (HR = 1.87), ejection fraction < 30% (HR = 1.69), and smoking (HR = 1.81).

Our data also revealed an inverse association of IgM aCL antibody levels with recurrent cardiac events. Thus, patients with elevated IgG aCL and low IgM aCL antibodies had the highest risk compared with all other aCL antibody groups.

The association between myocardial infarction and IgG aCL antibodies has been suggested in several smaller studies. With regard to noncardiac events, IgG aCL antibod-
Table 3. Hazard Ratios of aCL Antibodies in Multivariate Cox Model

<table>
<thead>
<tr>
<th>aCL Antibodies</th>
<th>HR*</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG (Q4,Q1)</td>
<td>1.63</td>
<td>1.11–2.38</td>
<td>0.01</td>
</tr>
<tr>
<td>IgM (Q4,Q1)</td>
<td>1.76</td>
<td>1.10–2.82</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Q4,Q1 indicates lower 3 quartiles; Q4 highest quartile.
Cutoff values for Q4: IgG aCL=12.5 GPL; IgM aCL=4.1 MPL.
Cox model includes adjustment for 6 clinical variables (P<0.1 to enter): age ≥60 y, diabetes mellitus, smoking, prior MI, pulmonary congestion, and ejection fraction ≥0.30.
*Ratio of the risk of having a cardiac event per unit time for patients in the top-risk partition vs patients in the lower-risk partition.

Table 4. Joint Risks for Patients in High- and Low-Risk Partitions of IgG and IgM aCL Antibodies

<table>
<thead>
<tr>
<th>IgG aCL Antibodies</th>
<th>IgM aCL Antibodies</th>
<th>Events/Patients, %</th>
<th>HR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q4</td>
<td>Q4</td>
<td>12/176 (6.8)</td>
<td>1.00*</td>
<td>...</td>
</tr>
<tr>
<td>Q4</td>
<td>Q1</td>
<td>76/686 (11)</td>
<td>2.35</td>
<td>0.01</td>
</tr>
<tr>
<td>Q4</td>
<td>Q1</td>
<td>11/81 (14)</td>
<td>2.91</td>
<td>0.01</td>
</tr>
<tr>
<td>Q4</td>
<td>Q1</td>
<td>30/207 (14)</td>
<td>3.32</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Q1,Q4 indicates lower 3 quartiles; Q4 highest quartile.
Cutoff values for Q4: IgG aCL=12.5 GPL; IgM aCL=4.1 MPL.
*Group with the lowest risk (reference group).

Table 5. Correlation Between aCL and aβ2GPI Antibodies by Genotype

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>IgM aCL</th>
<th>IgG αβ2GPI</th>
<th>IgM αβ2GPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG aCL</td>
<td>r=0.16</td>
<td>r=0.14</td>
<td>r=0.13</td>
</tr>
<tr>
<td>IgM aCL</td>
<td>r=0.08</td>
<td>r=0.50</td>
<td></td>
</tr>
<tr>
<td>IgM αβ2GPI</td>
<td>r=0.12</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

P<0.005 for all r correlation values.

antibodies detected in SLE are primarily directed against the so-called “cofactor proteins,” including β2GPI (the predominant cofactor), prothrombin, annexin V, thrombomodulin, and proteins C and S, among others.26,30 Such cofactors share the property of being phospholipid-binding proteins with intrinsic anticoagulant activity. Hence, binding of aCL antibodies may diminish the function of these anticoagulant systems. In addition, IgM aCL antibodies enhance platelet activation and thromboxane production27 and activate endothelial cells in the presence of β2GPI.28,29 The interplay among aCL antibodies, the anticoagulant system, and the endothelial cells could contribute to the prothrombotic state that is central to the pathogenesis of recurrent MI.16,31

A unique finding of our study is the reverse association of the IgM aCL antibodies with recurrent cardiac events, even in the presence of IgG aCL antibodies. Several non–mutually exclusive mechanisms could account for this observation. IgM aCL antibodies might represent protective natural autoantibodies32 or they could have rheumatoid factor activity and thus play a beneficial role in immune homeostasis.33 Alternatively, it could be postulated that the aCL antibody response in our patients starts with a primary IgM antibody response against nonpathogenic epitopes that subsequently, through epitope spreading, results in the recognition of previously ignored pathogenic epitopes by antigen-selected IgG antibodies.34 A hypothetical example of a protective initial IgM antibody response might be the binding and inhibition of the colonization of the vascular endothelium by infectious agents such as Chlamydia pneumoniae that may play a role in the development of atherosclerosis.35,36 The ability of aCL antibodies to recognize chlamydial antigens, or conversely, the ability of chlamydial infection to elicit aCL antibodies, remains to be tested. This model is appealing because it is also consistent with the lack of association of recurrent coronary events with αβ2GPI antibodies that was observed in our study. Indeed, aCL antibodies induced by infections do not recognize β2GPI,37 and these antibodies may be prevalent in our cohort of nonautoimmune patients.

It should be noted that aCL antibodies cross-react with oxidized LDL25 and that antibodies against oxidized LDL have been implicated in the development of atherosclerosis.22 When considered that both C pneumoniae and chlamydial hsp60, an inflammatory antigen that was recently localized in atheromas, can induce cellular oxidation of LDL,38 a reasonable link between chlamydial infection, aCL antibodies, and atherosclerosis emerges.

In summary, IgG aCL antibodies are an independent risk factor for recurrent cardiac events in this large population of nonautoimmune patients. Our findings contribute new data in the ongoing research for identification of additional coronary risk factors.39 At present, the use of IgG aCL antibodies to
stratify patients for coronary risk is limited by the heterogeneity of these antibodies,40 the available commercial kits,41 and the lack of standardization of the aCL antibody assays between the different laboratories.42 As we learn more about the different specificities of the aCL antibodies and more standardized assays develop, aCL antibodies may become useful for identifying patients at risk for coronary events. Although high levels IgG and low levels of IgM aCL antibodies convey independent risk, the predictive value of these antibodies is too low for use in risk-stratification application at this time.

Acknowledgments
This study was supported by National Institutes of Health grants HL-48259 and HL-30616 and by the University of Rochester School of Medicine and Dentistry. We thank Elsa Welch for technical support and Dr Elena Rey for advice with the ELISA tests. We thank Dr John Conradi for reviewing the manuscript.

References
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Circulation. 2000;102:1258-1263
doi: 10.1161/01.CIR.102.11.1258

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