Lipoprotein-associated phospholipase A2 activity and risk of heart failure: the Rotterdam Study

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Aims Evidence is accumulating that inflammation plays a role in the pathophysiology of heart failure. Lipoprotein-associated phospholipase A2 (Lp-PLA2) has pro-inflammatory properties. We investigated whether Lp-PLA2 activity is associated with heart failure.

Methods and results Lp-PLA2 activity was determined in a random sample of 1820 subjects from the Rotterdam Study, a population-based cohort study among persons aged 55 years and over. During a mean follow-up of 6.7 years, 94 heart failure cases occurred. We excluded participants with heart failure or coronary heart disease at baseline and we accounted for incident coronary heart disease during follow-up. We used Cox proportional hazard models to compute hazard ratios adjusted for age, sex, non-HDL cholesterol, HDL cholesterol, body mass index, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, smoking, and C-reactive protein. The hazard ratio per unit increase of Lp-PLA2 activity was 1.03 [95% confidence interval (95% CI 1.01–1.05); P for trend was 0.011. Hazard ratios for the second, third, and fourth quartiles were 1.06 (95% CI 0.55–2.04), 1.43 (95% CI 0.73–2.81), and 2.33 (95% CI 1.21–4.49), respectively, using the lowest quartile of Lp-PLA2 activity as the reference category.

Conclusion This study suggests that Lp-PLA2 activity is independently associated with incident heart failure.

KEYWORDS Heart failure; Inflammation; Epidemiology

Introduction

During the last 15 years, an interest has developed for the potential role of inflammatory mediators in the pathophysiology of heart failure. Associations have been found between elevated inflammatory markers, such as interleukin-6,¹ tumour necrosis factor-α,² and C-reactive protein,³ and congestive heart failure. It has been shown that inflammatory mediators may influence left ventricular remodelling, left ventricular function, and pulmonary oedema.⁴,⁵ Furthermore, a correlation has been found between high blood levels of these inflammatory markers and worsening functional NYHA class, increased hospitalization rates, and poorer survival of heart failure patients.⁶

Recently, several studies have found an independent association between the inflammatory marker lipoprotein-associated phospholipase A2 (Lp-PLA2) and risk of coronary heart disease.⁷–¹¹ Lp-PLA2 is an enzyme that circulates in the blood bound to low-density lipoprotein (LDL) cholesterol. The enzyme has pro-inflammatory properties because of its capacity to hydrolyse oxidized phospholipids.¹² However, it is also suggested to have anti-inflammatory properties because of its ability to hydrolyse platelet-activating factor.¹³,¹⁴ The relationship found between Lp-PLA2 and coronary heart disease suggests that the pro-inflammatory properties of Lp-PLA2 outweigh its anti-inflammatory properties.

To our knowledge, no studies have yet been conducted on Lp-PLA2 as a predictor of heart failure. Therefore, we investigated the association between Lp-PLA2 and risk of heart failure in the Rotterdam Study, a population-based cohort study among men and women aged 55 years and over.

Methods

Rotterdam Study

The Rotterdam Study is a population-based cohort study comprising 7983 men and women aged 55 years and over. Its overall aim is to assess the occurrence of and risk factors for chronic diseases in the elderly. A detailed description of the objectives and methods of the Rotterdam Study has been given elsewhere.¹⁵ All residents of a Rotterdam suburb aged 55 and over were invited to participate in the study and 78% participated. Baseline measurements started in 1990 and were completed in 1993.

The Medical Ethics Committee of Erasmus Medical Center, Rotterdam, approved the study. All participants gave written consent.

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informed consent. This study complies with the Declaration of Helsinki.

Study population
Lp-PLA2 activity was determined in a random subcohort of 1820 subjects. Prevalent heart failure cases at baseline (n = 47) were excluded for the current analysis. In addition, 183 subjects were excluded because they had a history of myocardial infarction, a history of coronary artery bypass grafting (CABG), or a history of percutaneous transluminal coronary angioplasty (PTCA) at baseline, leaving 1590 subjects, who were used for the analysis.

Measurement of Lp-PLA2 activity
Plasma aliquots prepared from non-fasting blood samples were collected at baseline and stored at −80 °C. Lp-PLA2 activity was measured with a high throughput radiometric activity assay, as described in detail previously.11 Lp-PLA2 activity was expressed as nano moles of platelet-activating factor hydrolysed per minute per millilitre of plasma samples.

Prior to analysis of plasma samples from the Rotterdam Study, a pre-study validation was conducted to determine the reliability of the LpPLA2 activity assay. Six plasma samples were tested in triplicate, and the coefficient of variation (CV) for intra-assay precision ranged from 3.51–8.96%. To assess inter-assay precision, six plasma samples were tested on three occasions, and CV ranged from 8.48–15.08%. Three cycles of freeze-thaw of frozen plasma did not result in appreciable loss of activity. The assay was therefore considered suitable for the analysis of the Rotterdam Study samples, which were tested in duplicate. Samples were re-tested if the replicate CV was >25%. The range of detection was 8–150 nmol/min per mL.

Measurement of covariates at baseline
At baseline, a trained interviewer visited all participants at home and collected information using a computerized questionnaire. The information obtained included current health status, medical history, drug use, and smoking behaviour. Additionally, established cardiovascular risk factors were measured at the research centre. Height and weight were measured, and the body mass index was calculated [weight (kg)/height² (m)]. Blood pressure was measured at the right brachial artery with a random-zero sphygmomanometer, with the participant seated. We defined hypertension as a systolic blood pressure ≥160 mmHg or a diastolic blood pressure ≥100 mmHg or the use of blood pressure-lowering medication with an indication for hypertension.

Non-fasting blood samples were drawn, and total cholesterol and high-density lipoprotein (HDL) cholesterol were measured within 2 weeks, as described previously.16 Non-HDL cholesterol was computed by subtracting HDL cholesterol from total cholesterol. LDL cholesterol was determined in fasting blood samples in 120 randomly selected subjects by use of an enzymatic method (Roche). We calculated Pearson’s correlation coefficient to compute the correlation of non-HDL cholesterol with LDL cholesterol, r = 0.97, P < 0.001. We defined diabetes mellitus as a random or post-load glucose level ≥11.1 mmol/L or the use of blood glucose-lowering medication.17 Using a nephelometric method (immage, Beckman Coulter), we measured C-reactive protein in blood samples kept frozen at −20 °C.

A 12-lead resting electrocardiography (ECG) was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs were processed by the modular ECG analysis system (MEANS) to obtain ECG measurements and interpretations.18 Myocardial infarction found on ECG was based on a comprehensive set of criteria that partly derive from the Minnesota code.19 A history of myocardial infarction was considered present in case of a self-report of myocardial infarction confirmed by ECG or additional clinical information, or the presence of an ECG characteristic of prior myocardial infarction.

In identifying incident myocardial infarctions (ICD-10 code I21), all available information, which included ECG, cardiac enzyme levels, and the clinical judgement of the treating specialist, was used. Revascularization procedures were identified by review of hospital discharge letters from the medical specialist.

Ascertainment of heart failure cases
Assessment of prevalent heart failure at the baseline examination in the Rotterdam Study has been described elsewhere in detail.20,21 Briefly, a validated score was used, similar to the definition of heart failure of the European Society of Cardiology.22 This score was based on the presence of at least two symptoms suggestive of heart failure (shortness of breath, ankle swelling, and pulmonary crepitations) or use of medication for the indication of heart failure, in combination with objective evidence of cardiovascular disease.

Information on the presence of heart failure at baseline was obtained for all participants, using one of the following three methods: interview questions on indication of cardiovascular medication and breathlessness, linkage of the Rotterdam Study database to a database containing hospital discharge diagnoses from all hospitals in the Rotterdam area as of 1 January 1991, and screening of all medical records in retrospect for the occurrence of heart failure in the majority of participants of the Rotterdam Study.

For the present study, follow-up started at the baseline examination, from 1990 till 1993, and was complete until 1 January 2000. Follow-up has been described in detail previously.21 Briefly, cases of incident heart failure were obtained by continuously monitoring participants of the Rotterdam Study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners. Each participant’s medical record was fully screened for incident heart failure. All available data on these events, such as hospital discharge letters and notes from general practitioners, were copied from the medical records.

Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area, as described earlier. The diagnosis of heart failure was classified as definite, probable, possible, or unlikely.23 Two research physicians independently classified all information on potential heart failure events. If there was disagreement, a consensus was reached in a separate session.

Finally, a cardiologist verified all probable and possible cases, and all cases in which the two physicians could not reach consensus. If the cardiologist disagreed with the research physicians, the cardiologist’s judgement was considered decisive. The research physicians and the cardiologist based their decisions on the same data. Only definite and probable cases were included in the analyses.

Statistical analysis
To compare the baseline characteristics of the random subcohort to the remainder of the Rotterdam Study, we used a χ² test for dichotomous variables, a t-test for continuous variables, and a Mann-Whitney test for C-reactive protein, because its distribution was skewed. We used ANCOVA to display age- and sex-adjusted baseline characteristics of the participants in different Lp-PLA2 activity quartiles. We log-transformed C-reactive protein because of its skewed distribution and we computed the geometric mean. We computed the standard deviation and interquartile range from the standard error. To compute P-value for trend for the baseline characteristics, we used logistic regression for dichotomous variables and linear regression for continuous variables. In both cases, continuous plasma values of Lp-PLA2 activity were used as the independent variable.

We used Cox proportional hazards models to evaluate the association of Lp-PLA2 activity with risk of heart failure. Subjects were censored at the time of occurrence of heart failure, death, or at
the end of the study period. Furthermore, we censored subjects at the time of occurrence of myocardial infarction, PTCA, or CABG if these took place before the occurrence of heart failure, to account for coronary heart disease. The proportional hazards assumption was tested by drawing log minus log plots of the survival function, which confirmed that the assumption was met. In model 1, we adjusted for age and sex. Lp-PLA2 is tightly associated with lipoproteins; in humans, it is predominantly located on LDL and, to a smaller extent, on HDL. Because these factors were most likely to be confounders, in model 2, we additionally adjusted for non-HDL cholesterol and HDL cholesterol. In model 3, we additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, hypertension, smoking status, diabetes mellitus, and C-reactive protein. First, we entered the continuous plasma values of Lp-PLA2 activity into the models to obtain the hazard ratio for heart failure per unit increase in Lp-PLA2 activity. By this means, we also obtained the \( P \)-value for trend. Second, to allow for the demonstration of a possibly non-linear association, we made quartiles of Lp-PLA2 activity with cutpoints 35.9, 42.9, and 50.8 nmol/min per mL plasma and used the lowest quartile as the reference category. To compare survival time until the occurrence of heart failure in the quartiles of Lp-PLA2 activity, C-reactive protein, and non-HDL cholesterol, we made event-free survival curves adjusted for age and sex.

We conducted a subgroup analysis to compare the association between Lp-PLA2 activity and heart failure in subjects with a non-HDL cholesterol level below and above the median (cutpoint 5.20 mmol/L). Lp-PLA2 was dichotomized in this analysis, using the median as a cutoff point (42.9 nmol/min per mL plasma). We adjusted for age, sex, HDL cholesterol, body mass index, systolic blood pressure, diastolic blood pressure, hypertension, smoking status, diabetes mellitus, and C-reactive protein. We did a similar subgroup analysis in the strata of C-reactive protein (cutpoint 1.79 mg/L), adjusting for non-HDL cholesterol instead of C-reactive protein. HDL cholesterol was lower in participants within the first quartile of Lp-PLA2 activity as the reference category. To test for interaction between Lp-PLA2 activity and non-HDL cholesterol and C-reactive protein, we entered interaction terms with the residuals were normally distributed with a constant variance. The characteristics of the random cohort were similar to the remainder of the Rotterdam Study. The characteristics of the random cohort were similar to the remainder of the Rotterdam Study, except for age, systolic blood pressure, and hypertension. Subjects in the random cohort were slightly younger (69.1 vs. 71.1 years), had a lower systolic blood pressure (138.2 vs. 139.9 mmHg), and had a lower prevalence of hypertension (33.1 vs. 37.1%). Table 1 shows the baseline characteristics of participants in different quartiles of Lp-PLA2 activity adjusted for age and sex (when appropriate) and the \( P \)-value for trend. In all linear regression models we used, the residuals were normally distributed with a constant variance. Quartiles with a higher Lp-PLA2 activity contained a higher percentage of men and hypertensive participants. They had a significantly higher body mass index, systolic blood pressure, non-HDL cholesterol, and C-reactive protein. HDL cholesterol was lower in participants within higher quartiles of Lp-PLA2 activity.

Lp-PLA2 activity was associated with risk of heart failure (Table 3). After adjustment for age and sex, the hazard ratio for heart failure per unit increase in Lp-PLA2 activity was 1.02 [95% confidence interval (CI) 1.00–1.03], \( P = 0.026 \). After additional adjustment for non-HDL cholesterol and HDL cholesterol, this was 1.02 (95% CI 1.00–1.04), \( P = 0.024 \), and after additional adjustment for known cardiovascular risk factors, 1.03 (95% CI 1.01–1.05), \( P = 0.011 \). Participants in the second, third, and fourth quartiles of Lp-PLA2 activity had a hazard ratio of 0.99 (95% CI 0.52–1.86), 1.27 (95% CI 0.68–2.40), and 1.93 (95% CI 1.09–3.42), respectively, for heart failure, using the first quartile of Lp-PLA2 activity as the reference category and adjusting for age and sex. Using model 2, this was 1.01 (95% CI 0.53–0.92), 1.34 (95% CI 0.69–2.60), and 2.16

<table>
<thead>
<tr>
<th>Variable</th>
<th>Random cohort (n = 1820)</th>
<th>Remainder Rotterdam Study (n = 6163)</th>
<th>( P )-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>69.1 ± 9.1</td>
<td>71.1 ± 9.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Men, %</td>
<td>38.3</td>
<td>39.1</td>
<td>0.55</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.2 ± 3.7</td>
<td>26.2 ± 3.8</td>
<td>0.3</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>138.2 ± 22.3</td>
<td>139.9 ± 22.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>73.3 ± 11.2</td>
<td>73.8 ± 11.8</td>
<td>0.12</td>
</tr>
<tr>
<td>Hypertension, %</td>
<td>33.1</td>
<td>37.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mmol/L</td>
<td>5.30 ± 1.24</td>
<td>5.24 ± 1.23</td>
<td>0.1</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.35 ± 0.38</td>
<td>1.34 ± 0.37</td>
<td>0.38</td>
</tr>
<tr>
<td>Diabetes mellitus, %</td>
<td>9.8</td>
<td>10.7</td>
<td>0.31</td>
</tr>
<tr>
<td>Smokers, %</td>
<td>Current: 23</td>
<td>22.5</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>Former: 41.7</td>
<td>40.4</td>
<td>0.34</td>
</tr>
<tr>
<td></td>
<td>Never: 35.3</td>
<td>37</td>
<td>0.18</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td>1.78 (0.90–3.59)</td>
<td>1.93 (0.92–3.71)</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as percentage.

*Median and interquartile range because of skewed distribution.
baseline and censored subjects with incident coronary heart disease during follow-up. This suggests that the association found between Lp-PLA2 activity and heart failure is independent of coronary heart disease.

To our knowledge, this is the first study performed on the association between Lp-PLA2 and risk of heart failure. The present study is a population-based prospective cohort study, which guards our study from selection and recall bias. Strengths of our study include the ability to account for possible confounding by incorporating established cardiovascular risk factors into the statistical models. Finally, we were able to account for prevalent and incident coronary heart disease in our analysis.

The pathophysiology of heart failure is complex. Heart failure was once considered to be merely a cardiocirculatory impairment, but now it is known that the neuroendocrine system is involved. Evidence is accumulating that inflammation also plays a direct role in the pathophysiology of heart failure. In former studies, several inflammatory markers, such as interleukin-6,\(^1\) tumour necrosis factor-\(\alpha\),\(^2\) and C-reactive protein,\(^3\) have been associated with incidence of heart failure. Inflammatory mediators have been found to affect left ventricular remodelling, left ventricular

(95% CI 1.13–4.11), respectively. Using model 3, this further increased to 1.06 (95% CI 0.55–2.04), 1.43 (95% CI 0.73–2.81), and 2.33 (95% CI 1.21–4.49), respectively.

The event-free survival curve according to quartiles of Lp-PLA2 activity shows that the survival time until the occurrence of heart failure was higher in the lowest quartile than in the highest quartile (Figure 1). The curve also illustrates that the difference in risk between quartiles 1 and 4 is rather consistent over time. Figures 2 and 3 show the event-free survival curve of C-reactive protein and non-HDL cholesterol, respectively. Although C-reactive protein was significantly related to the event-free survival time, no clear association was found for non-HDL cholesterol.

Figure 4 shows the results of our subgroup analyses. The hazard ratios for heart failure associated with Lp-PLA2 activity for the subgroups below and above the median of non-HDL cholesterol level were 1.89 (95% CI 1.05–3.39) and 1.77 (95% CI 0.83–3.79), respectively. The hazard ratio was somewhat larger in subjects with a non-HDL cholesterol below the median, but no significant interaction was found between Lp-PLA2 activity and non-HDL cholesterol (\(P\)-value for interaction = 0.817) in relation to risk of heart failure. The hazard ratio for the subjects with a C-reactive protein below the median was 3.83 (95% CI 1.64–8.93), which was higher than the hazard ratio for the subjects with C-reactive protein above the median, namely 1.26 (95% CI 0.71–2.23). However, the interaction term for Lp-PLA2 activity and C-reactive protein was not significant (\(P\)-value for interaction = 0.364).

### Discussion

In the present population-based cohort study, Lp-PLA2 activity was an independent predictor of heart failure. The association persisted after we adjusted for known cardiovascular risk factors and C-reactive protein. A significant trend was seen, and subjects in the highest quartile had no less than a doubled risk of developing heart failure compared with subjects in the lowest quartile, even though we excluded subjects with prevalent coronary heart disease at baseline and censored subjects with incident coronary heart disease during follow-up. This suggests that the association found between Lp-PLA2 activity and heart failure is independent of coronary heart disease.

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as percentage. All (geometric) means and percentages are adjusted for age and sex, except for age (only adjusted for sex) and sex (only adjusted for age).

a Geometric mean and interquartile range because of skewed distribution.
dysfunction, pulmonary oedema, fetal gene expression, and cardiomyopathy.4,5 Finally, a correlation has been found between high blood levels of inflammatory markers and poorer prognosis in heart failure patients.6

In the Atherosclerosis Risk in Communities study, an association between Lp-PLA2 and incident coronary heart disease was present after adjustment for age, sex, and race. After further adjustments for cardiovascular risk factors, the association was only present in subjects with a low LDL cholesterol.9 Our subgroup analysis showed that the association found between Lp-PLA2 activity and heart failure is present in subjects with a non-HDL cholesterol level below the median as well as in subjects with a non-HDL cholesterol level above the median, although in the latter group, the association lacked significance. The association between Lp-PLA2 and heart failure was much stronger in subjects with a C-reactive protein level below the median than in subjects with a C-reactive protein level above the median. We have no explanation for this difference in risk estimates. The interaction between Lp-PLA2 and C-reactive protein was not significant, so the difference may be due to chance.

Table 3 Hazard ratios for heart failure according to quartiles of Lp-PLA2 activity

<table>
<thead>
<tr>
<th>Lp-PLA2 (nmol/min per mL)</th>
<th>Cases/subjects</th>
<th>Hazard ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Model 1</td>
</tr>
<tr>
<td>Unit increase</td>
<td>94/1590</td>
<td>1.02 (1.00–1.03)</td>
</tr>
<tr>
<td>P-value for trend</td>
<td></td>
<td>0.026</td>
</tr>
<tr>
<td>Quartile 1</td>
<td>18/397</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Quartile 2</td>
<td>20/398</td>
<td>1.00 (0.52–1.86)</td>
</tr>
<tr>
<td>Quartile 3</td>
<td>21/398</td>
<td>1.27 (0.68–2.40)</td>
</tr>
<tr>
<td>Quartile 4</td>
<td>35/397</td>
<td>1.93 (1.09–3.42)</td>
</tr>
</tbody>
</table>

Note: Model 1 adjusted for age and sex; model 2 adjusted for age, sex, non-HDL cholesterol, and HDL cholesterol; model 3 adjusted for age, sex, non-HDL cholesterol, HDL cholesterol, body mass index, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, smoking status, and C-reactive protein.
Several studies have investigated the association between LDL cholesterol and heart failure. Although the Framingham Study found a positive relation, subsequent studies were not able to confirm this. In our study, we also failed to find a clear relation between non-HDL cholesterol and risk of heart failure. Lp-PLA2 is an enzyme bound to LDL cholesterol and therefore Lp-PLA2 activity is highly correlated with LDL cholesterol levels. In the present study, we found that the association of Lp-PLA2 with heart failure was independent of non-HDL cholesterol. We used non-HDL cholesterol for adjustment, since no measurements of LDL cholesterol were available. Because of the high correlation between LDL cholesterol and non-HDL cholesterol in a random sample of our cohort ($r = 0.97$, $P < 0.001$), we believe that residual confounding by LDL cholesterol cannot explain our results.

In conclusion, our findings suggest that Lp-PLA2 activity is independently associated with risk of heart failure. Our study provides further evidence that inflammation is involved in the aetiology of heart failure.

Acknowledgements

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Conflict of interest: none declared.
References


