Multivariate Analyses of Serum Apolipoproteins and Risk Factors in Relation to Acute Myocardial Infarction

Gunnar Fager, Olle Wiklund, Sven-Olof Olofsson, Lars Wilhelmsen, and Göran Bondjers

In 25 middle-aged infarction survivors and 76 corresponding controls, representative for a well-defined community, multivariate analysis was used to evaluate whether serum apolipoproteins were better discriminators of infarction survivors than serum lipids and other risk factors. Levels of serum cholesterol and triglycerides, alpha-lipoprotein cholesterol, apolipoproteins A-I, A-II, B, and D, as well as tobacco smoking and other risk factors, were included. In descending order, serum apo A-II levels ($t_b = -3.12, p = 0.002$), tobacco consumption ($t_b = 2.64, p = 0.010$), and serum triglycerides ($t_b = 2.06, p = 0.042$) contributed significantly to the multiple regression on myocardial infarction ($R = 0.53, p = 0.00001$). When entered into a discriminant function, these three variables gave a good separation between survivors and controls. Of the survivors, 50% were above the 90th percentile in the control group. The relative prevalence of infarction increased continuously with increasing values of the function from zero to more than 6 times the average. Serum apo A-II levels alone were almost as good in separating cases and controls. From this study, we concluded that, among apolipoproteins, apo A-II seems to be a more sensitive discriminator of infarction survivors than other risk factors. (Arteriosclerosis 1981; 1:273–279)
alcohol intemperance than were the controls. However, several variables were strongly intercorrelated. It seemed important, therefore, to evaluate which variables were the independent discriminators.

This paper reports the results of multivariate analyses carried out to evaluate the discriminative power for myocardial infarction of the apolipoprotein levels and of the established risk factors. It also presents a hypothetical model for the predictive power of the variables associated with infarction.

**Methods**

**Epidemiological Methods**

Since details have been published elsewhere, only a brief description of the selection of myocardial infarction survivors and random controls will be given here. The myocardial infarction group consisted of 25 men who survived a first myocardial infarction at the age of 40 to 44 years and who lived in the well-defined community of Göteborg, Sweden during 1975–1977. They comprised 71% of all first infarctions (86% of all survivors) registered in the population at risk (34,205 man-years of experience). This group is representative for infarction survivors, but may differ from the 17% who died. The incidence of first infarction was 102.3 per 100,000 men and year. Blood samples were drawn from the survivors at 6 months to 3½ years after infarction. At this time, four had been advised to make moderate adjustments in diet because of hyperlipidemia; one was also taking clofibrate. One patient was on a diet for mild diabetes, 19 (76%) were taking beta-blocking agents, one was being treated with quinidine, three were on digitalis, and two were on diuretics. The survivors did not differ significantly from the controls with regard to smoking habits or the levels of physical activity at the time of blood sampling.

A reference group was selected at random from the subpopulation from which the infarction survivors were derived. As shown in a previous study in Göteborg, the nonattendants in this sample (23.5%) may have differed from the 76 who attended. However, the observed differences between the groups could not be attributed to the dropout rate. Blood samples were obtained from controls simultaneously with the cases. Of the 76 controls, one man had had a myocardial infarction and was therefore also allocated to the infarction group; he was excluded from the reference group before the multivariate analyses. One man was on a diet for mild diabetes, and one was taking a beta-blocker.

Basic characteristics, including disease history and therapy as well as smoking habits and the levels of physical activity, were evaluated.
from standard questionnaires used in epidemiological studies in Göteborg. Different scores, increasing with tobacco consumption or the levels of physical activity, were used to characterize smoking habits and physical activity at work and during leisure time. Information about men registered for alcohol intemperance was obtained from the local temperance board. The records of the board provided a coarse means of selecting subjects with alcohol problems. Body weight index was calculated as body weight/(body height − 100) kg/cm. No blood pressure registrations were available from the time before infarction. The records of infarction in the survivors. Due to changes in blood pressure with myocardial infarction and with beta-blocking treatment, blood pressure was not included in the statistical calculations of the present study.

**Biochemical Methods**

The lipoprotein variables were quantitated in sera obtained after 12 hours of fasting and non-smoking and after 48 hours of abstinence from alcohol. Serum cholesterol and triglycerides, as well as alphalipoprotein cholesterol, were determined as previously described. Apo A-I, A-II, apo B, and apo D were determined with electroimmunoassay, as described elsewhere.

**Statistical Methods**

Correlations between study variables were evaluated with Spearman’s rank correlation coefficients. Coefficients of skewness and kurtosis were used to test deviations from normal distribution. Transformations according to log X (for alphalipoprotein cholesterol and apo B) or to log (log X times 100) (for serum triglycerides and apo D) were used to achieve a distribution that was not significantly different from normal prior to multivariate analyses. Serum cholesterol and apo A-I levels did not deviate significantly from a normal distribution. After logarithmic transformations, the distribution of apo A-II levels was still significantly different from normal. The apo A-II values were used without transformation in the calculations.

The association between study variables and myocardial infarction was at first tested with stepwise multiple regression. In multiple regression analyses, the infarction and reference groups were combined and calculations carried out with infarction/no infarction as a 1/0 dependent variable. The dependent variable was tested against 12 independent variables (body weight index, smoking score, physical activity score at work and during leisure time, registration at the temperance board, serum cholesterol and triglyceride levels, alphalipoprotein cholesterol levels, and the levels of apo A-I, A-II, apo B, and apo D). The significance of different variables for the prevalence of infarction was evaluated from the partial regression coefficients b, divided by their standard deviation, as t

The variables that were significant in multiple regression analyses were entered into discriminant analyses. An arbitrarily chosen constant was introduced in the discriminant function to render all values positive. The relative prevalence of infarction survivors in different discrete intervals of a variable was calculated according to the following: (no. of infarctions in the interval/total no. of infarctions)/(no. of controls in the interval/total no. of controls). All intervals created in this way contained seven or more controls, a number that was representative for more than 3000 man-years of experience.

All statistical calculations were carried out on an Olivetti P 6060 computer (Olivetti, Ivrea, Italy). Values of p < 0.05 (two-tailed tests) were considered statistically significant.

**Results**

**Correlations between Lipoprotein Variables and Risk Factors**

Spearman’s rank correlation coefficients are given in table 2. The serum levels of apo A-I and A-II as well as alphalipoprotein cholesterol were strongly correlated with each other but not so strongly with apo D levels. Serum apo B and cholesterol levels were strongly correlated with one another. Negative correlations were observed between apo D or alphalipoprotein cholesterol and serum triglyceride levels. As evident from table 2, several weaker correlations were also observed between other variables of the study. Among those, the negative correlations between tobacco consumption and alphalipoprotein cholesterol, apo A-I, or apo D may deserve particular notice.

**Association between Study Variables and Myocardial Infarction**

Three variables were found to correlate significantly with infarction by stepwise multiple regression (tested at F1 = F2 = 4) (table 3). As judged from their partial regression coefficients, apo A-II (t = -3.12, p = 0.002), tobacco consumption prior to infarction (t = 2.64, p = 0.010) and serum triglycerides (t = 2.06, p = 0.042) were the most important variables. The multiple correlation coefficient (R = 0.53) was highly significant (p = 0.00001) and explained (R²) 28% of the variation of the dependent variable. When calculations were repeated also to include variables of borderline significance (F1 = F2 = 2), registration at the temperance board (t = 1.83, p = 0.075) and the level of physical activity during leisure time (t = 1.83, p = 0.075) were added to
Table 2. Spearman's Rank Correlation Coefficients Between Some Risk Factors and Lipoprotein Variables in the Random Control Group (n = 76)

<table>
<thead>
<tr>
<th></th>
<th>Smoking</th>
<th>Physical activity</th>
<th>Body weight index</th>
<th>Serum cholesterol</th>
<th>Serum triglycerides</th>
<th>Alphalipoprotein cholesterol</th>
<th>Apo A-I</th>
<th>A-II</th>
<th>Apo B</th>
<th>Apo D</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoking</td>
<td>1</td>
<td>-0.27*</td>
<td>0.09</td>
<td>0.01</td>
<td>0.21</td>
<td>-0.35*</td>
<td>-0.25*</td>
<td>-0.15</td>
<td>0.17</td>
<td>-0.24*</td>
</tr>
<tr>
<td>Physical activity</td>
<td>1</td>
<td>0.12</td>
<td>0.12</td>
<td>0.00</td>
<td>0.20</td>
<td>0.01</td>
<td>-0.07</td>
<td>0.17</td>
<td>-0.10</td>
<td>-0.01</td>
</tr>
<tr>
<td>at work</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>during leisure time</td>
<td></td>
<td>-0.03</td>
<td>-0.04</td>
<td>-0.08</td>
<td>0.17</td>
<td>0.23*</td>
<td>0.23*</td>
<td>0.22</td>
<td>-0.02</td>
<td>0.21</td>
</tr>
<tr>
<td>Body weight index</td>
<td>1</td>
<td>0.25*</td>
<td>0.38*</td>
<td>-0.11</td>
<td>0.06</td>
<td>0.09</td>
<td>0.16</td>
<td>-0.07</td>
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<td></td>
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<tr>
<td>Serum cholesterol</td>
<td>1</td>
<td>0.24*</td>
<td>0.15</td>
<td>0.26*</td>
<td>0.25*</td>
<td>0.74*</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Serum triglycerides</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alpha-lipoprotein cholesterol</td>
<td>1</td>
<td>-0.46*</td>
<td>-0.18</td>
<td>-0.04</td>
<td>0.16</td>
<td>-0.30*</td>
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<td></td>
</tr>
<tr>
<td>Apo A-I</td>
<td>1</td>
<td>0.74*</td>
<td>0.07</td>
<td>0.37*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo A-II</td>
<td>1</td>
<td>0.03</td>
<td>0.38*</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Apo B</td>
<td>1</td>
<td>-0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apo D</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

*Significance levels: \( p = 0.05, r_s = 0.23; p = 0.01, r_s = 0.30; p = 0.001, r_s = 0.39.\)

Table 3. Results of Stepwise Multiple Regression Analyses

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>( F_1 = F_2 = 4 )</th>
<th>( F_1 = F_2 = 2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Constant ( = 0.53 ) (( p = 0.00001 ))</td>
<td>Constant ( = 1.08 )</td>
</tr>
<tr>
<td></td>
<td>( R = 0.57 ) (( p = 0.00001 ))</td>
<td>( R = 0.57 ) (( p = 0.00001 ))</td>
</tr>
<tr>
<td></td>
<td>( b ) ( t_b ) ( p )</td>
<td>( b ) ( t_b ) ( p )</td>
</tr>
<tr>
<td>Serum apo A-II levels</td>
<td>-1.64</td>
<td>-3.12</td>
</tr>
<tr>
<td>Smoking score</td>
<td>0.06</td>
<td>2.64</td>
</tr>
<tr>
<td>Serum triglycerides levels</td>
<td>0.71</td>
<td>2.06</td>
</tr>
<tr>
<td>Physical activity/leisure time</td>
<td>0.11</td>
<td>1.83</td>
</tr>
<tr>
<td>Alcohol intemperance</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Multiple \( R \) = multiple correlation coefficient. \( b \) = partial regression coefficient. \( t_b = b/SD_b \). \( p \) = probability value.

The possibility of discriminating between cases and controls was finally tested by discriminant analyses. One discriminant function (D3) contained serum apo A-II and triglyceride levels and tobacco consumption. In the other (D5), registered intemperance and physical activity during leisure time were also included. The functions are given in table 4. The distribution of values for D3 are given in figure 1A. Of the infarction survivors, 50% were above the 90th percentile in the reference group. More than 90% of the survivors were above the 50th percentile of the controls. Function D5 did not improve the discrimination, as seen in figure 1B.

In figure 2, the relative prevalence of infarction survivors is given for five discrete intervals of values of the discriminant functions D3 and D5. With increasing values of D3, the relative prevalence increased continuously from 0 to more than 6 times the average.

Table 4. Constants of Discriminant Functions D3, D5

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>D3</th>
<th>D5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum apo A-II levels</td>
<td>-11.95</td>
<td>-13.63</td>
</tr>
<tr>
<td>Smoking score</td>
<td>0.42</td>
<td>0.47</td>
</tr>
<tr>
<td>Serum triglycerides levels</td>
<td>5.19</td>
<td>5.21</td>
</tr>
<tr>
<td>Physical activity/leisure time</td>
<td>0.89</td>
<td>1.12</td>
</tr>
<tr>
<td>Alcohol intemperance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arbitrary constant</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
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Figure 1. Cumulative frequency distributions of values of discriminant function for acute myocardial infarction (•••) and control (o– –– o) groups. A. Calculations with three variables (D3) (serum apo A-II levels, triglyceride levels, and tobacco consumption). B. Calculations with five variables (D5) (serum apo A-II levels, triglyceride levels, tobacco consumption, registration at the temperance board, and physical activity during leisure time).

Figure 2. Relative prevalence of myocardial infarction plotted against five discrete intervals of values of the discriminant functions D3 (•••••) and D5 (o– – – o) (cf. figure 1).

Figure 3 gives a similar representation of the relative prevalence of infarction survivors for each variable included in the discriminant function D3. Apo A-II seems to be the best single variable to divide the material into low and high relative prevalences. The prevalence increased continuously from 0 to more than 18 times the average with decreasing apo A-II levels. Heavy smokers had a prevalence of 4 times the average. With increasing serum triglyceride levels, the relative prevalence increased from 0 to 5 times the average.

Figure 3. The relative prevalence of myocardial infarction plotted against five discrete intervals of serum apo A-II levels (•••••), triglyceride levels (o– – – o), and tobacco consumption (x– • • x).
Discussion

Serum apo A-II and triglyceride levels, as well as tobacco consumption, were found to contribute independently to the discrimination between infarction survivors and controls. Simple statistical analyses showed low levels of apo A-I and alphalipoprotein cholesterol in the infarction survivors; these low levels were not independently correlated to infarction, however, as judged from multivariate analyses in which the strong correlation between apo A-I, A-II, and alphalipoprotein cholesterol was taken into consideration. High apo B levels were also associated with infarction by simple statistics, but they did not contribute significantly to the discrimination of infarction survivors as evaluated from multivariate analyses.

High tobacco consumption was found to correlate strongly and independently to the development of infarction in several earlier studies. High levels of serum triglycerides have been regarded as an independent risk factor by some researchers, but not by others. Low levels of high density lipoprotein (HDL) or alphalipoprotein cholesterol, and high total serum cholesterol levels, were regarded as more significant for infarction at an older age than were serum triglycerides in the latter studies. Our results suggest that, when serum apolipoproteins A-I, A-II, D, and B are taken into consideration, serum triglycerides may contribute independently to the discrimination of infarction at a younger age, whereas serum cholesterol levels do not.

Low serum apo A-II levels have not been demonstrated to be an independent discriminator of infarction cases, although low levels have been found in infarction cases by simple statistics. The relevance of our findings must be interpreted against the background of possible errors. There have been no indications in the literature that any lipoprotein variable is changed by beta-blocking therapy regarding the lipoprotein families. The question arises as to what proportion of HDL is involved in the early development of infarction. In this study, we found no evidence for a significance of apo D in this process. We were recently able to isolate Lp A (containing the A-I and A-II polypeptides as protein moiety) and Lp A-I (containing only the A-I polypeptide) from HDL and to suggest the presence of these lipoprotein forms in native serum. We speculate whether variations in Lp A levels explain our results not only on apo A-II but also on apo A-I and alphalipoprotein cholesterol in relation to myocardial infarction.

The confidence limits of the relative prevalence of infarction in different strata of discrimination scores may well be rather wide. However, the proper way to test the relevance of the discriminant function would be in a separate study. This should be a prospective study in which the discriminant function is tested on a model to predict the possibility of males developing an infarction between 40 and 44 years of age.

Acknowledgment

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References


Index Terms: acute myocardial infarction · apolipoproteins · lipoproteins · risk factors
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