Serum Parameters as Discriminators between Normal and Coronary Groups

By Bernard S. Schlessinger, Ph.D., Fredrick H. Wilson, Jr., B.S., Lawrence J. Milch, Ph.D., and the Cardiovascular Research Group

Evaluation was undertaken of several measurements in discriminating between a group of clinically normal persons and a group of persons with definite atherosclerosis manifested by clinical myocardial infarctions. Various $S_r$ lipoprotein classes, the serum cholesterol, the beta lipoprotein cholesterol, the lipid phosphorus, and the "atherogenic index" were included in the study. The results are presented and their significance is discussed.

In the past 10 years, several biochemical and biophysical tests have been suggested as possible indicators of atherosclerosis and predictors of clinical complications of this disease.\(^1\)\(^-\)\(^6\) In 1950, the National Advisory Heart Council initiated a cooperative study involving 4 laboratories to evaluate 2 of these measurements as predictors of clinical complications of atherosclerosis. Serum cholesterol and lipoprotein measurements in a population (15,000) of clinically healthy men aged 40 to 59 were made among the laboratories, and the data were analyzed after a sufficient number of cases of coronary disease had occurred.

The results of the cooperative study were published in 1956.\(^7\) During the 6 year period, the Donner Laboratory had changed the methods of serum lipoprotein measurements to include additional lipoprotein classes\(^8\) and to correct for the effects of concentration on flotation rates.\(^8\) These changes, as well as a dispute over the definition of "new clinical events," resulted in a division of opinion as to definitive results.\(^7\) The Donner group claimed that the measurement of serum lipoproteins (with the refinements added in 1952) was a superior predictor of clinical complications of atherosclerosis, while the Cleveland, Harvard, and Pittsburgh groups stated that the cholesterol measurement was "at least as useful" as the lipoprotein determination.

This paper reports a preliminary study designed to evaluate the effectiveness of several serum measurements in discriminating between a group of clinically normal persons and one of persons with manifest atherosclerosis (clinical myocardial infarctions).

Methods

For this study, blood sera from a group of 24 male patients with myocardial infarction (MI) at Brooke Army Hospital, Fort Sam Houston, Texas, were utilized. The age distribution of this diseased group was determined and used as the criterion for selecting a comparable group of presumably normal individuals. This latter group (N) consisted of the 59 men between the ages of 30 and 66 taken from the total of 207 on whom satisfactory samples were received for routine analysis during the period from September 1, 1957 to January 15, 1958, and whose histories failed to indicate disease. Of the 59 normal men, 26 were persons in Pentagon executive positions, and 33 were members of the professional staff at the School of Aviation Medicine, USAF. The average age of the N group was 45 compared to an average age of 46 for the total (N + MI) population.

Each serum sample was analyzed ultracentrifugally for the concentration of $S_f^2$ 0-12, $S_f^2$ 12-20, and $S_f^2$ 20-400 lipoprotein classes by methods described elsewhere.\(^9\)\(^-\)\(^11\) The standard Gofman atherogenic index was calculated for each sample. Cholesterol was measured by a modification of the Bloor method\(^12\) and lipid phosphorus by a modified Fiske-Subbarow technique.\(^12\)

The $\beta$-cholesterol concentration was determined by a total cholesterol measurement on the top fraction of serum isolated after 15 hours of centrifugation at 79,000 $\times$ g at a medium density of 1.063 Gm./ml. Previous measurements of this
TABLE 1.—Serum Parameter Efficiency for Group Discrimination

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>Mean</th>
<th>Sample size</th>
<th>Standard deviation</th>
<th>Test statistic (t or z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>MI</td>
<td>331.47</td>
<td>23</td>
<td>82.45*</td>
<td>2.78†</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>280.85</td>
<td>59</td>
<td>45.78</td>
<td></td>
</tr>
<tr>
<td>β cholesterol</td>
<td>MI</td>
<td>219.84</td>
<td>24</td>
<td>74.46*</td>
<td>2.26§</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>183.32</td>
<td>58</td>
<td>42.06</td>
<td></td>
</tr>
<tr>
<td>Lipid P</td>
<td>MI</td>
<td>11.821</td>
<td>23</td>
<td>2.446*</td>
<td>2.46§</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>10.463</td>
<td>58</td>
<td>1.590</td>
<td></td>
</tr>
<tr>
<td>Δ cholesterol × 100</td>
<td>MI</td>
<td>65.31</td>
<td>23</td>
<td>11.69*</td>
<td>.18</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>N</td>
<td>64.83</td>
<td>58</td>
<td>7.32</td>
<td></td>
</tr>
<tr>
<td>Sβ 0-400</td>
<td>MI</td>
<td>724.25</td>
<td>24</td>
<td>222.50†</td>
<td>3.47</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>551.71</td>
<td>59</td>
<td>156.45</td>
<td></td>
</tr>
<tr>
<td>Sβ 0-12</td>
<td>MI</td>
<td>489.8</td>
<td>24</td>
<td>155.7*</td>
<td>3.17‡</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>381.82</td>
<td>50</td>
<td>92.8</td>
<td></td>
</tr>
<tr>
<td>Sβ 12-20</td>
<td>MI</td>
<td>65.7</td>
<td>24</td>
<td>37.1*</td>
<td>2.00§</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>47.9</td>
<td>50</td>
<td>22.5</td>
<td></td>
</tr>
<tr>
<td>Sβ 20-400</td>
<td>MI</td>
<td>168.75</td>
<td>24</td>
<td>117.95</td>
<td>1.94</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>121.90</td>
<td>59</td>
<td>91.72</td>
<td></td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>MI</td>
<td>87.88</td>
<td>24</td>
<td>28.93</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>N</td>
<td>67.92</td>
<td>59</td>
<td>22.55</td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>MI</td>
<td>27.98</td>
<td>23</td>
<td>3.42</td>
<td>1.40</td>
</tr>
<tr>
<td>Lipid P</td>
<td>N</td>
<td>26.93</td>
<td>58</td>
<td>2.88</td>
<td></td>
</tr>
</tbody>
</table>

* MI standard deviation significantly different from the normal standard deviation at the .01 level.
† MI standard deviation significantly different from the normal standard deviation at the .05 level.
‡ MI mean significantly different from the normal mean at the .01 level.
§ MI mean significantly different from the normal mean at the .05 level.
|| MI mean significantly different from the normal mean at the .001 level.

The data indicate a decrease with decreasing lipoprotein density (higher Sβ value) of the level of significance for differences in mean value between the 2 groups. In the Gofman atherogenic index, greater weighting is given to the lipoprotein moieties with higher Sβ values. Also, Sβ 0-400 lipoprotein concentration, with no weighting of any component class, is at the same level of significance as the atherogenic index. Both these factors indicate that a re-evaluation of the weighting factors in the calculation of the atherogenic index might lead to a more sensitive parameter.

RESULTS AND DISCUSSION

The data for the serum parameters are summarized in table 1.

Each variable was considered separately. A test of homogeneity of variances was performed on the N and MI groups to see if their variances were essentially equal. If this test indicated no significant difference, a t test (which assumes equal group variances and a normal distribution for each group) was used to test for differences in mean values. If the homogeneity of variance procedure showed a significant difference, a z test, assuming a normal distribution for each group but not equal group variances, was performed. The hypothesis tested on each variable was that "the means for the 2 groups were samples from the same population."

The only parameters in table 1 for which the differences of the means of the 2 groups are significant at the 0.001 level are Sβ 0-400 lipoprotein concentrations and the calculated atherogenic index. No individual lipoprotein class concentration measured showed a significant difference between the 2 groups at the .001 level as did the sum of all the β-lipoprotein concentrations, although the means of the N and MI groups for each separate class were significantly separated.

The parameter have been accomplished by alcohol fractionation methods and paper electrophoresis. The value for percentage of total cholesterol borne by the β-lipoprotein fraction reported here (66 per cent) compares favorably with the values reported by persons using alcohol fractionation technics (66 per cent by Oneley et al.,13 69 per cent by Barr et al.,14 70 per cent by Pearsall and Chanutin15). Using paper electrophoresis, Nikkila16 reported a value of 64 per cent for β-cholesterol, while Durrum's17 value, also with paper electrophoresis methods, was approximately 80 per cent.

In the case of Sβ 20-400, the value for t is on the threshold of significance at the .05 level.

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Total cholesterol discriminates between the 2 groups at a level of significance (0.01) less than either Sβ 0-400 concentration or atherogenic index (0.001), although cholesterol concentration is better than any of the other lipid parameters. Since only the β-lipoproteins are included in the calculation of the atherogenic index, it would seem that a measurement of β-cholesterol might be more effective than one of total cholesterol. Such
SERUM PARAMETERS AS DISCRIMINATORS

is not the case; β-cholesterol differentiates between the 2 groups at the 0.05 level of significance. These data indicate that some importance must be ascribed to the protein components of the lipoprotein moieties.

More significant discrimination of the β-lipoprotein parameters between diseased and normal persons may be due to the nature of the lipid-protein bond or the relative concentrations of the various constituents in the lipid-protein complex. These explanations do not require that any single lipid variable differentiate as well as the β-lipoproteins, but it would be expected that the lipid variables would show some significant separation between groups.

Neither the ratios of β to total cholesterol nor of total cholesterol to lipid phosphorus seem to discriminate the groups. The significance attached to the component parameters disappears when they are combined into a ratio.

It must be emphasized that these data (as well as most of the literature on this subject) relate to the ability of a serum parameter to discriminate between groups of MI and N subjects. The extrapolation of such data to the individual as a predictive measure is unwarranted except for calculation of the risk or of probability of disease in relation to other persons of the same age group. It may be, for instance, that the change in serum characteristics of the diseased patient, which well could account for the difference observed here, occurs after clinical manifestation of the disease. Only a “before and after” clinical study can accomplish an evaluation of “predictive” as opposed to “discriminatory” efficiency for any set of variables. Until such a clinical study is completed, any serum lipid or lipoprotein determination on an individual is only of incidental interest. Such a study, of the West Point class of 1956,18, 19 is in progress in this laboratory.

Summary

Several serum parameters were evaluated as discriminators between a normal (N) and a coronary disease (MI) group. The best discriminating parameters were $s_0^2$ 0-400 lipoprotein concentrations and the calculated atherogenic index. The means of the N and MI groups for each lipoprotein class were significantly separated. Total cholesterol was a less effective discriminator than total β-lipoprotein concentration or atherogenic index, but more effective than β-cholesterol or lipid phosphorus.

The data indicate that (a) some importance must be ascribed to the protein components of the lipoprotein moieties, and (b) a re-evaluation of the weighting factors in the calculated atherogenic index might lead to a more sensitive parameter.

Acknowledgement

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Summario in Interlingua

Plure parametras serologic esseva investigate con respecto a lor valor como criterios del differentiation de un grupo de subjectos normal (N) ab un grupo de subjectos con morbo coronari (MI). Le parametras le plus fidel pro ille differentiation esseva (1) le concentrationes de lipoproteina $s_0^2$ 0-400 e (2) le calculate indice atherogenic. Le valores medie pro omne le classes de lipoproteina esseva significativamente differente in le duo gruppos, i.e. le grupo N e le grupo MI. Le valor del cholesterol total esseva un minus efficace differentiator que le valor de lipoproteina beta o le indice atherogenic. Del altere latere, illo esseva un plus efficace differentiator que le valor de cholesterol beta o le valor de phosphoro lipidic.

Le datos indica (a) que un certe importantia debe esser ascribe al componentes proteinic in le subdivisiones de lipoproteina e (b) que un re-evaluation del factores de ponderation in le calculation del indice atherogenic poterea resultar in le obtention de un parametro de plus alte grades de sensibilitate.
REFERENCES


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