Correlation of Laboratory Tests and Clinical Evaluation in Phenotyping of Lipoproteinemias

James Winkelman, Donald R. Wybenga, and Frank A. Ibbott

Accuracy of phenotyping of lipoproteinemias by electrophoresis was assessed by comparing the interpretation of that laboratory test alone with the clinical diagnosis. Results were consistent with the clinical diagnosis in 88.7% of the series. Inconsistent results most frequently occurred with samples for which cholesterol and triglyceride determinations were not made or considered. Quantitative serum values for these substances obtained from another specimen or which had been determined by another laboratory and were not available to our laboratory, were the most common bases for the physician's conclusion that the laboratory report was incorrect. We conclude that cholesterol and triglyceride should be routinely determined in the laboratory testing for phenotyping of lipoproteinemia. The cholesterol and triglyceride analyses must be performed on the same specimen that is electrophoresed to provide satisfactory accuracy.

Additional Keyphrases cholesterol, triglyceride determination • cellulose acetate electrophoresis

SeveraL IMPORTANT FACTORS that determine the precision of laboratory tests for the phenotyping of hyperlipoproteinemias have been established in recent studies (1–3). Our experience showed that paper electrophoresis of lipoproteins (1) suffered from poor reproducibility due to the subjective interpretation of the position and intensity of lipoprotein bands. This factor plus the limited ability of the technique to separate β- and pre-β bands also led to discrepancies between the interpretation of the electrophoretograms and the quantitative serum levels of cholesterol and triglyceride that are characteristic of the hyperlipoproteinemia phenotypes. Furthermore, a large percentage of samples submitted for analysis could not be classified according to Fredrickson's criteria (4). Such samples came from either normal people or successfully treated patients.

A cellulose acetate electrophoresis (cae) technique was developed that largely overcame the problems of poor resolution of β- and pre-β bands, and a system of reporting was evolved that provided for the nondefinitive as well as the typical patterns (2). Quantitation of the stained lipoprotein bands of these various types of cae strips increased the precision of the technique, but not sufficiently to justify the incorporation of this step in routine laboratory practice (3).

Understanding and control of these factors permitted the errors attributable to purely technical aspects of the procedure to be minimized. It then became possible, and appeared highly desirable, to determine the accuracy of results so generated. The remaining factors that contributed to the lack of correlation between the phenotyping of lipoproteinemias by electrophoresis and the clinical diagnosis were identified in this study. Virtually all causes of inaccuracy were extrinsic to the electrophoretic technique itself.

We can now recommend a modified protocol that provides a highly satisfactory laboratory procedure for assessing lipoproteinemia phenotypes.

Materials and Methods

All specimens (866) submitted to Bio-Science Laboratories during 10 days with a request for phenotyping of lipoproteinemias were included in this study. In 45.7%, various other lipid analyses were also requested. Virtually none of the requests provided such ancillary information as the age of the patient, the clinical impression, the results of previous laboratory tests, etc.

The methods used for cae, cholesterol, tri-
glyceride, phospholipids, and total lipids have been described (2). The system of reporting is the same as that previously developed to accommodate the variety of electrophoretic strip appearances encountered in specimens from a similar population (2). Readings were made by visual evaluation of the CAE strip according to comparisons with known abnormal types (4). If cholesterol and triglyceride results were available, the strip readings were checked for consistency according to criteria evolved from our work (1). These were closely similar to those reported by others (5–7). Inconsistency was resolved either by rereading the strip, by repeating chemical and (or) electrophoretic analysis, or by analysis of a new specimen if it was believed that the original specimen was drawn from a nonfasting individual or had changed because of instability with aging (8).

Accuracy was determined entirely on the basis of the response of the clinician, who was asked whether the phenotyping result was consistent with his clinical diagnosis. If the result was inconsistent, further questions were asked to elicit the basis for his conclusion. The following format, printed on the reverse side of a duplicate report, was provided for the response:

A. ___ The lipoprotein phenotyping report is consistent with the clinical diagnosis
B. ___ The lipoprotein phenotyping report is not consistent with the clinical diagnosis
   1. ___ The wrong phenotype was reported. The correct phenotype is __I, __II, __III, __IV, __V
      a. The correct phenotype was established on the basis of other laboratory findings
      b. physical findings
      c. family or past history
      d. response to dietary or drug therapy
   2. ___ A definitive pattern was reported, but
      a. the patient is not hyperlipidemic
      b. the sample was not fasting
      c. the diet had not been normal

3. ___ A nondefinitive pattern was reported, but
   a. the patient is hyperlipidemic
   b. the patient previously was hyperlipidemic, and has been treated
   c. the patient previously was hyperlipidemic, and has not been treated
   d. the wrong borderline or treated type was listed as possible with the observed pattern

Since first respondents to a survey are possibly not representative of the entire group, a second mailing was made to all nonrespondents. Another copy of the original report was sent with the same response format on the reverse side, accompanied by a letter explaining the objectives and needs of the study. This was designed to reveal biases such as a tendency for immediate responses only in clear-cut consistent cases, but hesitation if data were not obviously compatible. By comparing responses to the first and second communications, we could assess possible differences owing to the type of report, presence or absence of other lipid analyses, level of requirements for diagnosis, or criticality of respondents.

Results

Table 1 summarizes the accuracy analysis survey. All reported tests and responses were categorized according to whether cholesterol and (or) triglyceride analyses were performed on the same specimen. From the combined response (64.2%) conclusions may be drawn for the entire series. A breakdown of results according to the reported lipoprotein phenotype showed an essentially identical distribution of results in the respondents as in the original sample. The percentage of all responses with "cholesterol and (or) triglyceride" among the respondents is also virtually identical to that of the entire population. Findings for each parameter between the responses to the first and second mail-

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**Table 1. Results of Phenotyping of Lipoproteinemia Accuracy Survey**

<table>
<thead>
<tr>
<th></th>
<th>All responses</th>
<th>Consistent responses</th>
<th>Inconsistent responses</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>866</td>
<td>556</td>
<td>63</td>
</tr>
<tr>
<td>Number</td>
<td>39.4</td>
<td>64.2</td>
<td></td>
</tr>
<tr>
<td>% of total sent</td>
<td>214</td>
<td>493</td>
<td>63</td>
</tr>
<tr>
<td>% of total responses</td>
<td>193</td>
<td>88.7</td>
<td>11.3</td>
</tr>
<tr>
<td>Number with</td>
<td>204</td>
<td>198</td>
<td></td>
</tr>
<tr>
<td>cholesterol and</td>
<td>289</td>
<td>125</td>
<td>2</td>
</tr>
<tr>
<td>triglyceride</td>
<td></td>
<td>73</td>
<td>1</td>
</tr>
<tr>
<td>% with cholesterol</td>
<td>33.4</td>
<td>41.6</td>
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</tr>
<tr>
<td>and triglyceride</td>
<td></td>
<td>37.9</td>
<td></td>
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<tr>
<td>Number with</td>
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<td>40.1</td>
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<tr>
<td>cholesterol or</td>
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<tr>
<td>triglyceride</td>
<td>107</td>
<td>29</td>
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<tr>
<td>% with cholesterol</td>
<td>10.2</td>
<td>2</td>
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<tr>
<td>or triglyceride</td>
<td>12.3</td>
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<td>CLINICAL CHEMISTRY, Vol. 16, No. 7, 1970  595</td>
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ings also showed no important differences; therefore, only the totals for each entry will be discussed.

The laboratory result of the phenotyping procedure was consistent with the clinical diagnosis in 88.7% of the cases. The most important feature differentiating those results from the inconsistent ones was that in the former the cholesterol and triglyceride values were much more frequently known, and had been considered in the formulation of the phenotyping report. Over 40% of consistent results were from such cases, whereas in only three instances, which comprised merely 4.8% of all inconsistent results, had cholesterol and triglyceride been determined. Although the numbers are smaller, there was a similar difference between consistent and inconsistent responses according to whether cholesterol or triglyceride had been analyzed.

The causes cited for the physicians' decisions that the laboratory result was inconsistent with the clinical diagnosis are listed in Table 2. A larger total number is tabulated than there were instances of inconsistency (Table 1) because several respondents stated more than one reason. The great majority of such conclusions were based on quantitative lipoprotein fractionation data from either some other source or some other specimen, which was not available to the laboratory when the lipoprotein CAE was performed and evaluated.

Discussion

The physician's clinical diagnosis was accepted without further review as the basis for evaluation of the laboratory results for the purpose of this study. Compatibility of the laboratory result with the diagnosis has thus been considered equivalent to accuracy. This assumption could be criticized since uniform diagnostic criteria were almost certainly not used by all respondents.

The widely accepted guidelines of Fredrickson et al. (4) are routinely provided with every laboratory report to facilitate the integration of the objectively described pattern of the CAE strip with other laboratory data and clinical findings. Despite this, for example, it is possible that responses of consistency were improperly weighted by acceptance of the laboratory report. Conversely, the judgment of inconsistency may have ignored or improperly weighted other factors. However, since no absolutely standardized criteria or independent measures are currently accepted or available for the phenotyping of hyperlipidemic patients and the requirements for the definitive identification of the phenotypes are still evolving, we believe that our approach was virtually the only one feasible.

The improvement in the accuracy of reported results that accrues from routinely performing cholesterol and triglyceride analyses on the same sample as that subjected to CAE and using those values as previously described in the interpretation of the phenotype (1) has led us to include those determinations as an intrinsic part of the test for phenotyping of lipoproteinemia. The benefits are much greater than those obtained after the use of quantitative measures of lipoprotein staining intensity (3), while the increase in overall cost is considerably less because of the availability of automated methods for those determinations.

References