The 16-Hour-Standing Test and Lipoprotein Electrophoresis Compared for Detection of Chylomicrons in Plasma

S. McNeely, K. Seatter, J. Yuhaniak, and M. L. Kashyap

We compared the 16-hour-standing plasma test to lipoprotein electrophoresis on agarose gel for detection of chylomicrons in 129 patients' samples with triglyceride values ≥4.00 g/L. Chylomicrons were observed in 12 samples (9.3%) by use of the standing-plasma test and in 58 samples (45.0%) by use of agarose-gel electrophoresis. Thus the standing-plasma test did not detect chylomicrons in 46 samples where they were observed by electrophoresis, or 79.3% of all cases where chylomicrons were present. Chylomicronemia was missed in the presence of lower triglyceride concentrations as well as at very high ones. We recommend that lipoprotein electrophoresis be routinely performed on plasma of patients with triglyceride concentrations >4.00 g/L to distinguish between types IV and V hyperlipoproteinemia, as well as to detect failure of a patient to fast before sample collection.

Additional Keyphrases: electrophoresis on agarose gel • triacylglycerols • hyperlipoproteinemia • chylomicronemia

In recent years, lipoprotein electrophoresis in conjunction with the routine determination of cholesterol and triglycerides in plasma has been de-emphasized. If increased lipoprotein concentrations are all that is being looked for, this is probably an acceptable omission. However, as Papadopoulos has pointed out (7), under certain conditions electrophoresis may reveal lipid abnormalities that may or may not be apparent from the cholesterol and triglyceride values. Several studies indicate that results of electrophoresis on agarose gel correlate well with those by analytical ultracentrifugation in chylomicron detection, in that material remaining at the origin during electrophoresis is identical with material with S<sub>r</sub> >400 (2-6), although the correlation is not good enough for electrophoresis to be used as a quantitative measure of chylomicrons (6).

Here we show that the commonly used 16-hour-standing plasma test fails to detect chylomicrons in nearly 80% of cases where they may be detected by electrophoresis. Accordingly, clinicians may not be aware of the presence of exogenous triglycerides (triacylglycerols) in a large proportion of patients, and so may be treating these patients for type IV hyperlipoproteinemia when treatment for type V hyperlipoproteinemia (7) would in fact be more appropriate. A further possibility is that type V hyperlipoproteinemia is more common than previously suspected. Finally, the presence of chylomicrons may be indicative of a non-fasting sample, giving a misleadingly high value for triglycerides. Decrease of triglyceride concentrations in subsequent samples on such a patient may be the result of fasting rather than a regression toward normal or an effect of treatment.

Materials and Methods

Patients' samples. Plasma was sampled from 129 patients whose triglyceride values had been found to equal or exceed 4.00 g/L after a 12-h fast, according to Lipid Research Clinics protocol (8). The age range for patients studied was 10–77 years (mean, 46.53); 57 were females and 72 males.

Standing plasma test. A 16-hour-standing plasma test was performed according to Lipid Research Clinics protocol (8): 2 mL of plasma was pipetted into a 10 × 75 mm glass culture tube and, after 16 h at 4 ºC, was examined under uniform lighting conditions; chylomicrons were noted as a supernatant creamy layer. In accordance with Lipid Research Laboratories Methods Committee recommendations, a wisp of cloudiness in the supernate was recorded as "chylomicrons absent."

Agarose-gel electrophoresis. Lipoprotein separation by electrophoresis was performed within seven days of sample collection according to the method of Elevich et al. (9). An agarose film/cassette system, along with an incubator oven for drying the films (Analytical Chemists, Inc., Palo Alto, CA 94303) was used to run the electrophoresis film. Thin agarose-gel films (10 g of agarose and 50 g of sucrose per liter of 75 mmol/L barbital buffer, pH 8.6; "Pol-E-Film," Pfizer Diagnostics, New York, NY 10017) were loaded with 1 µL of sample in each of eight wells, then placed in the electrophoresis cell and run for 30 min at 90 V in 50 mmol/L barbital buffer, pH 8.6. The films were then dried for 30 min at 70 ºC, stained with a methanolic solution of Fat Red 7B, cleared for 20 s with an equivolume mixture of absolute methanol and de-ionized water, rinsed with glycerol/water (2/98 by vol) for 15 s, and air dried at room temperature.

Chylomicrons were observed as a distinct ring of stained material surrounding the well in which the sample was loaded (origin). A trace of material at the origin was interpreted as no chylomicrons present, because this material is also observed in excessive trailing of very-low-density lipoprotein.

Results and Discussion

The observed range of triglyceride values was 4.00–97.60 g/L, with a mean of 11.23 (SD 11.7) g/L. Table 1 summarizes triglyceride values in relation to the observed presence or absence of chylomicrons.

Chylomicrons were observed in 12 of the 129 samples (9.3%) by the standing-plasma test, in 58 (45.0%) by lipoprotein electrophoresis. In 46 samples (35.7% of all samples, 79.3% of all chylomicron-positive samples) chylomicrons were detected by electrophoresis but not by the standing-plasma test; in no instance was chylomicronemia detected by the standing-plasma test but not by electrophoresis. When the standing-plasma test was performed after one week instead of 16 h on a subset of 112 samples, chylomicrons were detected in 27 samples by the standing-plasma test vs. 56 samples by electrophoresis, that is, in 48.2% of all chylomicron-positive samples.

By electrophoresis, chylomicrons were detected in the presence of triglyceride concentrations as low as 4.00 g/L, whereas they were first detected by the standing-plasma test.

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Table 1. Plasma Triglyceride Concentrations vs Presence of Chylomicrons

<table>
<thead>
<tr>
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<th>Triglyceride concn.</th>
<th>n</th>
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<tbody>
<tr>
<td>All samples</td>
<td>11.228 (11.766)</td>
<td>129</td>
</tr>
<tr>
<td>SPT negative</td>
<td>1.028 ( 1.118)</td>
<td>117</td>
</tr>
<tr>
<td>SPT positive</td>
<td>20.427 (13.813)</td>
<td>12</td>
</tr>
<tr>
<td>LPE negative</td>
<td>6.300 ( 2.470)</td>
<td>71</td>
</tr>
<tr>
<td>LPE positive</td>
<td>17.260 (15.366)</td>
<td>58</td>
</tr>
<tr>
<td>SPT neg., LPE pos.</td>
<td>16.433 (15.782)</td>
<td>46</td>
</tr>
<tr>
<td>SPT pos., LPE pos.</td>
<td>20.427 (13.813)</td>
<td>12</td>
</tr>
</tbody>
</table>

* SPT = 16-hour-standing plasma test.  b Negative = chylomicrons absent.
  c Positive = chylomicrons present.  d LPE = lipoprotein agarose-gel electrophoresis.

at 5.40 g/L. By electrophoresis, chylomicronemia was observed in 37 of 41 patients whose triglyceride concentrations exceeded 10.0 g/L but in only 8 of the 41 patients by the standing-plasma test.

The currently accepted practice of visual detection of chylomicrons by use of a 16-hour-standing plasma test is inadequate for determining the presence or absence of exogenous triglycerides. Increasing the length of time the plasma is left to stand results in some improvement, but more chylomicronemia is still detected by agarose-gel electrophoresis. As noted above, the standing-plasma test failed to detect chylomicrons not only in samples with lower triglyceride concentrations (<10.0 g/L), where theoretically there might be fewer chylomicrons to detect, but also in 29 of 37 (78.4%) of the cases with grossly above-normal triglycerides (>10.0 g/L).

We recommend that agarose-gel electrophoresis be routinely performed by laboratories on all samples with triglyceride concentrations exceeding 4.00 g/L, if patients are to be adequately characterized for treatment as having type IV or type V hyperlipoproteinemia, and that the possibility of increased triglyceride concentrations being due to non-fasting before sampling be considered in evaluation.

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References