References


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Screening for Dysbetalipoproteinemia by Plasma Cholesterol and Apolipoprotein B Concentrations, Dirk J. Blom,Frans H. O’Neill, and A. David Marais’ (Division of Lipidology, Department of Medicine, University of Cape Town, Cape Town, South Africa; * address correspondence to this author at: Lipid Laboratory, 5th Floor, Chris Barnard Bldg., UCT Faculty of Health Sciences, Anzio Road, 7925 Observatory, South Africa; fax 27-21-4066396, e-mail dmarais@capeheart.uct.ac.za)

Dysbetalipoproteinemia (type III hyperlipidemia) is a highly atherogenic mixed hyperlipidemia characterized by the accumulation of remnants of triglyceride-rich lipoproteins (chylomicrons and VLDL) (1). The binding of these remnants to hepatic lipoprotein receptors is mediated by apolipoprotein E (apoE). At the apoE gene locus, there are three common alleles: e2, e3, and e4 (2). ApoE2 binds poorly to hepatic lipoprotein receptors, leading to impaired remnant clearance. Consequently, remnants become enriched with cholesterol and migrate abnormally on electrophoresis. More than 90% of dysbetalipoproteinemic patients are homozygous for apoE2, but only a minority (1 in 20) of apoE2 homozygotes will be overtly hyperlipidemic. Remnant accumulation sufficient to cause hyperlipidemia usually occurs only when a second metabolic hit increases lipoprotein production (e.g., diabetes) or further decreases remnant clearance (e.g., hypothyroidism) (3).

Dysbetalipoproteinemia is highly atherogenic but responds well to lifestyle changes and lipid-modifying medications. Genetic counseling is important, particularly in areas where there is a high local prevalence of autosomal-dominant dysbetalipoproteinemia.

Composition (10). For cystatin C, we saw no systematic difference between venous and capillary blood samples. The small differences seen (Fig. 1) included the analytical imprecision of the assay, which in our hands performed according to NCCLS EP5 was 5% (CV) at 1.9 mg/L and 4% at 0.8 mg/L. Therefore, the scatter between venous and capillary concentrations appears clearly acceptable in clinical practice.

Our study had at least two limitations. The first limitation is that the cystatin C concentrations were within our laboratory’s reference range and thus our findings might not apply to patients with renal failure. The study suggested, however, good equilibration of cystatin C between the compartments potentially involved in capillary blood sampling, suggesting that this possibility is unlikely. The other limitation is that we did not study children. Previous studies comparing capillary and venous blood sampling of various analytes in children and adults did not suggest an effect of age on the agreement of results with these sampling techniques (11–13).

In conclusion, our findings indicate that cystatin C can be measured reliably in serum samples obtained by capillary finger puncture and with use of a commercially available particle-enhanced immunonephelometric assay for the protein.

We thank our colleague volunteers for participating in this study, Inge van Blerk for performing the cystatin C analyses, and Dade Behring (Marburg, Germany) for providing us with cystatin C tests free of charge.
mal dominant apoE mutations (4, 5). Dysbetalipoproteinemia is often suspected when both the total cholesterol (TC) and triglyceride (TG) concentrations are increased and the TC/TG molar ratio approximates 2:1. Clinically, pathognomonic palmar crease xanthomata are found in only ~20% of patients (4, 6, 7). There are no simple diagnostic tests for dysbetalipoproteinemia. Diagnostic tests are based either on the demonstration of remnant accumulation or characterization of apoE. Electrophoretic techniques include serum agarose gel electrophoresis, but a broad β band is found in less than one half of patients (8, 9). Ultracentrifugation is required to demonstrate β-migrating VLDL. Nondenaturing polyacrylamide gradient gel electrophoresis is an effective screening technique (9). Cholesterol-enriched VLDL is diagnostic of dysbetalipoproteinemia (10–12), but sample preparation requires ultracentrifugation, which is not available in routine diagnostic laboratories. ApoE phenotyping requires isoelectric focusing, and genotyping is usually done by PCR-based methods. ApoE characterization is not universally available, and standard techniques to identify the variance at codons 112 and 158 may misclassify or fail to diagnose autosomal dominant mutations in apoE.

Apo B100 (apoB) is the structural protein of atherogenic lipoproteins including VLDL, intermediate-density lipoprotein, and LDL. Each lipoprotein contains one apoB molecule, and plasma apoB concentrations reflect the total number of circulating atherogenic lipoproteins. Larger lipoproteins contain proportionally less apoB and more lipid than smaller lipoproteins.

ApoB is readily and reliably quantified by automated methods, and the test is widely available (13). We investigated whether apoB could be used as an initial screening test in patients with mixed hyperlipidemia in whom special investigations were undertaken to confirm dysbetalipoproteinemia. The patients attended the lipid clinic at Groote Schuur Hospital, a large public sector teaching hospital in South Africa.

We retrospectively identified all patients with mixed hyperlipidemia in whom the admitting clinician had suspected dysbetalipoproteinemia and for whom VLDL compositional analysis and apoE genotyping had been performed. Patients with incomplete data and those taking lipid-lowering medications at presentation were excluded. We compared the patients with definite dysbetalipoproteinemia with a sensitivity of 89% [95% confidence interval (CI), 78–96%] and a specificity of 97% (CI, 94–98%). Classifying these patients as dysbetalipoproteinemia with a sensitivity of 93–98%. Dysbetalipoproteinemic patients had higher lipid values (TG and TC) but lower apoB concentrations than the patients in whom the diagnosis had been excluded. Serum lipids in patients with indeterminate VLDL composition did not differ significantly from serum lipids in patients in whom dysbetalipoproteinemia had been confidently excluded, but TC was lower and apoB higher than in patients with dysbetalipoproteinemia. The apoB/TC ratios in the patients are shown in Fig. 1. When we compared the patients with definite dysbetalipoproteinemia with those in whom the diagnosis had been excluded, we found that a cutoff of <0.15 identified dysbetalipoproteinemia with a sensitivity of 89% [95% confidence interval (CI), 78–96%] and a specificity of 97% (CI, 94–98%). The area under the ROC curve was 0.98 (CI, 0.95–0.99). Although patients with indeterminate VLDL composition have serum lipids similar to patients in whom dysbetalipoproteinemia has been excluded, they remain difficult to classify confidently. If these patients are classified as not dysbetalipoproteinemic, the diagnostic specificity of a ratio <0.15 is reduced to 96% (CI, 93–98%). Classifying these patients as dysbetalipoproteinemic (despite the lack of confirmatory genotyping) reduces the sensitivity to 71% (CI, 60–80%). In our series of dysbetalipoproteinemic patients, the apoB/TC ratio did not exceed 0.2, and in patients with higher ratios, further investigations for dysbetalipoproteinemia are unlikely to be positive.

We also evaluated the diagnostic usefulness of the apoB/TG ratio, which was significantly lower [0.24 (0.16)]...
vs 0.39 (0.15); \( P < 0.001 \) in dysbetalipoproteinemic patients than in those in whom the diagnosis had been excluded. The values overlapped widely (data not shown), and this ratio was not diagnostically useful.

Remnant lipoproteins are larger and contain more lipid than LDL. Equivalent increases in serum cholesterol will therefore be associated with lower apoB concentrations when accumulations of remnants are compared with LDL accumulation. The apoB/TC ratio approximates the cholesterol content of circulating lipoproteins, although it does not take into account cholesterol circulating in non-apoB-containing lipoproteins such as HDL. However, we found that the apoB/non-HDL-C ratio was less discriminating than the apoB/TC ratio (data not shown). In our study, HDL-C was measured with a precipitation-based assay. These assays may overestimate HDL-C in hypertriglyceridemic samples (15), giving a falsely low non-HDL-C value. This would increase the ratio apoB/non-HDL-C, obscuring the low ratio characteristic of dysbetalipoproteinemia. In our cohort of patients with mixed hyperlipidemia, TG concentrations were higher in those with dysbetalipoproteinemia.

There are few screening tests for dysbetalipoproteinemia suitable for general use. Nondenaturing polyacrylamide gradient gel electrophoresis of plasma prestained for lipoproteins is both sensitive and specific (9). Although the technique is not technically demanding, it is not available in routine clinical laboratories. The apoB/apoE ratio has been evaluated previously in 40 dysbetalipoproteinemic patients and 48 controls matched for lipid values (16). The diagnostic sensitivity was 95% with a specificity of 88%. ApoE measurements can be automated in clinical laboratories, but many laboratories do not offer this test routinely. A precipitation-based screening method for dysbetalipoproteinemia has also been described (17). Dextran sulfate/MgCl\(_2\), used in first-generation direct LDL assays, precipitates both LDL and remnants. The combination of high precipitate cholesterol and TGs identifies dysbetalipoproteinemia with high sensitivity but has poor specificity (30%). Third-generation direct LDL-cholesterol (LDL-C) assays have largely replaced

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### Table 1. Clinical and biochemical data\(^a\) for patients with mixed hyperlipidemia evaluated for dysbetalipoproteinemia.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Dysbetalipoproteinemia</th>
<th>Dysbetalipoproteinemia excluded</th>
<th>Indeterminate VLDL composition</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>57</td>
<td>254</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>ApoE diagnostic criterion</td>
<td>( \varepsilon^2 ) homozygosity</td>
<td>( \varepsilon^3 ) or ( \varepsilon^4 ) allele present</td>
<td>All genotypes</td>
<td></td>
</tr>
<tr>
<td>VLDL composition criteria</td>
<td>VLDL-C/VLDL-TG &gt; 0.96 or VLDL-C/plasma-TG &gt; 0.69</td>
<td>VLDL-C/VLDL-TG &lt; 0.80 and VLDL-C/plasma-TG &lt; 0.57</td>
<td>VLDL-C/VLDL-TG range 0.80–0.96 or VLDL-C/plasma-TG range 0.57–0.69</td>
<td></td>
</tr>
<tr>
<td>Age at presentation, years</td>
<td>50.1 (9.3)</td>
<td>52.9 (11.8)</td>
<td>54.43 (16.32)</td>
<td>NS(^b)</td>
</tr>
<tr>
<td>M/F, n</td>
<td>29/28</td>
<td>101/153</td>
<td>7/15</td>
<td>NS</td>
</tr>
<tr>
<td>Diabetes, %</td>
<td>17.5</td>
<td>38.8</td>
<td>36.4</td>
<td>0.009</td>
</tr>
<tr>
<td>TC, mmol/L</td>
<td>11.75 (4.4)(^c,d)</td>
<td>7.94 (1.7)</td>
<td>8.61 (1.7)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TGs, mmol/L</td>
<td>5.86 (4.89–7.00)(^c)</td>
<td>4.32 (4.11–4.45)</td>
<td>4.19 (3.59–4.88)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HDL-C, mmol/L</td>
<td>1.20 (0.4)</td>
<td>1.14 (0.3)</td>
<td>1.05 (0.4)</td>
<td>NS</td>
</tr>
<tr>
<td>ApoB100, g/L</td>
<td>1.15 (0.48)(^c,d)</td>
<td>1.61 (0.37)</td>
<td>1.68 (0.52)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TC/TG ratio</td>
<td>2.1 (0.9)</td>
<td>1.9 (0.6)</td>
<td>2.2 (0.9)</td>
<td>NS</td>
</tr>
<tr>
<td>VLDL-C/VLDL-TG ratio</td>
<td>1.15 (0.31)(^c,d)</td>
<td>0.53 (0.11)(^d)</td>
<td>0.81 (0.05)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>VLDL-C/plasma-TG ratio</td>
<td>0.79 (0.20)(^c,d)</td>
<td>0.36 (0.09)(^d)</td>
<td>0.55 (0.08)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ApoB/TC ratio</td>
<td>0.10 (0.04)(^c,d)</td>
<td>0.20 (0.03)</td>
<td>0.19 (0.04)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

\(^a\) All data are the mean (SD), except for TGs, which are the geometric mean (95% CI).

\(^b\) NS, not significant.

\(^c\) \( P < 0.05 \) vs patients in whom dysbetalipoproteinemia has been excluded.

\(^d\) \( P < 0.05 \) vs patients with indeterminate VLDL composition.

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Fig. 1. ApoB (g/L)/TC (mmol/L) ratio in patients with mixed hyperlipidemia.

ApoB/TC is plotted for 57 patients with dysbetalipoproteinemia (○), 254 patients with mixed hyperlipidemia (●) in whom dysbetalipoproteinemia had been excluded, and 22 patients with indeterminate VLDL composition (△). The dashed line indicates the proposed diagnostic cutoff of 0.15. The solid horizontal bars are the group means.
first-generation assays and may overestimate LDL-C in patients with dysbetalipoproteinemia (18, 19). Some of our dysbetalipoproteinemic patients were initially evaluated in laboratories that perform direct LDL-C measurements (various methods), and in our experience (unpublished observation), their measured LDL-C is often much lower than the LDL-C calculated by the Friedewald formula. The Friedewald formula is known to overestimate LDL-C in dysbetalipoproteinemia because of abnormal VLDL composition.

It has been suggested that measuring TC adds little to cardiovascular risk assessment when HDL-C and LDL-C are measured directly (20). This approach is valid for most patients but will underestimate atherosclerotic risk in conditions in which abnormal nonmeasured lipoproteins such as remnants accumulate. ApoB concentrations are an important component of cardiovascular risk assessment (21, 22). Increased apoB is associated with higher numbers of circulating atherogenic lipoproteins and higher cardiovascular risk. In dysbetalipoproteinemia, the situation is somewhat reversed, and apoB concentrations below the 75th North American population percentile (23) are associated with very high cardiovascular risk. In dysbetalipoproteinemia, high TC and TG concentrations indicate high risk, once again highlighting that apoB and lipid measurements are not identical but complementary indices for risk assessment (24).

In conclusion, the apoB/TC ratio is a simple but effective screening test for dysbetalipoproteinemia in patients with mixed hyperlipidemia when lipid values are suggestive of dysbetalipoproteinemia. It best identifies patients that are unlikely to be dysbetalipoproteinemic, for whom additional testing likely will be nondiagnostic. The ratio should not be used in other lipid phenotypes. No additional laboratory work is required if apoB is included as part of the routine assessment of dyslipidemia.

References


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Effect of Two Common Polymorphisms in the ATP Binding Cassette Transporter A1 Gene on HDL-Cholesterol Concentration, Pettor S. Woll, Naomi Q. Hanson, Valerie L. Arends, and Michael Y. Tsai (Department of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN 55455; * address correspondence to this author at: 420 Delaware Street SE, Mayo Mail Code 609, Minneapolis, MN 55455-0392; fax 612-625-5622, e-mail tsai001@tc.umn.edu)