min D status). The clinical decision values for circulating 25(OH)D (see Table 1) were provided. The survey asked which patients should receive treatment with vitamin D. We e-mailed the survey to providers who were selected because they had ordered 1 or more 25(OH)D determinations in the preceding year. Only practicing healthcare providers received this survey; physicians-in-training were excluded.

In one scenario, the total 25(OH)D result was 16 ng/mL; all responding healthcare providers (45 physicians, 12 physicians assistants and nurse practitioners) correctly identified this as vitamin D deficiency in need of vitamin D treatment (Table 1). However, for the scenario reporting 25(OH)D3 < 5 ng/mL and 25(OH)D2 40 ng/mL, 13 (23%) interpreted these results to indicate either vitamin D deficiency or vitamin D3 deficiency requiring vitamin D treatment (Table 1).

The important objective in assessing vitamin D status is to obtain a measure of the total circulating 25(OH)D, whether from a validated RIA, HPLC, or LC-MS assay. To achieve this objective, there is no advantage to reporting circulating 25(OH)D2 and 25(OH)D3 separately. Moreover, the results of this survey suggest that reporting 25(OH)D2, 25(OH)D3 and total 25(OH)D can confuse practicing healthcare providers, thereby leading to incorrect clinical decisions. We believe that, until a specific advantage for separate reporting of these metabolites is identified, laboratory reports should be limited to total circulating 25(OH)D or, if individual metabolites are listed, such reports must indicate clearly that the total circulating 25(OH)D is the measurement that should be used in clinical decision-making about vitamin D status.

The above recommendation does not diminish the important observation of D2 and D3 differing effects on maintenance of circulating 25(OH)D. Moreover, we acknowledge that there may be physiologic differences between D2 and D3. However, such theoretical differences have not been established and current dogma is that circulating 25(OH)D2 and 25(OH)D3 have equal clinical efficacy.

Dr. Hollis is a consultant for the Diasorin corporation.

Table 1. Potential for clinical misinterpretation when both 25(OH)D2 and 25(OH)D3 are reported.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Vitamin D deficiency, treated with vitamin D, n (%)</th>
<th>Vitamin D2 deficiency treated with vitamin D, n (%)</th>
<th>Vitamin D3 deficiency treated with vitamin D, n (%)</th>
<th>Optimum vitamin D concentrations, no vitamin D treatment necessary, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>25(OH)D3 = 32 ng/mL</td>
<td>1 (2)</td>
<td>7 (12)</td>
<td>0 (0)</td>
<td>49 (86)</td>
</tr>
<tr>
<td>25(OH)D2 &lt; 5 ng/mL</td>
<td>52 (93)</td>
<td>2 (4)</td>
<td>2 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>25(OH)D3 = 16 ng/mL</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>11 (19)</td>
<td>44 (77)</td>
</tr>
<tr>
<td>25(OH)D2 &lt; 5 ng/mL</td>
<td>3 (5)</td>
<td>0 (0)</td>
<td>11 (19)</td>
<td>44 (77)</td>
</tr>
<tr>
<td>25(OH)D3 = 40 ng/mL</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>11 (19)</td>
<td>44 (77)</td>
</tr>
</tbody>
</table>

Data are presented as n (%)—number of health care providers responding and percentage of total responding. This electronic survey depicted a hypothetical 82-year-old patient with a hip fracture for whom 3 scenarios of serum 25(OH)D results were reported. Four possible interpretations and treatment plans were presented for each scenario.

For all 3 scenarios, the following text accompanied the result: Vitamin D, 25-OH clinical reference values, measured as (a) < 10 ng/mL, severe deficiency; (b) 10–24 ng/mL, mild to moderate deficiency; (c) 25–80 ng/mL, optimum concentrations; and (d) > 80 ng/mL, toxicity possible.

References


Falsely Low LDL Cholesterol Results and Cholestasis

To the Editor:

LDL cholesterol (LDL-C) is commonly measured for evaluation and management of hypercholesterolemia. The Friedewald formula [LDL-C = (total cholesterol)−(HDL cholesterol)−triglycerides/2.2 for mmol/L] is commonly used to determine LDL-C, but this method has some well-established shortcomings and may not meet the National Cholesterol Education Program criteria of total error <12% (1–3). Another approach is the use of homogeneous methods for direct quantification of LDL-C. In our laboratory, we use the
Kyowa Medex® method 2nd-generation assay on a Roche Cobas Integra 800® analyzer (1, 3, 4).

We report a case of falsely low LDL-C results in a man 69 years of age with type IIa dyslipidemia according to the Frederickson classification. The patient was admitted to the hospital for coronary heart disease and weight loss of 8 kg over a 3-week period. On admission, we performed a lipid evaluation on the Cobas Integra 800. The results revealed a discrepancy between the total cholesterol (TC; 9.18 mmol/L), and the sum of direct LDL-C (3.55 mmol/L) and HDL-C (0.27 mmol/L). Therefore, direct LDL-C and LDL-C calculated according to the Friedewald formula (8.31 mmol/L) did not match (see Fig. 1 in the Data Supplement that accompanies the online version of this Letter at http://www.clinchem.org/content/vol52/issue11). This discrepancy occurred consistently for 3 days. The patient then died of a myocardial infarction 3 days after admission.

To investigate the LDL-C measurement discrepancy, we performed various tests with the approval of our hospital ethics board.

We diluted the patient’s plasma in bovine serum albumin solution and found that this did not decrease the difference. We checked whether the error was the result of the TC or the HDL-C methods by performing the same measurements with various devices (Vitros 750®, Ortho Clinical Diagnostics; AU 600®, Olympus; RXL®, Dade Behring), and we obtained results similar to our own (see Fig. 1 in the online Data Supplement). TC results were comparable: 8.7 mmol/L with Vitros 750, 9.4 mmol/L with AU 600, and 9.1 mmol/L with RXL. HDL-C measured with the AU 600 was relatively high (0.93 mmol/L) compared with measurements made with other devices (0.23 mmol/L with Cobas Integra 800, 0.19 mmol/L with Vitros 750, and 0.21 mmol/L with electrophoresis) that may have been linked directly to the Daichi method.

Then, we performed lipoprotein electrophoresis (Hydragel 15 LIPO®, Sebia), which revealed an LDL-C fraction of 93.3% (7.16 mmol/L), indicating that LDL-C represented 93.3% of the lipoproteins quantified (LDL-C + HDL-C + VLDL-C + chylomicrons). We found no abnormal lipoproteins such as lipoprotein X or lipoprotein-a, which are reported to influence some homogeneous assays (5). We concluded that the Cobas Integra 800 LDL-C result was falsely low.

We found no reports in the medical literature or in data provided by Roche of interference occurring with the use of treatments the case patient had received, such as Fenofibrate 300®, Aldalix®, Verapamil®, Aspecig 250®, or Trunitrine.

Several publications have reported a lack of specificity leading to false-positive results for intermediate (I) DL-C, rich apolipoprotein E HDL-cholesterol measured with the homogeneous LDL-C assays (1, 3, 6, 7), but we did not detect any abnormal lipoprotein fraction. Furthermore, laboratory results for our case patient showed serum TG at 1.33 mmol/L and total bilirubin at 50 μmol/L, values below the manufacturer-reported interference limits of 13.7 mmol/L for TG and 684 μmol/L for total bilirubin. Bile acids <200 μmol/L and lipoprotein X do not affect the direct LDL-C assay, and cholesterol associated with lipoprotein-a particles is not measured with this assay (7).

The only physiologic abnormality noted in our patient was cholestasis, indicated by an alkaline phosphatase concentration of 191 units/L (reference interval, 40–129 units/L) and a gamma glutamyl transferase (GGT) concentration of 568 units/L (reference interval, 8–61 units/L). There was also moderate liver cytolysis, with transaminases activity 3 times the reference value.

Because we did not find an obvious explanation for this interference, we reintroduced the Friedewald formula to screen for similar interferences in other patients. Eight cases were reported with transient falsely low or undetectable LDL-C, with reductions in LDL-C of 30%–100%. In each case, a period of cholestasis (defined as GGT >50 units/L and alkaline phosphatase >120 units/L) was noted. We considered the possible influence of cholestasis, as suggested by Fei et al. (6). We did observe that correlation coefficients for results obtained with the Friedewald formula (X) and direct analysis (Y) were lower (r² = 0.73; Y = 0.986X + 0.143; n = 5197) among patients with than among those without cholestasis (r² = 0.96; Y = 0.803X + 0.286; n = 593).

Homogeneous assays for LDL-C are commonly used, but these assays may not be suitable for all patients. Because we have, currently, no explanation for the observed discrepancies, further investigation is needed of the various causes of cholestasis and their potential implications in these falsely low LDL-cholesterol results. Other abnormalities that must be considered include paraproteins, which were detected in 1 of our 8 study patients and have been reported as associated with artifactual undetectable HDL-C (8).

We thank Mrs. Henhache, Sebia® Laboratory.

References

To the Editor:
We read with interest the comments of Lippi et al (1) who questioned the reproducibility and difficulty of standardization when we (2) used platelet-rich plasma (PRP) in the thrombin generation assay (TGA). We evaluated the use of TGA to identify patients needing further specific thrombophilic risk factor testing (2). For this, we used frozen-thawed (ft-PRP). PRP was obtained by centrifugation (190g for 12 min), adjusted to 150×10⁹/L, and immediately frozen at −80 °C. Samples were immediately thawed before the assay. TGA was triggered by the addition of 0.5 pmol/L of recombinant human tissue factor. To test for thrombophilia, we evaluated the TGA in the presence of activated protein C (APC) (25 nmol/L). This methodology had been described and evaluated earlier by Regnault et al. (3–5).

We agree that the nature of the biological sample is a key point. We also agree that when TGA is performed with platelet poor-plasma (PPP), it is mandatory to avoid residual platelets or platelet debris. This was stressed recently by 2 other groups (6, 7). Chantarangkul et al. (6) proposed 2 alternatives to overcome the role of residual platelets when PPP was used: a 0.22-μm filtration before freezing plasma or the use of phospholipids in a concentration >1.5 μmol/L. Gerotziafas et al. (7) also reported the importance of the concentration of phospholipids when PPP was used, which in their experimental condition had to be >4 μmol/L to overcome the bias induced by nonfiltered PPP.

To test for thrombophilia, we chose to use a frozen-thawed (ft-)PRP (2). The 2 theoretical reasons for this choice were to perform TGA in a physiological environment, i.e., in the presence of autologous platelet phospholipids, and to use a biological material compatible with multicenter studies, i.e., a frozen sample. In preliminary experiments, we compared ft-PPP with ft-PRP. When TGA was performed in ft-PPP, synthetic phospholipids were added at a concentration of 4 μmol/L. When APC was used at a concentration of 25 nmol/L, thrombin generation triggered by tissue factor was fully inhibited in ft-PPP, in both normal and factor V Leiden plasma. On the contrary, when the assay was performed in ft-PRP, the test was highly sensitive to APC resistance (Fig. 1). Furthermore, we previously reported on the low imprecision of the assay in ft-PRP (2).

We agree that standardization of the procedure is a crucial point and needs to be evaluated and validated in a large, multicenter scale.

References