

## A Patient with Primary Biliary Cirrhosis and Elevated LDL Cholesterol

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### CASE DESCRIPTION

A 47-year-old woman was referred to us for follow-up of primary biliary cirrhosis (PBC).<sup>4</sup> She reported nausea, malaise, weight loss, and new-onset jaundice. The patient had recently had an episode of melena with anemia requiring blood transfusions, but upper endoscopy and colonoscopy did not reveal a bleeding source. She reported a smoking history of 30 years and was currently smoking. The patient's family history included rheumatoid arthritis, hyperlipidemia, and cardiovascular disease. Her medical history included fibromyalgia and treated Hashimoto thyroiditis.

A diagnosis of PBC had been made elsewhere 5 years previously on the basis of positive antimitochondrial antibody and liver biopsy findings showing stage 1 PBC. The patient was started on ursodeoxycholic acid, but she did not tolerate this medication. Four years later, she remained unable to tolerate ursodeoxycholic acid and was referred to our clinic.

When the case patient presented to our clinic 1 year ago her laboratory studies showed alkaline phosphatase 1416 U/L, aspartate aminotransferase 120 U/L, alanine aminotransferase 81 U/L, total bilirubin 18.9  $\mu\text{mol/L}$  (1.1 mg/dL), direct bilirubin 10.3  $\mu\text{mol/L}$  (0.6 mg/dL), albumin 35 g/L (3.5 g/dL), total cholesterol 17.2 mmol/L (665 mg/dL), triglycerides 2.01 mmol/L (178 mg/dL), HDL cholesterol (HDL-C) 0.67 mmol/L (26 mg/dL), and calculated LDL-C 15.6 mmol/L (603 mg/dL). Ursodeoxycholic acid treatment was recommended, and the patient's lipids were to be rechecked in 3 months. The patient returned 1 year later, off medication, after the episode of gastrointestinal bleeding. Physical examination showed scleral icterus, jaundice, and sclerodactyly, as well as a palpable liver 2 cm below the costal

margin and multiple firm, whitish dermal papules on her left forearm. The most recent laboratory results are shown in Table 1. Computed tomographic enterography of her abdomen was negative for a bleeding source in the small bowel. In addition, extensive atherosclerotic disease, especially in the aortoiliac vasculature, and focal ectasia in the distal right common iliac artery were noted. Because of the patient's high LDL-C concentration and family history of atherosclerosis, she was referred for a cardiology evaluation.

### DISCUSSION

#### LABORATORY EVALUATION OF INCREASED LDL-C

This patient's marked elevations in total cholesterol and LDL-C, far above optimal ranges as defined by the National Cholesterol Education Program Adult Treatment Panel III guidelines, were of particular interest. The total cholesterol of 27.4 mmol/L (1060 mg/dL) and LDL-C of 25.9 mmol/L (1002 mg/dL), measured on a Roche-Hitachi modular system, prompted further evaluation. The automated Roche direct method uses a multidetergent enzymatic system for HDL quantification, and LDL-C was determined by use of the Friedewald equation: total cholesterol - (HDL-C + triglyceride/5). We also performed the triglyceride assay with an automated, enzymatic, colorimetric method on the Roche-Hitachi modular system. A cardiovascular risk-marker panel, which included lipoprotein (Lp) electrophoresis, was performed. Agarose Lp electrophoresis (SPIFFE<sup>®</sup> Lp electrophoresis, Helena Laboratories) and polyacrylamide gel electrophoresis (Quantimetrix Lipoprint<sup>®</sup> LDL Subfractions Kit) were performed (Fig. 1). Abnormal electrophoresis results prompted additional testing using an alternate method. Ultracentrifugation with  $\beta$ -quantification of LDL-C and HDL-C determined by precipitation of the lower fraction with dextran sulfate and  $\text{Ca}^{++}$  was performed (Table 1). We did not detect Lp(a) or chylomicrons.

We observed discrepancies between the initial automated lipid concentration measurement results and the concentrations obtained by using the ultracentrifugation method. Ultracentrifugation gave substantially lower, although still markedly increased, LDL-C

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<sup>4</sup> Nonstandard abbreviations: PBC, primary biliary cirrhosis; C, cholesterol; Lp, lipoprotein; apo, apolipoprotein.

**Table 1. Patient laboratory results.**

Most recent laboratory results		Reference interval
Alkaline phosphatase	1328 U/L	39–100 U/L
Aspartate aminotransferase	122 U/L	8–43 U/L
Alanine aminotransferase	80 U/L	7–45 U/L
Total bilirubin	3.0 mg/dL	0.1–1.0 mg/dL
Direct bilirubin	1.9 mg/dL	0.0–0.3 mg/dL
Albumin	2.6 g/dL	3.5–5.0 g/dL
Total-C	1060 mg/dL	<200 mg/dL (optimal)
Triglycerides	169 mg/dL	<150 mg/dL (optimal)
HDL-C (Roche)	24 mg/dL	40–60 mg/dL
LDL-C (calculated)	1002 mg/dL	<100 mg/dL (optimal)
ApoB	247 mg/dL	44–148 mg/dL
<b>Lipid measurements with ultracentrifugation method</b>		
Total-C	1060 mg/dL	<200 mg/dL (optimal)
Triglycerides	169 mg/dL	<150 mg/dL (optimal)
HDL-C (ultracentrifuge)	201 mg/dL	40–60 mg/dL
LDL-C (ultracentrifuge)	740 mg/dL	<100 mg/dL (optimal)
VLDL-C	119 mg/dL	<15 mg/dL (optimal)

measurement results compared to the calculated concentration from the Roche-Hitachi instrument. In contrast, ultracentrifugation with precipitation of the lower fraction gave a substantially higher HDL-C concentration. Agarose electrophoresis showed an atypical pattern that did not resolve with dilution (Fig. 1A).

LDL-C appeared to be present on the agarose gel, but the dark, smearing nature of the gel with slight reverse migration was consistent with the presence of LpX. Neither Lp(a) nor HDL-C was visible on the gel. The electrophoresis findings suggest that the ultracentrifugation result of 5.21 mmol/L (201 mg/dL) for HDL-C was erroneous, but we cannot rule out the possibilities that the HDL-C was in some way modified, resulting in a change in migration, or that HDL-C is not bound to LpX particles. We also performed LDL-C electrophoresis on polyacrylamide gel, followed by densitometry (Fig. 1B). Although apolipoprotein (apo)B was increased, this was unlikely due to LDL-C, because apoB was not detected on this gel. ApoB is found on chylomicrons (apoB-48), VLDL, IDL, LDL, and Lp(a) but is not reported to be contained on LpX.

## DIAGNOSIS: PBC WITH LpX

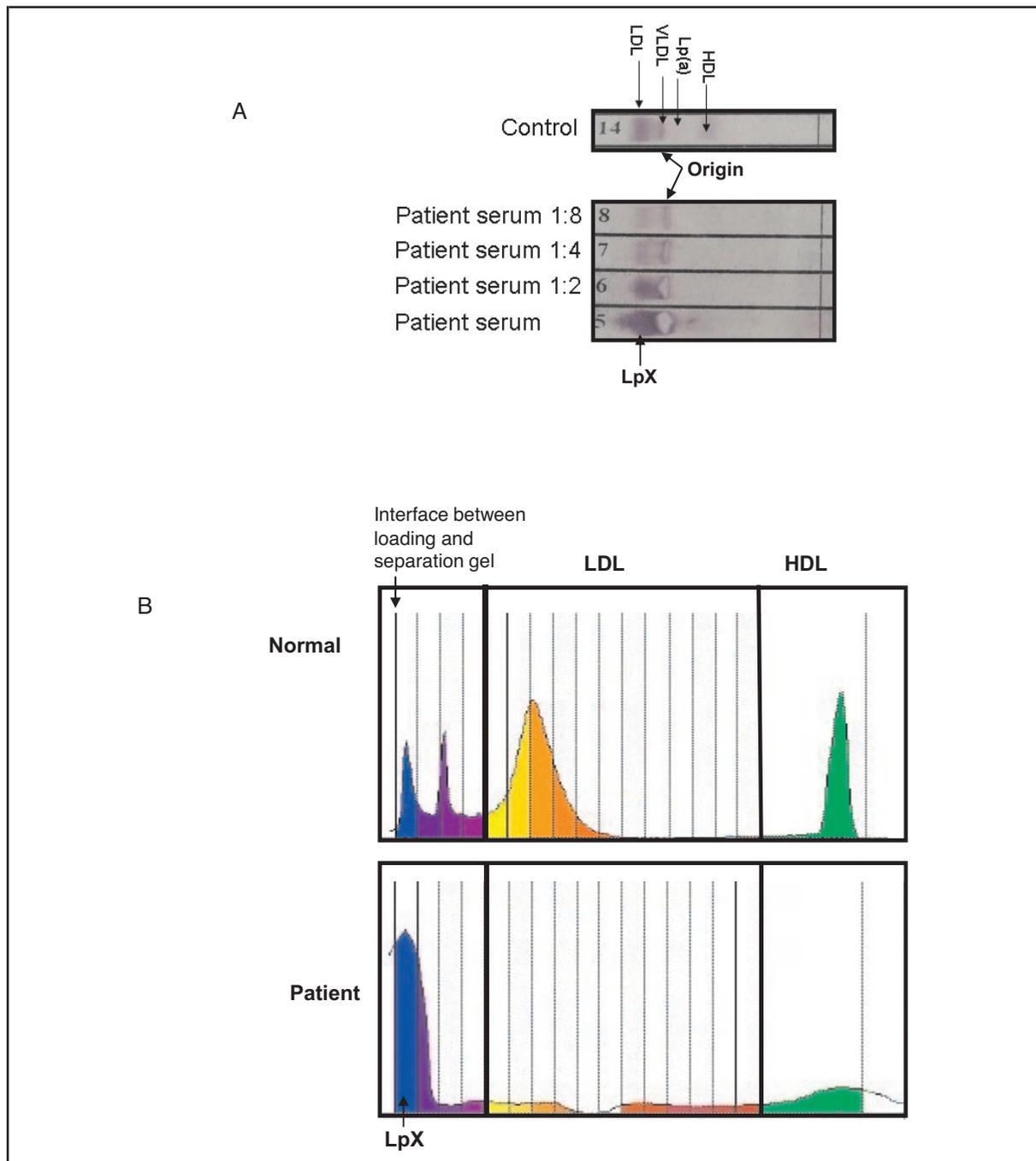
### OVERVIEW OF LpX

Although not uncommonly present in patients with cholestatic liver diseases such as PBC, LpX is seldom discussed or considered by the clinical laboratorian or

physician. The term LpX was coined in 1969 by Seidel and colleagues after they demonstrated the presence in patients with PBC of an abnormal Lp that was immunohemically distinct from Lp(a) and Lp(B) (1). Previously, this Lp had been referred to as Lp “d < 1.063,” based on its density as determined via centrifugation, and OLP (obstruction Lp). LpX is a unique Lp characterized by a vesicular structure that consists of a lipid bilayer of 30–70 nm that encloses an aqueous compartment. LpX has a uniquely high content of unesterified cholesterol and phospholipids and carries apoC, apoE, apoA-I, and albumin but is devoid of the structural protein apoB (2, 3). Although the density of LpX is in the LDL range, the physical size is in the range of VLDL or larger. Like VLDL, LpX is large enough to scatter light and cause visual turbidity. This atypical Lp migrates with  $\beta$ -globulins. The mechanism of formation of LpX remains poorly understood. LpX is also seen in lecithin-cholesterol acyltransferase deficiency and may contribute to nephropathy related to lecithin-cholesterol acyltransferase deficiency (4). Interestingly, LpX is not thought to be very atherogenic. The risk of coronary artery disease does not seem to be increased in patients with PBC (5). Some researchers believe LpX has antiatherogenic properties and may in fact decrease atherosclerotic risk (6).

### INTERACTION OF LpX WITH LDL-C AND HDL-C MEASUREMENTS

Homogeneous assays for LDL-C are known to be markedly affected by LpX (7, 8). In the case we report,



**Fig. 1. Agarose Lp and polyacrylamide gel electrophoresis.**

(A), Patient's serum after agarose gel electrophoresis (SPIFE system). No HDL was observed on the gel, and the LDL band was obscured but stained darkly. The slight reverse migration observed is consistent with the presence of LpX. (B), Densitometry results after polyacrylamide gel electrophoresis of patient's serum (Quantimetrix Lipoprint LDL Subfractions Kit). Note the abnormal pattern and absence of substantial HDL and LDL in the patient's sample. LpX, like VLDL, is too large to enter the 3% polyacrylamide gel, thus it is observed as a large band at the interface between the loading and separation gel. Lipoproteins migrating in the region between the interface and LDL are thought to be IDL and/or small VLDL particles.

lipid analysis with ultracentrifugation gave substantially lower LDL-C and higher HDL-C concentration results than those obtained from the automated Roche-Hitachi instrument. The ultracentrifugation (modified  $\beta$ -quantification) procedure requires precipitation of LDL in the bottom fraction with dextran sulfate and  $\text{Ca}^{2+}$ , followed by enzymatic measurement of the remaining HDL-C. We reason that LpX present in the bottom fraction was not fully precipitated along with LDL and thus was measured as HDL-C. The HDL-C concentration determined this way was 4.58 mmol/L (177 mg/dL) higher than that found with the automated Roche direct HDL method. Because LDL-C is determined by subtracting HDL-C from the total cholesterol in the bottom fraction, the LDL-C concentration obtained by ultracentrifugation (740 mg/dL) was lower than the calculated LDL-C from the Roche-Hitachi instrument (1002 mg/dL). By either method, the LDL-C determinations produced inaccurate results when LpX was present at high concentrations, because most of the LDL-C measured is derived from the LpX particles. Because we do not fully understand the composition of the LpX particle and the nature of its interactions with other Lps, we cannot accurately assess LDL status in a patient with increased LpX. Although apoB measurement is one potential option, we cannot rule out the possibility that samples with high concentrations of LpX interfere with the methods for apoB determination.

#### IMPORTANCE OF LpX

It is important for clinical chemists to note that high concentrations of LpX may affect several laboratory tests. In addition to the effects on Lp analyses described here, high LpX may result in pseudohyponatremia (9) and falsely increased laboratory measurement of Lp(a) (10). These effects can have important clinical implications for treatment recommendations.

#### DIAGNOSIS

On the basis of the electrophoresis patterns observed and the diagnosis of PBC, we concluded that the discrepant results in this patient were due to the presence of LpX. Results from the Roche-Hitachi analyzer were released with the comment that LpX was present in the sample and that it accounted for the majority of cholesterol present. The true LDL-C concentration in the sample could not be calculated, owing to the presence of LpX, but both LDL-C and HDL-C appeared to be markedly decreased when the gels were examined.

#### CASE RESOLUTION

Although LpX is not thought to be atherogenic, this patient had marked atherosclerosis revealed on com-

puted tomographic examination and was referred for a cardiology consultation. The patient was counseled on smoking cessation, and further testing was ordered to assess coronary calcification. Specific antilipemic medications were not thought to be necessary until reassessment of the patient's lipid status after appropriate treatment and improvement of her cholestasis. At the time of this report the patient was scheduled to return for further laboratory testing after 3 months of therapy with ursodeoxycholic acid.

#### CONCLUSION

LpX is often present in association with PBC and cholestatic liver disease. In the presence of LpX, lipid and Lp analyses cannot be expected to yield reliable results. HDL-C may be falsely increased or decreased, depending on the method used, and LDL-C may be falsely and markedly increased. It is not common knowledge to either the clinician or the clinical laboratorian that these abnormalities and inaccuracies in measurement are usually the result of LpX. If the presence of LpX is suspected, confirmation by electrophoresis is recommended. In addition to the laboratory abnormalities observed for lipid testing, other laboratory tests may also be affected. Further characterization of LpX and its effects on laboratory testing is warranted.

#### POINTS TO REMEMBER

- Consider PBC in the differential diagnosis for patients with hypercholesterolemia. PBC patients often have increased total cholesterol.
- LpX is an atypical Lp found in patients with PBC or cholestasis and can account for a major fraction of the total serum cholesterol.
- LpX interferes with routine lipid panel measurements. It can falsely increase the laboratory measurement of Lp(a) as well as direct and calculated LDL-C. Depending on the method used, LpX can lead to falsely decreased or increased HDL-C measurement results.
- In PBC patients with hypercholesterolemia, treatment of the cholestasis is typically addressed first; antilipidemic drugs (such as statins) are not typically first-line treatments.
- LpX is not thought to be proatherogenic, but there are limited data concerning this characteristic.

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## Commentary

James Otvos

The case study by Foley et al. calls needed attention to the existence of an unusual lipoprotein called LpX. Our experience measuring lipoproteins nontraditionally by nuclear magnetic resonance spectroscopy suggests that LpX occurs in patients more often than recognized. Normal lipoproteins (VLDL, LDL, HDL) are structurally similar, with cholesterol esters and triglycerides in a spherical “core” surrounded by a “shell” of phospholipids, unesterified cholesterol, and various apolipoproteins. Because of their common structures, reliable inferences can be made about lipoprotein concentrations and their associated cardiovascular risk by measuring concentrations of cholesterol in plasma or in particular lipoprotein fractions.

But all bets are off when a patient has LpX, because much or most of the cholesterol is not in atherogenic LDL or antiatherogenic HDL particles, but in seemingly atherogenically neutral vesicular structures con-

taining almost nothing but unesterified cholesterol and phospholipids and no apoB. Patients with LpX commonly have very high total cholesterol and low HDL-C, a lipid phenotype suggesting high cardiovascular risk and a need for aggressive LDL lowering. If LpX is not suspected, which is usually the case, such patients would typically be treated with statins, which would be ineffective in lowering the cholesterol (because LpX does not undergo LDL receptor-mediated clearance) and might even exacerbate an underlying liver disorder.

In the reported case, the laboratory performed confirmatory electrophoresis testing only because of the patient's history of PBC. Our laboratory measures lipoprotein profiles by nuclear magnetic resonance spectroscopy without access to patient information and has flagged many cases of LpX over the years by virtue of the unique spectroscopic signature of LpX. When notified of the presence of LpX, clinicians generally admit to no understanding of LpX. Follow-up testing for markers of cholestatic liver disease is frequently inconclusive, with a suggestion in many cases of drug-induced cholestasis, which resolves after therapy modification. We therefore suspect that LpX goes unrecognized much of the time, with possibly significant clinical consequences.

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# Clinical Case Study

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