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CASE REPORT

Diagnostic Pitfalls in Primary Biliary Cholangitis: Delta Bilirubin and Lipoprotein-X Interferences

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SUMMARY

Background: **Cholestasis in primary biliary cholangitis (PBC) induces delta bilirubin and lipoprotein-X (LpX), complicating biochemical interpretation.**
Methods: **Comparative wet/dry chemistry analyses, total cholesterol (TC)/apolipoprotein B (Apo B) ratio calculation, and clinical-laboratory integration were utilized.**
Results: **Delta bilirubin (87.4 µmol/L) masked true bilirubin levels, while LpX falsely elevated LDL-cholesterol (LDL-C) (23.98 mmol/L) and induced pseudohyponatremia (Na^+: 135 → 142 mmol/L).**
Conclusions: **Integrated methodologies and clinician-laboratory collaboration are essential to mitigate diagnostic pitfalls in PBC.**
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KEYWORDS

primary biliary cholangitis, delta bilirubin, lipoprotein-X, method interference

INTRODUCTION

Primary biliary cholangitis (PBC) is a chronic autoimmune liver disease characterized by progressive intrahepatic bile duct destruction and cholestasis [1]. Biochemical markers, such as alkaline phosphatase (ALP) and anti-mitochondrial M2 antibodies (AMA-M2), are pivotal for diagnosis and monitoring. However, cholestasis-induced metabolic derangements, notably delta bilirubin and lipoprotein-X (LpX), introduce analytical interferences that confound routine laboratory tests [2, 3]. Delta bilirubin, a covalently albumin-bound fraction, accumulates in prolonged cholestasis, while LpX, an abnormal lipoprotein, disrupts lipid and electrolyte assays [4,5]. These interferences may lead to misinterpretation of disease severity and inappropriate therapeutic decisions. This case underscores the necessity of integrating advanced methodologies and fostering clinician-laboratory dialogue to navigate these challenges effectively.

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Table 1. Wet chemistry results.

Parameters	Results	Reference values
Total protein (TP) (g/L)	70.7	65.0 - 85.0
Albumin (ALB) (g/L)	37.7	40.0 - 55.0
Alanine aminotransferase (ALT) (U/L)	197	7 - 40
Aspartate aminotransferase (AST) (U/L)	201	13 - 35
ALP (U/L)	1,134	50 - 135
GGT (U/L)	1,737	7 - 45
Total bilirubin (TBIL) (µmol/L)	190.5	0.0 - 23.0
DBIL (µmol/L)	151.1	0.0 - 8.0
Creatinine (CREA) (µmol/L)	22.0	41.0 - 81.0
Potassium (K^+) (mmol/L)	3.65	3.50 - 5.30
Sodium (Na^+) (mmol/L)	135	137 - 147
Chloride (Cl^-) (mmol/L)	98.9	99.0 - 110.0
TC (mmol/L)	29.91	< 5.2
Triglyceride (TG) (mmol/L)	2.13	< 1.7
HDL-cholesterol (HDL-C) (mmol/L)	1.14	> 1.04
LDL-C (mmol/L)	23.98	< 2.82
Order additional laboratory tests		
Apolipoprotein AI (Apo AI) (g/L)	0.80	1.00 - 1.60
Apo B (g/L)	1.06	0.60 - 1.20
TC/Apo B (mmol/g)	28.2	4.0 - 7.7 (female), 3.8 - 6.3 (male) [6]

Table 2. Dry chemistry results.

Parameters	Results	Reference values
TBIL (µmol/L)	204.8	3.0 - 22.0
Bc (µmol/L)	92.5	0.0 - 5.0
Unconjugated bilirubin (Bu) (µmol/L)	24.8	0.0 - 19.0
Delta bilirubin (µmol/L)	87.4	0.0 - 3.0
K^+ (mmol/L)	3.89	3.50 - 5.30
Na^+ (mmol/L)	142	137 - 147
Cl^- (mmol/L)	104.8	99.0 - 110.0

CASE PRESENTATION

A 55-year-old woman with a six-month history of PBC presented with persistent jaundice and pruritus. Laboratory investigations demonstrated markedly elevated hepatic enzymes: alkaline phosphatase (ALP) 1,134 U/L (reference range: 50 - 135 U/L) and gamma-glutamyl transferase (GGT) 1,737 U/L (reference range: 7 - 45 U/L), accompanied by hyperbilirubinemia (total bilirubin 190.5 µmol/L, normal: 0.0 - 23.0 µmol/L). Immunological workup revealed strongly positive AMA-M2 (4.40; normal < 1.00 Antibody Index) and anti-centromere protein B autoantibodies (Anti-CENP-B) (1.76; < 1.00 Antibody Index). Comprehensive viral hepatitis serology (HAV, HBV, HCV, HDV, HEV) and testing for cytomegalovirus (CMV) and Epstein-Barr virus (EBV) were negative. Abdominal magnetic resonance imaging (MRI) demonstrated hepatic cysts, gallbladder wall thickening, and lymphadenopathy. Chronic therapy included prednisone (45 mg/day), azathioprine (50 mg/day), and ursodeoxycholic acid (15 mg/kg/day).

Initial wet chemistry analysis (Table 1) revealed pseudohyponatremia (Na^+: 135 mmol/L; reference: 137 - 147 mmol/L) and extreme hypercholesterolemia (TC: 29.91 mmol/L; reference: < 5.2 mmol/L). Suspecting methodological interference, dry chemistry reanalysis (Table 2) identified delta bilirubin (87.4 µmol/L; reference: 0.0 - 3.0 µmol/L), constituting 42.6% of total bilirubin, and corrected electrolyte values (Na^+: 142 mmol/L; Cl^-: 104.8 mmol/L). The TC/Apo B ratio (28.2 vs. reference: 4.0 - 7.7) confirmed LpX interference, explaining the artifactual LDL-C elevation (23.98 mmol/L; reference: < 2.82 mmol/L) [6].

DISCUSSION

In cholestasis, impaired biliary excretion promotes delta bilirubin formation, a stable albumin-bound fraction with a prolonged half-life [2]. Traditional wet chemistry methods fail to differentiate delta bilirubin from conjugated bilirubin (Bc), leading to overestimated direct bilirubin (DBIL) levels (151.1 µmol/L vs. dry chemistry Bc: 92.5 µmol/L) [7]. This discrepancy misrepresents disease activity, as delta bilirubin does not reflect acute hepatocellular injury. Dry chemistry, employing multilayer film technology, quantifies delta bilirubin separately, aligning laboratory results with clinical stability [8]. Clinicians must recognize this pitfall to avoid unnecessary interventions based on misleading bilirubin trends.

LpX, a phospholipid-rich lipoprotein devoid of ApoB, accumulates in cholestasis due to Lecithin-Cholesterol Acyltransferase (LCAT) deficiency and disrupted cholesterol metabolism [9]. Its presence invalidates Friedewald-calculated LDL-C and causes false elevations in direct LDL-C assays, as observed here (LDL-C: 23.98 mmol/L) [10]. The TC/apoB ratio, a cost-effective surrogate for LpX detection, exceeded reference ranges 4-

fold (28.2 vs. 4.0 - 7.7), corroborating LpX interference [6]. Additionally, LpX interacts with indirect ion-selective electrode (ISE) methods, displacing sodium ions and inducing pseudohyponatremia. Direct ISE (dry chemistry) circumvents this artifact by measuring ion activity in an aqueous phase, as demonstrated by corrected sodium levels (142 mmol/L) [11].

To mitigate diagnostic errors in PBC-associated cholestasis, laboratories should prioritize method-specific validation by adopting dry chemistry or high-performance liquid chromatography (HPLC) for accurate bilirubin fractionation and utilizing direct ISE methods to avoid pseudohyponatremia in hyperlipidemic samples [8]. Additionally, unexplained elevations in LDL-C or electrolyte imbalances in PBC patients warrant immediate clinical-laboratory collaboration, including TC/Apo B ratio analysis and method verification to confirm LpX interference [6]. Importantly, therapeutic strategies must be tailored to cholestatic mechanisms: statins are contraindicated in LpX-mediated hypercholesterolemia due to their limited efficacy and potential hepatotoxicity, emphasizing the need for alternative management approaches [12]. This integrated approach ensures accurate diagnosis and optimizes patient care in complex cholestatic scenarios.

Clinicians managing PBC patients should maintain vigilance for delta-bilirubin presence. Concurrent hypercholesterolemia should prompt consideration of LpX, which interferes with LDL-cholesterol quantification and electrolyte measurements. Comprehensive diagnostic evaluation requires integrated analysis of the TC/Apo B ratio, dry chemistry methods, and clinical context. Multidisciplinary collaboration between clinicians and laboratory specialists is critical for identifying analytical interference and optimizing therapeutic decision-making.

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Declaration of Interest:
All authors declare that they have no competing interests.

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