

False-Positive Acetaminophen Results in a Hyperbilirubinemic Patient

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Background: Acetaminophen was falsely detected in the plasma of a severely jaundiced patient, and a methodologic interference from bilirubin was suspected.

Methods: Acetaminophen was measured by an enzymatic method (GDS Diagnostics). The putative bilirubin interference was investigated in 12 hyperbilirubinemic specimens and in bilirubin linearity calibrators. The analytical method was modified to correct for background absorbance at a second wavelength. Hyperbilirubinemic specimens were fortified with acetaminophen to assess the effect of the interference on acetaminophen measurements.

Results: Acetaminophen was detected in 12 specimens from hyperbilirubinemic patients without a history of recent acetaminophen exposure. Dilution of hyperbilirubinemic specimens produced a nonproportional decrease in apparent acetaminophen concentrations, and no acetaminophen was detected when bilirubin was <50 mg/L. Background correction at a second wavelength failed to compensate for the interference. Although erroneous acetaminophen concentrations were detected in all specimens with high bilirubin, acetaminophen measurements in fortified specimens were accurate.

Conclusion: The data are consistent with bilirubin interference in the enzymatic and/or chromogenic reactions involved in the acetaminophen method.

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In the clinical evaluation of fulminant hepatic failure, the laboratory has a prominent role in narrowing the range of diagnostic possibilities. Drug toxicity is the third most common cause of acute liver failure, comprising up to 25% of fulminant hepatic necrosis (1), and the widespread use of acetaminophen places this drug high on the list of

suspected hepatotoxic agents when history and serology have eliminated alcoholic and viral causes of hepatic disease. Acetaminophen is hepatotoxic in doses sufficient to deplete glutathione reserves, permitting accumulation of the highly reactive intermediate *n*-acetyl-*p*-benzoquinoneimine, which binds to the hepatocellular membrane, and the resulting injury precipitates centrilobular necrosis (2). After acetaminophen overdose, symptoms of liver failure may not appear for 24–48 h (3). In addition, susceptibility to hepatotoxicity may vary with age (4–6), race (7), and in certain diseases (8–10). Prompt treatment with the glutathione precursor *N*-acetylcysteine can minimize or prevent hepatocellular damage, but its effectiveness diminishes rapidly 12–24 h after acetaminophen exposure (11). Measurement of plasma acetaminophen can establish whether *N*-acetylcysteine treatment is indicated in seronegative patients for whom a reliable history is not available.

Case Report

A severely jaundiced 17-year-old male patient presented in the emergency department with abdominal pain. Transaminase enzymes were significantly increased, and total bilirubin was 198 mg/L (reference interval, 3–12 mg/L). Serology was negative for hepatitis A, B, and C. The apparent plasma acetaminophen concentration was 34 mg/L, prompting the clinical suspicion of intentional or inadvertent overdose at 12–24 h before presentation, but the patient denied using any medications containing acetaminophen within the previous week. The clinician contacted the laboratory to question the accuracy of the acetaminophen measurement, which was confirmed by reanalysis. Review of the product literature accompanying the acetaminophen test assay revealed an interference at bilirubin concentrations exceeding 250 mg/L. Therefore, the possibility of a falsely increased acetaminophen concentration in this patient attributable to hyperbilirubinemia was investigated.

Materials and Methods

Acetaminophen was measured by an enzymatic method (GDS Diagnostics) in which hydrolysis of acetaminophen

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to *p*-aminophenol is catalyzed by arylacylamidase. Condensation with *o*-cresol in the presence of periodate forms the blue indophenol chromophore. The acetaminophen method was adapted to a Modular™ chemistry analyzer (Roche Diagnostics Corporation) configured to measure absorbance at 600 nm and using a two-point rate calculation from read points at 13 and 19 s. Background-corrected acetaminophen measurements were made by modifying the Modular settings to include subtraction of the absorbance at 800 nm and read points at 19 and 23 s. The specimen volume for both procedures was 10 μ L. Bilirubin was measured on the Modular analyzer by a modified diazotization method using 2,5-dichlorophenyl-diazonium tetrafluoroborate as the source of diazonium ion (Roche Diagnostics Corporation). Normal saline was used for dilution experiments. For standard additions, a 300 mg/L acetaminophen calibrator (GDS Diagnostics) was mixed with serum pools in ratios of 1:1, 1:2, and 1:5. Lin-Trol® total and direct bilirubin linearity calibrators were purchased from Sigma Chemical Company.

The University of Florida Institutional Review Board determined that the laboratory investigation of this case was exempt from their review.

Results

To determine whether the presumed interference was an isolated occurrence, we selected 12 hyperbilirubinemic plasma specimens (total bilirubin, 159–338 mg/L) from patients without recent history of acetaminophen ingestion. Apparent acetaminophen concentrations of 5–18 mg/L were detected in these specimens (Fig. 1). Serial dilutions with normal saline (1:1 and 1:3) of seven of these specimens revealed a nonlinear decrease in apparent acetaminophen concentration, approaching zero when total bilirubin was diluted to <50 mg/L (Fig. 2). Spectrophotometric background correction at a wavelength of 800 nm decreased the magnitude of, but did not eliminate, the false increases in acetaminophen. Measurement of acetaminophen in bilirubin linearity calibrators revealed

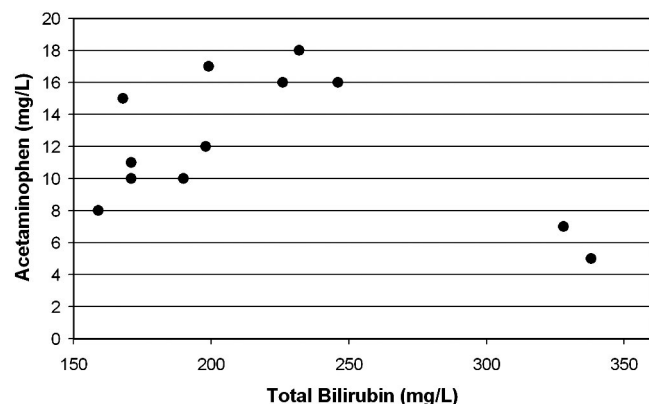


Fig. 1. Apparent acetaminophen concentrations in 12 plasma specimens with total bilirubin concentrations of 159–338 mg/L.

None of the specimens were from patients with a recent history of acetaminophen ingestion.

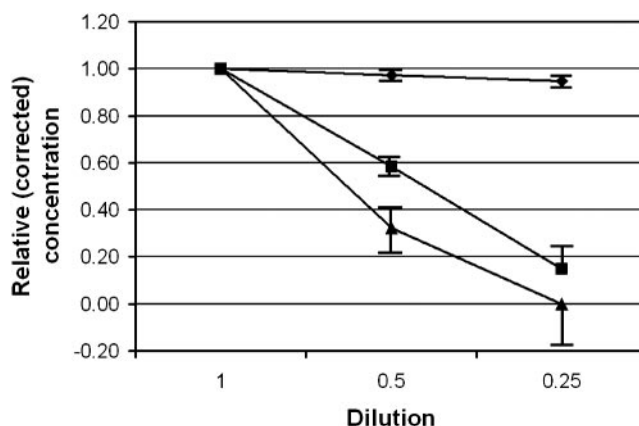


Fig. 2. Mean dilution-corrected relative concentrations of bilirubin (◆) and acetaminophen (■) measured in five hyperbilirubinemic plasma specimens.

Results of background-corrected acetaminophen (▲) measurements are also shown. The mean total bilirubin was 179 mg/L (range, 159–198 mg/L), the mean (apparent) acetaminophen concentration was 27 mg/L (range, 17–34 mg/L), and the mean background-corrected (apparent) acetaminophen concentration was 11.9 mg/L (range, 8–17 mg/L) in undiluted specimens. Error bars, SD.

relative increases in apparent acetaminophen concentrations, although the pattern reversed in uncorrected acetaminophen measurements at bilirubin concentrations >250 mg/L (Fig. 3).

To determine whether bilirubin influenced the measurement of actual acetaminophen concentrations, five hyperbilirubinemic specimens (mean bilirubin, 207 mg/L; range, 165–344 mg/L) were mixed with a 300 mg/L acetaminophen calibrator to produce acetaminophen concentrations of 50, 100, and 150 mg/L. Although the five specimens without added acetaminophen produced apparent acetaminophen measurements of 16–29 mg/L (2–10 mg/L background-corrected), specimens with added acetaminophen produced accurate acetaminophen measurements, within the limits of analytical variance (Fig. 4).

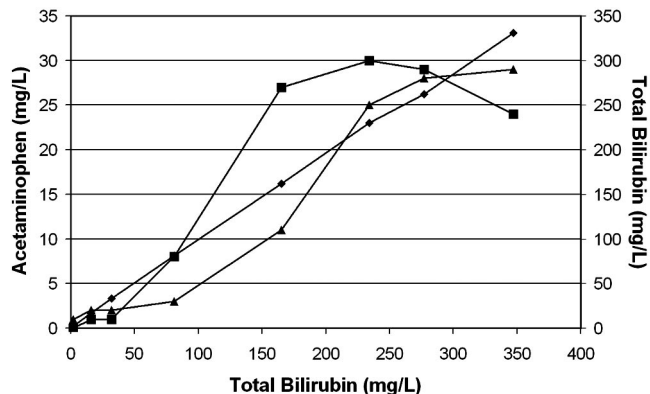


Fig. 3. Uncorrected (■) and background-corrected (▲) acetaminophen measurements and total bilirubin (◆) concentrations in Lin-Trol total and direct bilirubin linearity calibrators.

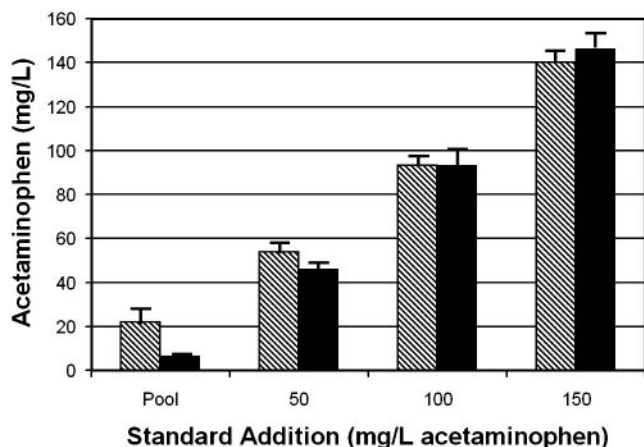


Fig. 4. Mean uncorrected (▨) and background-corrected (■) acetaminophen measurements in five hyperbilirubinemic serum pools (mean bilirubin, 207 mg/L; range, 165–344 mg/L) undiluted (*Pool*) and mixed with a 300 mg/L acetaminophen calibrator to produce final drug concentrations of 50, 100, and 150 mg/L.

Error bars, SD.

Discussion

Most automated methods for measuring acetaminophen are either immunoassays (e.g., fluorescence polarization, enzyme-multiplied immunoassay, turbidimetric inhibition) or enzymatic (arylacylamidase). Responses to a recent College of American Pathologists acetaminophen proficiency survey suggest that the participating laboratories are nearly evenly split between immunoassay and enzymatic approaches to acetaminophen measurement. In the same survey, 37 of the 2799 participating laboratories reported using the GDS method.

Bilirubin has considerable potential for interfering with spectrophotometric measurements because of its broad, intense absorbance in the ultraviolet and visible regions of the electromagnetic spectrum. Rate or blanked methods compensate, to a large degree, for the background absorbance from bilirubin in serum and plasma specimens. The GDS acetaminophen method is based on enzymatic conversion of acetaminophen to *p*-aminophenol and subsequent reaction with *o*-cresol to form the blue-colored indophenol, which is measured by the change in absorbance at 600 nm. An increase in background absorbance at 600 nm caused by the presence of bilirubin may contribute to a false increase in the acetaminophen measurement, but this mechanism also predicts that the magnitude of the false increase is directly proportional to the bilirubin concentration. Dilution of a hyperbilirubinemic specimen by a factor of 2 should, therefore, reduce the factitious acetaminophen concentration by a corresponding amount.

In this study, dilution of hyperbilirubinemic specimens by a factor of 2 produced a nearly fourfold reduction in the apparent acetaminophen concentration. This observation is not consistent with an interference that derives exclusively from the contribution to background absor-

bance, but instead suggests a mechanism involving the reaction of bilirubin with components of the reagent system to produce a chromophore that absorbs at or near the analytical wavelength. Electronic correction for absorbance at a second wavelength did not adequately compensate for the interference, which also suggests a non-spectral mechanism.

Bilirubin has substantial reducing activity, and a mechanism of interference involving the periodate-promoted *o*-cresol condensation reaction was also considered. Most consistent with the observed data is a mechanism involving the reaction of periodate with bilirubin to produce a product that absorbs more strongly at 600 nm than does unreacted bilirubin. Although this mechanism does not account for the plateau effect observed at high bilirubin concentrations, the lack of interference with measurement of endogenous acetaminophen could be attributable to the comparatively favorable kinetics of the *p*-aminophenol/periodate reaction.

The results of acetaminophen measurements in bilirubin linearity calibrators were consistent with the results obtained for hyperbilirubinemic specimens. There was a general correlation between increasing bilirubin and apparent acetaminophen concentration, but the relationship appeared to reverse at very high (>250 mg/L) bilirubin concentrations. Wavelength-corrected acetaminophen results did not display this reversal at high bilirubin concentrations, indicating that background absorbance may contribute, at least in part, to the overall interference. In these experiments, falsely detected acetaminophen in hyperbilirubinemic specimens appeared to plateau at 25–30 mg/L, when bilirubin concentrations were 20–30 mg/L.

Although the false detection of acetaminophen in hyperbilirubinemic specimens was consistent and reproducible, the accuracy of actual acetaminophen measurements between 50 and 150 mg/L did not seem to be affected. Mean analytical recoveries of an acetaminophen calibrator added to hyperbilirubinemic specimens varied from 93% to 108% (92–97% with the background-corrected method). This observation is consistent with an interference resulting from nominal cross-reactivity of bilirubin with the arylacylamidase or periodate-catalyzed reaction. It is possible that, in the presence of acetaminophen, the competitive disadvantage of bilirubin in either or both of these reactions minimizes production of an interfering chromophore, although the data do not confirm this explanation.

Acetaminophen was detected in specimens with bilirubin concentrations as low as 80 mg/L, contrasting the manufacturer's advice that acetaminophen measurements may be falsely increased at "extremely high concentrations of bilirubin" (GDS Diagnostics Enzymatic Acetaminophen Reagent package insert). There was no evidence of erroneous detection of acetaminophen in specimens with bilirubin concentrations within the reference interval. There has been a previous report of bilirubin interference

with acetaminophen measurements (12), but the analytical method was nonenzymatic and the characteristics of the interference differed substantially from those in the present investigation. It is possible that adjusting the read points in the enzymatic rate method may reduce or eliminate the interference and that this may have been partly responsible for the reduced effect observed when measurements were corrected for absorbance at a second wavelength because the read points were lengthened as well. Although this investigation did not involve extensive comparisons with other acetaminophen methods, for a small number of hyperbilirubinemic specimens two other enzymatic assays were attempted. One of the assays (Emit *tox* Acetaminophen Assay; Dade Behring Inc.) was not affected by high bilirubin concentrations, whereas the other (Roche Diagnostics) was.

Clinicians should exercise caution in interpreting apparent acetaminophen concentrations in jaundiced patients unless the potential bilirubin interference has been ruled out by method-validation studies in the laboratory. Questionable acetaminophen results should be confirmed by a nonenzymatic method or dilution studies.

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