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False positive acetaminophen concentrations in icteric serum

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ABSTRACT

Introduction: Serum concentrations of acetaminophen are measured to predict the risk of hepatotoxicity in cases of acetaminophen overdose and to identify acetaminophen use in patients with acute liver injury without a known cause. The acetaminophen concentration determines if treatment with N-acetyl cysteine, the antidote for acetaminophen poisoning, is warranted.

Description: A 49-year-old woman was admitted to our hospital with a hepatic encephalopathy and a total serum bilirubin concentration of 442 μmol/l. The acetaminophen concentration of 11.5 mg/l was measured with an enzymatic-colorimetric assay, thus treatment with N-acetyl cysteine was started. Interestingly, the acetaminophen concentration remained unchanged (11.5–12.3 mg/l) during a period of 4 consecutive days. In contrast, the acetaminophen concentration measured by HPLC, a chromatographic technique, remained undetectable

Discussion: In the presented case, elevated bilirubin was the most likely candidate to interfere with acetaminophen assay causing false positive results. Bilirubin has intense absorbance in the ultraviolet and visible regions of the electromagnetic spectrum and for that reason it causes interference in an enzymatic-colorimetric assay.

Conclusion: False positive acetaminophen laboratory test results may be found in icteric serum, when enzymatic-colorimetric assays are used for determination of an acetaminophen concentration. Questionable acetaminophen results in icteric serum should be confirmed by a non-enzymatic method, by means of ultrafiltration of the serum, or by dilution studies.

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1. Introduction

Acetaminophen is widely accessible and a major cause of overdose and overdose-related liver failure and death [1]. Intoxications with acetaminophen are potentially lethal if not adequately treated with the antidote N-acetylcysteine [1]. Treatment with N-acetylcysteine is generally decided upon based on an elevated acetaminophen serum concentration.

At therapeutic doses, acetaminophen is metabolized mainly in the liver by sulfate and glucuronide conjugation. Less than 5% is metabolized by cytochrome P450 2E1 to N-acetyl-p-benzoquinoneimine (NAPQI), a highly reactive and toxic intermediate [2]. NAPQI is usually reduced to a nontoxic mercaptate or cysteine conjugate by glutathione. In case of an acetaminophen overdose, the sulfation and glucuronidation pathways become saturated. Consequently, more acetaminophen is

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metabolized by cytochrome P450 2E1 and an excessive amount of NAPQI is created. The increase in NAPQI depletes the reserves of glutathione [3]. NAPQI binds to the hepatocellular membrane and causes liver cell necrosis [4]. Treatment with the glutathione precursor N-acetylcysteine thereby prevents hepatocellular damage [5]. Here, we describe a case of a patient with severe liver damage and an elevated acetaminophen concentration for which she was treated with N-acetylcysteine. However, the acetaminophen concentration proved to be a false positive result because of the interference of bilirubin. The liver damage was found to be due to alcohol abuse.

Chromatographic techniques, immunoassays and spectrophotometric techniques are the most frequently used methods for the determination of acetaminophen serum concentrations. Chromatographic methods, such as High-Performance Liquid Chromatography (HPLC), have proved to be reliable and accurate methods for the bioanalysis of acetaminophen [6]. A disadvantage of this method is the long time of analysis. More recently, automated immunoassays have become available, but they are generally more expensive and have not yet been adopted widely [6]. The most common used method is the enzyme-coupled colorimetric method. This is a spectrophotometric technique that, for example, the Cobas and the Architect use for determining an acetaminophen serum concentration. This case report reveals a disadvantage of this most commonly used method, which all medical laboratories should be aware of.

2. Case

A 49 year-old comatose woman was presented at the Emergency Department. She was transferred to the hospital by a friend who claimed she had been comatose for 4 days. Initially he had assumed that she was drunk, before presenting her. Her medical history consisted of alcohol abuse, but no any other underlying disease. Physical examination revealed a Glasgow Coma Score of E1M1V1 (in total 3). She had a blood pressure of 75/55 mmHg, heart rate 92 bpm and a body temperature of 35.8 °C. She had evident jaundice and abdominal examination revealed dilated veins and signs of ascites. She was admitted to the Intensive Care Unit (ICU).

Laboratory tests showed elevated liver biochemistry; a bilirubin total 442 μmol/l (ref: < 17.0 μmol/l), alkaline phosphatase (AF) 456 U/l (ref: < 125 U/l), gamma-glutamyl transferase (GGT) 1182 U/l (ref: < 50 U/l), aspartate transaminase (ASAT) 129 U/l (ref: < 40 U/l). Alanine aminotransferase (ALAT) was remarkable low; 43 U/l (ref: < 45 U/l), which could be a sign of very extensive liver damage. Liver function tests were also profoundly disturbed with a prothrombin time of 30.7 s (ref: 11–14 s.), lactate of 3.9 mmol/l (ref: 0.5–1.7 mmol/l) and albumin of 15 g/l (ref: 29–49 g/l). The ammonia concentration was 81 μmol/l (ref: 10–45 μmol/l), possibly explaining her comatose state. She also had kidney failure with a creatinine of 562 μmol/l (ref: 45–80 μmol/l), most likely prerenal because of dehydration or as part of the hepatorenal syndrome.

The friend revealed that the patient had been taking an undefined amount of acetaminophen. How much acetaminophen and when it was taken was not clear. The acetaminophen concentration measured on the Cobas 6000 was 11.5 mg/l, and consequently treatment with N-acetyl cysteine was initiated. Surprisingly, during the following days the acetaminophen concentration did not decrease at all (Table 1). After 4 days, the acetaminophen concentration was still in the same range and therefore false-positive values were suspected. To analyze this, the acetaminophen concentration was measured with two other devices, using the Architect C8000 and a HPLC measurement. The Architect C8000 showed similar levels as measured using Cobas 6000. The acetaminophen concentration measured by HPLC remained undetectable. False positive levels using the Cobas 6000 were proven and treatment with N-acetyl cysteine was stopped.

Liver failure based on alcohol abuse was diagnosed and our patient clinically worsened rapidly. The only option for survival would have been a liver transplantation, but due to recent alcohol abuse she was not accepted as a candidate. A palliative treatment was initiated and shortly thereafter she passed away.

3. Discussion

Serum concentrations of acetaminophen are routinely measured to predict the risk of hepatotoxicity in cases of an acetaminophen overdose and to identify acetaminophen use in patients with idiopathic acute liver injury [7]. In cases of acetaminophen overdose, the Rumack-Matthew nomogram is helpful in determining the likelihood of hepatotoxicity as a function of the acetaminophen-concentration and post-ingestion time [8]. The acetaminophen concentration determines if

Table 1
Bilirubin en acetaminophen concentrations.

Analytical method	Test	Day 1	Day 2	Day 3	Day 4	Day 5
	Bilirubin total (μmol/l)	357	380	354	388	429
Cobas 6000	Acetaminophen (mg/l)	11.5	12.3	11.6	12.2	13.0
Architect C8000	Acetaminophen (mg/l)	–	–	–	7.5	–
HPLC	Acetaminophen (mg/l)	–	–	–	< 1.0[a]	–

– Not measured.
[a] Not detected.

treatment with N-acetyl cysteine, the antidote for acetaminophen poisoning, is necessary.

Bilirubin has a considerable potential for interfering with spectrophotometric measurements because of its broad and intense absorbance in the ultraviolet and visible regions of the electromagnetic spectrum. The enzyme-coupled colorimetric method is based on the hydrolysis of acetaminophen to yield p-aminophenol. With the Cobas, the p-aminophenol is converted to the blue-colored indophenol in the presence of o-cresol and a periodate catalyst. With the Architect, the p-aminophenol is reacting with 8-hydroxyquinoline-5-sulfonic acid in the presence of manganese ions to form a colored compound, 5-(4 iminophenol)-8-quinolone. The production of indophenol and 5-(4 iminophenol)-8-quinolone are followed colorimetrically. The change in absorbance at 600 nm is directly proportional to the quantitative drug concentration in serum. An increase in the background absorbance at 600 nm caused by the presence of bilirubin, however, may contribute to a false increase in acetaminophen [6,9]. Unconjugated and conjugated bilirubin have an absorption peak between 390 and 460 nm [10]. Therefore, the fact that the Cobas and the Architect use a primary or secondary wavelength (600–800 nm) higher than 460 nm lends additional support to the idea that not bilirubin itself, but byproducts of bilirubin are the cause of the increased acetaminophen values. The byproducts could be formed through a reaction of bilirubin with components of the reagent [7,9].

The impact of the interference depends on the bilirubin levels and the acetaminophen concentration. The degree of interference may vary by device and is described in the product information. During the validation of an assay this information should be stated in the standard operating procedure (SOP).

In this case we show that measured serum concentrations of acetaminophen can be influenced by high bilirubin levels and can even give false positive values. The consequence could be the unjust initiation of treatment with N-acetyl cysteine, whereas this is not without potential serious side-effects. Gastrointestinal effects are very common, but even more important, in up to 64% of patients, anaphylactoid reactions are seen, sometimes critical [11]. Furthermore, due to false-positive acetaminophen concentrations, treating physicians can wrongly assume that the cause for acute liver failure is found, making them less eager in pursuing other causes. Patients are therefore at risk of missing needed treatment for a diagnosis never found.

For the abovementioned reasons, medical laboratories should be aware for the possibility of false positive acetaminophen concentrations in icteric patients presenting with acute liver failure. In any doubt, acetaminophen concentrations should be verified (if the assay is not validated for the interference of high bilirubin levels), which can be done in different ways. The concentrations can be measured using a non-enzymatic method as a HPLC measurement, like used in the present case. Another method would be using ultrafiltration for the removal of interfering substances as protein bound bilirubin, hemoglobin and lipoprotein [6,12]. Last of all, a dilution study can be done to ascertain the impact of bilirubin [9].

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