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False positive acetaminophen concentrations in patients with liver injury

Julie Polson[a], **Frank H. Wians Jr.**[b], **Paul Orsulak**[b], **Dwain Fuller**[f], **Natalie G. Murray**[c], **Jonathan M. Koff**[d], **Adil I. Khan**[b], **Jody A. Balko**[b], **Linda S. Hynan**[e,f], **William M. Lee**[a,*], and **Acute Liver Failure Study Group**

[a]Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, United States

[b]Department of Pathology, University of Texas Southwestern Medical Center, Dallas, United States

[c]Baylor University Medical Center, Dallas and Baylor All Saints Medical Center, Fort Worth, United States

[d]Walter Reed Army Medical Center, Washington DC, United States

[e]Department of Clinical Sciences, University of Texas Southwestern Medical Center, Dallas, United States

[f]Department of Psychiatry University of Texas Southwestern Medical Center, Dallas, United States

Abstract

Background—Acetaminophen toxicity is the most common form of acute liver failure in the U.S. After acetaminophen overdoses, quantitation of plasma acetaminophen can aid in predicting severity of injury. However, recent case reports have suggested that acetaminophen concentrations may be falsely increased in the presence of hyperbilirubinemia.

Methods—We tested sera obtained from 43 patients with acute liver failure, mostly unrelated to acetaminophen, utilizing 6 different acetaminophen quantitation systems to determine the significance of this effect. In 36 of the 43 samples with bilirubin concentrations ranging from 1.0-61.5 mg/dl no acetaminophen was detectable by gas chromatography-mass spectroscopy. These 36 samples were then utilized to test the performance characteristics of 2 immunoassay and 4 enzymatic-colorimetric methods.

Results—Three of four colorimetric methods demonstrated ‘detectable’ values for acetaminophen in from 4 to 27 of the 36 negative samples, low concentration positive values being observed when serum bilirubin concentrations exceeded 10 mg/dl. By contrast, the 2 immunoassay methods (EMIT, FPIA) were virtually unaffected. The false positive values obtained were, in general, proportional to the quantity of bilirubin in the sample. However, prepared samples of normal human serum with added bilirubin showed a dose-response curve for only one of the 4 colorimetric assays.

Conclusions—False positive acetaminophen tests may result when enzymatic-colorimetric assays are used, most commonly with bilirubin concentrations >10 mg/dl, leading to potential

*Corresponding author. Department of Internal Medicine/Digestive and Liver Diseases, UT Southwestern Medical Center, 5959 Harry Hines Blvd. Dallas, TX 75390-8887, United States. Tel.: +1 214 645 6110; fax: +1 214 645 6114. William.Lee@UTSouthwestern.edu (W.M. Lee). .

clinical errors in this setting. Bilirubin (or possibly other substances in acute liver failure sera) appears to affect the reliable measurement of acetaminophen, particularly with enzymatic-colorimetric assays.

Keywords

Acetaminophen toxicity; Immunoassay; Colorimetric assay; Bilirubin

1. Introduction

Blood concentrations of acetaminophen [or *N*-acetyl-*para*-aminophenol (APAP)] are used routinely to predict the likelihood of hepatotoxicity in the setting of large acetaminophen overdoses and to identify acetaminophen use in patients with acute liver injury when the history is unclear. In cases of acetaminophen overdose at a single point in time, the Rumack-Matthew nomogram is helpful in determining the likelihood of significant hepatotoxicity as a function of acetaminophen concentration and time post-ingestion [1] In patients admitted with acute liver failure (ALF), hepatic encephalopathy often precludes an accurate history and the acetaminophen assay has been used to ascertain whether acetaminophen has been taken [2]. In such patients, any concentration of acetaminophen might signal a possible overdose and imply that *N*-acetyl cysteine, the antidote for acetaminophen poisoning, should be given [3]. In either situation, rapid accurate determinations of acetaminophen concentration are required to assess the need to give NAC in a timely fashion.

We recently identified 2 patients with positive acetaminophen concentrations (i.e., greater than the lower limit of detection (LLD) of the acetaminophen assay used) in whom severe liver damage was ultimately found to be due to other causes. In both cases, acetaminophen concentrations were later attributed to the possible interference of bilirubin in the assay used to quantify acetaminophen concentration. 2 recent case reports have shown similar results [4,5].

In this study, we evaluated systematically the likelihood of false positive values of serum acetaminophen concentration by testing 36 acute liver failure sera, once gas chromatographymass spectrometry (GC-MS) had excluded the presence of any amount of the drug, utilizing 6 commonly used acetaminophen methods. We further examined whether purified bilirubin *per se*, when added to normal serum, affected the assay in similar fashion.

2. Case 1

A 71-y-old woman complaining of nausea, vomiting and jaundice for 2 weeks was admitted to hospital with a total serum bilirubin concentration of 34.8 mg/dl. She denied taking any acetaminophen while she was fully alert. Serologic testing indicated acute hepatitis A (positive anti-HAV IgM) as the cause of her ALF. An acetaminophen concentration was measured initially at 14.2 μg/ml; however, the acetaminophen concentration did not decline but remained in the same range (12.8–16.8 μg/ml) on daily testing over a week's time. A 1:1 dilution of the patient's serum sample with physiological saline yielded an undetectable acetaminophen result, confirming that her previous acetaminophen concentrations likely represented false positive values. Despite her history, she was given NAC on the basis of the 'detectable' acetaminophen concentration; she recovered slowly over the next 14 days.

3. Case 2

A 49-y-old man with ALF of unknown cause was admitted to Walter Reed Army Medical Center with altered mentation and a total serum bilirubin concentration of 28 mg/dl. Based

on an acetaminophen concentration of 30 μg/ml he was given NAC. The patient did not improve and continued to have positive acetaminophen concentrations on several occasions over a 3 week hospitalization. Based on liver biopsy, he was diagnosed with severe autoimmune hepatitis and begun on prednisone and azathioprine therapy with good improvement. Similar to Case 1, repeat acetaminophen testing using a 1:1 dilution of this patient's serum sample yielded no detectable acetaminophen.

4. Patients and methods

4.1. Specimens and subjects

The Acute Liver Failure Study Group began in 1998 to collect detailed prospective information and serum samples on patients with this rare condition [6]. Serum samples from 43 patients enrolled in the U.S. Acute Liver Failure Study (including case 1 above) were selected for study to represent patients with varying severity of liver injury, varying diagnoses and a range of bilirubin concentrations. All samples and clinical data had been obtained after informed consent from next of kin according to local IRB guidelines at sites participating in the ALF Study Group. Sera had been stored at −70 °C prior to use in this analysis. Samples selected were from patients with acute viral hepatitis (n=25), acetaminophen-induced hepatotoxicity (n=7), ischemic hepatopathy (n=1), idiosyncratic drug-induced liver injury, (n=5), or of indeterminate cause (n=5) using previously defined clinical criteria. Patients with acetaminophen-induced hepatotoxicity and acute liver failure had a well-defined history of acetaminophen overdose (large ingestions reported) and very high aminotransferase concentrations. All represented late presentations with severe liver failure as opposed to early after ingestion, so acetaminophen concentrations would be expected to be low or undetectable. Bilirubin concentrations in the overall group ranged from 1.0 mg/dl to 61.5 mg/dl and included 34 with bilirubin concentrations >10 mg/dl (Table 1).

4.2. Acetaminophen testing

All 43 serum samples were tested for acetaminophen initially by gas chromatography with nitrogen/phosphorous flame ionization detection (GC-NPD). The identification of acetaminophen in the positive samples was confirmed by gas chromatography-mass spectrometry (GC-MS). Both GC-NPD and GC-MS procedures were developed and validated for this study. Using this technique, seven sera were determined to contain acetaminophen above the limit of detection for the GC assays (1 μg/ml) and excluded from further study. Subsequently, the 36 remaining acetaminophen-negative samples were submitted for testing utilizing 6 different assay techniques at hospitals affiliated with or in close proximity to our center. The methods/instruments used were: EMIT-AU400 (Olympus America Corp, Irving, TX), Hitachi (Roche Diagnostics, Indianapolis, IN), AxSym (Abbott, Abbott Park, IL), Vitros 950 (Ortho-Clinical Diagnostics, Rochester, NY), Dimension RXL (Dade Behring, Newark, DE), and Stanbio (Stanbio Laboratory, Boerne, TX)-AU400 (Table 2). The characteristics of the assays are shown in detail in Table 2.

4.3. Preparation of samples supplemented with bilirubin

To specifically evaluate the role of bilirubin concentration as the cause of the detectable apparent acetaminophen concentration in the various acetaminophen assays, we prepared normal human serum samples supplemented with unconjugated bilirubin (Sigma, Baltimore, MD) to achieve final bilirubin concentrations at or near 0, 1, 2, 5, 9, 13, 18, 26, 34 and 38 mg/dl. We then submitted these samples for testing by the 4 colorimetric assays. Calculated bilirubin concentrations obtained in the sera tested were independently confirmed by standard biochemical testing for the samples used (modified diazo method for quantification

of bilirubin concentration in the Olympus AU400 chemistry analyzer, with a lower limit of linearity=0.08 mg/dl.

4.4. Statistical methods

Pearson product moment correlational analysis was used to predict apparent acetaminophen concentration from the total bilirubin concentration when the relationship between these variables appeared linear. Spearman rank order correlation (rho) was used when the relationship was non-linear. We used box-and-whisker plots to evaluate the effect of a total bilirubin concentration >10 mg/dl on apparent acetaminophen concentration; outliers in box-and-whisker plots were defined as values that were smaller than the lower quartile minus 1.5 times the interquartile range, or larger than the upper quartile plus 1.5 times the interquartile range (inner fences of central box in box-and-whisker plot). Mann–Whitney tests were used to study the influence of total bilirubin concentration >10 mg/dl on apparent acetaminophen concentration. SPSS V14 was used to perform all statistical analyses and box-and-whisker plots were made with MedCalc software (Mariakerke, Belgium).

5. Results

5.1. Acetaminophen concentration by GC-NPD and GC/MS

Acetaminophen was undetectable by GC-NPD in 36/43 samples. Among the 7 samples that were positive for acetaminophen, 5 were from patients with acetaminophen-induced ALF, and 2 were from patients with ALF secondary to acute hepatitis B. Among the 7 acetaminophen patients diagnosed clinically as acetaminophen-related, 5 were positive as noted, and 2 were negative by GC-MS, possibly due to the long interval (~72 h) between ingestion and obtaining serum for storage. The 7 samples that were positive for acetaminophen by GC-NPD and GC-MS were excluded from further testing.

5.2. Effect of ALF sera on measured acetaminophen concentration by various methods/instruments

Four of the six assay methods demonstrated frequent false positive results (i.e., values of acetaminophen>the method-specific LLD), varying from 4/36 to 27/36 positive samples depending upon the method employed (Fig. 1). The EMIT (immunoassay) and AAA-INDP-D methods (colorimetric) were essentially unaffected (Fig. 1A and B). The FPIA method (immunoassay) demonstrated a very low concentration (<3 μg/ml apparent acetaminophen concentration), and therefore clinically insignificant, likely non-specific interference in 15/36 (42%) serum samples (Fig. 1D). The three acetaminophen methods most affected by increased concentrations of bilirubin in serum samples from patients with ALF were colorimetric methods (Stanbio, 23 positive, AAA-INDP-H 27 positive, and AAA-THQ 14 positive); Each of these 3 methods includes an oxidizing agent (i.e., periodate or ferricyanide) as a chromogen activator (Table 1 and Fig. 1C, E and F). Fig. 2 demonstrates the extent to which the acetaminophen assay was affected in the 3 remaining colorimetric acetaminophen methods. The Mann-Whitney test was used to compare concentrations of total bilirubin concentrations (<10 mg/dl versus >10 mg/dl for each method (Table 3). Total bilirubin concentration did not significantly influence apparent acetaminophen concentration by the EMIT, FPIA and AAA-INDP-D assays, whereas the apparent acetaminophen concentration by the AAA-THQ, AAA-INDP-H, and Stanbio methods appeared to be influenced greatly by hyperbilirubinemia particularly those samples containing >10 mg/dl total bilirubin concentration (Fig. 2). The effect of increasing bilirubin concentration on acetaminophen values by the AAA-THQ method was linear, highly correlated (r=0.96), and consistent with a 27% increase in apparent acetaminophen concentration for every 1 mg/dl increase in bilirubin concentration (Fig. 1C and Table 4). The Spearman rank order

correlations for AAA-INDP-H (rho=0.87, $p<0.0001$) and FPIA (rho=0.65, $p<0.0001$) with bilirubin concentration were also found to be highly correlated.

5.3. Effect of bilirubin on acetaminophen concentration in healthy human sera

Of the four assay systems tested with the bilirubin-supplemented samples, the Dimension assay did not report any positive acetaminophen concentrations at increasing concentrations of bilirubin in healthy human sera, with the exception of a barely detectable concentration (just below the lower limit of detection) at the highest concentration of bilirubin (38 mg/dl). The Hitachi and Stanbio assays, both of which showed false positive acetaminophen concentrations in many ALF sera, did not report a single positive acetaminophen concentration in these healthy human sera regardless of bilirubin concentration. However, the Vitros assay reported false positive acetaminophen concentrations which increased in proportion to increasing concentrations of bilirubin in the normal human sera as follows: no acetaminophen detected up to 13 mg/dl bilirubin, 10 μg/ml APAP detected at 18 mg/dl bilirubin, 13 μg/ml APAP at bilirubin 26 mg/dl, 16 μg/ml APAP at bilirubin 34 mg/dl, and 20 μg/ml APAP at bilirubin 38 mg/dl.

6. Discussion

Our study suggests that false positive acetaminophen results occur quite commonly when testing serum samples from patients with severe liver injury. Bilirubin appeared to be the most likely candidate to interfere with acetaminophen assays causing false positive results and the association with the colorimetric assays we used would seem to confirm this. However, the nature and performance characteristics of this interference have not been fully determined. We chose 6 different assays commonly in use that, according to a College of American Pathologists external proficiency survey, represent the methods of choice in approximately 70% of U.S. clinical laboratories [7]. All were readily available in hospital laboratories in and around the UT Southwestern medical complex. We frequently observed positive values in our 36 proven negative samples with all 4 colorimetric methods to varying degrees, depending on the method. However, the impact of increasing total bilirubin concentration on reported acetaminophen concentration in ALF sera was linear only for 1 method: AAA-THQ on the Vitros instrument (Fig. 1C). This method is the only one employing the ferricyanide reagent. It may be that ferricyanide plus serum components (bilirubin or others) yield a suitable byproduct that absorbs at or near the primary wavelength (λ=670 nm) of the intended “dye” product (undefined in the manufacturer’s product insert), formed during the AAA-THQ reaction. This same assay was the only one that performed in a nearly linear fashion when healthy human sera with added bilirubin were tested, supporting the concept that bilirubin (or its byproducts) may be the interfering factor in acetaminophen measurement at least for this particular assay. Unconjugated and conjugated bilirubin have an absorption peak between 390 and 460 nm [8]. Therefore, the fact that the chemical methods most affected by bilirubin interference use a primary or secondary acetaminophen measurement wavelength [600–800 nm (Table 2)] considerably higher than 460 nm lends additional support to the idea that bilirubin byproducts, and not bilirubin (unconjugated or conjugated) itself, are a likely cause of the increased acetaminophen values observed in acetaminophen assays most affected by bilirubin interference.

That the 3 other assays did not demonstrate as robust linearity and did not seem affected when normal serum with added bilirubin was tested, suggests that other interfering substances are important in these three assays systems although a partial role for bilirubin cannot yet be excluded.

As might be expected if bilirubin were the culprit, immunoassay methods (EMIT, FPIA) that do not involve a color reaction were unaffected by samples containing high concentrations (up to 60 mg/dl) of bilirubin; we did not test them further with the spiked bilirubin sample technique (Fig. 1). Since a chemical reaction is not required in these assays, immunoassay methods are less prone to non-specific interference from endogenous substances either present in human serum or created as reaction byproducts in the colorimetric methods that might absorb at or near the primary and/or secondary absorbance wavelengths. Our data shows that the extent to which byproducts are either present or created varies between serum samples from different patients with ALF because in several instances samples from 2 different patients with nearly identical total bilirubin concentrations produced disparate results (one positive, one negative) when each was tested by the same acetaminophen assay (Fig. 1). This provides further support that for the conclusion that other as yet unidentified non-bilirubin substances must be present in serum from patients with ALF to explain these findings.

As noted, a great deal of variation in the effect on apparent acetaminophen concentration was observed between the 4 colorimetric methods with increasing total bilirubin concentration in ALF sera. For example, the AAA-INDP method in the Dimension instrument showed no apparent effect of increasing total bilirubin concentration (Fig. 1B) while the Hitachi instrument showed a substantial effect (Fig. 1E). The difference between these 2 methods may be due to varying role of the oxidizing agent, periodate, with *o*-cresol and varying samples volumes between the 2 methods (Table 2). For example, the AAA-INDP method in the Dimension instrument did not appear to be affected by increasing total bilirubin concentration because, compared to the AAA-INDP method in the Hitachi instrument, Dimension uses a relatively small sample volume (4 μl), the *o*-cresol reagent in this method is not combined with periodate; in addition, the reaction mode is endpoint instead of rate. It is of interest that the AAA-INDP acetaminophen method most affected by increased concentrations of bilirubin in ALF sera was the Stanbio method, which uses periodate and the highest serum sample volume (100 μL) of all acetaminophen methods that we tested (Table 2). Similarly, the use of an oxidizing agent (i.e., ferricyanide), a chromogen activator (i.e., THQ) other than *o*-cresol-periodate, and the highest primary measurement wavelength (λ=670 nm) of all acetaminophen methods that we tested may explain the differences and substantial effect of increasing total bilirubin concentration on apparent acetaminophen concentration between the AAA-THQ method and other methods that are also based on the AAA reaction (Table 2).

Interestingly, neither the Hitachi nor the Stanbio assays (both colorimetric methods, both apparently affected by increased bilirubin concentrations in ALF sera) reported false positive APAP concentrations with increased bilirubin concentrations in healthy human sera. This finding seems to confirm that, at least for these assays, that bilirubin itself may not be the interfering factor, but rather may simply correlate with the concentration of another substance or substances present in sera from patients with severe liver injury.

In ALF samples, we observed 2 distinct outliers with the AAA-INDP-D method; however, visual inspection of the data in Figs. 1B and 2 for the AAA-INDP-D acetaminophen method clearly indicates that the bulk (34/36 data points) of the apparent acetaminophen values by this method was less than the lower limit of detection (2.0 mg/l) for this method. Although it is difficult to explain the 2 outlier values for apparent acetaminophen concentration in Fig. 1B, both of these values occurred in samples containing relatively low (1.0 mg/dl and 2.0 mg/dl) total bilirubin concentration and the magnitude of the apparent acetaminophen concentration in these samples was also low (1.8 μg/ml and 4.3 μg/ml) and clinically insignificant. The presence of other interfering substances in larger quantities in these 2 samples would explain these results. Moreover, 2 of the acetaminophen methods (AAA-

INDP-D and AAA-INDP-H) (Fig. 1B and E) that we evaluated were based on the same reaction principle, yet only one of these methods (AAA-INDP-H; Fig. 1E) demonstrated a substantial effect of bilirubin on apparent acetaminophen concentration.

Our findings confirm the results of prior papers reporting the potential for false positive acetaminophen results when hyperbilirubinemic sera are assayed by various methods, [4,5] especially colorimetrically-based assays, and they broaden the understanding of this phenomenon in several ways. First, we tested serum samples from a large number of patients with liver injury from a variety of causes and a broad range of bilirubin concentrations up to ~60 mg/dl. We demonstrated that the relationship between apparent acetaminophen concentration and bilirubin concentration can approach linearity with some assays but is much less predictable using most methods. We demonstrated that 3 of the 6 most commonly used acetaminophen assays were markedly affected by sera from patients with liver injury and increased bilirubin concentrations. This finding underscores the potential for the widespread occurrence of false positive acetaminophen concentrations that at least correlate with the presence of, but are not necessarily caused by, high serum bilirubin concentrations. The finding that only one of the assays appears to be affected specifically by bilirubin, as opposed to other substances present in acute liver injury sera, raises the intriguing question of what these other substances may be.

The limitations of this study were that we did not examine the effect of adding acetaminophen to hyperbilirubinemic sera; however, most patients with acetaminophen overdoses have a normal or only mildly increased bilirubin concentration at the time of presentation [7]. In this way, the quantitation of acetaminophen in cases of severe acute toxicity is not likely to be affected, since bilirubin concentrations immediately following the acute ingestion are normal or minimally increased. Bertholf et al. demonstrated a small but measurable effect of bilirubin (<10%) on the acetaminophen concentration observed in the toxic (50–150 μg/ml) dose range, and this is mentioned in some package inserts for these assays [4]. Secondly, our study does not address true prevalence of false positive acetaminophen results in patients with liver injury and hyperbilirubinemia. Nevertheless, our findings demonstrate that false positive acetaminophen results occur commonly but vary considerably between assays, ranging from 0% (EMIT) to 69% (Stanbio).

Since virtually all patients seen within the first 48 h of an acute acetaminophen overdose have only mildly increased bilirubin concentrations [9], it is unlikely that false positive acetaminophen results will occur in the setting of early acute acetaminophen toxicity, where the quantitative assay is most useful. However, in any patient with acute liver injury of unknown etiology and no clear history of acetaminophen use, clinicians may wish to, but should not rely on, a low-positive acetaminophen concentration as indicative of acetaminophen-induced liver injury, particularly when serum bilirubin concentrations are high. Use of *N*-acetyl cysteine, though relatively safe, has not been shown to be effective to date in non-acetaminophen liver failure—a randomized blinded trial is currently under way using intravenous *N*-acetyl cysteine for non-acetaminophen patients [10]. A search for etiologies other than acetaminophen toxicity should continue to be pursued in patients with signs and symptoms of acute hepatic dysfunction and hyperbilirubinemia who have low-positive acetaminophen concentrations particularly when based upon an enzymatic-colorimetric assay.

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Anne M. Larson, Hao Do, University of Washington, Seattle WA

Jeffrey S. Crippin, Laura Gerstle, Washington University School of Medicine, St. Louis MO;

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M. Edwyn Harrison, Rebecca Rush, Mayo Clinic, Scottsdale, Scottsdale AZ

Adrian Reuben, Nancy Huntley, Medical University of South Carolina, Charleston SC

Santiago Munoz, Chandra Misra, Albert Einstein Medical Center, Philadelphia PA

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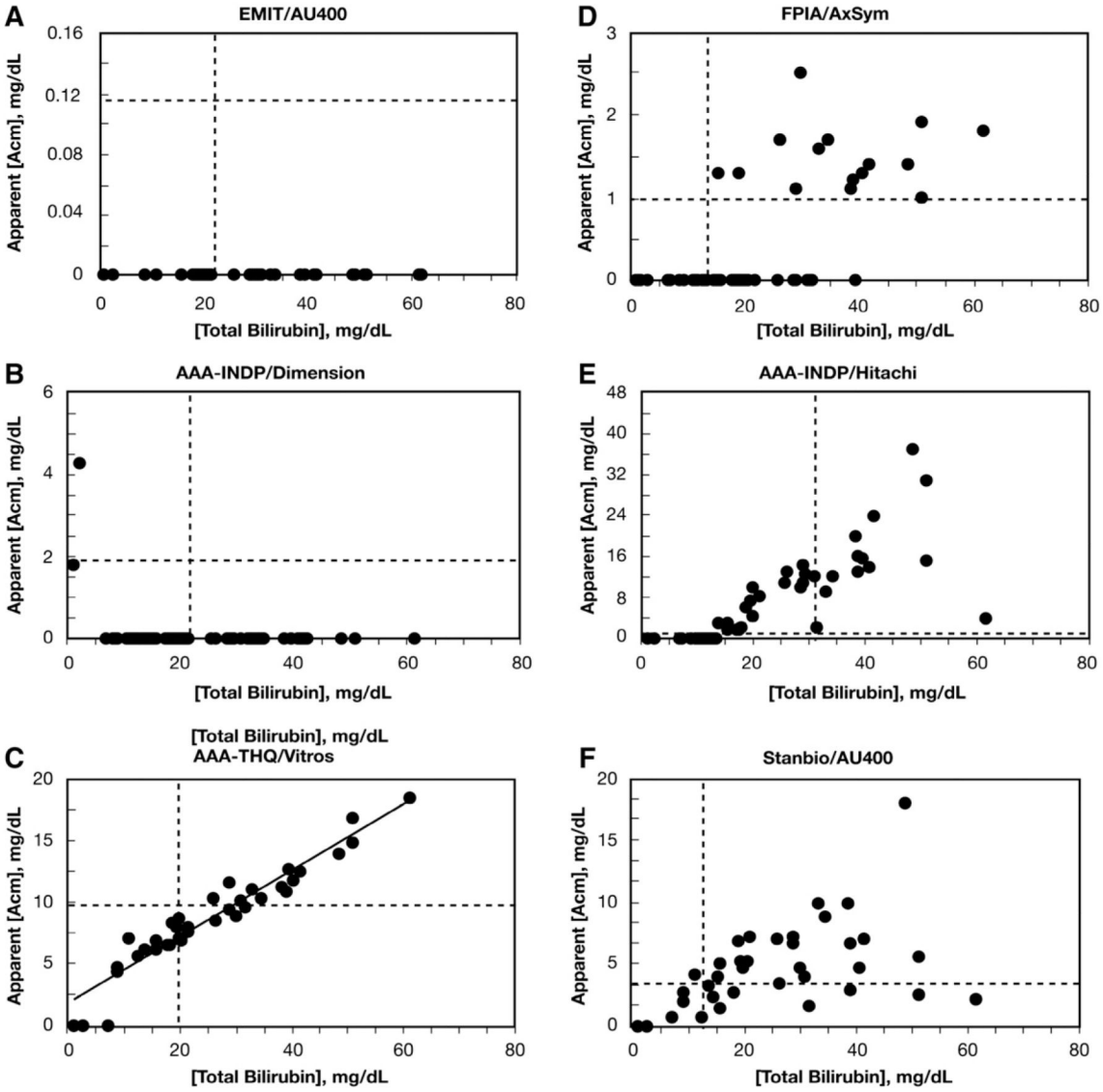

Fig. 1.

Apparent effect of total bilirubin concentration on acetaminophen measurements in serum samples from 36 patients with acute liver failure assayed using various methods/instruments: EMIT/AU400 (A); AAA-INDP/Dimension (B); AAA-THQ/Vitros 250 (C); FPIA/AxSym (D); AAA-INDP/Hitachi (E); Stanbio/AU400 (F). Abbreviations are the same as in Table 1. Horizontal dotted line represents LLD for each acetaminophen assay; vertical dotted line represents total bilirubin concentration cited by manufacturer as providing <10% error in measured acetaminophen concentration; and, solid line in panel C is the linear least-squares regression line (y=0.2707x+1.7125; r=0.9566). Note differences in scale of y-axis between panels.

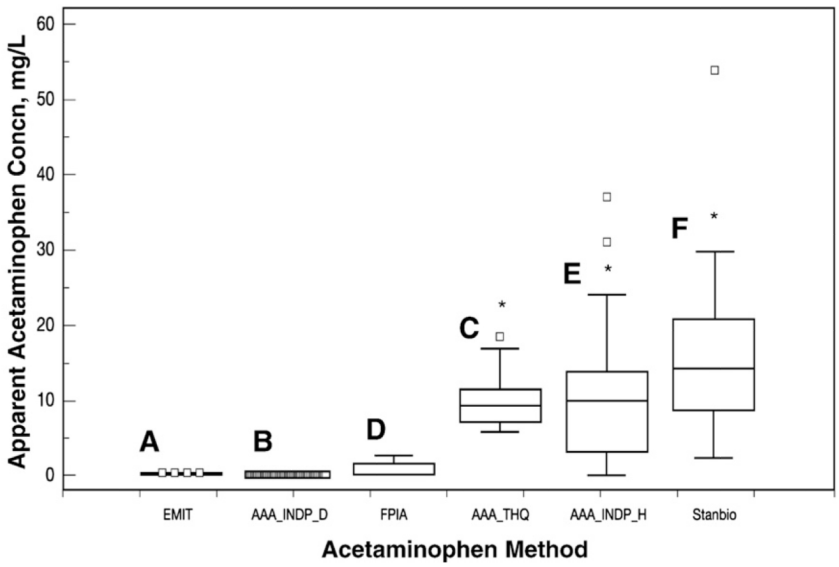

Fig. 2.

Extent of increased acetaminophen measurements in serum samples (n=36) from patients with acute liver failure. Abbreviations are the same as in Table 1. The lower and upper lines in the central box represent the 25th and 75th percentile values of the data set, while the middle line represents the median value. The vertical line extends from the lowest to the highest value in the data set, excluding outliers. Outliers are displayed as separate points (*empty squares*). The asterisk* denotes statistically significant effect.

Table 1

Overall study group characteristics, n=43

Age (median, range)	39, 20–71
Gender (%M/%F)	51/49
Admission total bilirubin, mg/dl (median, range)	18.3, 1.4–61.5
Admission ALT, IU/l (median, range)	1.154, 29–9.967
Admission INR (median, range)	2.6, 1.2–10.1
Survival (%)	72
Transplanted (%)	28*

*No transplanted patients died during the study period

Table 2

Comparison of acetaminophen assay characteristics

Characteristic	Acetaminophen method					
	EMIT	**INDP-H**	**FPIA**	**THQ**	**INDP-D**	**Stanbio**
Instrument	AU400	Hitachi	AxSym	Vitros 950	Dimension RXL	AU400
Assay type	EMIT	Colorimetric	FPIA	Colorimetric	Colorimetric	Colorimetric
Reaction mode	Rate	Rate	Endpoint	Endpoint	Endpoint	Rate
Enzyme	G6PDH	AAA	n.a.	AAA	AAA	AAA
Chromogen activator[a]	n.a.	*o*-cresol-periodate	n.a.	Ferricyanide	*o*-cresol	*o*-cresol-periodate
Sample volume, μL	3	10	150	10	4	100
Reaction						
Time, Min	1.2	10	14	5	10	10
Temp, °C	37	37	34	37	37	37
Reaction product	NADH	INDP	n.a.	Dye	INDP	INDP
Primary λ, nm	340	600	n.a.	670	600	615
Secondary λ, nm	410	800	n.a.	n.a.	700	n.a
No. of calibrators (concentration range)	6 (0–200)	2 (0, 300)	6 (0–200)	3 (10, 95, 330)	3 (0, 75, 300)	1 (300)
LLD, μg/ml	0.12	1.2	1.0	10	2.0	10
No interference[b] at [TBili, mg/dl]>	30	28	10	20	20	10

Abbreviations: EMIT, enzyme-multiplied immunoassay technique; G6PDH, glucose-6-phosphate dehydrogenase; AAA, arylacylamidase; NADH, nicotinamide adenine dinucleotide (reduced form) INDP, indophenol; H, Hitachi; FPIA, fluorescence polarization immunoassay; THQ, tetrahydroquinoline; D, dimension; temp, temperature; LLD, lower limit of detection; [TBili, mg/dl], total bilirubin concentration in units of mg/dl; λ, wavelength; n.a., not applicable.

[a]We prefer the term “chromogen activator” over “catalyst,” the term used in the manufacturer's product insert for reactions involving enzymes-substrates because enzymes are catalysts.

[b]Less than 10% error in measured acetaminophen concentration.

Table 3

Comparison of bilirubin concentrations (≤10 mg/dl versusN10 mg/dl) for 6 assay methods

Assay method	Bilirubin concentration								Mann–Whitney
	≤10 mg/dl (*n*=5)				>10 mg/dl (*n*=31)				
	Average rank	Median	Minimum	Maximum	Average rank	Median	Minimum	Maximum	*p*-value
EMIT	17.2	0	0	0.02	18.71	0	0	0.05	0.7902
INDP-H	4.5	0	0	0	20.76	10	0	37	0.0002
FPIA	11	0	0	0	19.71	0	0	2.5	0.0911
THQ	3	0	0	4.6	21	9.3	5.7	18.5	<0.0001
INDP-D	24.7	0	0	4.3	17.5	0	0	0	0.161
Stanbio	4.8	2	0	7.4	20.71	14.1	2.3	53.8	0.0004

Table 4

Spearman rank order correlations (except where noted) of 6 assay methods with bilirubin concentration (*N*=36)

Method	Correlation	*p*-value
EMIT	−0.34	0.0402
INDP-H	0.87	<0.0001
FPIA	0.65	<0.0001
THQ	0.96	<0.0001[a]
INDP-D	−0.40	0.0167
Stanbio	0.52	0.0011

[a]Pearson product moment correlation.