Journal of Medical Toxicology
https://doi.org/10.1007/s13181-019-00705-2

ORIGINAL STUDY

The Relationship Between Circulating Acetaminophen-Protein Adduct Concentrations and Alanine Aminotransferase Activities in Patients With and Without Acetaminophen Overdose and Toxicity

Steven C. Curry[1,2,3] · Angela Padilla-Jones[1] · Anne-Michelle Ruha[1,2,3] · Ayrn D. O'Connor[1,2,3] · A Min Kang[1,2,3] · Diana G. Wilkins[4] · Hartmut Jaeschke[5] · Kelly Wilhelms[3] · Richard D. Gerkin[1] · The Acetaminophen Adduct Study Group[1]

Received: 30 November 2018 / Revised: 28 February 2019 / Accepted: 4 March 2019

Abstract

Introduction Measurement of serum acetaminophen-protein adducts (APAP-CYS) has been suggested to support or refute a diagnosis of acetaminophen (APAP)-induced hepatotoxicity when ingestion histories are unreliable or unavailable and when circulating APAP concentrations are low or undetectable. Non-APAP overdose patients commonly have used APAP products in non-toxic quantities and, thus, will have measurable APAP-CYS concentrations, even when hepatic injury results from other causes, such as ischemic hepatitis. The relationship between alanine aminotransferase (ALT) activity and APAP-CYS concentration might assist in distinguishing between toxic and non-toxic APAP doses in patients suspected of drug overdose.

Methods We measured serial levels of serum APAP-CYS and ALT activities in 500 overdose patients in whom APAP toxicity was suspected on inpatient admission, but who were then classified at time of discharge and before results of APAP-CYS concentrations were available into three groups: 1) definite APAP group; 2) definitely not APAP group; and 3) indeterminate group. Subjects in the definite and definitely not APAP groups were selected in whom a plasma ALT activity was measured within ± 4 h of a serum APAP-CYS concentration. Regressions with correlation coefficients between APAP-CYS and ALT were calculated for repeat measures in the 335 subjects (908 blood samples) in the definite APAP group and 79 subjects (231 samples) in the definitely not APAP group, with an emphasis on APAP-CYS concentrations and calculation of 95% prediction intervals when ALT was ≥ 1000 IU/L.

Results A strong correlation was found between APAP-CYS and ALT in the definite APAP group over all ALT activities ($r = 0.93$, $p < 0.001$; $N = 335$), and when ALT was > 1000 IU/L ($r = 0.82$, $p < 0.001$, $N = 144$). In the 79 definitely not APAP subjects,

✉ Steven C. Curry
steven.curry@bannerhealth.com

Angela Padilla-Jones
angela.padilla-jones@bannerhealth.com

Anne-Michelle Ruha
michelle.ruha@bannerhealth.com

Ayrn D. O'Connor
ayrn.oconnor@bannerhealth.com

A Min Kang
aaron.kang@bannerhealth.com

Diana G. Wilkins
diana.wilkins@utah.edu

Hartmut Jaeschke
hjaeschke@kumc.edu

Kelly Wilhelms
kelly.wilhemls@sonoraquest.com

Richard D. Gerkin
richard.gerkin@bannerhealth.com

[1] Department of Medical Toxicology, Banner–University Medical Center Phoenix, Phoenix, AZ, USA

[2] Divisions of Medical Toxicology and Precision Medicine, and Clinical Data Analytics and Decision Support, Department of Medicine, University of Arizona College of Medicine–Phoenix, Phoenix, AZ, USA

[3] Center for Toxicology, Pharmacology, Education and Research, University of Arizona College of Medicine–Phoenix, Phoenix, AZ, USA

[4] Center for Human Toxicology, University of Utah, Salt Lake City, UT, USA

[5] Department of Pharmacology, Toxicology and Therapeutics, University of Kansas Medical Center, Kansas City, KS, USA

Published online: 12 April 2019

no significant correlation was found when ALT exceeded 1000 IU/L ($r = 0.04$; $p = 0.84$, $N = 32$). All subjects in the definitely not APAP group displayed APAP-CYS concentrations < 3 μM. In definitely not APAP subjects, the great majority of APAP-CYS levels were below the 95% prediction interval for APAP-CYS concentrations in definite APAP group subjects when ALT was ≥ 1000 IU/L. However, some definitely not APAP group subjects who had ingested non-toxic doses of APAP displayed APAP-CYS concentrations as high as 2.8 μM in the face of ALT elevation from ischemic hepatitis.

Conclusion The interpretation of serum APAP-CYS concentrations must always be made in light of detailed clinical information and the population being tested, especially because of some overlap in APAP-CYS levels in subjects with and without APAP toxicity.

Keywords Acetaminophen · Overdose · Acetaminophen-protein adducts · Alanine aminotransferase · Hepatotoxicity

Introduction

Hepatotoxicity following acetaminophen (APAP) overdose is explained by metabolism of APAP by cytochrome P450 enzymes to N-acetyl-p-benzoquinone imine (NAPQI), an electrophile that covalently binds to intracellular proteins, most commonly to cysteine residues, to form APAP-protein adducts [1]. There exists strong support for NAPQI's binding to mitochondrial proteins as being essential for subsequent development of hepatocellular necrosis in animals and man [2, 3].

Measurement of circulating APAP-protein adducts as 3-cysteinyl-acetaminophen (APAP-CYS) has been described for more than 30 years. It is recognized that even therapeutic, non-hepatotoxic doses of APAP result in measurable APAP-CYS concentrations in serum, with levels remaining below about 1.1 μM [4]. In patients with known APAP overdose, serum APAP-CYS levels greater than 1.1 μM have been reported to correlate with serum ALT activity greater than 1000 IU/L [5]. We use the terms APAP-CYS, APAP-protein adducts, and adducts synonymously in this paper.

Serum APAP-CYS levels remain elevated after serum APAP concentrations have become unmeasurable [1, 6]. Therefore, measuring serum APAP-CYS concentrations may aid in the recognition of APAP as being responsible for liver injury or failure in patients who cannot or do not provide accurate histories concerning drug ingestion and in whom elevated serum APAP levels are no longer detectable [6]. Khandelwal and colleagues reported that about 18% of patients with acute liver failure from indeterminate cause after extensive histories and diagnostic studies possessed elevated APAP-CYS concentrations in serum, indicating APAP may be the cause of liver failure in this subset of patients [7].

Acetaminophen is found in hundreds of over-the-counter and prescription medications. It was estimated in a 2002 study that each week about 23% of adults in the USA (> 57 million) consumed an APAP-containing product [8]. Patients may take nontoxic doses of APAP incidentally or as part of a multidrug overdose and display measurable serum APAP and/or APAP-CYS concentrations, but have liver injury or failure from other causes, including hepatic ischemia from shock and/or hypoxia. Furthermore, ischemic hepatitis causes increased release of APAP-CYS into the circulation in animals receiving non-toxic doses of APAP [9].

It is important that the performance of a diagnostic biomarker be studied in relevant patient populations. No previous study has reported a detailed systematic examination of serum APAP-CYS concentrations in a large population of adults initially suspected of acetaminophen overdose in order to describe serum adduct levels in patients in whom APAP overdose was present, and in those in whom it was determined that APAP had not contributed to illnesses. Such a study would be especially important given the widespread use of APAP products and various factors that may influence circulating adduct concentrations. Therefore, we prospectively measured serial serum APAP-CYS concentrations and gathered detailed clinical information in 500 toxicology patients in whom there was initial suspicion for acute or chronic APAP overdose in order to describe the range of serum APAP-CYS concentrations observed at different ALT values in patients with and without APAP-induced hepatotoxicity. Such information should assist in interpreting serum APAP-CYS concentrations when attempting to assign causality to APAP in patients with liver injury and failure.

Methods

This prospective observational cohort study took place between 2010 and 2017 at a single academic medical center with active medical toxicology, hepatology, and liver transplantation services, and which served as a referral center for toxicology patients and for patients with acute liver failure from any cause. The study was approved by our Institutional Review Board. Data from subjects enrolled in this study have been partially reported in previous publications for purposes unrelated to this study [2, 10–17].

Subject Selection

All inpatients ≥ 18 years of age under the care of medical toxicologists and in whom APAP toxicity from acute or chronic excessive ingestions was initially suspected by referring physicians or medical toxicologists at our center were considered for recruitment upon arrival or on first contact with a physician toxicologist using convenience sampling, if pregnancy had been excluded and if informed consent could be obtained. Prisoners were excluded.

Data and Blood Collection

After obtaining informed consent, blood for measurement of serum APAP-CYS was drawn into a clot tube upon study entry, again 12 h later, and then once daily until day ten, after which blood was drawn every 48 h until the time of discharge or for a total of 3 weeks. Serum was separated using centrifugation and then frozen. On rare occasions, plasma was used for APAP-CYS measurement when serum was unavailable, and plasma was separated and frozen in a similar manner.

Data on each subject were prospectively collected into a registry and included demographic information, history of ingestion, whether the ingestion history was considered reliable, laboratory and imaging results, date and time of each specimen collection, treatment, and outcome. Intravenous N-acetylcysteine (NAC) was infusing in almost all subjects during the initial blood draw and was continued beyond 24 h in those with worsening liver function tests or with acute liver failure until prothrombin time was clearly improving and less than about 25 s, in the absence of hepatic encephalopathy.

Group Assignment

At the time of discharge and before results of APAP-CYS concentrations were available, subjects were classified regarding APAP's role in their illness. Two authors reviewed a final assemblage of records that included emergency medical services charts; previous and current hospital records; laboratory results, including results of comprehensive urine drug screening (including gas chromatography/mass spectrometry) and evaluations for other causes of liver disease; imaging studies; prescription records; psychiatric histories and assessments; and interviews with patients, friends, and family members both on admission and just prior to discharge. If required, prehospital personnel, referring emergency physicians, and psychiatrists were contacted to obtain more detailed information from the scene, at initial presentation, or from final psychiatric assessments. Subjects were then classified into three groups with regard to whether or not an excessive dose of APAP had definitely been ingested and whether any ALT rise above normal (> 50 IU/L) was definitely from APAP:

1. Definite APAP group. Investigators were confident:
   a. Supratherapeutic dose(s) of APAP had been taken, and
   b. Elevated ALT values, if present, resulted from APAP toxicity.
2. Definitely not APAP group. Investigators were confident:
   a. A supratherapeutic dose of APAP was not ingested and/or
   b. Any elevation in ALT activity, when present, was unrelated to APAP in the event APAP was ingested in any quantity.
3. Indeterminate APAP group. Investigators were:
   a. Unsure whether a toxic dose of APAP had been ingested or
   b. Unsure whether rises in ALT activity were due, at least in part, to APAP toxicity, when APAP was believed to have been taken.

In the event of disagreement in classification, a treating medical toxicologist for each subject was contacted and any discrepancy in classification was discussed along with review of records, and a unanimous agreement was made in every case, even if classified as indeterminate.

The cohort for this study comprised subjects who were in groups 1 and 2; definite APAP and definitely not APAP groups. The cohort was further restricted to those subjects in whom at least one plasma ALT activity was obtained ± 4 h of a serum APAP-CYS concentration. Subjects classified as having indeterminate APAP overdose at time of discharge were excluded.

Definitions

An APAP overdose could represent acute or chronic excessive doses of APAP. An acute APAP overdose meant the history suggested all APAP was ingested in less than a 3-h period. This included subjects in whom serum APAP concentrations were above the treatment line of the Rumack-Matthew nomogram, or in whom the nomogram could not be applied. All other excessive ingestions (> 4 g APAP per day) were labeled as being chronic, whether staggered over several hours, days, or weeks. Renal failure was defined as a serum creatinine concentration > 2 mg/dL more than 24 h after hospital admission, or as the necessity for renal replacement therapy in the form of hemodialysis or continuous veno-venous hemodiafiltration. Hepatic encephalopathy was deemed present when the subject received treatment for such, including lactulose, rifaximin, or efforts to reduce intracranial pressure (e.g., hypothermia, hypertonic saline, mannitol,

pentobarbital). Hepatotoxicity was defined as plasma ALT activity ≥ 1000 IU/L as a result of APAP overdose. Death from APAP meant that death resulted from cerebral edema or refractory shock secondary to acute liver failure, or that death resulted within days of improvement in liver function, but from a consequence of liver failure, such as persistent irreversible cerebral injury or septic shock from gut bacterial translocation.

Measurement of APAP-CYS and ALT

Quantification of APAP-CYS was performed as described previously using high-performance liquid chromatography-tandem mass spectrometry, with a limit of quantification of 0.01 μM [10, 18] within 6 months of collection. Evaluation of paired samples found no statistically significant difference between serum and plasma APAP-CYS concentrations. ALT activity was assayed as part of normal inpatient laboratory chemistries using the Dimension Vista® ALTI method (Siemens Corporation, Washington DC).

Statistical Analyses

Because blood sampling for measurement of APAP-CYS and ALT was not always simultaneous, paired values of APAP-CYS and ALT values were utilized in which the chosen ALT activity was within 4 h of determination of the serum APAP-CYS concentration. That is, paired samples comprised serum APAP-CYS concentration and ± 4 h serum ALT activity. In instances in which more than one ALT value was within the 4-h window, the value closest in time to when the adduct level was obtained was used.

The type of relationship between APAP-CYS concentrations and ALT activities was determined using scatterplots. Log transformations were performed, and the final form of data was determined by curve fitting (IBM SPSS Statistics for Macintosh v 25.0, IBM Analytics, Armonk, NY) and selecting the relationship with the highest F statistic. Correlations between ALT and adduct values were determined using a mixed model (SAS 9.4m4, SAS Institute, Cary, NC) with a random statement to account for repeated measures between subjects. In order to determine predicted values of serum adduct concentration from ALT (predictor), regression lines were calculated using a mixed model with random intercepts for each subject. Pearson correlation coefficients were calculated on subjects in both groups. All serum adduct values below the limit of quantification (0.01 μM) were assumed to be 0.01 μM for purposes of calculating correlation coefficients. Since the major interest is in determining the presence or absence of APAP toxicity in subjects in whom ALT values exceed 1000 IU/L, correlations were also examined when paired adduct and ALT values were restricted to those with ALT activities ≥ 1000 IU/L, and relevant 95% prediction intervals for serum APAP-CYS concentrations were calculated.

All p values are two-tailed and nominal, and those < 0.05 were considered significant.

Results

Of 500 subjects entered into the study, 449 subjects provided 1242 blood samples in which a serum APAP-CYS concentration could be paired with a ± 4-h plasma ALT activity. Of these, 335 subjects were placed in the definite APAP group and 79 were placed in the definitely not APAP group. Thirty-five subjects in the indeterminate APAP group were excluded (Fig. 1).

Definite APAP Group

Descriptive data are summarized in Table 1. Of 335 subjects in the definite APAP group, 241 (72%) were classified as having acutely ingested and 94 (28%) as having chronically ingested APAP. Seventy-one of 335 subjects (21%) ingested APAP, alone, while 264/335 (79%) subjects ingested various drugs and/or ethanol in addition to APAP. Serum APAP concentrations at presentation ranged from 0 (late presentations) to 867 mg/L. Values for ALT ranged from 8 to 13,207 IU/L, and serum APAP-CYS concentrations ranged from below limit of quantification (< 0.01) to 37.3 μM during hospitalization. Renal failure developed in 48/335 (14%) subjects; 25 subjects with acute, and 23 with chronic ingestions. Renal failure represented acute tubular necrosis and resulted from combinations of factors, including APAP, hypotension/shock, and rhabdomyolysis. Thirty-five subjects (10%) developed hepatic encephalopathy. Eighteen subjects died, and 14 deaths (4%) were from APAP-induced liver failure and/or resultant complications. No patient underwent liver transplantation. Physician investigators caring for the subjects found the history of time of ingestion(s) to be reliable in only 42 of 335 (12%) instances. In only 18 instances (5%) did investigators find the history concerning the amount of APAP ingested to be considered reliable. One hundred forty-four definite APAP subjects (43%) developed a peak ALT ≥ 1000 IU/L.

The 335 subjects in the definite APAP group provided 908 samples of paired serum adduct concentration and ± 4-h ALT activity (mean 2.7, range 1–15 samples per subject) during their hospitalizations. The mean ± standard error of the mean (SEM) time difference (absolute value) between time of blood draws for adduct levels and ALT was 0.73 ± 0.04 h. The relationship between APAP-CYS concentration and ALT activity was best fit by a log-log curve (Fig. 2). When considering all ALT values, there was a strong correlation between serum APAP-CYS concentration and ALT activity ($r = 0.93$, $p < 0.001$; Fig. 2). In Fig. 2 and other figures, a dotted horizontal line at an adduct concentration of 1 μM is provided as a

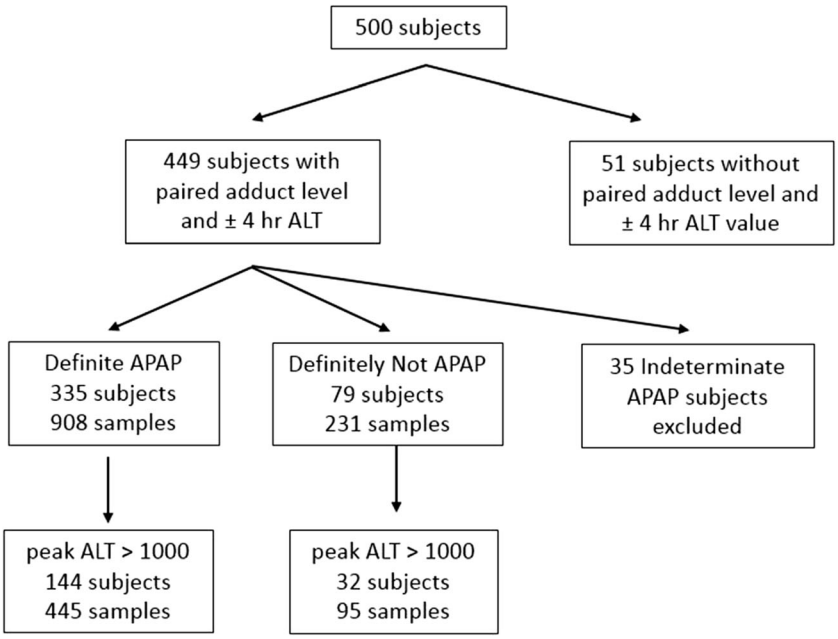

Fig. 1 Numbers of study subjects and blood samples.

reference since therapeutic doses of APAP have, to date, resulted in APAP-CYS levels that have remained ≤ 1.1 μM, and an adduct concentration ≥ 1.1 μM has been claimed to be predictive of an ALT activity ≥ 1000 IU/L in patients with known APAP toxicity [5]. There was no apparent difference in the relationship between serum adduct levels and ALT activity when comparing samples from subjects with acute ingestions to subjects with chronic ingestions, so no effort was made to analyze these groups separately.

Table 1 Descriptive parameters in study cohorts for all subjects, regardless of ALT activity

Parameter	Definite APAP	Definitely not APAP
Total number of subjects	335	79
# samples (data points)	908	231
Age yr. (mean (range))	37 (18–82)	43 (18–69)
Female	235 (70%)	44 (56%)
Male	100 (30%)	35 (44%)
Acute	241 (72%)	–
Chronic	94 (28%)	–
Renal failure	48 (14%)	26 (33%)
Hepatic encephalopathy	35 (10%)	5 (6%)
Death	18 (5%)	6 (7%)
	14 (4%) from APAP	0 from APAP
Peak ALT > 1000 IU/L	144 (43%)	32 (40%)

All values represent numbers of subjects (%), except for age, which is in years. APAP, acetaminophen; ALT, alanine aminotransferase. Renal failure was defined as a serum creatinine concentration > 2 mg/dL more than 24 h after admission and/or need for renal replacement therapy. Hepatic encephalopathy was deemed present when the subject received treatment for such, including lactulose, rifaximin, or efforts to reduce intracranial pressure (e.g., hypothermia, hypertonic saline, mannitol, pentobarbital).

A strong and significant correlation persisted in the definite APAP group when analysis was restricted to 445 data points from 144 subjects in which ALT activity exceeded 1000 IU/L ($r = 0.81$, $p < 0.001$; Fig. 3; Table 2). The mean ± SEM time difference between time of blood draws for adduct levels and ALT in these subjects was 0.67 ± 0.05 h. The 95% prediction interval in Fig. 3 represents where 95% of values would be expected to fall in the face of APAP toxicity for a given ALT activity.

Fifty-nine data points from 36 subjects are found in Fig. 3 with ALT values ≥ 1000 IU/L and serum APAP-CYS concentrations < 1 μM. Of these 36 subjects, 26 subjects had earlier peak adduct levels > 1 μM, and adduct levels had fallen below 1 μM while ALT values persisted above 1000 IU/L. Ten of the remaining 36 subjects (18 data points) displayed peak adduct levels < 1 μM, despite ALT > 1000 IU/L. Five of these ten subjects demonstrated peak ALT < 2000 IU/L. Of the remaining five subjects, four presented with ALT levels between 2000 and 4000 IU/L and with serum APAP-CYS concentrations < 1 μM with subsequent rapid declines in both parameters. APAP-CYS concentrations certainly may have been higher earlier in these four subjects. The remaining subject presented an unknown time following acute APAP ingestion with an ALT activity of 21 IU/L and serum APAP level of 188 mg/L. NAC was not commenced in the outlying hospital for 8 h after presentation. ALT peaked at 3541 IU/L, and serum APAP-CYS peaked at 0.74 μM.

Thirty-seven subjects provided 68 data points with an adduct concentration > 1 μM and corresponding ALT value < 1000 IU/L (Fig. 2). Of these, 24 subjects had measured peak ALT activities > 1000 IU/L during their hospital course.

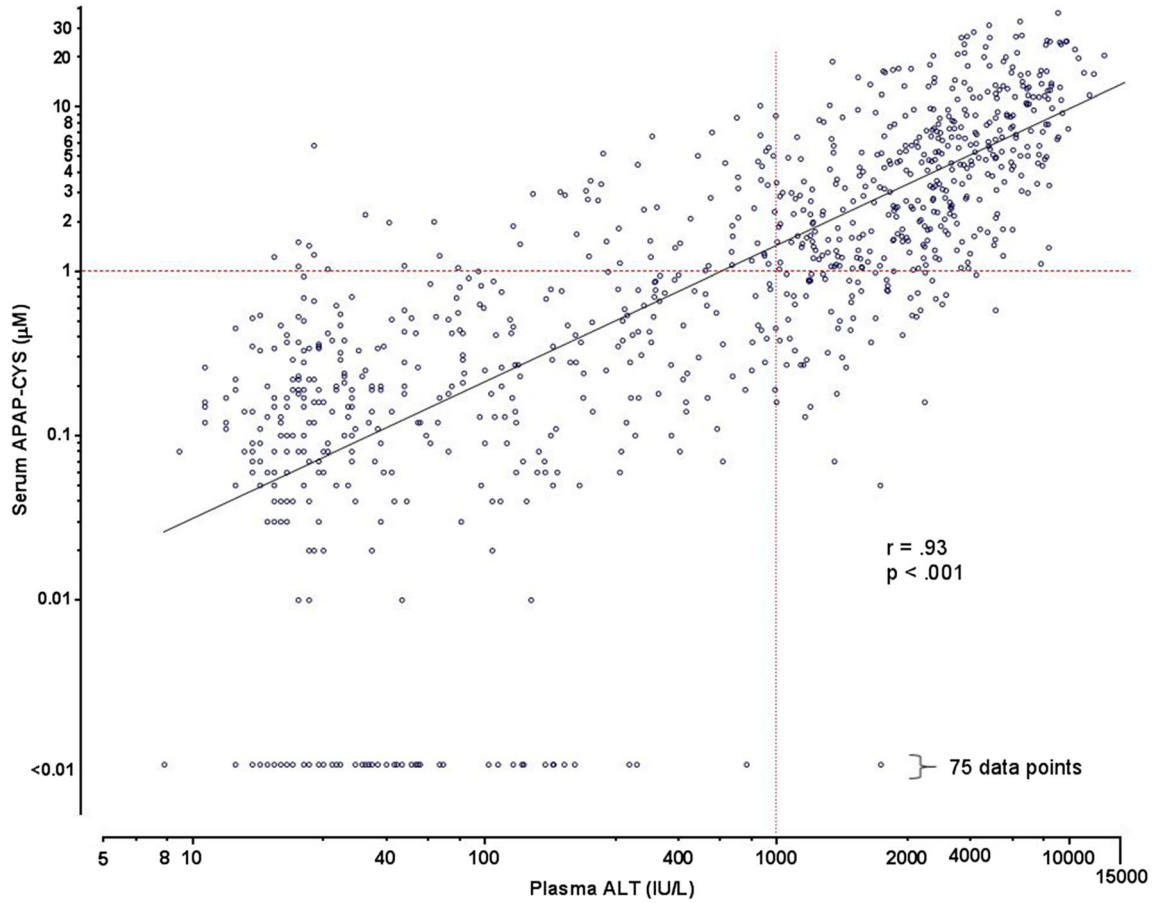

Fig. 2 Definite APAP group. Serum APAP-CYS concentration versus plasma ALT activity. Nine hundred eight data points from 335 subjects during the course of their hospitalizations are shown. Seventy-five samples contained serum APAP-CYS concentrations < 0.01 μM.

Thirteen of the 37 subjects with 26 data points had APAP-CYS concentrations > 1 μM with measured peak ALT < 1000 IU/L (Fig. 2).

Definitely Not APAP Group

In the definitely not APAP group (Table 1), 62 of 79 subjects (78%) had undetectable APAP in serum, and 17 subjects had serum APAP concentrations ranging from 2 to 36 mg/L. ALT values ranged from 18 to 9104 IU/L. Serum adduct levels were below limit of detection at all times of measurement in 37 of 79 subjects. In the remaining 42 subjects, APAP-CYS concentrations ranged from below limit of quantification to 2.86 μM. Sixty-three of 79 subjects (80%) had overdosed on substances other than APAP, while 16 subjects were determined at the time of discharge to not have overdosed. Diagnoses in this latter group included shock from sepsis, gastrointestinal hemorrhage, or cardiomyopathy; sickle cell crisis; cholecystitis; hepatitis A infection; heat stroke; hypothermia; and baclofen withdrawal. Renal failure from acute tubular necrosis developed in 26/79 (33%) subjects secondary to shock and/or rhabdomyolysis. Thirty-two subjects (40%) developed peak ALT ≥ 1000 IU/L. Six subjects (7%) died.

The 79 subjects in the definitely not APAP group provided 231 samples of paired serum APAP-CYS concentration and ± 4 h ALT activity (mean 2.9, range 1–10 samples per subject; Table 2). The mean ± SEM time difference (absolute value) between time of blood draws for adduct levels and ALT was 0.93 ± 0.08 h. Again, the relationship between APAP-CYS concentration and ALT activity best fit a log-log curve. When examining all 79 subjects, there was a weak to moderate correlation ($r = 0.5$, $p < 0.001$) between ALT and APAP-CYS concentration (Fig. 4). But when restricting the correlation to samples in which ALT exceeded 1000 IU/L (32 subjects and 95 data points), the correlation disappeared ($r = 0.04$, $p = 0.84$; Fig. 5). Thus, the correlation found with the entire 79 subjects was largely explained by the weak to moderate correlation when ALT values remained below 1000 IU/L ($r = 0.52$; $p < 0.001$). The 95% prediction interval in Fig. 5 represents where 95% of values would be expected to fall in the absence of APAP toxicity for a given ALT activity.

All subjects in the definitely not APAP group displayed a serum APAP-CYS concentration < 3 μM (highest value 2.86 μM, Fig. 4). Eleven samples from six subjects in this group displayed serum APAP-CYS concentrations > 1 μM when ALT exceeded 1000 IU/L (Fig. 5), and only four

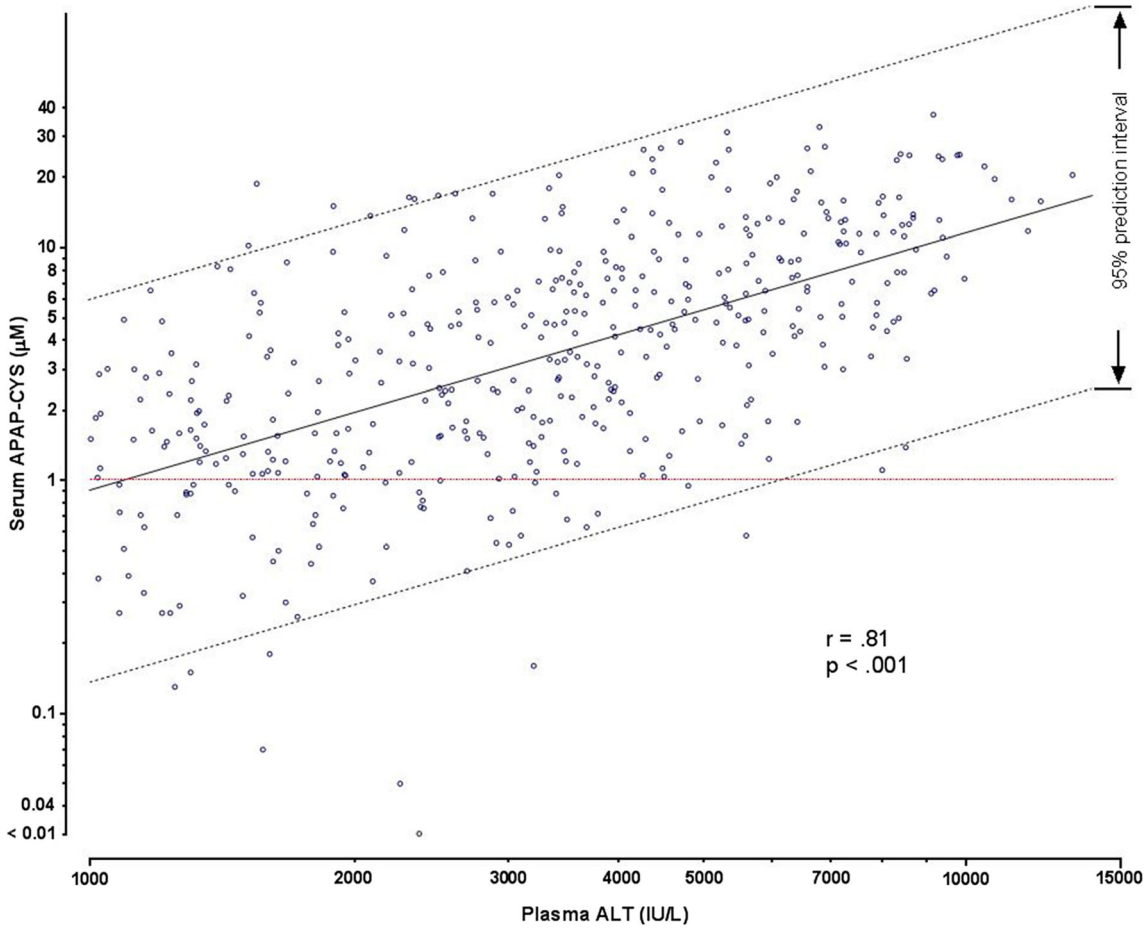

Fig. 3 Definite APAP group. Four hundred forty-five data points from 144 subjects when ALT ≥ 1000 IU/L. The 95% prediction interval illustrates where 95% of serum APAP-CYS concentrations would be expected to fall from APAP-induced elevation in plasma ALT ≥ 1000 IU/L during hospitalization of patients with APAP-induced hepatotoxicity. Also see Fig. 6 for areas of overlap with non-APAP overdose subjects.

Table 2 Descriptive parameters for cohort subjects with ALT > 1000 IU/L

Parameter	Definite APAP	Definitely not APAP
Total number of subjects	144	32
# Samples (data points)	445	95
Age yr. [mean (range)]	39 (18–79)	44 (18–69)
Female	98 (68%)	17 (53%)
Male	46 (32%)	15 (47%)
Acute	241 (72%)	–
Chronic	94 (28%)	–
Renal failure	41 (28%)	21 (65%)
Hepatic encephalopathy	34 (24%)	5 (16%)
Death	16 (11%)	3 (9%)
	14 (10%) from APAP	0 from APAP

All values represent numbers of subjects (%) except for age, which is in years. APAP, acetaminophen. ALT, alanine aminotransferase. See legend for Table 1 for definitions of renal failure and hepatic encephalopathy.

samples from three of the six subjects reached or exceeded 2 μM. These six subjects are described in more detail.

Subject A reported consumption of 3 g APAP over four days for relief of flu-like symptoms before presenting with shock, tachycardia, and hypoglycemia. Initial laboratory studies showed ALT 3365 IU/L; creatinine 2.1 mg/dL; total bilirubin 6.7 mg/dL; and serum APAP 12 mg/L. It was unclear exactly when the last APAP dose had been taken, but overdose, depression or suicidal ideation were denied. Intravenous NAC and antibiotics were begun as a precaution. Blood cultures from admission quickly grew *Salmonella*, and acalculous cholecystitis was recognized as the infectious source. Septic shock continued to worsen with development of multiple organ system failure. The subject was discharged after a prolonged hospital stay with diagnoses of septic shock and multiple organ system failure with cholestasis along with ischemic hepatitis and acute tubular necrosis. An initial serum APAP-CYS concentration was 2.86 μM and was drawn about 20 h after presentation (ALT 1485 IU/L) and continued to decline.

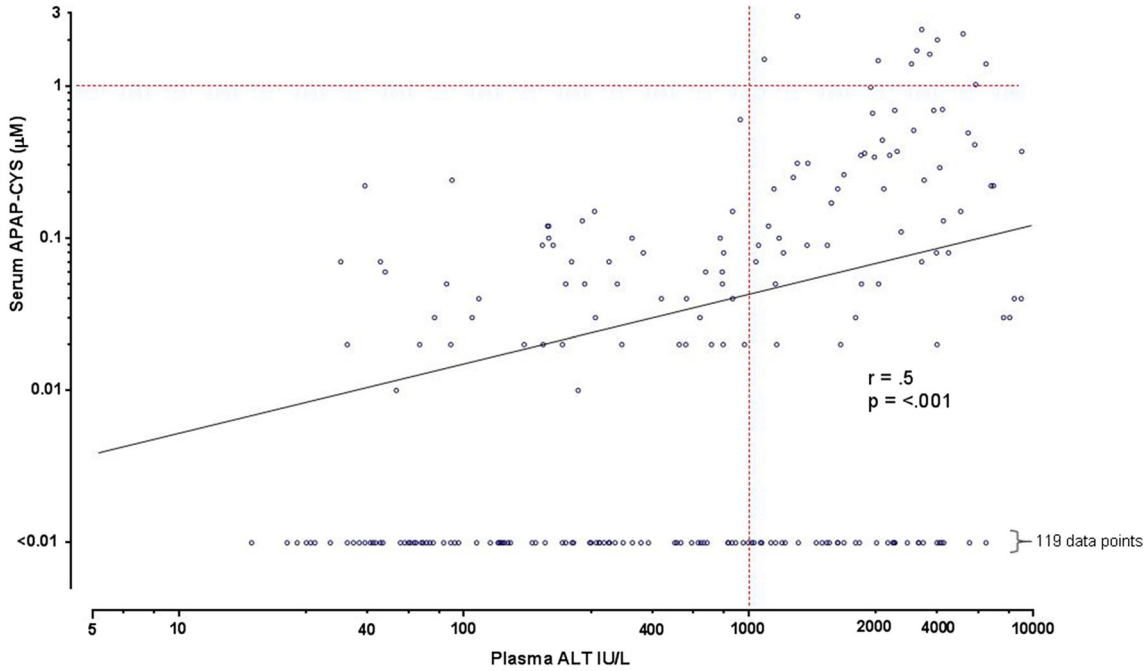

Fig. 4 Definitely not APAP group. Serum APAP-CYS concentration versus plasma ALT activity. Two hundred thirty-one data points from 79 subjects during the course of their hospitalizations are shown. One hundred nineteen samples displayed serum APAP-CYS concentrations < 0.01 μM. See Fig. 5 and text regarding the correlation for ALT values > 1000 IU/L.

Subject B, with obstructive sleep apnea and requiring continuous positive airway pressure (CPAP) during sleep, underwent extremity surgery and was discharged with a prescription for oxycodone and also had APAP/oxycodone at

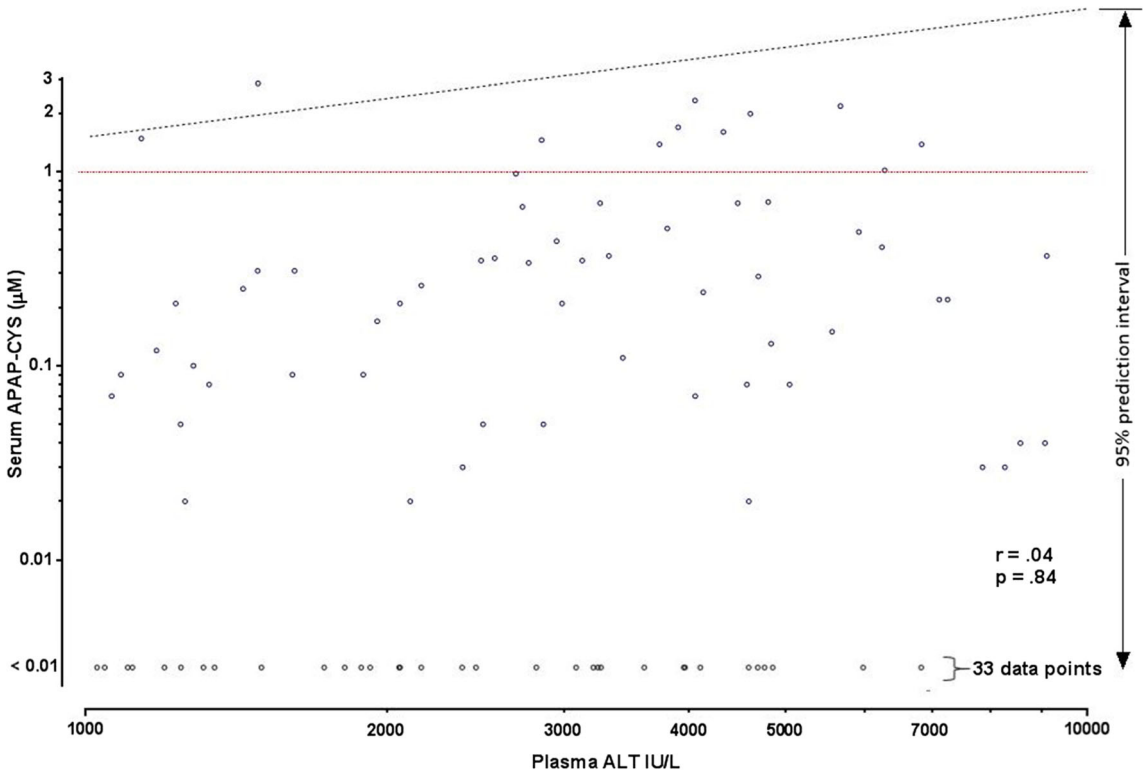

Fig. 5 5 Definitely not APAP group. Ninety-five data points from 32 subjects with plasma ALT ≥ 1000 IU/L. The 95% prediction interval encompasses all values below the dotted black line.

home. ALT had been normal on admission. Two days after discharge she was found lethargic and dehydrated in bed, where she had been lying for at least a day without using her CPAP apparatus. Paramedics found hemoglobin oxygen saturations about 70%, and oxygen administration and ventilation caused her to quickly become alert. Initial laboratory tests showed ALT 4198 IU/L, creatinine 2.6 mg/dL, total bilirubin 1.2 mg/dL and undetectable serum APAP. Urine contained APAP and oxycodone. Serum ALT peaked at 4385 IU/L and serum creatinine at 2.8 mg/dL the following day and then progressively declined. Discharge diagnoses included obstructive sleep apnea, hypovolemia, hypoxia, ischemic hepatitis, and acute tubular necrosis. The serum APAP-CYS level was first measured at 2.34 μM on day 2 when ALT was 4056 IU/L and then declined.

Subject C was found unresponsive and hypoxemic and awakened after administration of naloxone. Oxycodone, tramadol, and over-the-counter APAP had been used for pain following hospital discharge for surgery 3 days earlier. She denied overdosing or taking more medication than instructed by prescription. Initial laboratory studies showed ALT 5691 IU/L, total bilirubin 2.2 mg/dL; and undetectable serum APAP. Urine contained tramadol, oxycodone, and APAP. Rhabdomyolysis and acute renal failure were diagnosed, and liver function studies improved over the next 8 days. Ischemic cerebral infarctions were noted in the distribution of the posterior circulation as a consequence of prehospital hypoxia. Discharge diagnoses included hypoxemia-induced hepatic injury and strokes, rhabdomyolysis, and acute tubular necrosis. The first serum adduct concentration was 2.19 μM (ALT 5665 IU/L) and progressively fell.

Subject D underwent extremity surgery and was discharged with a prescription for APAP/hydrocodone. Four days later she presented with gastrointestinal bleeding from a duodenal ulcer and gave a history of using APAP/hydrocodone and over-the-counter naproxen for pain, as instructed. Admission laboratory results showed ALT 11 IU/L and serum APAP 5 mg/L. Hemorrhagic shock complicated by ischemic colitis and additional hemorrhage ensued during hospitalization with a rise in ALT to 4826 IU/L. Liver function studies normalized over about 10 days. A diagnosis of ischemic hepatitis from hemorrhagic shock was made. The serum APAP-CYS concentration was first measured at 1.7 μM when ALT was about 3900 IU/L, and decreased thereafter.

Subject E, with heart failure and atrial fibrillation, complained of chest pain to her caregiver (who did not give medication) and then was found minimally responsive. Paramedics found her obtunded and hypotensive, and she suffered an 18-min cardiac arrest in the field that was treated with cardiopulmonary resuscitation. Initial laboratory studies showed ALT 1988 IU/L; total bilirubin 0.3 mg/dL; creatinine 2.5 mg/dL; and serum APAP 6 mg/L. A urine drug screen was negative for APAP, causing suspicion that the serum level of 6 mg/L, near the lower limit of reporting, may have represented a false positive. Serum ALT peaked 6 h later at 4329 IU/L, at which time the serum APAP-CYS concentration was 1.6 μM. Both ALT and APAP-CYS levels then declined. The subject awoke on day 2 and denied any overdose (as did the caregiver), and was discharged after 17 days with diagnoses that included bacterial pneumonia, myocardial infarction, cardiogenic shock, ischemic hepatitis, and renal failure (acute tubular necrosis).

Subject F was found unresponsive with low hemoglobin oxygen saturations and awoke after administration of naloxone. She had been taking oxycodone and cyclobenzaprine for abdominal pain. On arrival, initial laboratory studies showed ALT 6823 IU/L, creatinine 3.5 mg/dL, total bilirubin 0.9 mg/dL, and an undetectable serum APAP level. Urine contained cyclobenzaprine and oxycodone; APAP was not detected. A cardiomyopathy with marked left ventricular dysfunction was found. The patient was discharged after 30 days with diagnoses that included ischemic hepatitis from cardiomyopathy complicated by respiratory depression from oxycodone, and acute tubular necrosis from shock/hypoxia and rhabdomyolysis. The initial serum adduct level was 1.39 μM (ALT 6823 IU/L) and then declined.

Data from subjects in both the definite APAP group and the definitely not APAP group are shown together for ALT values ≥ 1000 IU/L in Fig. 6.

Discussion

Histories from patients who have acutely or chronically overdosed are commonly unreliable, and patients with impaired mental status may not or cannot be forthcoming about APAP doses and times of ingestion. Histories obtained from friends or family members are generally even of lower quality [19]. That more than 57 million adults use an APAP-containing product weekly also means that APAP or metabolites, including APAP-CYS, may be identified in patients who have overdosed on other substances or have liver injury/failure for other reasons [8]. Examining the relationship between APAP-CYS concentrations and ALT activity may help distinguish between patients who have ingested toxic or nontoxic amounts of APAP.

Serum APAP-CYS concentrations are reported to remain below 1.1 μM, and usually below 0.5 μM, following therapeutic doses of APAP [4]. Animal experiments, supported by human hepatocyte cell culture studies, demonstrate that release of adducts with therapeutic APAP doses occurs in the absence of markers of hepatocyte injury or lysis [9, 20]. Following APAP-induced hepatic necrosis, adducts are also released as a result of hepatocyte lysis.

Distinguishing between patients who present with liver injury due to APAP overdose from patients with liver injury

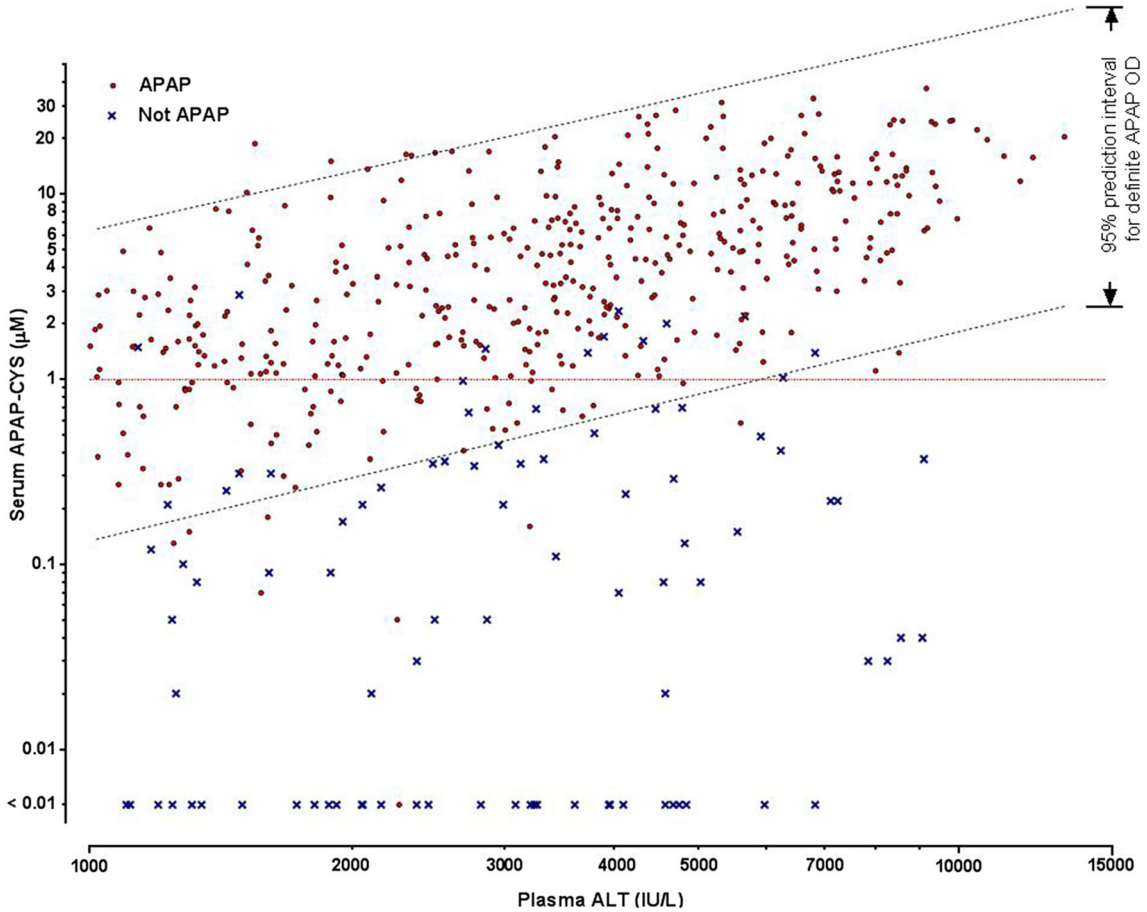

Fig. 6 Combined data points from the definite APAP group and from the definitely not APAP group for all ALT ≥ 1000 IU/L. The single value for a definite APAP subject (red circle) below the limit of detection represents an undetectable APAP-CYS concentration in a subject 3 weeks after admission; earlier adduct levels had been dramatically higher.

from other causes, but who were using APAP-products, might seem straightforward, with the non-overdose APAP patient displaying marked elevation of ALT, but with APAP-CYS levels typical of therapeutic dosing, less than about 1.1 μM. However, the relationship between APAP-CYS concentration and ALT activity may be influenced by additional factors. Renal failure prolongs APAP-CYS elimination half-life [10]. In mice receiving a nontoxic APAP dose, hepatic ischemic injury increases serum APAP-CYS concentrations more than six times over control animals, and ischemic hepatitis certainly occurs in known and suspected overdose patients [9]. This is in keeping with dramatic rises in postmortem APAP-CYS concentrations from tissue redistribution in persons who have taken APAP and have no evidence of hepatic necrosis [11].

We examined serum APAP-CYS concentrations in overdose patients in whom the possibility of acute or chronic APAP overdose was initially entertained. We expect this to be the most common scenario in which measurement of serum adduct levels might be used clinically to detect and/or confirm APAP-induced hepatotoxicity if testing becomes available for routine use. We performed regressions and examined the correlations between serum APAP-CYS levels and ± 4 h ALT activities in subjects with definite APAP overdose or toxicity, and in those who were believed at time of discharge to definitely not have experienced toxicity from excessive ingestion of APAP. In the definite APAP group, we found a strong correlation between APAP-CYS concentration and ALT activity when assuring that analysis was restricted to samples in which adduct concentration was paired to an ALT value obtained within 4 h. While a correlation was evident when ALT values were high or low, the predictive value of APAP-CYS concentration for APAP-induced liver injury is mainly of interest when ALT values exceed 1000 IU/L, as shown in Fig. 3, with a 95% prediction interval for APAP-CYS levels. As an example of applying this prediction interval, finding a serum APAP-CYS concentration of 1.4 μM when corresponding ALT activity was 12,000 IU/L would be unexpected in APAP-induced hepatic necrosis.

Subjects in the definite APAP group with serum APAP-CYS concentrations <1 μM, but with ALT values >1000 IU/L, most commonly had experienced higher adduct levels

previously, with a decline in adduct levels below 1 μM before ALT had declined to <1000 IU/L. Several subjects in whom this pattern could not be documented presented with established, but improving, hepatotoxicity and with falling ALT activities and adduct concentrations. Serum APAP-CYS levels were probably higher earlier.

In the definitely not APAP group, when ALT values exceeded 1000 IU/L, there was no significant correlation between serum adduct concentration and ALT activity (Fig. 5). This is expected to be the case if ALT values were elevated from an hepatic process such as ischemic hepatitis rather than from APAP, but APAP had been taken in non-toxic doses, if at all (many subjects had unmeasurable APAP-CYS). All APAP-CYS concentrations in this group were less than about 3 μM (≤ 2.86 μM).

Figure 6 illustrates that most all serum adduct concentrations in the definitely not APAP group fell below the 95% prediction intervals for those subjects in the definite APAP group. But there clearly was an area of overlap between the two groups, as described above. Eleven samples from six subjects in the definitely not APAP group had serum adduct concentrations greater than 1 μM at some time during their course when ALT was ≥ 1000 IU/L. These APAP-CYS levels may be explained by the presence of the aforementioned factors that may influence serum adduct concentrations, including increased APAP-CYS release into the circulation as a consequence of ischemic hepatitis following non-toxic doses of APAP. The possibility of misclassification of a subject can never be excluded with absolute certainty. However, the presence of several subjects with serum adduct values between 1 and 2.86 μM for whom strong evidence existed that APAP was not the cause for elevated ALT supports the observation of such APAP-CYS concentrations in the absence of APAP toxicity as being valid.

In the past, the relationship between ALT activities and serum APAP-CYS concentrations have been examined in various manners and/or in populations other than adults initially suspected of acute or chronic drug overdose. In 2008, James and colleagues reported APAP-CYS concentrations and ALT activities in children and adolescents with APAP overdose [21]. They reported a significant correlation between peak APAP-CYS level and peak ALT activity, but did not report correlations for non-peak values. As in our study, adduct levels did not appear to be influenced by whether the ingestion was considered to be acute or chronic.

James and coauthors separately addressed APAP-CYS concentrations in 235 samples from 53 adults with acute liver failure due to APAP overdose [5]. These investigators described correlations between serum APAP-CYS levels and aspartate aminotransferase (AST) activities on hospital days 3, 4, and 5, with r values ranging from 0.66 to 0.84, but it was not their purpose to report on overdose patients with lesser degrees of APAP toxicity, on non-APAP overdose patients, or serial ALT measurements. They did report a correlation between peak adduct concentration and peak ALT activity ($r = 0.72$), though it is not clear that transaminase activities were necessarily measured at a time near sampling for APAP-CYS measurement apart from being on the same day. In this population of APAP-induced acute liver failure patients, there was no relationship between the peak adduct level and reported APAP dose, and this is in keeping with our finding of lack of confidence in the history of dose ingested in the great majority of subjects in our study.

In the methods section of the same report, James and colleagues illustrated a receiver-operator characteristic (ROC) curve created from "an existing set of samples from patients with APAP overdose" [5]. While the number and characteristics of these patients and number of samples were not reported, the authors wrote that a single cut point of serum APAP-CYS concentration of 1.1 μM was highly sensitive and specific for predicting an ALT value ≥ 1000 IU/L. It is important to recognize, though, that the ROC curve did not describe a diagnostic cut point for distinguishing between patients with and without APAP toxicity.

In 2010, Heard and others stressed the importance of studying the performance of an assay for acetaminophen-protein adducts in patients who are similar to patients with the diagnosis of interest, as we have done [4]. Peak ALT activities were compared to peak adduct levels, but adduct levels and ALT activities could not always be measured near the same time.

Roberts and colleagues examined APAP-CYS concentrations in relatively small groups of selected subjects, including those with acute liver failure from various causes, some from APAP [6]. A serum APAP-CYS concentration was matched to an ALT value drawn on the same day, but there were no data with regard to how close in time sampling took place beyond this. Results from patients with suspected APAP overdoses but without transaminase elevations were not included in their various patient groups.

Important observations from the present study when ALT values ≥ 1000 IU/L include:

1. Every subject with serum APAP-CYS concentrations that exceeded about 3 μM had been classified as definitely suffering from APAP toxicity. Every subject in the definitely not APAP group had a serum adduct concentration < 3 μM. However, because several of these latter subjects had declining serum APAP-CYS concentrations at the time of presentation, it is possible that higher levels would have been found if they had presented earlier.
2. Some subjects in the definitely not APAP group displayed adduct values as high as 2.86 μM in the presence of ischemic or hypoxic hepatitis and APAP use. In suspected

overdose patients, there clearly is a range in which serum APAP-CYS concentrations overlap between those with APAP toxicity and those in whom non-toxic doses of APAP may have been taken, but elevated ALT activities result from other causes.

3. If serum adduct concentrations are measured in samples when ALT values are declining, APAP-CYS levels may sometimes fall below 1 μM in patients who still have ALT values somewhat > 1000 IU/L from APAP-induced hepatotoxicity.

Our study has several strengths. Large numbers of subjects, all of whom were initially suspected of drug overdose, were included, and serial adduct and ALT values for each subject were obtained, when possible. Subjects were not chosen because of the presence or absence of liver injury or failure, but because they were initially suspected of suffering from APAP overdose. This allowed us to examine APAP-CYS concentrations across a range of ALT values, including subjects with very high serum APAP concentrations, but who received NAC therapy early and experienced no hepatotoxicity. Serum APAP-CYS concentrations were only used if they could be matched to a serum ALT activity obtained within ± 4 h to assess a valid and clinically useful correlation between ALT activity and adduct level. Group assignment was performed before results of serum APAP-CYS levels became available. That investigators cared for all of the subjects allowed prospective detailed analysis of causality that included interviews with family members, psychiatrists, and patients; examination of paramedic records and discussions with prehospital personnel as to what was found at the scene; conversations with referring physicians, and use of results from comprehensive urine drug screens capable of detecting APAP along with hundreds of other drugs and drug metabolites. Unlike previous studies, we were able to calculate a 95% prediction interval to assist in interpreting a serum APAP-CYS concentration for a given ALT value. We used statistical methodology that adjusted for repeat measures among subjects in calculating regressions and prediction intervals.

There exist several potential weaknesses in our study. It would have been ideal to create an ROC curve to choose the best cutoff for distinguishing between subjects with elevated ALT activities from APAP from those with elevated ALT values from other causes. However, the method for creating a valid ROC curve using data with repeated measures, in which a single subject could provide numerous different data points over time, has not been convincingly described in the literature, especially when one subject may provide a single data point, while another might provide ten or more. The possibility of misclassification of a subject to a group can never be completely excluded. The best we can do when assessing causality is consider all clinical information at time of discharge and, using the experience of authors, who have collectively cared for thousands of patients with known APAP toxicity over several decades, arrive at conclusions as to whether or not APAP was definitely ingested in toxic quantities and whether it was responsible for rises in transaminases and in other outcomes. Provision of an indeterminate category allowed us to avoid forcing a subject into definite or definitely not APAP categories when we were not completely confident. That there was no correlation between adduct levels and ALT activities with ALT ≥ 1000 IU/L in subjects assigned to the definitely not APAP group, and the fact that several subjects demonstrated serum APAP-CYS concentrations greater than 1 μM, despite convincing evidence of lack of APAP overdose, supports the concept that ischemic hepatitis can raise serum adduct concentrations above those that might occur with non-toxic doses of APAP, alone, as demonstrated in animals [9]. Nevertheless, it certainly must be possible to suffer both from APAP-induced rises in ALT as well as ischemic hepatitis, and we made no attempt to recognize such patients.

Conclusion

Our study expands the knowledge regarding the correlation between circulating APAP-CYS concentrations and ALT activities in suspected overdose patients. If and when routine and relatively rapid measurements of serum APAP-CYS levels become available to assist in clinical care, interpretation of adduct levels for given ALT values should be enhanced with the use of a 95% prediction interval as we have provided, and with the recognition of potential for elevation of serum adduct concentrations > 1 μM in the absence of APAP-induced hepatotoxicity. The interpretation of serum APAP-CYS concentrations must always be made in light of detailed clinical information and the population to which testing is applied. This is especially true because of some overlap in serum APAP-CYS levels in subjects with and without APAP toxicity.

Acknowledgments Acetaminophen Study Group

Kimberlie A. Graeme, Frank Lovecchio, Daniel E. Brooks, Hannah Malashock, Rachel D. Levitan, Joshua Canning, Aaron B. Skolnik, Carrie A. Truitt, Adam R. Bosak, Robert N.E. French, An Tran, C. Will Heise, Jerry W. Snow, Michael Levine, David C. Watts, Meghan B. Spyres, Eleanor R. Oakley, Elissa C. Moore, Lauren M. Porter, Erik S. Fisher, Laura E. Tortora

Funding This investigator-initiated study was supported, in part, by a grant from McNeil Consumer Healthcare.

Dr. Curry, Dr. Jaeschke, and Dr. Wilkins have had research activities supported by grants from McNeil Consumer Healthcare.

Compliance with Ethical Standards

Conflict of Interest Dr. Curry, Dr. Jaeschke, and Dr. Wilkins have had research activities supported by grants from McNeil Consumer Healthcare.

References

1. McGill MR, Jaeschke H. Mechanistic biomarkers in acetaminophen-induced hepatotoxicity and acute liver failure: from preclinical models to patients. Expert Opin Drug Metab Toxicol. 2014;10(7):1005–17.
2. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest. 2012;122(4):1574–83.
3. Barbier-Torres L, Iruzubieta P, Fernández-Ramos D, Delgado TC, Taibo D, Guitierrez-de-Juan V, et al. The mitochondrial negative regulator MCJ is a therapeutic target for acetaminophen-induced liver injury. Nat Commun. 2017 Dec;8(1):2068.
4. Heard KJ, Green JL, James LP, Judge BS, Zolot L, Rhyee S, et al. Acetaminophen-cysteine adducts during therapeutic dosing and following overdose. BMC Gastroenterol. 2011 Mar;11:20.
5. James LP, Letzig LG, Simpson PM, Capparelli E, Roberts DW, Hinson JA, et al. Pharmacokinetics of acetaminophen protein adducts in adults with acetaminophen overdose and acute liver failure. Drug Metab Dispos. 2009;37(8):1779–84.
6. Roberts DW, Lee WM, Hinson JA, Bai S, Swearingen CJ, Stravitz RT, et al. An immunoassay to rapidly measure acetaminophen protein adducts accurately identifies patients with acute liver injury or failure. Clin Gastroenterol Hepatol. 2017;15(4):555–62.
7. Khandelwal N, James LP, Sanders C, Larson AM, Lee WM. Unrecognized acetaminophen toxicity as a cause of indeterminate acute liver failure. Hepatology. 2011;53(2):567–76.
8. Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. Recent patterns of medication use in the ambulatory adult population of the United States: the Slone survey. JAMA. 2002;287(3):337–44.
9. McGill MR, Lebofsky M, Norris HR, Slawson MH, Bajt ML, Xie Y, et al. Plasma and liver acetaminophen-protein adduct levels in mice after acetaminophen treatment: dose–response, mechanisms, and clinical implications. Toxicol Appl Pharmacol. 2013;269(3):240–9.
10. Curry SC, Padilla-Jones A, O'Connor AD, Ruha AM, Bikin DS, Wilkins DG, et al. Prolonged acetaminophen-protein adduct elimination during renal failure, lack of adduct removal by hemodiafiltration, and urinary adduct concentrations after acetaminophen overdose. J Med Toxicol. 2015;11(2):169–78.
11. Thomas KC, Wilkins DG, Curry SC, Grey TC, Andrenyak DM, McGill LD, et al. Detection of acetaminophen–protein adducts in decedents with suspected opioid–acetaminophen combination product overdose. J Forensic Sci. 2016;61(5):1301–6.
12. Weemhoff JL, Woolbright BL, Jenkins RE, McGill MR, Sharpe MR, Olson JC, et al. Plasma biomarkers to study mechanisms of liver injury in patients with hypoxic hepatitis. Liver Int. 2017;37(3):377–84.
13. Woolbright BL, McGill MR, Staggs VS, Winefield RD, Gholami P, Olyaee M, et al. Glycodeoxycholic acid levels as prognostic biomarker in acetaminophen-induced acute liver failure patients. Toxicol Sci. 2014;142(2):436–44.
14. Ward J, Kanchagar C, Veksler-Lublinsky I, Lee RC, McGill MR, Jaeschke H, et al. Circulating microRNA profiles in human patients with acetaminophen hepatotoxicity or ischemic hepatitis. Proc Natl Acad Sci U S A. 2014;111(33):12169–74.
15. McGill MR, Cao M, Svetlov A, Sharpe MR, Williams CD, Curry SC, et al. Argininosuccinate synthetase as a plasma biomarker of liver injury after acetaminophen overdose in rodents and humans. Biomarkers. 2014;19(3):222–30.
16. McGill MR, Li F, Sharpe MR, Williams CD, Curry SC, Ma X, et al. Circulating acylcarnitines as biomarkers of mitochondrial dysfunction after acetaminophen overdose in mice and humans. Arch Toxicol. 2014;88(2):391–401.
17. Tortora L, Ruha M, Ramos K, Jaeschke H, Rumack B, Kang M, et al. Pharmacogenomic analysis of a patient with severe hepatotoxicity and hemolysis after acetaminophen overdose despite early N-acetylcysteine therapy. Clin Toxicol. 2018;56(10):983–4.
18. Cook SF, King AD, Chang Y, Murray GJ, Norris HR, Dart RC, et al. Quantification of a biomarker of acetaminophen protein adducts in human serum by high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry: clinical and animal model applications. J Chromatogr B Analyt Technol Biomed Life Sci. 2015;985:131–41.
19. Gregory B, Larson AM, Reisch J, Lee WM, Acute Liver Failure Study Group. Acetaminophen dose does not predict outcome in acetaminophen-induced acute liver failure. J Investig Med. 2010;58(5):707–10.
20. McGill MR, Yan HM, Ramachandran A, Murray GJ, Rollins DE, Jaeschke H. HepaRG cells: a human model to study mechanisms of acetaminophen hepatotoxicity. Hepatology. 2011;53(3):974–82.
21. James LP, Capparelli EV, Simpson PM, Letzig L, Roberts D, Hinson JA, et al. Acetaminophen-associated hepatic injury: evaluation of acetaminophen protein adducts in children and adolescents with acetaminophen overdose. Clin Pharmacol Ther. 2008 Dec;84(6):684–90.