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ORIGINAL ARTICLE

Differentiating Ischemic Hepatitis from Acetaminophen Overdose in Acute Liver Failure: Role of Acetaminophen Adducts—Ischemic Hepatitis vs Acetaminophen Overdose

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Abstract

Background and Aims Acetaminophen (APAP) hepatotoxicity and ischemic hepatic injury (IH) demonstrate remarkably similar biochemical patterns. Deciding between these two etiologies in the setting of acute liver failure (ALF) can be challenging. We reviewed all cases in the Acute Liver Failure Study Group (ALFSG) registry where these diagnoses were considered, to determine reasons for, and frequency of, difficulties making these diagnoses. We hypothesized that the newly developed APAP-CYS adduct assay could help in discerning the correct diagnosis.

Methods Among 3364 patients with ALF or acute liver injury (ALI: INR $\geq$ 2.0 but without encephalopathy) between 1998 and 2019, 1952 (58%) received a final diagnosis of either APAP (1681) or IH (271). We utilized a review committee of senior hepatologists as well as the APAP-CYS assay (where sera were available), measuring the presence of toxic by-products of APAP injury to optimize adjudication.

Results With these methods, a total of 575 adduct positive APAP cases included 488 recognized APAP, as well as an additional 87 patients previously diagnosed as other etiologies. Nine cases initially attributed to IH were deemed combination APAP-IH injuries. Conversely, 215 of the 280 IH subjects tested for adducts disclosed 173 confirmed as IH with adduct testing below the toxicity threshold, while 9 cases were revised from APAP to the IH-APAP combination phenotype, where both hypotension and APAP likely played a role.

Conclusions Discerning APAP from IH can be difficult—in rare cases, combined injury is observed (18/1952). APAP-CYS testing resulted in revising the diagnosis in 14.6% of cases.

Keywords Hepatic necrosis · Shock liver · Acetaminophen overdose · Cardiac dysfunction · Hypoxia

Abbreviations

APAP	Acetaminophen
IH	Ischemic hepatitis
ALF	Acute liver failure
ALFSG	Acute Liver Failure Study Group
ALI	Acute liver injury

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DILI	Drug-induced liver injury
ALT	Alanine aminotransferase
APAP-CYS	Acetaminophen-protein adducts
GSH	Hepatic glutathione
NAPQI	*N*-Acetyl-p-benzoquinone imine
HPLC-ED	High-performance liquid chromatography with electrochemical detection
INR	International normalized ratio of prothrombin time
TFS	Transplant-free survival
LT	Liver transplantation
HIPAA	Health Insurance Portability and Accountability Act
CV	Coefficient of variation
T Bili	Total bilirubin
AST	Aspartate aminotransferase
COPD	Chronic obstructive pulmonary disease
NAC	*N*-Acetylcysteine

Introduction

Acetaminophen (APAP) overdose is a major cause of acute liver failure (ALF) globally; in the United States, it accounts for 46% of all ALF admissions [1–3]. Ischemic hepatic injury (IH) is a recognized cause of ALF but impacts a much smaller number of individuals, ~ 8% of those diagnosed with ALF. In the absence of a reliable history, accurately distinguishing between APAP and IH can be a challenge due to remarkably similar biochemical patterns of injury [4]. IH is caused by hepatic hypo-perfusion in the setting of cardiac, circulatory or respiratory failure/hypoxia, and typically results in marked but transient elevation of aminotransferases, similar in level, timing and evolution to APAP injury [4–7]. A study by Weemhoff et al. investigating the mechanisms of injury during 'hypoxic hepatitis' confirmed that cell death is mainly caused by necrosis, similar to APAP toxicity. Mitochondrial injury is thought to represent the key event in liver cell death in both these settings [8, 9]. Both are classified as 'hyperacute' since injury evolves predictably within 24 h of initial insult, peaking at approximately 72 h following initial APAP ingestion or ischemic insult. Thus, the clinical diagnosis of ischemic hepatitis (IH) necessitates exclusion of other causes of acute liver injury, particularly APAP overdose. However, the presence of hypotension is not always recognized or present such that the development of ischemic hepatitis may go un-noticed. In a recent systematic review of ischemic hepatitis cases, only 52.9% of patients had had a documented hypotensive event [10].

Acetaminophen-protein (APAP-CYS) adducts are specific biomarkers of APAP-related hepatotoxicity that can be measured in tissue or blood samples [11]. After large-dose APAP ingestions, when hepatic glutathione (GSH) is depleted, the toxic intermediate metabolite *N*-acetyl-p-benzoquinoneimine (NAPQI) binds with cysteine sulfhydryl groups on cellular proteins to form APAP-CYS adducts, released into the peripheral circulation following hepatocyte lysis [12]. The short half-life of the APAP parent compound limits its use in detecting ALF cases attributable to APAP toxicity once sufficient injury has occurred: APAP parent compound was already undetectable in serum from 52% of patients at time of admission with ALF [12]. The presence of APAP-CYS in blood can be quantified through high-performance liquid chromatography with electrochemical detection (HPLC-ED) [13]. The median elimination half-life of acetaminophen-protein adducts in adults with ALF is ~ 42 h (range 22.6–61.2 h), greatly exceeding that of APAP, the parent drug, with a median plasma half-life of 5.4 h (range 0.8–119.7) [12]. APAP-CYS adducts are detectable in blood in 100% of known acetaminophen ALF patients and in none of the ALF patients with other defined causes [6], while 19% of indeterminate ALF cases demonstrated adducts in serum, providing a cogent diagnosis. In patients with well characterized APAP toxicity and an ALT value > 1000 IU/L, an adduct concentration of ≥ 1 nmol/L has high sensitivity and specificity for APAP toxicity [13].

The aim of this study was to analyze in detail all cases of presumed ischemia or APAP toxicity in the ALFSG registry using standard criteria developed by a review committee of senior hepatologists while evaluating the additional tool of the APAP-CYS adduct assay as an aid in validating the correct diagnosis. We hypothesized that the adduct assay would improve accuracy of diagnosis in this setting.

Methods

Study Design and Inclusion Criteria

The ALFSG was a consortium of 23 tertiary academic referral centers with interest and expertise in ALF that was funded by the National Institute of Diabetes and Digestive and Kidney Diseases from 1997 to 2023 (NCT00518440). A total of 3,364 patients were enrolled prospectively in the ALFSG registry between January 1998 and August 2019. Enrollment criteria for ALF included the presence of coagulopathy with an international normalized ratio of prothrombin time (INR) ≥ 1.5 and any level of hepatic encephalopathy within 26 weeks of illness onset in a person with no known underlying liver disease. A separate category of severe acute liver injury (ALI) was also utilized after 2010, with the following criteria: an INR ≥ 2, absence of hepatic encephalopathy and an illness of ≤ 26 weeks' duration in patients without known chronic liver disease [14]. Primary outcomes assessed at 21 days from enrollment were: transplant-free survival (TFS), liver transplantation (LT), or death [15, 16].

Written informed consent was obtained from subjects or legal next-of-kin. All centers adhered to local Institutional Review Boards' requirements and the Health Insurance Portability and Accountability Act (HIPAA). The study population consisted of all ALF/ALI patients with presumed APAP toxicity or hepatic ischemia enrolled between January 1998 through August 2019.

Measurement of Serum APAP (Parent Compound) Levels

APAP (parent compound) levels were reported at each site, typically from the initial emergency department evaluation prior to study enrollment, and recorded in the case report forms. A variety of methods are used across study sites to measure APAP levels, but most are reproducible and reliable [1, 12]. The presence of any detectable APAP level *in a patient who has already developed severe liver injury*, given its remarkably short half-life is associated with high likelihood of toxicity [11–13].

Selection of Sera for Study

The presence of APAP in plasma in significant amounts and/or a strong history of APAP overdose were used to exclude many APAP overdose patients from analysis for APAP adducts, with a low threshold to include patients where any question existed. By contrast, we examined all IH patients where serum samples were available, given the greater uncertainty in this cohort of exposure to significant amounts of APAP. Thus, we submitted samples from a total of 575 out of 1681 APAP subjects and 215 of 280 IH subjects for adduct testing.

Measurement of Serum APAP-CYS Adduct Concentration

Serum samples collected at admission to study or on day 2 after study enrollment were aliquoted and stored at -80 degrees Celsius and subsequently analyzed in a blinded fashion for APAP-CYS adducts using a previously reported high-performance liquid chromatography with electrochemical detection (HPLC-ED) method that is highly sensitive and specific for APAP-CYS [11, 12]. In brief, serum samples were treated with gel filtration to remove small molecules such as APAP, APAP metabolites, and *N*-acetylcysteine. The resulting sample was treated with protease and the resulting peptides analyzed for APAP-CYS adducts by HPLC-ED with a reverse-phase C18 column and detected by oxidation at 155 and 280 mV [12]. The range of linearity for the method was 0.03 to 30.0 nmol APAP-CYS adducts per mL of serum. The coefficients of variation (CVs) for the assay were consistently < 15% at concentrations of 0.03, 1.0, 6.0 and 30.0 nmol/mL APAP-CYS adducts. Based on the CVs for the standard curve for the assay, the lower limit of quantitation was defined as 0.03 nmol/mL APAP-CYS. In previous studies, adduct values of ≥ 1.0 nmol adducts/mL serum in patients with clinically defined APAP overdose and a simultaneously drawn ALT value > 1000 IU/L had a sensitivity of 96.8% and a specificity of 95% [11, 12]. For the purposes of this study, based on previous studies evaluating APAP-CYS in a variety of toxic and non-toxic exposures, a toxic adduct level was defined as a value of APAP-CYS > 1.0 nmol/mL, while levels of 0.1 but less than 1.0 nmol/mL were defined as non-toxic (or therapeutic) [6, 7, 9, 11, 12]. Trace amounts may be detected between 0.03 nmol and 0.01, (considered barely detectable).

Adjudication of Etiology and Causality Assessment

Each case was given a diagnosis by the site investigator at the time the patient was reported by the local site to the data coordinating center. All cases in the ALFSG database were subsequently reviewed by a causality adjudication committee of 9 experienced hepatologists. Specific diagnostic criteria for each etiology were established by the committee prior to the start of the study as follows:

ALF due to acetaminophen.
APAP-CYS adducts positive at a toxic level (> 1.0 nmol/L) OR a history of APAP ingestion (any amount) OR detectable APAP (parent compound) level; AND ALT > 1000 IU/L and total bilirubin (T Bili) < 10 mg/d L [17].
ALF due to Shock/Ischemia.
Negative APAP history and/or APAP-CYS adducts not (or below pre-defined toxic level, < 1.0 nM/L) detectable,

AND

- History of suspected or documented hypotension or acute cardiopulmonary incident or hypoxia for any reason

AND one of the following:

- Rapid recovery time course (Peak to ~ 50% reduction in aspartate aminotransferase (AST) by 24 h after peak value recorded) or
- ALT ≥ 1000 IU/L and T Bili ≤ 5.0 mg/dL.

For the purpose of the present study, the review committee focused on those initially diagnosed as either APAP or ischemia, (Fig. 1). Having available serum samples from most patients allowed for measurement of APAP-CYS adducts to further aid adjudication. For very obvious cases based on history or parent compound levels, adduct levels

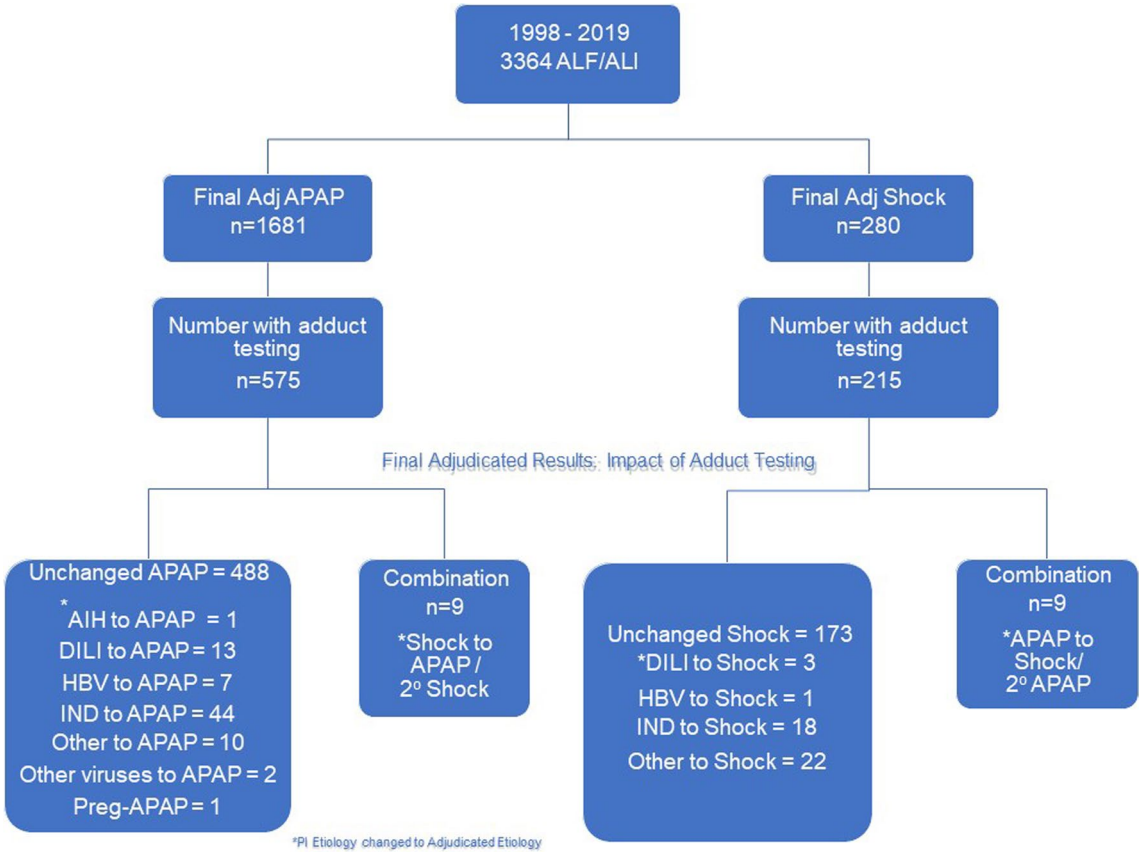

Fig. 1 Consort diagram showing results after final adjudication for all patients in the study

were not always obtained; those patients whose sera were sent for adduct level measurement were mainly those without a clear history of APAP ingestion or available positive APAP level results. Three committee members reviewed each case and came to consensus after discussion on teleconference calls.

Additional laboratory testing (beyond APAP-CYS adducts) included HEV testing [18], viral sequencing via microarray analysis and metagenomics next-generation sequencing [19] performed on sera when available and as indicated to further clarify diagnoses.

Results

From a total of 3,364 patients enrolled during the entire study, 1672 received a final etiology adjudication of APAP, and 251 were attributed to IH, while an additional 18 were considered to have a combined injury. A relatively small number of patients initially deemed by the site investigator to be a certain diagnosis were changed by the committee as shown in Fig. 1. In the left-hand portion, all those whose final diagnosis was APAP are represented: most initial diagnoses was APAP remained as APAP or while others were changed to APAP as a result of the adjudication (including APAP-CYS testing) or to APAP-IH combined. In some instances, the initial diagnosis such as hepatitis B, became a secondary diagnosis if APAP was determined to be the primary reason for ALF/ALI, (e.g., chronic hepatitis B plus APAP overdose). Among a total of 575 patients with final APAP adjudications, 488 confirmed the initial APAP diagnosis, while an additional 87 were revised from other diagnoses to APAP based on adduct values $\geq$ 1.0 nmol/ml. Among these, 78 represented other initial diagnoses than APAP or ischemia (Fig. 1, Table 1), while 9 initially deemed as ischemia received a final adjudication of APAP-ischemia (see below).

Similarly, among the 251 subjects that were adjudicated finally as ALF/ALI due to ischemia, most (215) had been tested for APAP adducts and found to be undetectable or with very low levels of APAP-CYS adducts. Likewise, other specific diagnoses, including APAP were adjudicated to ischemia in the right circumstances (history supporting ischemia and/or negative or below toxic adduct levels) while 9 subjects with initial diagnosis of APAP were changed to combined injury. Thus, there were 18 apparent combination

Table 1 Comparison of APAP and IH groups with the combined APAP/IH injury group

Median (min–max) *	APAP $n=566$	IH $n=206$	Combined $n=18$	p-value
Age*	36 (17–79)	53 (17–84)	53 (22–83)	< 0.001
Female (%)	404 (71.4)	101 (49.0)	9 (50.0)	< 0.001
Cauc/AA/Other (%)				0.002
Caucasian	472 (84.3)	159 (77.2)	10 (55.6)	
African American	59 (10.4)	26 (12.6)	6 (33.3)	
Other	30 (5.3)	21 (10.2)	2 (11.1)	
Ethnicity non-Hispanic (%)	534 (94.3)	184 (89.3)	18 (100)	0.076
Peak AST*	8960 (45–38546)	4948 (374–34790)	3592 (363–12774)	0.004
24 h AST ($n=542$)*	2916 (23–21020)	2600 (50–32870)	2194 (163–8932)	< 0.001
Peak ALT*	5325 (144–27440)	3288 (241–17000)	3294 (584–10459)	< 0.001
24 h ALT ($n=539$)*	2853 (47–9987)	2178 (123–9129)	2338 (500–5447)	0.389
Peak T Bili*	7.4 (1.0–70.0)	5.4 (1.0–69.0)	4.5 (2.0–12.0)	0.002
24 h T Bili ($n=377$)*	4.9 (0.4–58.0)	3.5 (0.4–45.5)	2.8 (1.2–10.9)	< 0.001
Transplant-free survival (%)	367 (64.7)	151 (73.3)	15 (83.3)	0.03
Death (%)	124 (21.9)	47 (22.8)	3 (16.7)	0.827
Transplanted (%)	35 (6.2)	4 (1.9)	0 (0)	0.034

* Median (min-max)

Clinical and laboratory results for APAP ($N=566$), Ischemia Hepatic Injury ($N=206$) and Combined Injury (18), after APAP-CYS adduct testing, highlighting median laboratory values (initial, peak and 24 h after peak) and outcomes for each category. APAP aminotransferase levels were higher in general than IH levels

injuries, most of whom were found unresponsive with hypotension or had an alternative reason for hypotension in addition to an earlier APAP overdose (Table 2 and case examples below).

Table 2 Combined group—individual patient results

Initial Dx	Second Dx	AST (IU/L)			ALT (IU/L)			T Bilirubin (mg/dL)			Adducts
		Initial	Peak	24 hr PP	Initial	Peak	24 hr PP	Initial	Peak	24 hr PP	nmol/L
APAP	Shock/Isch	3941	10805	3941	5682	5682	2983	1.0	1.6	1.2	0.411
APAP	Shock/Isch	536	2200	536	531	1300	531	5.4	12.2	10.9	0.186
APAP	Shock/Isch	1080	1080	623	493	584	542	5.1	5.1	4.7	0.181
APAP	Shock/Isch	1351	4300	1351	821	2047	821	1.8	6.7		2.151
APAP	Shock/Isch	524	1528		1927	1927	1404	3.3	3.3	2.7	0.000
APAP	Shock/Isch	1977	1977	1678	3771	3771	2576	3.0	3.0	2.6	0.000
APAP	Shock/Isch	2600	2600	2600 / 540	2724	3575	2724	2.98	5.61	5.2	5.16
APAP	Shock/Isch	1191	2885	1191	500	626	500	1.9	1.9		0.627
APAP	Shock/Isch	5338	10000	5338	3534	6150	3534	0.8	1.6	1.4	0.271
Shock/Isch	APAP	363	363	163	1079	1079	774	4.5	4.5	3.6	1.352
Shock/Isch	APAP	270	770	270	2127	3013	2127	1.2	1.5	1.2	1.396
Shock/Isch	APAP	5254	12348	5254	5447	10459	5447	3.2	5.7	5.1	4.642
Shock/Isch	APAP	1473	1473	335	1318	1318	677	1.8	2.3	2.2	2.89
Shock/Isch	APAP	8932	12774	8932	3679	4956	3676	2.0	2.6	2.0	5.235
Shock/Isch	APAP	6825	9064	2411	4994	4994	3344	0.8	1.9		1.206
Shock/Isch	APAP	6562	7000	6562	4297	6800	4297	6.2	7.4		5.671
Shock/Isch	APAP	4107	8294	3739	1268	2145	1263	0.8	5.9		2.759
Shock/Isch	APAP	6763	9066	6763	2548	4590	2548	7.5	7.5		4.951

Individual laboratory values (initial, peak (highest value recorded) and 24 h after peak), and APAP-CYS adduct levels, for each of the 18 combination APAP/IH case. Nine were initially considered APAP but finally concluded to be a combination and similarly, 9 were considered IH but ultimately considered an APAP/IH combination. Colors signify samples were the same, that is, Initial and peak or 24 h PP (post peak) were the same values/same sample

Median peak lab values for both overall groups were similar: APAP: AST 8960 IU/L, ALT 5,325 IU/L, T Bili 7.4 mg/dL vs. Ischemia: AST 4,948 IU/L, ALT 3288 IU/L, T Bili 5.4 mg/dL (Table 1). Outcomes were similar in all the groups: for death, 21.9% APAP, 22.8 ischemia and 16.7% for the combined group; for transplantation, 6% APAP and 1.9% ischemia. Among the deaths within the APAP group, nearly half were noted to be related to cerebral edema and uncal herniation and a similar number due to multi-organ failure/sepsis. Within the ischemia cohort, there were 48 deaths: 31 attributed directly to the underlying cardiac cause, while 9 were thought secondary to multi-organ failure or sepsis, five to direct neurological issues and in three the cause of death was unknown. Note that the rapid upstroke/rapid recovery of AST particularly but also ALT reflects the similar underlying mechanism of necrosis/injury between the APAP and IH etiologies (Figs. 2, 3). Overall, there were 789 of 1961 (40%) of the APAP cases who had a AST > ALT on admission to the study; however, the timing of each patient's study entry varied so this finding is of uncertain significance.

Comparison of APAP and IH Biochemistry Patterns

We sought to compare the injury patterns observed in the APAP and IH groups. For both etiologies, the durations of recovery were remarkably similar, with calculated elimination half-lives for AST and ALT as shown in Table 3. In general, as has been noted previously, AST has a shorter half-life than ALT. In the present study, we also separated the intentional overdoses from the unintentional ones–unintentional overdoses demonstrated slightly longer half-lives for ALT. By contrast, AST and ALT half-lives observed in ischemia are somewhat longer than the AST or ALT values observed in most APAP patients. Figures 2 and 3 emphasize how closely these patterns resemble one another. Adduct

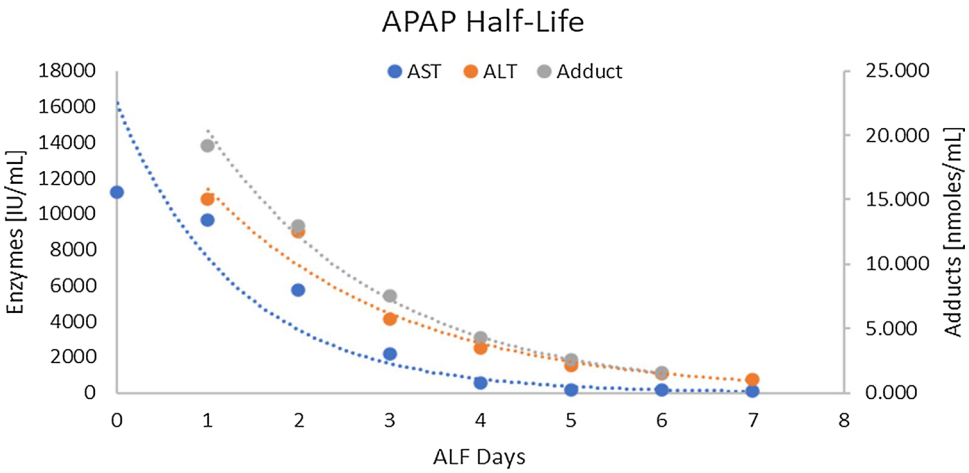

Fig. 2 Lab values over 7 days for a single APAP patient. AST, ALT and APAP-CYS adducts are plotted for this 26-year-old woman who took 21 gm APAP 29 h prior to presentation. She received oral and intravenous *N*-acetylcysteine (NAC) on day 1; AST half-life = 1.4 days, ALT half-life = 2.6 days, adduct half-life = 2.5 days

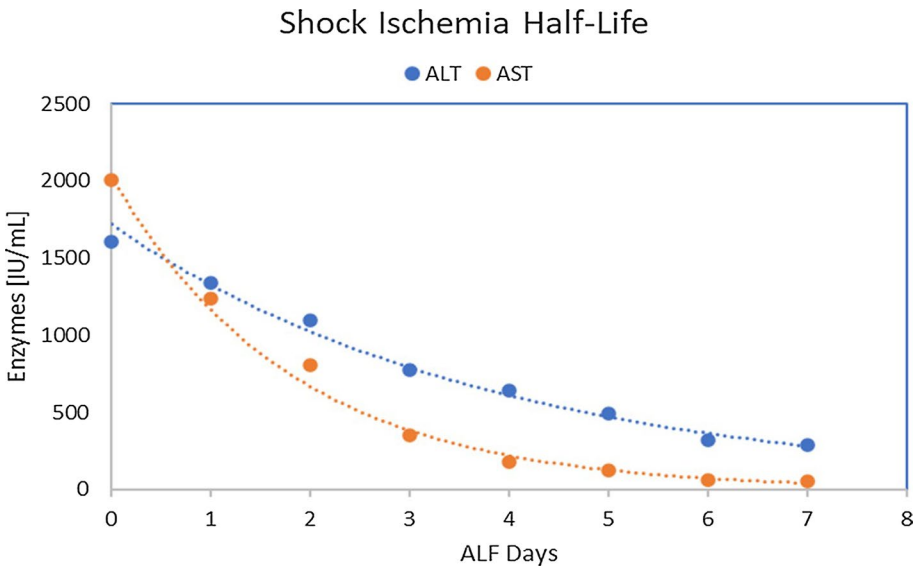

Fig. 3 Lab values over 7 days for an ischemic hepatic injury patient. Plot is similar to Fig. 2; AST and ALT values decline over a similar time frame in this 80-year-old man with COPD and congestive heart failure; AST half-life = 2.3 days, ALT half-life = 2.9 days

Table 3 AST, ALT and APAP-CYS Half-lives

Category	Half-life days		
	AST*	ALT*	APAP-CYS**
APAP intentional $n=16$*/$n=24$**	1.3 (0.5–2.1)	2.1 (1.4–2.7)	2.7 (2.1–3.4)
APAP unintentional $n=15$*/$n=5$**	1.7 (1.1–2.3)	2.4 (1.8–3.1)	2.8 (2.6–3.0)
Ischemia $n=10$	2.0 (1.3–2.7)	3.2 (2.7–3.6)	–
Figure 2 APAP	1.4	2.6	2.5
Figure 3 Ischemia	2.3	2.9	–

* Median (min-max)

Half-lives for AST, ALT and APAP-CYS for APAP and AST, ALT half-lives only for Ischemia

level decline paralleled most closely the resolution of ALT (Fig. 2).

Combined Group

In 18 patients we were unable to clearly delineate between APAP and IH, and ultimately described these as combined injury. Interestingly, there were 9 initially considered to have APAP and ultimately adjudicated as IH-APAP combination and 9 changed from IH to APAP-IH combination, based on careful review and the availability of the APAP-CYS adduct testing. The committee was unable to differentiate these 18 into one or the other category. Those initially considered APAP all had high/toxic adduct levels, while those initially considered to be ischemic had clearer evidence of hypotension but also in most cases had at least detectable APAP adducts or a history of some APAP ingestion. The individual results including adduct levels for this group of 18 are presented in Table 2 with several exemplary case histories below:

Case 1

A 49-year-old male with history of resection of a squamous cell cancer of the tongue and recurrent alcohol-associated pancreatitis was admitted obtunded with multifocal necrotic pneumonia and septic shock and was discovered to have acute liver failure. He had been prescribed APAP/Hydrocodone for his cancer and for pain associated with chronic pancreatitis. ALF initially attributed to ischemia and later adjudicated to APAP-induced based on elevated adduct level (2.58 nmol/L). In this case, it was impossible to separate ischemic injury to the liver in the setting of septic shock from liver toxicity due to APAP overdose. It is notable that the adduct level helped to identify the (at least) partial contribution of APAP to this patient's clinical course as APAP use was not initially obtained from this patient's history.

Case 2

This 54-year-old male was admitted with bilateral mandibular fractures after an assault, for which he was prescribed acetaminophen in uncertain amounts. He was confused, hypothermic, and hypotensive on arrival. His WBC $> 20 \times 10^9$ mm^3, lactate 15 pg/mL. After 7 L of intravenous fluid resuscitation as well as norepinephrine, he recovered nicely. Subsequently he was determined to have a highly toxic APAP-CYS level (5.160 nmol/L), consistent with excessive APAP in the setting of jaw pain. Although initially diagnosed as being in shock and thus IH, both the adduct level and the overall picture favored a multifactorial explanation rather than any singular etiology for this patient's ALF.

Case 3

This 48-year-old female with history of anxiety, depression, PTSD, opioid dependence and alcohol use disorder was found unresponsive at home by her son. She was intubated for hypercapnic respiratory failure. Her chest Xray was concerning for pneumonia; she became progressively hypotensive requiring vasopressor support and IV fluids. APAP parent compound level negative initially. ALF etiology adjudicated from IH to a combination injury, based on APAP-CYS adduct level of 1.206. In this case, the committee was unable to completely eliminate the role of septic shock to this patient's ALF. The toxic APAP-CYS level indicated that APAP was likely the initiating cause of her ALF, but given her respiratory failure requiring vasopressor support, a combined injury, with hypotension/IH intervening due to her having been comatose for unknown time at home.

Case 4

This 56-year-old woman with chronic obstructive pulmonary disease (COPD), hypertension, morbid obesity status post gastric bypass surgery presented with chronic back and knee pain and had been using oxycodone. When she ran out of oxycodone, she started taking her husband's APAP-hydrocodone, as many as 100 tablets estimated over 5 days. She was noted to be somnolent 2 days prior to admission and was intubated for airway protection shortly after admission. Peak AST and ALT were 2200 IU/L and 1300 IU/L respectively and T bilirubin was initially 5.4 mg/dL, rising to a peak of 12.2 mg/dL. Her APAP-CYS adduct level surprisingly was only 0.186 nmol/L (not toxic). By history, this seemingly obvious APAP overdose turned out to be more

likely pulmonary failure and hypoxia/ischemia on that basis or a combination thereof.

Discussion

Overall, 1961/3364 (58%) of ALFSG registry cases represented hyper-acute presentations, either IH or APAP-related injury with a very small number (18) representing an apparent combination injury. The vast majority (1672) had evident APAP toxicity, more than 6 times the number (251) of IH cases. Correctly choosing between the diagnoses of APAP and IH is important since management of each condition differs, though the enzyme patterns for the two are so similar. Thus, the clinical diagnosis ultimately relies on historical findings for either diagnosis (history of an APAP ingestion or a history of hypotension/cardiomyopathy/volume depletion), plus APAP levels and, as a novel research tool, APAP-CYS adduct quantitation. Choice of therapy for ischemia or for APAP toxicity depends in part on identifying the cause rapidly. In many instances, treatment for both possibilities can be considered if uncertainty about etiology is present.

The APAP-CYS assay provides convincing evidence for APAP overdose long after the APAP parent compound is undetectable and can be particularly helpful when the history is uncertain or unobtainable. Importantly, the APAP-CYS assay is negative in patients with routine APAP use in the absence of liver injury. In the present study, among 790 tested for APAP-CYS adducts, 87 additional APAP cases were identified by having toxic adduct levels. By contrast, very few instances of APAP were clarified/changed to IH as a result of undetectable or very low adduct levels. Of 14 subjects initially designated as APAP, but with negative or very low adduct levels, two were adjudicated as IH, one as indeterminate and the remainder were deemed late presentations, since their ingestion apparently had occurred more than 8 days prior to study entry. Thus, APAP-CYS testing corrected the diagnosis to APAP in 14.6% of the uncertain case group (87/575).

We were particularly interested in those cases where the diagnosis was adjudicated from APAP-induced ALI/ALF or ischemia induced ALI/ALF initially to a combination injury and looked for reasons behind these discrepancies. For the most part, these cases were based on clinical history and presenting features plus biochemistries that could be attributed to either condition, and did not clearly fit into a single etiology. Persons found unconscious with high enzyme levels are commonly diagnosed as syncope/hypotension, in the absence of a clear drug history. APAP toxicity is common and co-ingestion of sedatives or opioids (e.g., hydrocodone) may also result in prolonged obtundation and even muscle compression injuries.

The 18 combination cases summarized in Table 3 describe a rare occurrence, where IH appears as a result of APAP toxicity (prolonged unresponsiveness resulting in hypoxia and/or hypotension or elements of both). This might also include patients who have taken APAP during an episode of sepsis. The presence of one would make the patient more susceptible to injury from the other and might portend a more severe course of illness. Patients with either APAP-associated ALI/ALF or ischemic injury typically have better short-term transplant-free survival than non-APAP/ALF patients [14, 20, 21]; there was no apparent worsening of outcomes in these combination cases, although the number of subjects in the combination group was quite small. An additional 21 cases were adjudicated as shock/ischemia but had therapeutic or low-level toxic adduct levels, indicating possible additional combination injuries, but with an antecedent history and documentation strongly favoring IH.

Strengths and weaknesses were as follows: we had access to a very large cohort of ALF patients' historical data and serum samples. Nevertheless, we lacked sera to test on 23% of IH subjects. We also did not choose to test every APAP subject when the diagnosis seemed secure. The 575 of 1681 (34%) APAP subjects whose sera were tested were those where there was some degree of uncertainty about the diagnosis. APAP-related ALF is readily diagnosed when elevated parent APAP compound and/or a strong ingestion history are present. The value of determining which patients display AST > ALT on admission as a marker for distinguishing ischemic injury from APAP or of comparing LDH levels for that matter seems of uncertain value in this setting [22].

While detection of the APAP-CYS adducts helps clarify the role of APAP toxicity in ALF cases where the history is inadequate, the utility of the clinical course and clinician assessment cannot be overstated. For the most part, the initial evaluation of patients with very high enzymes and low bilirubin levels (the hyperacute presentation) still involves a high suspicion for APAP hepatotoxicity with a sizeable minority representing ischemic hepatitis.

Conclusions

The interchangeable clinical scenarios as well as the virtually identical biochemical pattern of liver injury makes it difficult on some occasions to distinguish ischemia from APAP-induced liver injury. Upon analysis of ALFSG cases where adjudicated etiology was based on expert opinion plus inclusion of the APAP-CYS adduct assay, we corrected the etiology in 14.6%, and identified 18 subjects with combined injury. Availability of a rapid point-of-care assay for APAP-CYS adducts [23] will further facilitate early identification of APAP cases masquerading as IH

(and vice versa) and allow for more directed use of *N*-acetylcysteine in these settings.

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Author's contribution W.M.L, F.A. and J.A.R. wrote the main manuscript text. L.J. developed and ran the APAP-CYS adducts assay. J.A.R. prepared tables 1, 2 and 3 and Figs. 1, 2 and 3. S.T., N.L.S., J.L.R., D.G., N.L.B., A.R. A.M.L. and T.S. were members of the Causality Committee. All authors reviewed the manuscript.

Data availability No datasets were generated or analyzed during the current study.

Declarations

Conflict of interest WML consults for Genentech, SeaGen, GSK and Veristat and receives research support from Gilead, Alexion, Vivet, Camurus, Lipocine, Madrigal and Akero, none related to the current article. LPJ is a part owner of Acetaminophen Toxicity Diagnostics and has submitted a patent for Acetastat®. NLS is employed by Durect but has no conflict with the current article. The remaining authors have no conflict.

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