Comparison of Prothrombin Time and Aspartate Aminotransferase in Predicting Hepatotoxicity After Acetaminophen Overdose

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Abstract Despite decades of experience with acetaminophen (APAP) overdoses, it remains unclear whether elevated hepatic transaminases or coagulopathy develop first. Furthermore, comparison of the predictive value of these two variables in determining hepatic toxicity following APAP overdoses has been poorly elucidated. The primary objective of this study is to determine the test characteristics of the aspartate aminotransferase (AST) and the prothrombin time (PT) in patients with APAP toxicity. A retrospective chart review of APAP overdoses treated with IV N-acetylcysteine at a tertiary care referral center was performed. Of the 304 subjects included in the study, 246 with an initial AST less than 1000 were analyzed to determine predictors of hepatic injury, defined as an AST exceeding 1000 IU/L. The initial AST >50 was 79.5 % sensitive and 82.6 % specific for predicting hepatic injury. The corresponding negative and positive predictive values were 95.5 and 46.3 %, respectively. In contrast, an initial abnormal PT had a sensitivity of 82.1 % and a specificity of 63.6 %. The negative and positive predictive values for initial PT were 94.9

and 30.2 %, respectively. Although the two tests performed similarly for predicting a composite endpoint of death or liver transplant, neither was a useful predictor. Initial AST performed better than the initial PT for predicting hepatic injury in this series of patients with APAP overdose.

Keywords Acetaminophen · Coagulopathy · Hepatic failure · Overdose

Background

Acetaminophen (APAP) is one of the most commonly encountered medications implicated in overdose in the USA [1, 2]. While APAP is quite safe in therapeutic dosing, when taken in supratherapeutic dosing, there is a risk of hepatotoxicity [3, 4]. Nearly half of all cases of acute liver failure in the USA and Great Britain are due to APAP [3]. Laboratory evidence of hepatic injury including elevated transaminases and coagulopathy can be detected within 24 h of ingestion [4]. It has been suggested that the aminotransferases are the first laboratory marker to become abnormal following acetaminophen ingestion [5]. In fact, references and textbook chapters state that the aspartate aminotransferase (AST) is not only the most sensitive marker for hepatotoxicity but that AST elevations also precede the evidence of actual liver dysfunction, including a prolonged prothrombin time (PT) [5, 6]. However, such statements are often unreferenced. The purpose of this study is to evaluate the test characteristics (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and positive and negative likelihood ratios) of the PT and AST for predicting hepatic injury or death/liver transplant in patients with APAP toxicity. A secondary objective is to determine if coagulation abnormalities occurred earlier than previously posited by the medical literature.

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Methods

This retrospective case series was performed at a 663-bed, tertiary care medical center in Phoenix, Arizona. The hospital serves as a regional referral center for poisoned patients. The study received approval from the institutional review board.

The Department of Medical Toxicology maintains a logbook of all consecutive patient encounters treated by toxicologists. Patients were identified via a review of the logbook which lists all toxins encountered on each patient. Adult patients (age ≥ 14 years) were eligible if they were treated with N-acetylcysteine (NAC) for an APAP overdose between 1 January 2006 and 31 December 2010. Patients were considered to have an APAP overdose only if proven by serum APAP concentration or in those with a self-reported history of an APAP ingestion more than 24 h prior who had APAP detected in the urine on comprehensive drug testing via gas chromatography mass spectrometry (GC/MS) and had elevated transaminases, despite a negative quantitative serum APAP concentration. Patients were excluded if they presented with a reported acute overdose of a drug other than APAP and had negative APAP concentrations but were treated with NAC because of elevated liver function tests. Similarly, patients were excluded if APAP toxicity was suspected, but not confirmed by analytical testing. Additional exclusion criteria included use of warfarin, previously documented elevated transaminases, pre-hospital cardiac arrest, or rhabdomyolysis.

Study Definitions

The following were defined a priori. Rhabdomyolysis was defined as a CK exceeding 1000 IU/L. Because patients often had their initial laboratory studies performed at different hospitals, and there is significant variability in the upper limit of normal for PT, it was considered to be “prolonged” if it exceeded the upper limit of normal for the performing lab. An elevated AST was defined as an AST greater than 50 IU/L, while liver injury was defined as an AST exceeding 1000 IU/L based on historical precedent. The composite endpoint of death or orthotopic liver transplant was created to identify the population with most severe acetaminophen toxicity. Ethanol consumption was considered daily only if explicitly written in the social history. Thus, subjects who reportedly consumed ethanol “socially” and those in whom no ethanol history was obtained were not considered to be daily ethanol consumers.

Data Abstraction

The data were abstracted onto a pre-designed data abstraction form by two investigators (ML, APJ) and subsequently entered into a spreadsheet (Excel, Microsoft, Redmond, WA). Before performing data abstraction, each reviewer received a

standardized training in systematic chart review. Ten percent of the charts were randomly selected by a second investigator to verify the accuracy of the abstraction process. A *kappa* statistic was performed to determine inter-rater reliability.

The data abstracted from the medical records included demographic information (age, sex), initial acetaminophen concentration, initial and maximal AST, PT, creatinine, and maximal CK (if performed). In addition, it was recorded if the blood for the initial PT was obtained before or after NAC administration. Lastly, past medical history, treatment rendered, alcohol consumption, length of stay, and outcome were recorded.

Data Analysis

Continuous variables were reported as means and standard deviations (SD) as well as median and interquartile range (IQR) to highlight that the data was non-normalized. Categorical variables were reported as percentages. Fisher exact tests were used to compare categorical variables and Mann-Whitney *U* tests for continuous variables. Test characteristics such as sensitivity and specificity were calculated along with their 95 % confidence intervals (95 % CI). Receiver operator curves (ROC) were constructed to determine the area under the curve (AUC) of predictors. To compare AUC values between predictors, a method was used as described by Hanley and McNeil [7]. A two-tailed $p < 0.05$ was considered significant. Additional subgroup analysis was performed to determine if stratification by whether initial PT was drawn before or after NAC administration and by ethanol use was necessary. A Breslow-Day test was used to determine if odds ratios were homogeneous. Data analysis was performed using IBM SPSS Statistics for Macintosh (Version 21.0, Armonk, NY: IBM Corp).

Results

During the study period, a total of 304 subjects met the inclusion criteria. The *kappa* score for each variable measured was 0.8 or greater. The median (IQR) age was 28 (19–42) years. One hundred (33 %) subjects were male. The median (IQR) acetaminophen concentration was 100 (44–179) mcg/mL. Acetaminophen was detectable in the serum in 262 (86 %) of the subjects on initial presentation. The time of ingestion was known and able to be plotted on the Rumack-Matthew nomogram in 158 (52 %) patients. Forty-four (14 %) subjects presented with chronic and/or sub-acute APAP ingestions. Thirty-nine (13 %) subjects had a single acute ingestion more than 24 h prior to presentation. The remaining 63 (21 %) patients had an unknown time of ingestion. Among those subjects who presented within 24 h of their ingestion (with a known time of ingestion), the median (IQR) time until the first

APAP concentration was obtained was 6 (4–10.5) hours. Thirteen (4 %) subjects died or underwent orthotopic liver transplant.

All but five patients had coagulation studies performed. Among these five patients who did not have a PT obtained, four had a normal AST, while one had a missing value for AST.

The mean (SD) and median (IQR) initial and maximal AST, along with initial and maximal PT are presented in Table 1. Initial PT was considered “prolonged” in 162 (54 %) subjects, while the maximal PT was considered “prolonged” in 235 (78 %) subjects. The initial PT was the maximal PT in 26 % of subjects. Initial AST >50 IU/L occurred in 124/303 (40.9 %) subjects, including 57 whose initial AST was >1000 IU/L.

Hepatic injury developed in 96 (31 %) subjects. Among these 96, 5 (5 %) had an initially normal AST but had an initially elevated PT. An additional five (5 %) subjects had an initially normal PT, but elevated initial AST. And three (3 %) had both initially normal AST and PT. The initial PT was obtained after starting NAC in 61/304 (20 %) subjects.

Test characteristics for predicting hepatotoxicity were calculated for the 247 patients whose initial AST was <1000 IU/L. An initially elevated PT was associated with a sensitivity (95 % CI) of 82.1 % (66.5–92.5 %) and a specificity of 63.5 % (55.7–69.5 %) for predicting hepatic injury. Thus, a normal initial PT had a 94.2 % (88.5–97.4 %) negative predictive value and a 30.2 % (21.7–39.9 %) positive predictive value. In contrast, an initially elevated AST was associated with a sensitivity of 79.5 % (63.5–90.7 %) and a specificity of 82.6 % (76.7–87.5 %) for predicting hepatic injury. The negative and positive predictive values were 95.5 % (91.4–98.1 %) and 46.3 % (34.0–58.9 %), respectively. These test characteristics along with positive and negative likelihood ratios are provided in Table 2.

An ROC was constructed to determine the AUC (or c statistic) for the initial AST and initial PT to determine their value in predicting hepatotoxicity (Fig. 1). The AUC for the initial AST was 0.864 (95 % CI 0.794–0.935) and for initial PT was 0.725 (95 % CI 0.624–0.827) for predicting hepatotoxicity ($p<0.001$ for the difference between these AUCs; 95 % CI 0.027–0.251) (Fig. 1a, b).

The test characteristics of all patients ($n=304$) for the initial and maximal PT along with the initial AST for predicting the

composite endpoint of death or orthotopic liver transplant are listed in Table 3. None of the predictors has test characteristics that make them clinically useful.

Subgroup Analysis

To address concerns that the measurement of PT can be affected by the administration of NAC, analysis was performed to determine if stratification by whether initial PT was drawn before or after NAC administration was necessary. A Breslow-Day test for homogeneity of odds ratios found that there was no significant difference ($p=0.565$) between the two groups. This demonstrates that the effect of drawing the initial PT after NAC administration did not significantly impact the performance in predicting hepatotoxicity.

Since ethanol consumption can affect transaminases, an analysis was performed to compare the frequency of initial AST >50 IU/L in those with and without a history of daily ethanol consumption. There was a significant difference between these groups. There was initial AST elevation in the ethanol group 55 % of the time, while in those not using ethanol, the elevation occurred only 37 % of the time, $p=0.010$, 95 % CI of difference 0.04–0.33. However, further analysis was performed to determine if stratification by ethanol use was important in the predictive value of initial AST elevation for hepatotoxicity. A Breslow-Day test for homogeneity of odds ratios found that there was no significant difference ($p=0.189$) between the two ethanol groups, demonstrating that stratification by ethanol use was not necessary.

Discussion

The PT is a measure of the extrinsic pathway of coagulation which is particularly sensitive to certain vitamin K dependent factors (II, VII, and X) and is an excellent indicator of the synthetic capacity of the liver. It is established as a useful prognostic indicator in patients with fulminant hepatic failure. The King's College Criteria established that PT >100 s was a poor prognostic indicator conferring high risk of mortality and potential need for transplantation [8, 9]. However, there is little in the literature that characterizes the utility of PT early in the course of acetaminophen toxicity. The primary focus of this study was to evaluate the test characteristics of PT in comparison to those of AST in patients treated with IV NAC for acetaminophen toxicity and to determine if meaningful prognostic information could be obtained in predicting hepatic injury, death, or orthotopic transplant.

Evaluating the likelihood ratios and ROC curves for initial AST and initial PT allows for the most direct and meaningful comparison regarding their respective performance in predicting hepatic injury. The AUC for initial AST was better at 0.864, which is in the excellent range for predicting an

Table 1 Mean and median values for AST and PT

	Mean (SD)	Median (IQR)
Initial AST (IU/L)	1208 ($\pm$ 3186)	37 (22–217)
Maximal AST (IU/L)	2426 ($\pm$ 4429)	48.5 (25–2991)
Initial PT (seconds)	17.4 ($\pm$ 13.2)	13.8 (11.8–16.4)
Maximal PT (seconds)	21.7 ($\pm$ 17.7)	15.4 (13.8–21)

Table 2 Test characteristics for predicting hepatic injury (in patients with initial AST <1000)

	Sensitivity	Specificity	PPV	NPV	+LR	−LR
Initial AST elevation	79.5	82.6*&	46.3*&	95.5	4.6*&	0.25
Initial PT elevation	82.1	63.5	30.2	94.9	2.3	0.28
Maximal PT elevation	100	31.5	21.9	100	1.5	0

Only those with initial AST <1000

*Different from initial PT elevation, $p<0.05$ &Different from maximal PT elevation, $p<0.05$

outcome. For comparison, the ROC for initial PT has an AUC of 0.725 for hepatotoxicity for a statistical ($p<0.001$) difference in the two curves. Neither initial AST nor initial PT was useful in predicting the composite endpoint of death or orthotopic liver transplant.

When comparing the test characteristics of PT in this study to those previously reported, there is a paucity of data in the literature. An abstract by Van der Steeg et al. revealed similar NPV for initial PT in 190 patients treated with IV NAC for APAP toxicity. They divided the patients into three groups based on time of PT after ingestion, <8 h, ≥8 but <16, and ≥16 h, the NPV was 96, 95, and 96, respectively [10]. These findings are similar to the NPV of 94 in our cohort of patients for initial PT. Van der Steeg et al. found initial PT to have a PPV of 41 overall (20 for <8 h, 28 for ≥8<16 h, and 60 for ≥16 h) which is comparable to our finding of a PPV of 30 overall. Consequently, both studies support that an initially normal PT is predictive of a good outcome in patients with APAP toxicity treated with IV NAC but it appears that initial

PT holds little value in predicting who will develop hepatic injury.

There are several concerns and potential confounders that may affect the accurate interpretation of PT early after APAP overdose in predicting likelihood of hepatotoxicity. Several authors have reported APAP's ability to prolong PT in the absence of hepatotoxicity [10–13]. Whyte et al. provided evidence that factor VII levels were depleted and INR elevated following APAP overdose in the absence of hepatotoxicity in both a prospective and retrospective study [12]. The retrospective study of 143 patients admitted for APAP ingestion without evidence of hepatotoxicity found that 50 % had an elevated INR typically between 12 and 16 h after ingestion [12]. The prospective study looked at INR, functional and antigenic Factor VII, and functional factor IX and VIIIc levels in 30 patients with APAP overdoses who did not develop hepatotoxicity in comparison to 8 controls. The authors concluded that there was a time-dependent increase in INR that mirrored the findings of the retrospective study and was significantly

Fig. 1 ROC curves for the initial AST (1A) and the initial PT (1B) to determine their value in predicting hepatotoxicity

Table 3 Test characteristics for predicting composite endpoint (death or orthotopic liver transplant)

	Sensitivity	Specificity	PPV	NPV	+LR	–LR
Initial PT elevation	100 (75.3–100) %	47.2 (41.2–53.2) %	8 (4.3–13.3) %	100 (97.3–100) %	1.9	0
Maximal PT elevation	100 (75.3–100) %	22.4 (17.7–27.3) %	5.5 (3.0–9.3) %	100 (94.4–100) %	1.3	0
Initial AST elevation	100 (75.3–100) %	61.7 (55.9–67.3) %	10.4 (5.7–17.3) %	100 (98–100) %	2.6	0

higher than the control group. The authors also found that coagulation factor VII was lower in the exposed patients compared to that in the controls and the observed fall in factor VII coincided with the observed rise in INR. The effect on factor IX was less pronounced, but levels were lower in the exposed group compared to that in the control group. There was no difference observed in Factor VIIIc levels between the two groups. The authors conclude that APAP may inhibit the gamma carboxylation of vitamin K-dependent factor activation thereby causing an elevation in INR [12].

The assertion that APAP affects vitamin K-dependent factor activation is further supported by several other studies that found that APAP increased the INR in patients previously on stable warfarin therapy. This typically occurred when daily doses of APAP exceed 2–4 g/day for several days [14–17]. There was significant debate in the literature regarding these findings as there did not appear to be a plausible explanation based on pharmacokinetic and cytochrome P450 mechanisms. Thijssen et al. provided valuable insight when they performed an in vitro study that provided an explanation for this well-documented interaction of warfarin and APAP. They demonstrated that N-acetyl-p-benzoquinone imine (NAPQI), the well-known toxic metabolite of APAP, is an inhibitor of vitamin K-dependent gamma-carboxylase. They also report that NAPQI inhibits vitamin K epoxide reductase although at higher concentrations [18]. This may explain why early albeit mild elevations in PT have been reported in the setting of APAP overdose [19].

To further complicate matters, others have suggested that intravenous NAC also independently increases prothrombin time [19, 20]. Interestingly, Thijssen et al. also confirmed this in their in vitro study discussed above, in which they demonstrated that at concentrations of approximately 1 mM, NAC was found to inhibit vitamin K epoxide reductase activity by 57 % [18]. Pizon and colleagues also performed an in vitro study with human plasma samples and demonstrated that the introduction of NAC led to a dose-dependent increase in PT. They found a mean increase of 3.5 s in PT when concentrations of 1000 mg/L of NAC were added to human plasma specimens. This concentration of NAC was chosen as it approximates that achieved in standard antidotal dosing (150 mg/kg) of NAC for APAP toxicity. The same group performed a second in vitro study in which they measured concentrations of coagulation factors II, VII, IX, and X in

plasma samples containing NAC at a 1000 mg/L and those without. All samples containing NAC had a statistically significant reduction in all factor concentrations compared to controls [21]. Jepsen and Hansen performed an in vivo and in vitro study using six healthy volunteers who were given NAC IV over 32 h for a total dose of 320 mg/kg (10 mg/kg loading dose followed by 10 mg/kg/h); the authors report a decrease of 30–40 % in the prothrombin ratio which would correspond to an increase in prothrombin time. Similarly, they found a slight decrease in Factor II, VII, and X activity after administration of IV NAC [22]. Other authors have asserted that NAC results in an increase in PT. However, this occurred in the setting of APAP toxicity. Therefore, the magnitude of effect cannot be fully ascribed to NAC given the effect of APAP's metabolites on gamma carboxylation of vitamin K-dependent coagulation factors [23, 24].

In addition to the effects of APAP metabolism and NAC on PT described above, several other factors may result in elevation of PT, such as failing to adequately fill the vacutainer tube, prolonged use of a tourniquet before drawing a blood sample, and vitamin K deficiency [25, 26]. All of these factors may make early and mild elevations of PT difficult to accurately interpret in the setting of APAP overdose.

Several limitations of the study need to be acknowledged. First, the retrospective nature of our study carries certain limitations, and the conclusions are limited by the completeness and quality of the data. In an effort to minimize the effect of this limitation, we primarily abstracted continuous variables (e.g., AST, PT) and dichotomous variables (e.g., AST above or below 1000 IU/L). These choices of data reduced abstractor bias and minimized some of the limitations of a retrospective review [27]. The most important limitation to highlight is that the test characteristics for PT and AST in this study population included only those patients treated with IV NAC for their identified APAP toxicity and therefore should not be used in order to discern who may or may not warrant therapy with NAC. An additional limitation may be that patients with pre-existing liver dysfunction were inadvertently included in the study, although exclusion criteria (use of warfarin, previously documented elevated transaminases, pre-hospital cardiac arrest, or rhabdomyolysis) were established to eliminate these patients and remove those that may have abnormally prolonged PT or elevated transaminases for reasons other than APAP toxicity. We also recorded whether the initial PT was

obtained before or after administration of NAC given concerns for the interference discussed above. Subgroup analysis resulted in non-significant differences in the test characteristics of initial PT between the two groups. Therefore, stratification based on whether the initial PT was drawn before or after NAC administration was not necessary. Additionally, excessive ethanol consumption can cause elevation of AST independent of APAP toxicity. Although there was a statistically significant difference in the frequency that initial AST was >50 IU/L when comparing those with daily ethanol consumption to those without, further analysis revealed that stratification by ethanol was not required as it did not affect the initial AST's predictive value for hepatotoxicity. Also, stratification of initial PT and AST by the time since ingestion was not analyzed. So characterization regarding the timing of PT elevation in patients with APAP toxicity cannot be made. Finally, the issue of using initial AST as a predictor of hepatotoxicity defined as AST >1000 IU/L must be acknowledged. This is a widely accepted definition of hepatotoxicity and is based on the literature and historical precedent; consequently, only those patients whose initial AST was <1000 IU/L could be used in calculating and comparing test characteristics in predicting hepatotoxicity [28].

This study supports an initially normal PT is predictive of a good outcome in patients treated with IV NAC for APAP toxicity. However, in this heterogeneous cohort of patients, the test characteristics of initial PT are inferior to those of initial AST in predicting hepatotoxicity. This evidence, coupled with the concerns outlined above regarding factors that may threaten the accuracy of initial PT, should prompt clinicians to exercise caution to ensure accurate interpretation of initial PT measurements. Although the initial PT was not as useful as initial AST in predicting hepatotoxicity in this cohort, a larger prospective study evaluating the test characteristics of PT and its ability to predict hepatotoxicity may be useful. Ideally, analyzing populations receiving antidotal therapy compared to those not receiving treatment in order to provide clinicians additional guidance in determining who requires antidotal therapy would be helpful. However, such a study in humans is unlikely to occur. In the meantime, obtaining serial PTs in patients with APAP toxicity may remain useful in monitoring the progression and severity of hepatic injury and is still recommended.

Conclusion

The initial AST is superior to the initial PT in predicting hepatotoxicity in patients treated with IV NAC for acetaminophen toxicity.

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