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The Role of Carbamoyl Phosphate Synthetase 1 as a Prognostic Biomarker in Patients With Acetaminophen-induced Acute Liver Failure

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Supplementary Material

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Conflicts of interest

The authors disclose no conflicts.

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Abstract

BACKGROUND & AIMS: Carbamoyl phosphate synthetase 1 (CPS1) is a highly abundant mitochondrial urea cycle enzyme that is expressed primarily in hepatocytes. CPS1 is constitutively and physiologically secreted into bile but is released into the bloodstream upon acute liver injury (ALI). Given its abundance and known short half-life, we tested the hypothesis that it may serve as a prognostic serum biomarker in the setting of acute liver failure (ALF).

METHODS: CPS1 levels were determined using enzyme-linked immunosorbent assay and immunoblotting of sera collected by the ALF Study Group (ALFSG) from patients with ALI and ALF (103 patients with acetaminophen and 167 non-acetaminophen ALF etiologies). A total of 764 serum samples were examined. The inclusion of CPS1 was compared with the original ALFSG Prognostic Index by area under the receiver operating characteristic curve analysis.

RESULTS: CPS1 values for acetaminophen-related patients were significantly higher than for non-acetaminophen patients ($P < .0001$). Acetaminophen-related patients who received a liver transplant or died within 21 days of hospitalization exhibited higher CPS1 levels than patients who spontaneously survived ($P = .01$). Logistic regression and area under the receiver operating characteristic analysis of CPS1 enzyme-linked immunosorbent assay values improved the accuracy of the ALFSG Prognostic Index, which performed better than the Model for End-Stage Liver Disease, in predicting 21-day transplant-free survival for acetaminophen- but not non-acetaminophen-related ALF. An increase of CPS1 but not alanine transaminase or aspartate transaminase, when comparing day 3 with day 1 levels was found in a higher percentage of acetaminophen transplanted/dead patients ($P < .05$).

CONCLUSION: Serum CPS1 determination provides a new potential prognostic biomarker to assess patients with acetaminophen-induced ALF.

Keywords

Biomarker; Liver Injury; Mitochondria

A cute liver failure (ALF) is defined as severe liver injury occurring in patients without preexisting liver disease and is accompanied by life-threatening complications related to coagulopathy and hepatic encephalopathy (HE).[1,2] Acetaminophen (N-acetyl-p-aminophenol/APAP) is the leading cause of ALF in the United States, accounting for ~46% of cases and nearly 450 deaths annually.[3,4] A multi-national analysis in Europe showed that APAP represents one-sixth of ALF cases registered for transplantation.[5] Because of low spontaneous survival (~56%),[6] accurate decision-making regarding proceeding with liver transplantation early on is critical for preventing mortality in patients with ALF.[7–9]

A variety of biomarkers and prognostic models have been developed to estimate outcomes of patients with ALF; however, these models have several limitations. For example, the

King's College Criteria (KCC) is the earliest and commonly applied prognostic model, but its limited sensitivity and negative predictive value curtail its use.[10] The Model for End-Stage Liver Disease (MELD) score, as compared with KCC, improves the sensitivity for predicting ALF patient deaths, but has low specificity for predicting survival.[11] Moreover, MELD is limited by inter-laboratory variability of its score parameters.[12] The ALF Study Group Prognostic Index (ALFSG PI) utilizes clinical parameters and widely available laboratory parameters of total bilirubin and international normalized ratio (INR) values that have improved performance characteristics compared with the KCC and MELD scores. However, the predicted value of ALFSG PI for different etiologies that lead to ALF still needs to be defined.[7] In terms of circulating biomarkers, M30 antibody reactivity, which recognizes caspase-mediated cleavage of the abundant epithelial cell cytoskeletal keratin-polypeptide-18, is a commonly used cell apoptosis marker.[13–15] Other biomarkers for ALF have been studied including micro-RNAs,[16] high mobility group box 1,[17] and cytochromes P450,[18] but their clinical utility remains to be validated.

Carbamoyl phosphate synthetase-1 (CPS1), the major mitochondrial urea cycle enzyme, accounts for nearly 20% of the mitochondrial matrix protein in hepatocytes.[19,20] CPS1 catalyzes the first step of the urea cycle by converting ammonia into carbamoyl phosphate.[19] Patients and animal models with CPS1 deficiency develop high circulating ammonia levels and encephalopathy.[21] Notably, CPS1 is constitutively and physiologically secreted into bile but is released into the bloodstream during acute liver injury (ALI) in humans and mice.[20,22] CPS1 is taken up by circulating monocytes, which then triggers M2 polarization to anti-inflammatory cells and homing to the liver.[22] The abundance of CPS1, its preferential expression in hepatocytes, and its short serum half-life ($T_{1/2}$ of 67−126 minutes in rodents[20,23]) make CPS1 an ideal serum biomarker for ALI.[20,22] We previously detected CPS1 in sera from 3 patients with APAP-related ALF who had recovered and noted a rapid drop in their CPS1 detectability as determined by immunoblotting.[20] This led us to hypothesize that CPS1 may be used as a prognostic biomarker if tested in patients with APAP- and non-APAP-related ALF. Therefore, we carried out a longitudinal study using sera collected by the ALFSG from patients with APAP or non-APAP ALF/ALI etiologies.

Methods

Enzyme-linked Immunosorbent Assay and Immunoblot Testing

A monoclonal antibody to CPS1 was generated to carry out immunoblotting of CPS1 (Supplementary Figure 1). A standard curve using 5 full-length recombinant CPS1 protein amounts (25/50/100/150/200 ng) were selected to generate the standard curve. Patient sera were diluted in 4 × Laemmli sample buffer. Immunoblot band intensities were quantitated by the Image-J software (Supplementary Figure 2). For enzyme-linked immunosorbent assay (ELISA) testing, we tested 5 commercial kits: Wuhan EIAab Science (ABIN1125223), Biomatik (EKC32977), Mybiosource (MBS7209206, MBS2000341), and Abbexa Ltd (abx384664). The ABIN1125223 kit provided the most sensitive results and was used for the ELISA serum analysis (Supplementary Figure 3).

Study Population

Samples from 270 ALFSG patients were randomly selected to represent 2 ALI etiologies. Of these, 103 patients had ALF and ALI injury due to APAP toxicity, and 167 patients had ALF and ALI injury due to non-APAP etiologies (autoimmune hepatitis, 45 patients; drug-induced liver injury [DILI], 72 patients; hepatitis B virus [HBV] infection, 50 patients). The etiology has been approved by the ALFSG Etiology Committee. Among the total 270 patients, 191 presented with ALF (70.7%), and 79 presented with ALI (29.3%). A total of 764 sera samples were examined that were collected upon admission and, in some cases during their hospitalization, by both ELISA and immunoblotting to measure CPS1 levels.

Typically, the etiology of ALF and ALI is made based on laboratory results and medical history. Patients with ALI had hepatic impairment (increased serum transaminases) and impaired hepatic function (jaundice and INR ≥2.0) but without HE and prior known liver disease.[2] ALF diagnosis is made upon development of severe ALI, accompanied by HE and INR ≥1.5 in patients without prior known liver disease.[2,24] A diagnosis of APAP hepatotoxicity is based on history of APAP intake, whereas a diagnosis of non-APAP excludes acetaminophen use.[10]

Data Management and Integrity

Patient demographics, clinical characteristics, and medical history were collected at enrollment. Sera were obtained as clinically necessary, and laboratory results were collected after admission to the hospital. All patients underwent a 21-day prognostic assessment for spontaneous survival (categorized by us as ‘Alive’), liver transplantation (TX), or death.

Statistical Analyses

Comparison of proportions was done using the χ^2 or Fisher exact tests, whereas comparison for continuous variables was done using the Wilcoxon rank-sum test. Logistic models were assessed using area under the receiver operating characteristic curve (AUROC) and compared with the DeLong test. Statistical significance was defined as 2-sided *P*-value < .05. Statistical analyses were performed using SAS version 9.4.

Results

Study Cohort Demographics and Clinical Characteristics

Patients with ALF or ALI (N = 270) were analyzed. Demographics and clinical characteristics were compared between patients with APAP- and non-APAP-related liver injury (Table 1). Patients with APAP were younger (38 vs 42 years; $P < .01$) and more likely white individuals than those with non-APAP (77.7% vs 55.1%; $P < .001$). The majority of the patients were female (68% and 67.1%) and overweight (body mass index, 26.5 and 27.4) in the APAP and non-APAP groups, respectively; however, non-APAP patients were more likely to have diabetes mellitus (24% vs 10.7%; $P < .01$).

Admission labs showed that APAP patients had significantly higher serum alanine aminotransferase (ALT) (3949.5 vs 796.5 IU/L; $P < .01$), aspartate aminotransferase (AST) (4264 vs 644 IU/L; $P < .01$), and creatinine (1.5 vs 0.9 mg/dL; $P < .01$) than non-APAP

patients, whereas alkaline phosphatase and bilirubin levels were significantly lower (as expected). The INR, an important ALF predictor, was higher in APAP patients (3.1 vs 2.7; $P > .05$) without significance difference to the non-APAP patients. For the 21-day outcome, more non-APAP patients were ever listed (45.2% vs 21.4%; $P < .01$) or received liver transplant (31.1% vs 12.6%; $P < .01$) and had lower spontaneous survival (42.5% vs 65.1%; $P < .01$).

Early Serum CPS1 Levels are Higher in APAP Than Non-APAP Patients

The early time point serum samples were the first blood draw of the patients admitted to the hospital, and most were from the first day (Supplementary Table 1). For ELISA testing, we examined 5 commercial kits (Supplementary Figure 3). The ABIN1125223 kit (Supplementary Figure 3B) provided the most sensitive results (validated by immunoblotting (Supplementary Figure 3A) and was subsequently used for all the ELISA serum analyses. APAP patients had higher serum CPS1 levels than non-APAP patients at early time points with ELISA (37.7 vs 11.0 ng/mL; $P < .0001$) (Figure 1A) and immunoblotting (170.7 vs 19.03 ng/μL; $P < .0001$) (Supplementary Figure 4A). Further analysis in non-APAP patients showed that both HBV and DILI TX/dead patients had higher CPS1 levels than autoimmune hepatitis (AIH), but only HBV had significantly higher values than AIH (13.79 vs 8.87 ng/mL; $P < .05$) (Table 2; similar results in immunoblot [Supplementary Table 2]).

Comparison of CPS1 in TX/Dead or 21-day Spontaneous Survival (Alive) Patients

Analysis of sera from early time points was performed to monitor CPS1 values over time. ELISA results showed that TX/dead APAP patients had significantly higher CPS1 levels than the alive patients (54.8 vs 28.5 ng/mL; $P < .01$), whereas no significant difference was detected (12.36 vs 9.19 ng/mL; $P = .11$) in non-APAP patients (Figure 1B). The difference in APAP patients remained significant after excluding 80 patients with ALI ($P < .01$) (Supplementary Figure 6B). Although most patients with APAP ALF (92.3%) received the standard treatment of N-acetylcysteine (NAC) upon admission, the TX/dead patients still had significantly higher serum CPS1 levels than alive patients (61.33 vs 27.03 ng/mL; $P = .01$) (Supplementary Table 3). We were not able to discern the timing of NAC administration as related to the first reading of CPS1, and, given the small number of patients who did not receive NAC with a higher day-3 than day-1 CPS1 values, we are not able to conclusively determine the direct impact of NAC on CPS1 levels. We also compared CPS1 ELISA levels in intentional (usually a single time point dose) and non-intentional (usually involves >1 day of dosing, or indeterminate) APAP overdoses. CPS1 levels were significantly higher in intentional than non-intentional patients, and higher in non-intentional TX/dead 21-day status than in alive patients when examining all patients (with ALI + ALF) and with ALF APAP (Supplementary Figure 5).

Serum CPS1 levels from days 1 to 7 after hospital admission were also analyzed. A decreasing trend was found in APAP patients, particularly in alive patients (Figure 1C). No decreasing trend was found in non-APAP patients, although their ELISA levels were lower than APAP patients overall (Figure 1D).

The Trend of CPS1 but not ALT or AST Levels During the First 3 Days Predict the 21-day Outcome of APAP Patients

We further compared the slope of CPS1, ALT, and AST during the first 3 days of hospitalization to obtain a better predictive value of CPS1. For alive patients, CPS1 dropped significantly faster than ALT levels (slope of −23.4 vs 5.64; $P < .005$) (Figure 2A; see comparison data in Supplementary Table 4). CPS1 also dropped faster than ALT in the TX/dead patients, but with no significance (Figure 2B). Only the percentage of patients with increased CPS1, but not ALT or AST in day 3 than day 1, was significantly higher in TX/dead than alive patients (16.1% vs 3.5%; $P < .05$) (Figure 2C). These significant CPS1 differences were not found in non-APAP patients (Figure 2D).

CPS1 Improves AUROC of ALFSG PI

We performed logistic regression and ROC analysis to compare the predictive accuracy of the ALFSG PI (base model) alone and together with early time point serum CPS1 and MELD score (Figure 3, Supplementary Figure 7). Only in APAP-related patients did combining CPS1 values improve the performance of the ALFSG PI, which, in turn, was more predictive of 21-day transplant-free survival than the MELD score (Figure 3B). However, when applied to all patients with ALF (Figure 3A) or only to non-APAP patients (Figure 3C), CPS1 did not improve the prediction model. The AUC values and prediction confidence evaluations are shown in Figure 3D.

Discussion

CPS1 was studied as a potential prognostic serum biomarker in patients with ALF. The first reading of CPS1, typically day 1 of hospitalization (available in 78.9% of the patients we studied) (Supplementary Table 1), paralleled the type of liver injury. Specifically, patients with APAP, as contrasted with non-APAP (HBV/AIH//DILI), had >3-fold ELISA CPS1 levels (Figure 1) ($P < .0001$). As we observed, ALT is much higher in APAP vs non-APAP (3889 vs 715 IU), which raises the question whether CPS1 levels remain significant in patients with comparable ALT (as a control for hepatocellular injury). Comparison of such groupings showed that CPS1 levels were still significantly higher in APAP vs non-APAP patients (Supplementary Table 5). CPS1 levels were strikingly different in APAP Tx/dead patients compared with the alive patients but were not as different in non-APAP patients (Figure 1). A faster decline of serum CPS1 as compared with ALT and AST in APAP Alive patients was observed, as well as an increase in day 3 vs day 1 levels of CPS1 but not ALT or AST in APAP TX/dead as compared with alive patients (Figure 2). This is consistent with the more rapid decline of CPS1 compared with ALT in experimental ALI when measured in the same mice over time[20] and with the reported shorter half-life of AST as compared with ALT (47 hours for ALT, 17 hours for total AST).[25] More detailed analysis, with a larger number of patients with ALF and follow-up of daily serum levels, will be needed. In our cohort, there is no significant ammonia level difference between APAP and non-APAP patients (Table 1) even though serum CPS1 levels differ markedly; however, ammonia levels do not always correlate with outcomes in APAP-related ALF.[26]

In terms of potential practical clinical use, early detection of CPS1 (first day of hospitalization) with follow-up daily monitoring for 3 or more consecutive days may help predict the prognosis of patients with APAP ALF. ELISA is widely used for clinical diagnosis and usually provides results within 24 hours[27]; of note, our assay was completed within 5 to 6 hours and may even be completed in less time, although this was not tested formally. Therefore, a morning blood draw should provide the CPS1 test result by mid-afternoon and offer potential assistance in clinical decision-making regarding the need for TX or closer monitoring of patients with persistently elevated or increasing CPS1 levels. Determination of appropriate cutoff values will need to be confirmed in follow-up validation studies.

We posit that the lower CPS1 in non-APAP is reflected by different cell death mechanisms in APAP and non-APAP injury. Unlike the physiologic constitutive release of CPS1 into bile,[22] CPS1 release from mitochondria into serum may be triggered by the APAP toxic metabolite N-acetyl-p-benzoquinone.[28,29] Mitochondrial damage and dysfunction are central to the molecular mechanism of APAP-induced liver injury,[28,29] with mitochondrial membrane permeability transition (MPT) leading to mitochondrial proteins being released from ruptured mitochondria and after necrotic cell death, like glutamate dehydrogenase.[30] The liver damage in DILI, which accounts for 43.1% of our non-APAP patients, is caused primarily by reactive metabolites through direct toxicity or immune reactions, with both triggering secondary mitochondrial damage. However, the mechanism of mitochondrial damage varies depending on the specific drug toxicity, including respiration and/or beta-oxidation inhibition, effecting mitochondrial DNA transcription, or effecting mitochondrial membrane disruption through membrane permeability transition.[30,31] APAP typically affects mitochondrial membrane disruption, which we posit results in more CPS1 bloodstream release than drugs that induce other mitochondrial damage types. For patients with HBV, the mechanism of hepatocyte injury for this etiology is generally linked with apoptosis or immune-mediated injury,[32,33] as is the case for autoimmune hepatitis, thereby providing a different injury mechanism compared with APAP-mediated ALF. Validation and additional cohorts of APAP/non-APAP will be needed to have a better understanding of how different ALF etiologies might trigger CPS1 release.

Although 2 other studies have described CPS1 detection in sera of patients with chronic hepatitis C[34] or in serum exosomes from patients with ALF linked to hepatitis E virus (HEV) infection,[35] these studies remain to be validated due to potential confounders. Specifically, the ELISA kits utilized in both studies were not validated for sensitivity/specificity using purified full-length CPS1 (eg, Supplementary Figure 3), and it is not clear if exosome isolation captured the entire serum CPS1 as characterized previously.[22] In addition, serum HEV nucleic acid was detected in 17% of the ALF cases attributed to HEV,[35] yet 85% of patients with HEV had detectable serum viral nucleic acid in another study that examined the dynamics of HEV viremia and fecal shedding.[36] Optimal HEV diagnosis typically includes viral nucleic acid detection coupled with serology assessment.[37,38]

Overall, our findings (Figure 4) provide clear evidence that CPS1 is readily detected in sera of patients with ALF, particularly in patients with APAP-related ALF. The findings herein,

which require validation and establishment of high-risk cut-off ranges, suggest that initial high levels of CPS1 of patients with ALF due to APAP may provide an added predictive outcome benefit to the current ALFSG PI. Also, continued in-hospital monitoring of serum CPS1 may distinguish APAP patients who are likely to require transplantation based on findings of higher CPS1 day 3 vs day 1 levels. Further studies that evaluate daily CPS1 levels in patients with APAP and non-APAP ALF are warranted; also, testing of non-APAP ALF subgroups will require further investigation with larger cohorts. The short half-life of CPS1, its mitochondrial localization and polarized release to the biliary system rather than blood (ie, limited if any detection in serum under physiologic conditions although diverse healthy populations will require testing), its abundance, and its substantial release from mitochondria after APAP injury are relevant factors that contribute to its likely utility as a dynamic biomarker of APAP-related ALI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper:

AIH	autoimmune hepatitis
ALF	acute liver failure
ALFSG	Acute Liver Failure Study Group
ALFSG PI	Acute Liver Failure Study Group prognostic index
ALI	acute liver injury
ALT	alanine aminotransferase
APAP	acetaminophen (N-acetyl-p-aminophenol)
AST	aspartate aminotransferase
AUROC	area under the receiver operating characteristic
CPS1	Carbamoyl phosphate synthetase 1
DILI	drug-induced liver injury
ELISA	enzyme-linked immunosorbent assay

HBV	hepatitis B virus
HE	hepatic encephalopathy
HEV	hepatitis E virus
INR	international normalized ratio
KCC	King's College Criteria
MELD	Model for End-Stage Liver Disease
NAC	N-acetylcysteine
TX	liver transplantation

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What You Need to Know

Background

Carbamoyl phosphate synthetase 1 (CPS1), a hepatocyte mitochondrial enzyme, is physiologically secreted into bile. It is not normally found in serum but is released in the bloodstream upon acute liver injury. We hypothesized that CPS1 may serve as a prognostic serum biomarker in acute liver failure (ALF).

Findings

We analyzed serum CPS1 levels by enzyme-linked immunosorbent assay in 270 patients with acute liver injury and ALF. The first reading of CPS1 and its increase in day 3 vs day 1, but not alanine transaminase or aspartate transaminase, were significantly higher in acetaminophen (APAP)-related than non-APAP patients, particularly in those who were liver transplanted or died. CPS1 improved the ALF Predictive Index accuracy of transplant-free survival in APAP patients.

Implications for patient care

Serum CPS1 measurement is a promising test that could predict transplant-free survival or need for transplantation in APAP-related ALF.

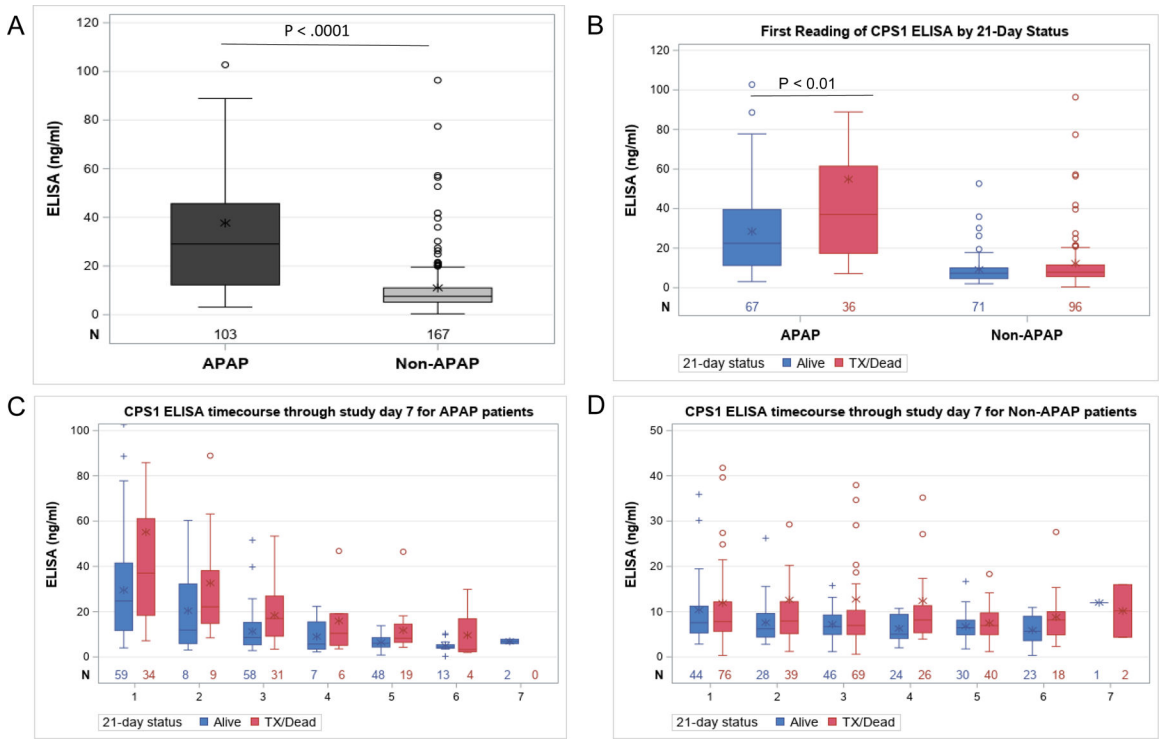


Figure 1.

Comparison of CPS1 between TX/dead and alive APAP and non-APAP patients. (*A*) ELISA analysis of initial hospitalization sera from APAP and non-APAP patients. (*B*) ELISA of first reading serum samples from TX/dead and alive APAP and non-APAP patients. (*C*–*D*) ELISA results of sera from days 1 to 7 hospitalization of APAP (*C*) and non-APAP (*D*) patients. *Represents the mean CPS1 values (ng/mL); the line within the box is the median.

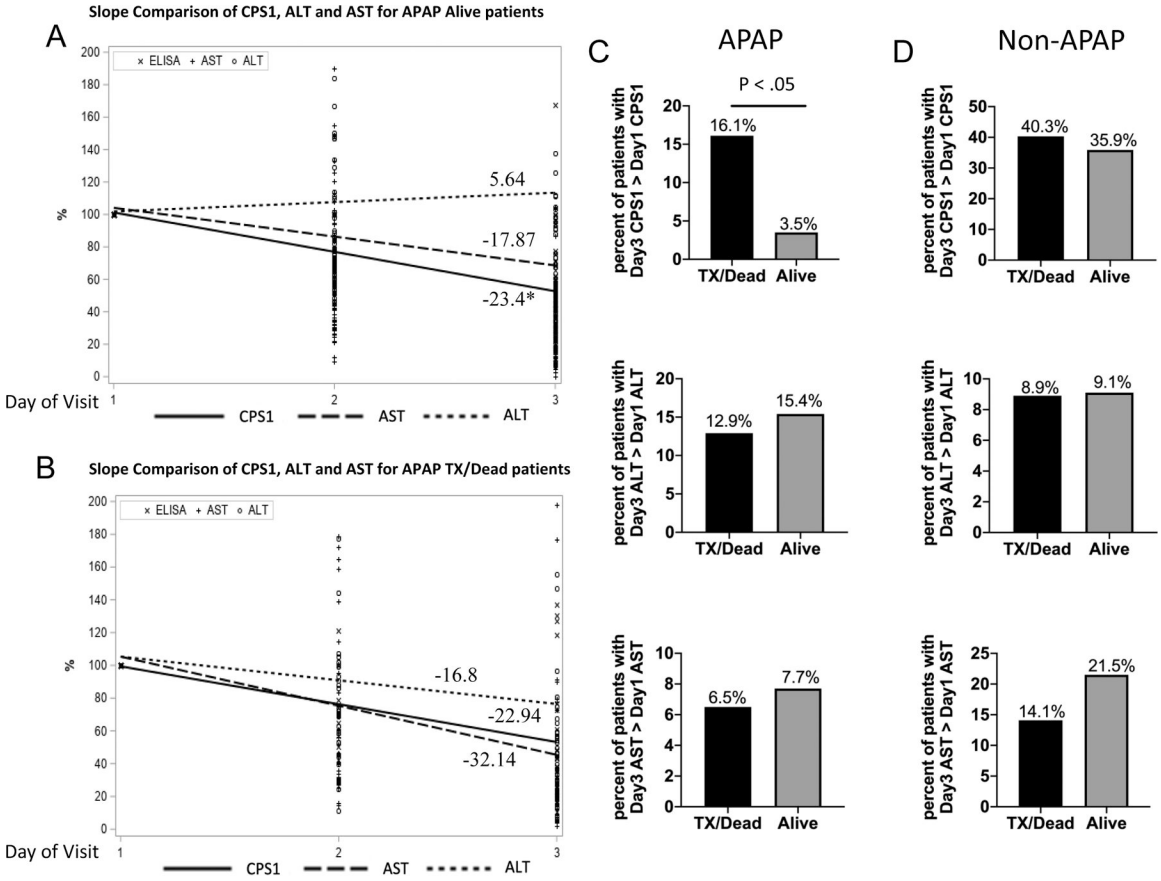
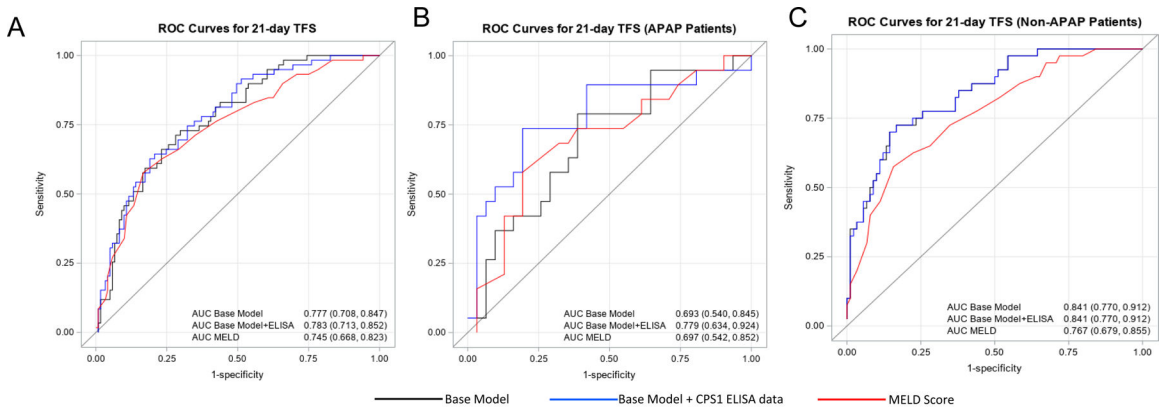


Figure 2.

Comparison of overall CPS1, ALT, and AST levels from sera collected on days 1, 2, and 3. The slope of the change (day 1, 2, and 3) of CPS1, ALT, and AST in APAP alive patients (*A*) and TX/dead patients (*B*). Each "x" represents CPS1 ELISA value from individual patients, whereas "+" and "o" represent individual sera AST and ALT levels, respectively. The percentages of TX/dead and alive APAP patients (*C*) and non-APAP patients (*D*) who had higher CPS1, ALT, and AST levels at day 3 compared with day 1 are shown.



D

	Model	AUC	Cutoff	Sensitivity	Specificity	PPV	NPV
TFS	Base	0.777 (0.708, 0.847)	0.24	81.4	48.8	43.6	84.3
	Base +ELISA	0.783 (0.713, 0.852)	0.24	81.4	50.4	44.4	84.7
	Meld	0.745 (0.668, 0.823)	0.24	83.1	44.2	42.2	84.1
APAP	Base	0.693 (0.540, 0.845)	0.24	84.2	16.1	38.1	62.5
	Base +ELISA	0.779 (0.634, 0.924)	0.24	78.9	41.9	45.5	76.5
	Meld	0.697 (0.542, 0.852)	0.24	84.2	29	42.1	75
Non-APAP	Base	0.841 (0.770.0.912)	0.24	77.5	62.2	47.7	86.2
	Base +ELISA	0.841 (0.770.0.912)	0.24	77.5	61.1	47.0	85.9
	Meld	0.767 (0.679, 0.855)	0.24	77.5	48.3	40.3	82.7

Figure 3.

AUROC plot comparisons of predictive models for 21-day transplant-free survival. The AUROC base model alone is compared with the addition of CPS1 ELISA data or with the MELD score. (*A*-*C*) AUROC analysis was performed in all transplant-free survival (TFS) patients (*A*), APAP patients (*B*), and non-APAP patients (*C*). Panel *D* shows details of the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

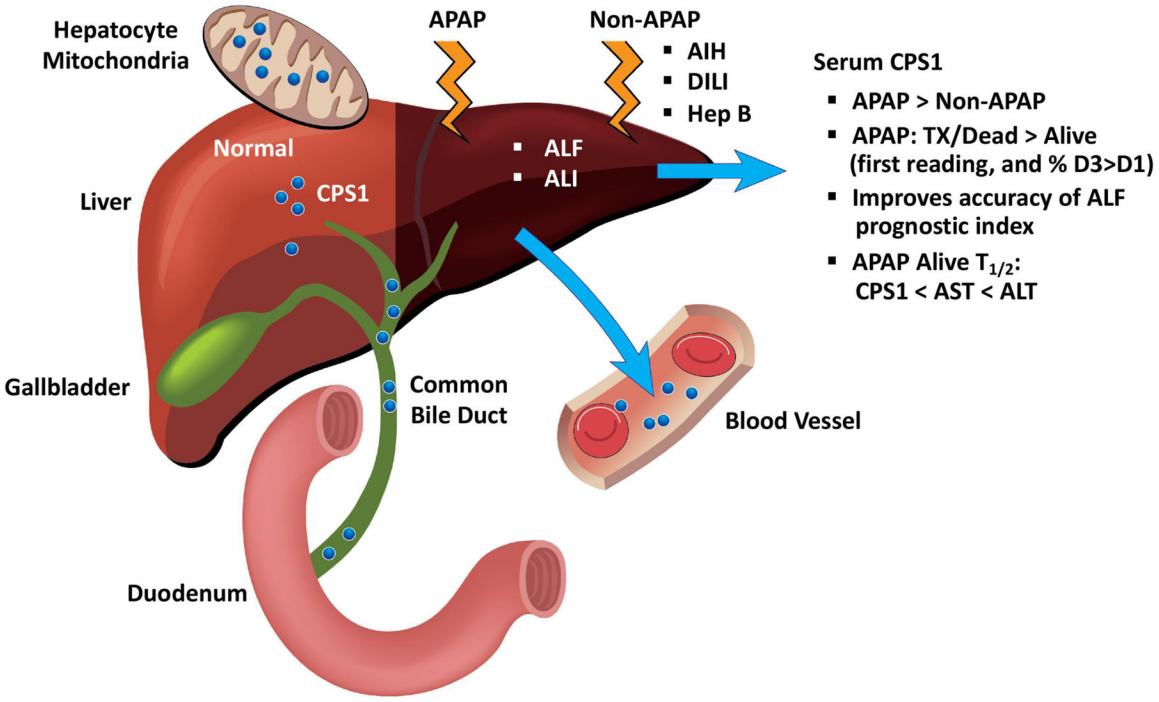


Figure 4.

Schematic model of CPS1 as a serum biomarker in ALI. In normal liver, hepatocyte mitochondrial CPS1 (*blue circles*) is constitutively released into bile. Upon APAP and non-APAP ALI or ALF, hepatic CPS1 is released into blood (*blue arrow*). Higher levels of serum CPS1 are detected in APAP vs non-APAP patients ($P < .0001$), and more CPS1 is found in APAP TX/Dead compared with spontaneously alive patients ($P < .01$). Also, more percentage of patients with higher D3 CPS1 levels than D1 was found in Tx/dead group ($P < .05$). CPS1 improves the accuracy of the ALFSG PI of 21-day transplant-free survival. In the alive patients, the serum half-lives (T1/2) can be extrapolated to be least for CPS1 then AST then ALT.

Table 1.

Comparison of Demographics and Clinical Characteristics of the study cohort

Variable	n	Non-APAP (n=167)	APAP (n=103)	P Value
Age, years	270	42.0 (24.0)	38.0 (18.0)	**
Female (%)	270	112 (67.1)	70 (68.0)	0.8946
Caucasian (%)	270	92 (55.1)	80 (77.7)	**
African American (%)	270	52 (31.1)	16 (15.5)	
Other races (%)	270	23 (13.8)	7 (6.8)	
Body Mass Index (BMI)	270	27.4 (10.4)	26.5 (7.2)	0.0527
Diabetes (% yes)	270	40 (24.0)	11 (10.7)	**
Admission labs				
Alanine aminotransferase (IU/L)	264	796.5 (1333.0)	3949.5 (3768.0)	**
Aspartate aminotransferase (IU/L)	262	644.0 (1337.5)	4264.0 (7236.0)	**
Alkaline phosphate (IU/L)	261	151.0 (87.0)	119.5 (63.0)	**
Bilirubin (mg/dL)	263	18.5 (12.3)	4.0 (3.7)	**
Creatinine (mg/dL)	267	0.9 (0.8)	1.5 (2.1)	**
International normalized ratio (INR)	260	2.7 (2.2)	3.1 (2.2)	0.1007
Platelet count (×109/L)	262	154.0 (108.0)	135.0 (107.0)	*
Leukocyte (WBC) count (×109/L)	263	9.0 (7.0)	9.4 (6.9)	0.8621
Arterial Ammonia (μmol/L)	39	85.0 (112.0)	108.0 (128.0)	0.2099
Venous Ammonia (μmol/L)	145	81.0 (67.0)	72.0 (67.0)	0.3418
Clinical parameters at admission				
Hepatic Encephalopathy (HE) Grade	189	2.0 (2.0)	3.0 (3.0)	*
Coma Grade (% 3/4)	187	45 (33.1)	28 (54.9)	**
Pressors (% yes)	269	13 (7.8)	20 (19.4)	**
Rapid Response Team (RRT) (% Yes)	269	13 (7.8)	23 (22.3)	**
Ventilator (% yes)	269	38 (22.9)	34 (33.0)	0.0887
Model for End-Stage Liver Disease (MELD) Score	254	32.0 (11.0)	30.0 (14.0)	0.0504
Clinical parameters during hospitalization				
Peak coma grade during hospitalization	188	3.0 (2.0)	4.0 (1.0)	**
Pressors during hospitalization (% yes)	269	42 (25.3)	30 (29.1)	0.5711

Variable	n	Non-APAP (n=167)	APAP (n=103)	P Value
RRT during hospitalization (% yes)	269	29 (17.5)	32 (31.1)	*
Ventilator during hospitalization (% yes)	269	67 (40.4)	45 (43.7)	0.6125
21-day outcome				
Ever listed for transplant (% yes)	269	75 (45.2)	22 (21.4)	**
Liver Transplant within 21 days (% yes)	270	52 (31.1)	13 (12.6)	**
Spontaneous survival (% yes)	270	71 (42.5)	67 (65.1)	**

Data expressed as median (interquartile range) for continuous variables and n (%) for categorical variables. The *p* value is calculated by the Fisher's exact text.

* $p < 0.05$,

** $p < 0.01$

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Table 2.

Comparison of CPS1 ELISA levels in non-APAP patients

Comparison of first reading of ELISA CPS1 between subgroups of Non-APAP patients					
21 days status	**Patient number**		**CPS1 (ng/ml)**	**Comparison**	**p value**
TX/Dead	Autoimmune hepatitis	29	8.87	Autoimmune hepatitis vs DILI	0.1700
	Hepatitis B	37	13.79	Autoimmune hepatitis vs Hepatitis B	0.0466
	DILI	30	13.95	DILI vs Hepatitis B	0.6008
				DILI vs Non-DILI *	0.7014
Alive	Autoimmune hepatitis	16	6.55	Autoimmune hepatitis vs DILI	0.2010
	Hepatitis B	13	8.64	Autoimmune hepatitis vs Hepatitis B	0.1095
	DILI	42	10.37	DILI vs Hepatitis B	0.8819
				DILI vs Non-DILI	0.4436
All	Autoimmune hepatitis	45	8.05	Autoimmune hepatitis vs DILI	0.0753
	Hepatitis B	50	12.45	Autoimmune hepatitis vs Hepatitis B	0.0075
	DILI	72	11.86	DILI vs Hepatitis B	0.3213
				DILI vs Non-DILI	0.6827

* Non-DILI includes both autoimmune hepatitis and Hepatitis B patients.