Testing the Effect of Acetaminophen Overdose on the Liver and the Role of Biomarkers to Predict Death or Survival

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Background

Acetaminophen overdose is the leading cause of acute liver injury with a third of cases being unintentional. At therapeutic doses, Acetaminophen (APAP, Tylenol, Paracetamol) is a safe and effective analgesic. Yet, in the US, APAP overdose is the cause of over 500 deaths and 50k hospitalizations annually. Patients may overdose intentionally or by accidentally combining several APAP-containing medications. Overdose occurs when a patient supersedes therapeutic doses, depleting their glutathione (GSH) stores and causing heptatocyte death. Symptoms occur between 18 and 36 hours; these include nausea, jaundice, delirium, and loss of consciousness. Treatment with N-Acetylcysteine (N-Ac) is effective between 12 and 24 hours at certain overdose levels. In the case where N-Ac is ineffective, survivability is contingent upon a liver transplant.

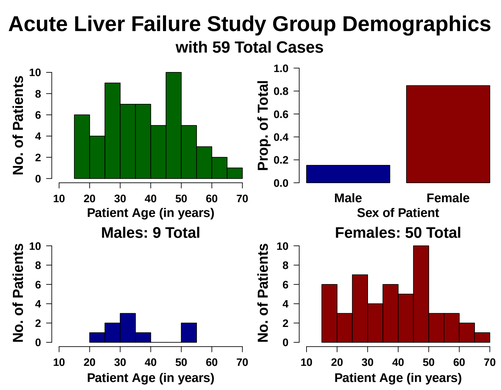
Project Aims

- The current model for assessing liver health, The King's College Criteria (KCC), cannot predict APAP dosage or time of overdose.
- The goal of the extended MALD is to create a more precise diagnostic tool to aid physicians in determining a patient's need for a liver transplant by estimating the best fit values for dosage and time of overdose.
- Testing the extended MALD, which includes a fourth biomarker, with a larger dataset as more cases containing values for APAP-protein adduct become available.

Acute Liver Failure Study Group

We validated our model using a multi-center, national dataset, the Acute Liver Failure Study Group (ALFSG). We selected 59 patient with acute liver failure (ALF) and acute liver injury (ALI) due to APAP overdose. Each case includes a patient's serum concentration values for the four biomarkers used in our model: AST, ALT, INR, and adduct. The data also includes a patient's duration of treatment, actual result (recovery, transplant, or death), recorded alcohol use, age, sex, weight, and height. Our study includes 50 females and 9 males, with ages ranging between 17 and 68 years.

Due to alcohol use potentially increasing liver-damage during APAP overdose, we also validated the model with patients with recorded alcohol use removed. After their removal, 39 females and 6 males remain. Since recipients of liver transplants would have otherwise died due to liver failure, their results are counted as death for the sake of model validation.



		All n = 59	No Alcohol Use n = 45	Recorded Alcohol Use n = 14
Ratio	females to males	50:9	39:6	11:3
Age	mean ± SD, years	38.6 ± 13.1	38.7 ± 13.6	38.4 ± 11.8
Weight	mean ± SD, kg	69.1 ± 38.6	69.8 ± 18.3	66.5 ± 16.8
BMI	mean ± SD, kg/m^2	26.0 ± 6.20	26.85 ± 6.51	23.36 ± 4.48
Result	recovered, patients	48	35	13
	death or transplant, patients	11	10	1

APAP Metabolism

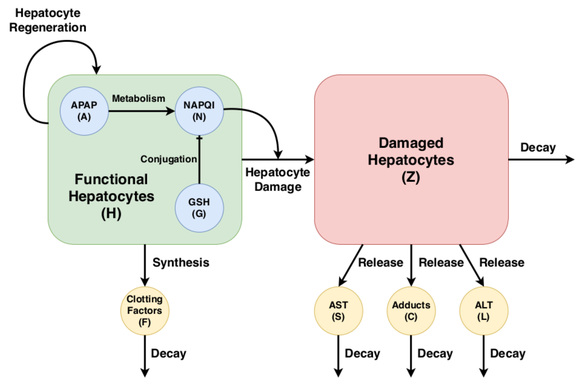


Fig. 2: APAP (A) is metabolized by the liver where it is converted to N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI (N) is conjugated by GSH (G) and safely excreted, until GSH stores are depleted. Through regeneration, functional hepatocytes produce clotting factors (F). As GSH stores are depleted, NAPQI causes damage to functional hepatocytes (H). These damaged hepatocytes (Z) then release AST (S) and ALT (L). NAPQI also covalently binds to APAP-protein adducts (C), which are released during lysis.

Mathematical Model

The Model of Acetaminophen-Induced Liver Damage:

$$\text{APAP} \quad \frac{dA}{dt} = -\frac{\alpha}{H_{max}}AH - \delta_A A.$$

$$\text{NAPQI} \quad \frac{dN}{dt} = \frac{qp\alpha}{H_{max}}A - \gamma NG - \delta_N N.$$

$$\text{GSH} \quad \frac{dG}{dt} = \kappa - \gamma NG - \delta_G G.$$

$$\text{Functional Hepatocytes} \quad \frac{dH}{dt} = rH\left(1 - \frac{H+Z}{H_{max}}\right) - \eta NH.$$

$$\text{Damaged Hepatocytes} \quad \frac{dZ}{dt} = \eta NH - \delta_Z Z.$$

$$\text{ALT} \quad \frac{dL}{dt} = \frac{\delta_Z \beta_L}{\theta H_{max}}Z - \delta_L(L - L_{min}).$$

$$\text{AST} \quad \frac{dS}{dt} = \frac{\delta_Z \beta_S}{\theta H_{max}}Z - \delta_S(S - S_{min}).$$

$$\text{Clotting Factor} \quad \frac{dF}{dt} = \beta_F\left(\frac{H}{H_{max}} - F\right).$$

$$\text{Adduct} \quad \frac{dC}{dt} = \frac{\delta_Z \beta_C}{\theta H_{max}}Z - \kappa_C C.$$

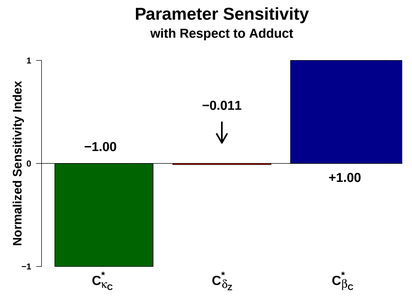
where INR (I) is given by:

$$I = \left(\frac{1-F_{min}}{F+F_{min}}\right)^4.$$

- The Model for APAP-Induced Liver Damage (MALD) uses dynamic system of differential equations to model liver injury.
- Currently, MALD uses three bio-markers: aspartate aminotransferase (AST), alanine aminotransferase (ALT), and international normalized ratio (INR).
- These biomarkers are indicative of hepatocyte death but are not specific to APAP.
- In our work we have modeled a fourth biomarker, APAP-protein adduct.
- APAP-protein adduct is a biomarker which is specific to the biosynthesis of APAP within the liver.
- This extended MALD predicts outcome based on admission values for the four biomarkers, which are compared to actual outcomes.

Sensitivity Analysis for κ_C, δ_Z and β_C

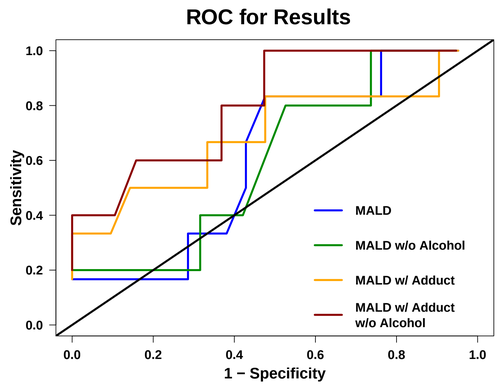
To analyze their relative importance to the model, we consider the normalized sensitivity indices (elasticity) of the equation for serum adduct concentration (C) with respect to the parameters for the elimination rate of APAP-protein adduct from the liver (κ_C), the damaged heptatocyte lysis rate (δ_Z), and the total amount of adduct in a healthy liver at therapeutic doses (β_C).



- We estimated β_C as 175 $\mu mol/L$. Based on literature, κ_C is set to $0.396/day$ Based on prior works, we set δ_Z at $5/day$.
- As κ_C is increased by 1%, C decreases by 1% and as β_C is increased by 1%, C increases by 1%.
- C is least sensitive to δ_Z and equally sensitive to β_C and κ_C.

ROC Curve to Compare Results of Each Case

The ROC curves compare the sensitivity and specificity of the model's estimated values verses the actual results (from data). Here, we compare the ROC curves for MALD with and without the addition of adduct. We also compare the two models where patients with reported alcohol use are removed.



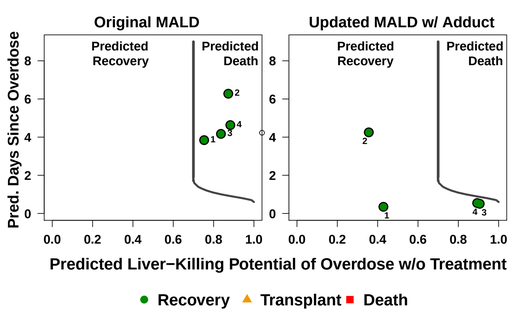
- The addition of adduct to MALD results in a 13.6% increase in the AUC.
- When cases with reported alcohol use are removed from the dataset, the addition of adduct to MALD results in a 34.2% increase in the AUC.

Summary of ROC Results:

Description	Sensitivity	Specificity	PPV	NPV	Accuracy
Original MALD	66.7%	72.7%	91.4%	33.3%	67.8%
Updated MALD w/ Adduct	81.3%	27.3%	83.0%	25.0%	71.2%
Original MALD w/ Alcohol Removed	65.7%	80.0%	92.0%	40.0%	68.9%
Updated MALD w/ Adduct & Alcohol Removed	85.7%	30.0%	81.1%	37.5%	73.3%

Improvements of Patient Fits for MALD with Adduct

For demonstration, four cases are shown below in which the addition of adduct to MALD increases accuracy in predicting the recovery of patients. The model estimates the number of days since overdose and the proportion of hepatic necrosis for 4 patients with known APAP overdose.



- Looking at the figure to the left, the gray line indicates time since overdose with a threshold of 70% hepatic necrosis. For patients to the right and above the gray line the model predicts death.
- For these 4 patients, the original model predicts death, whereas the model updated with APAP-protein adducts correctly predicts recovery.

Patient Information:

Patient	Sex	Age	BMI	Adduct	ALT	AST	INR	ALI/ALF	Result
Patient 1	Female	17	21	1.809	5434	5496	3.82	ALF	Recovery
Patient 2	Female	51	NA	4.691	2508	812	1.90	ALF	Recovery
Patient 3	Male	28	24	8.087	5374	4397	6.00	ALF	Recovery
Patient 4	Female	20	28	8.92	4069	3959	7.10	ALI	Recovery

Conclusion

The inclusion of APAP-protein adducts in the model improved the its sensitivity by 21.9%, with an overall improvement to the AUC of the ROC curve of MALD by 13.6%. Upon removal of cases with reported alcohol use, the sensitivity increased by 30.4% and the ROC curve improved by 34.3%. We found the addition of APAP-protein adducts increases the predictive quality for the model.

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