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DRUG-INDUCED LIVER INJURY: A PREDICTIVE MODEL, MITOCHONDRIAL TOXICITY MECHANISMS RISK, AND ANTIPSYCHOTIC MEDICATIONS RISK IN A REAL-WORLD SETTING

Payal Rana

University of Rhode Island, payal.m.rana@pfizer.com

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DRUG-INDUCED LIVER INJURY: A PREDICTIVE MODEL, MITOCHONDRIAL
TOXICITY MECHANISMS RISK, AND ANTIPSYCHOTIC MEDICATIONS RISK IN A
REAL-WORLD SETTING

BY
PAYAL RANA

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE
REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

IN
PHARMACEUTICAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2020

DOCTOR OF PHILOSOPHY DISSERTATION

OF

PAYAL RANA

APPROVED:

Dissertation Committee:

Major Professor Stephen J. Kogut

Xuerong Wen

Fatemeh Akhlaghi

Michael D. Aleo

Brenton DeBoef

DEAN OF THE GRADUATE SCHOOL

UNIVERSITY OF RHODE ISLAND

2020

ABSTRACT

Drug-induced liver injury (DILI) is one of the leading reasons for clinical candidate termination during drug discovery and development. Similarly, many drugs have received a black-box warning or withdrawn from the market due to DILI. It is reported that DILI is the 4th leading cause of liver disease leading to liver failure. It is estimated that the DILI has an annual incidence rate of 13.9 ± 2.4 cases per 100,000 people, translating to about 44,000 liver injury patients in the United States each year. Despite tremendous research in the predictive field, liver injury prediction using in vitro and in vivo models remains a substantial challenge. In this dissertation, I use the three-manuscript format to address the literature gap based on the drug's toxicological properties and its ability to cause mitochondrial dysfunction. I also evaluated the risk of liver injury among patients newly treated with atypical antipsychotics.

Manuscript 1 combined physicochemical properties and in vitro cytotoxicity assays, including mitochondrial dysfunction, to build organ-specific univariate and multivariable logistic regression models to derive odds ratios for the prediction of clinical hepatotoxicity, nephrotoxicity, and cardiotoxicity for 215 marketed drugs. The multivariable hepatotoxic predictive model showed an odds ratio of 6.2 or 7.5 for mitochondrial inhibition or drug plasma $C_{max} > 1 \mu\text{M}$ for drugs associated with liver injury, respectively. The multivariable nephrotoxicity predictive model showed an odds ratio of 5.8, 6.4, or 15.9 for drug plasma $C_{max} > 1 \mu\text{M}$, mitochondrial inhibition, or hydrogen bond acceptor atoms greater than 7 for drugs associated with kidney injury, respectively. Conversely, drugs with a total polar surface area $\geq 75\text{\AA}^2$ were 79% less likely to be associated with kidney injury. Based on this study, I found that combining in silico physicochemical properties descriptors along with in vitro toxicity assays can be used to build predictive toxicity models to select small molecule therapeutics with

less potential to cause liver and kidney toxicity. Therefore, I recommend a blended approach of utilizing readily calculated physicochemical properties combined with in vitro toxicity assessments to select small molecules with less potential for clinical organ toxicity for the liver and kidney.

There is a growing need for characterization of age-differences in the susceptibility and frequency of DILI caused by hepatotoxicants according to their ability to cause mitochondrial dysfunction. Manuscript 2 investigated the relationship between liver injury reports submitted to the Food and Drug Administration Adverse Event Reporting System with drugs associated with hepatotoxicity via mitochondrial mechanisms compared with non-mitochondrial mechanisms of toxicity. This study provides evidence that a higher proportion of reports of severe liver injury adverse events among drugs are associated with mitochondrial mechanisms of toxicity compared with non-mitochondrial mechanisms of toxicity. Furthermore, I found that reports of liver injury were 2.2 times more likely to be associated with older patient age than reports involving patients ages under 65 years. This finding agreed with the theory that age is a susceptibility factor in liver injury via mitochondrial mechanisms of toxicity. The findings from this study align with mitochondrial mechanisms of toxicity being an important cause of DILI, and this should be further investigated in real-world studies with robust designs.

Compared to typical antipsychotics, atypical antipsychotics (AAP) medications have improved safety profiles and rarely cause liver injury. However, no retrospective cohort studies have evaluated AAP's liver injury risk using an administrative database in the United States. In manuscript 3, I conducted a retrospective cohort study using an administrative claims database to estimate the cumulative incidence rate and identify risk factors for liver injury in patients taking AAP medications. Univariate and multivariate logistic regression analyses were utilized to identify risk factors associated

with liver injury. The study estimated the cumulative incidence rate of liver injury 15.2 to 25.1 per 1000 persons per year among patients newly treated with AAP medications. During the first months of treatment, quetiapine, as compared to aripiprazole, was associated with a 22% increased risk of liver injury in patients 75 years and younger. Our study suggests that patients receiving quetiapine AAP should be monitored for liver enzyme elevations frequently during therapy initiation. Patients with comorbidities including alcohol abuse, hypertension, obesity, and hyperlipidemia were also found to be at higher risk and these patients should be monitored closely for liver injury. Further prospective real-world studies are warranted to substantiate these findings.

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PREFACE

This dissertation is written in the three-manuscript format and formatted based on the guideline provided by the University of Rhode Island Graduate School. These three manuscripts encompassed important aspects of Drug-induced liver injury: most influential descriptors for a predictive model (manuscript I), mitochondrial toxicity mechanism risk (manuscript II), and liver injury among patients newly treated with atypical antipsychotics mediations (manuscript III).

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MANUSCRIPT I

Title: Most influential physicochemical and in vitro assays descriptors for hepatotoxicity and nephrotoxicity prediction

Payal Rana^{*a, b}, Stephen Kogut^b, Xuerong Wen^b, Fatemeh Akhlaghi^b, and Michael D.
Aleo^a,

^aDrug Safety Research & Development, Pfizer, Eastern Point Road, Groton, CT
06340, USA

^b University of Rhode Island, College of Pharmacy, Kingston, RI 02881, USA

*Corresponding author: Address: Payal Rana, Drug Safety R&D, Eastern Point Road,
Groton, CT 06340, USA; email: payal.m.rana@pfizer.com

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1.1 ABSTRACT

Drug-induced organ injury is a major reason for drug candidate attrition in preclinical and clinical drug development. The liver, kidneys, and heart have been recognized as the most common organ systems affected in safety-related attrition or the subject of black box warnings, and post-market drug withdrawals. In silico physicochemical property calculations and in vitro assays have been utilized separately in the early stages of the drug discovery and development process to predict drug safety. In this study, we combined physicochemical properties and in vitro cytotoxicity assays including mitochondrial dysfunction to build organ-specific univariate and multivariable logistic regression models to derive odds ratios for the prediction of clinical hepatotoxicity, nephrotoxicity, and cardiotoxicity for 215 marketed oral drugs. The multivariable hepatotoxic predictive model showed an odds ratio of 6.2 (95% Confidence Interval (CI)-1.7-22.8) or 7.5 (95% CI-3.2-17.8) for mitochondrial inhibition or drug plasma C_{max} >1 μ M for drugs associated with liver injury, respectively. The multivariable nephrotoxicity predictive model showed an odds ratio of 5.8 (95% CI-2.0-16.9), 6.4 (95% CI-1.1-39.3), or 15.9 (95% CI-2.8-89.0) for drug plasma C_{max} >1 μ M, mitochondrial inhibition, or hydrogen bond acceptor atoms greater than 7 for drugs associated with kidney injury, respectively. Conversely, drugs with a total polar surface area $\geq 75 \text{\AA}^2$ were 79% (odds ratio 0.21, 95% CI-0.061-0.74) less likely to be associated with kidney injury. Drugs belonging to the Extended Clearance Classification System (ECCS) class 4, where renal secretion is the primary clearance mechanism (low permeability drugs that are bases/neutrals), were 4 (95% CI-1.8-9.5) times more likely to cause kidney injury with this data set. Alternatively, ECCS class 2 drugs, where hepatic metabolism is the primary clearance (high permeability drugs that are bases/neutrals), were 77% less likely (odds ratio 0.23 95%CI-0.095-0.54) to cause

kidney injury. A cardiotoxicity model was poorly defined using any of these drug physicochemical attributes. Combining in silico physicochemical properties descriptors along with in vitro toxicity assays can be used to build predictive toxicity models to select small molecule therapeutics with less potential to cause liver and kidney organ toxicity.

1.2 INTRODUCTION

The high rate of safety-related attrition and the enormous cost of developing new drugs have encouraged the pharmaceutical industry to move safety evaluation earlier into the drug discovery process when structure-toxicity relationship determination is feasible.¹ This can be easily done before more expensive toxicity testing is conducted in small (rodent) and large (non-rodent) animal species an advance of clinical trials that may ultimately be poorly predictive of human drug-induced organ injury.²⁻⁴ Hence, various in silico tools, such as structural alerts, physicochemical properties,⁵⁻⁷ and in vitro toxicity assays^{8, 9} have been proposed to accelerate drug development with adequate safety and at a lower cost.

The association of physicochemical properties with animal and human toxicity has been widely accepted and published in the literature.¹⁰⁻¹⁴ Hughes et al. demonstrated that highly lipophilic, less polar drug candidates are more likely to cause safety issues in animal exploratory toxicity studies using 245 small molecules.¹⁰ The utilization of physicochemical properties combined with general cytotoxicity assays to progress potential clinical candidates into animal toxicity studies has been explored.¹¹⁻¹³ For human liver organ toxicity, Leeson (2018) successfully utilized physicochemical properties to predict "Most-DILI-" from "No-DILI-" concern drugs in 1841 marketed small molecule therapeutic drugs.¹⁴ The author demonstrated high value of lipophilicity and fraction of carbon atoms that are sp³ hybridized (Fsp³) when combined with maximum applied daily dose for separating these binary (categorical) predictions of human liver toxicity.¹⁴

There has been utilization of absorption, distribution, metabolism, elimination (ADME) drug attributes in the prediction of human DILI as well.¹⁵⁻²⁰ Some researchers have analyzed DILI relationships based on the Biopharmaceutics Drug Disposition Classification System (BDDCS), a method used to predict the drug disposition as well

as potential drug-drug interactions (DDI).¹⁵⁻¹⁷ As expected, the majority of drugs that cause human DILI undergo extensive hepatic metabolism¹⁸ and, in the absence of knowledge of an applied daily dose, it was suggested that avoiding the development of BDDCS class 2 molecules (low solubility, extensive mechanism) could be used to prevent clinical DILI.¹⁹ Using this approach, Chan et al. stated there was little additional value in adding in vitro toxicity assays over the BDDCS classification system.²⁰ Similar to BDDCS classification, some researchers have utilized the Extended Clearance Classification System (ECCS) for early predictions of rate-determining clearance mechanisms.²¹ ECCS has been utilized to understand ADME attributes, and enabling victim DDI predictions in chemical design and molecule selection.²²

From a biological perspective, several assays related to hepatotoxicity have also been used for predictions.²³ These involve measurements of cytotoxicity, or mitochondrial dysfunction, and/or bile acid efflux inhibition.²³ Some studies have utilized mitochondrial toxicity assessments alone²⁴ or in combination with other assays to highlight drugs of Most-DILI-concern.²⁵ Although, these approaches have been useful in highlighting drugs with Most-DILI-concern potential, neither of these later approaches considered the value of combining physicochemical properties, or ECCS classifications, with relevant in vitro mechanistic assays.

Aleo et al. recently showed the value of combining major physicochemical properties with in vitro assays relevant to liver safety for discriminating DILI compounds across and within categories of liver injury (Most-, Less- and No-DILI-concern) along with their FDA label for liver safety (no mention, adverse reactions, warnings and precautions, black box warnings and withdrawn).²⁶ This hybrid approach offered value for new chemical entities in clinical development that were discontinued for significant liver transaminase elevations. With this success, however, we believe considerable

efforts should be made to develop better predictive models of clinical organ toxicity beyond the liver. To our knowledge, there is a significant gap in the literature for integrating both physicochemical properties with in vitro mechanistic assays for predicting major organ system toxicities beyond the liver, such as kidney and heart.

Because mitochondrial dysfunction is a universal safety concern across multiple organ systems, the utilization of mitochondrial toxicity assays and physicochemical properties for the prediction of organ toxicity was explored by Rana et al. using 228 drugs.²⁷ It was concluded that testing drugs in mitochondrial assays at 100 times the maximum total plasma concentration (C_{max}) provided the highest sensitivity for predicting hepatotoxicity. Furthermore, in-depth physicochemical properties space was explored for the drugs that were positive in the mitochondrial assays compared with those that were tested negative. Drugs testing positive in mitochondrial assays possessed different physicochemical properties in terms of lipophilicity and were prone to be in either acid or neutral ionization states compared with drugs that tested negative.²⁷ In this study, we proposed to expand the concept of combining physicochemical properties with in vitro assays to explore the major organ system toxicity other than hepatotoxicity. Furthermore, we expanded the combination of the most predictable characteristics of in vitro assays and physicochemical properties to build human organ toxicity predictive models using univariate and multivariable logistic regression modeling. We hypothesized that there are different sets of in vitro assays, and physicochemical properties can be predictive of toxicity in selected organs (liver, kidney, and heart). We examined this relationship quantitatively using the odds ratio for the prediction of clinical organ toxicity.

The overall goal of this study is to help build better predictive models for hepatotoxicity, nephrotoxicity, and cardiotoxicity using the least number of input variables necessary for discrimination to optimize the process for efficiency and cost

savings. Drug physicochemical properties (hydrogen bond donor and acceptor count, Fsp³, calculated log partition coefficient (cLogP), total polar surface area (TPSA), molecular weight (MW), and Cmax values), ECCS class, and effects using in vitro mechanistic assays (Transformed Human Liver Epithelial (THLE) cytotoxicity assays, glucose/galactose assays, and mitochondrial respiration inhibitory/uncoupling assays) of 215 pharmaceutical drugs were investigated via utilization of multivariable logistic regression methods.

1.3 METHODS

Data Source

The physicochemical properties, ECCS classes, and in vitro results of the marketed drugs were utilized from previously published methods.^{21, 27-32} The in vitro assay results were previously published by Rana et al. or Shah et al.^{13, 23, 27}

Inclusion criteria for the drugs

The present study included 215 drugs (70 hepatotoxic drugs, 44 cardiotoxic drugs, 45 nephrotoxic drugs, and 56 drugs not known to cause human organ toxicity). These drugs were small molecules, represented diverse target pharmacology, and belonged to multiple indications and therapeutic areas. Hepatotoxic drugs and drugs not known to have organ toxicity (Most-, Less- and No-DILI-concern) were annotated using the United States National Center for Toxicological Research Liver Toxicity Knowledge Base (NCTR-LTKB).^{33, 34} Nephrotoxic and cardiotoxic drug classification were adapted from previously published literatures from Lin and Will, and Rana et al.^{27, 30} Lin and Will (2012) classified nephrotoxic and cardiotoxic drugs via automated queries from published literature on drug-induced toxicity; this information was curated manually.²⁷ All the compounds selected for three categories of organ toxicity were reported in the previous publications or had literature evidence of specific organ black box warning or withdrawal.^{13, 23, 27, 30, 35}

To build a separate model for each organ toxicity group using a univariate and multivariable logistic regression model, a drug was classified as either positive (1) or negative (0) outcome of organ toxicity.³⁶ For drugs where multiple papers reported two or more organ toxicities, drug adverse event counts (using FAERS database) were utilized to include a drug in one organ toxicity category. The method of calculating drug adverse event counts has been described previously.^{27, 37}

Selection of independent variables

Medicinal chemists in the pharmaceutical industry have utilized Lipinski's Rule of 5 as the first step to eliminate molecules with poor physicochemical properties for oral bioavailability.³⁸ More recently, ECCS computational modeling has been employed to facilitate the early assessment of the rate-determining step in drug clearance, pharmacokinetic variability, and proposed metabolism/excretion pathway based on basic physicochemical properties and passive membrane permeability assessments.^{21, 22} This differs slightly from estimates of BDDCS classification, which focuses on the extent of drug elimination and solubility.²¹

Physicochemical properties such as TPSA and cLogP are indicative of drug polarity and lipophilicity. Drugs with high lipophilicity and low polarity (cLogP $\geq$ 3 and TPSA $<$ 75 Å²) had significantly increased toxic outcomes in animal studies compared with drugs that had both lower lipophilicity and higher polarity (cLogP $<$ 3 and TPSA $\geq$ 75 Å²).^{10, 12, 39} Along with higher lipophilicity, lower Fsp³ has been reported with predicting drugs with high potential of liver injury.¹⁴ MW is essential in demonstrating on- and off-target drug toxicity.¹³ MW greater than 400 Da has been associated with toxicity and considered as a less desirable characteristic in an orally administered drug.^{14, 21} A previous study reported that reducing the number of heteroatoms, especially hydrogen-bond donors and acceptors, has been used to decrease P-glycoprotein transporter efflux.⁴⁰ The hydrogen bond donor (greater than 5) and acceptor (greater than 7) counts have been used for optimal drug exposure.³⁹ Finally, drugs with higher Cmax have been demonstrated in several studies with a higher risk of organ injury.^{14, 23, 27, 41}

This study utilized Respirometric Screening Technology (RST), a high throughput screening mitochondrial assay that has been used to measure effects on respiration (inhibition or uncoupling) in isolated rat liver mitochondria along with cellular assessments of mitochondrial toxicity. Mitochondrial dysfunction at the cellular level

was determined using the previously published glucose/galactose model.^{28, 31, 42-44} Furthermore, this study also included a general cytotoxicity assay that measures the effect of a compound on Transformed Human Liver Epithelial (THLE) cells viability. This assay has been reported for being a valuable tool to select compounds for progression into in vivo studies.^{12, 13}

In the present study, we have used independent variables including physicochemical properties (hydrogen bond donor & acceptor count, Fsp³, cLogP, TPSA, MW, and Cmax values) and in vitro assay results (THLE cytotoxicity assay, glucose/galactose assays, and mitochondrial respiration inhibitory/uncoupling assays) to develop predictive models for liver, kidney, and heart organ toxicity. The study further incorporated the ECCS based on clearance, metabolism, permeability, and drug ionization states to evaluate their association with the organ toxicity group.

Dependent variable

For this study, the dependent variable was whether a drug was associated with an increased risk of hepatotoxicity, nephrotoxicity, or cardiotoxicity (yes or no).

Statistical Analysis

This study intended to use binary outcome models to build separate predictive models for each organ toxicity drug group. There has been evidence in the literature for utilizing this approach to develop risk based adjustment models.³⁶ Each toxic drug group (hepatotoxic, nephrotoxic, and cardiotoxic) was compared against a no organ toxic drug group. The differences in the continuous variables of drug characteristics between the comparison groups were assessed using the Student t-test for independent samples. All continuous variables were dichotomized using clinically-relevant criteria available in the literature (as shown in Table 1). Collinearity among independent variables was assessed by examining variance inflation factors, condition

indices, and variance proportions. Highly correlated variables were identified, and the less important variables were removed from further inclusion in univariate and multivariable logistic regression modeling.

The study first specified a univariate logistic regression model for each toxicity group (against no organ toxic group) and calculated the odds ratios, 95% confidence intervals, and p-values. The p-values less than 0.2 (less stringent with 80% probability of association) in the univariate logistic regression models were included for further multivariable logistic regression analyses. Afterward, a stepwise multivariable logistic regression model via backward elimination was utilized to choose the most predictive variables after removing insignificant variables. The odds ratios for final fitted models, 95% confidence intervals, and p-values were calculated in this multivariable logistic regression model after removing the insignificant variables with $p \geq 0.2$. The univariate analysis evaluated the unadjusted association between organ toxicity outcome and a single variable. In contrast, the multivariable analysis assessed the adjusted association between organ toxicity outcome with several variables simultaneously.⁴⁵

The Hosmer and Lemeshow goodness-of-fit test was performed to determine if the model fit adequately, where $p > 0.05$ indicated that the model fit appropriately.⁴⁵ The AUC-ROC (Area Under Curve – Receiving Operating Curve) method was used to evaluate performances of different predictive models, and to identify the optimized model with the most influential predictors of the organ toxic drugs. AUC-ROC is a plot of sensitivity vs. 1-specificity.⁴⁶ AUC-ROC evaluates the discrimination power of the model; an AUC-ROC value close to 1 suggests that selected variables were able to separate organ toxic drugs from no organ toxic drugs. Whereas, an AUC-ROC value close to 0.5 indicates that selected variables were not able to separate organ toxic drugs from no organ toxic drugs. All analyses were performed with SAS version 9.4

(SAS Institute, Cary, NC), and all statistical tests were two-sided with a significance level at 0.05.

1.4 RESULTS

Characteristics of physicochemical properties and in vitro assay results of organ toxicity drugs

The physicochemical properties (hydrogen bond donor and acceptor count, Fsp³, cLogP, TPSA, MW, and Cmax values) and in vitro assay results (THLE cytotoxicity assay, glucose/galactose assays, and RST mitochondrial respiration inhibitory/uncoupling assays) of each organ toxicity drug groups were compared with the no organ toxicity group (Table 2). For hepatotoxic drugs, there was a statistically significant (p-value < 0.05) difference for Cmax, THLE cytotoxicity assay, and RST mitochondrial inhibitory/uncoupling assays from the no organ toxic drugs. For nephrotoxic drugs, there was a statistically significant difference for hydrogen donor & acceptor count, TPSA, MW, and Cmax values from the no organ toxic drugs. For cardiotoxic drugs, there was a statistically significant difference for cLogP and THLE cytotoxicity assay from the no organ toxic drugs.

The collinearity assessment found that the results of both HepG2 glucose and HepG2 galactose in vitro assays were highly correlated (via variance inflation factors, condition indices, and variance proportions). These two variables are cell-based assays measuring mitochondrial health as cell viability in two different media, glucose, and galactose. HepG2 galactose assay along with the ratio of the cytotoxicity results allow the detection of drugs causing mitochondrial toxicity in cell-based assays.^{31, 47} In galactose media, HepG2 cells are forced to use mitochondrial oxidative phosphorylation (circumventing the Crabtree effect) instead of glycolysis making them more prone to drugs causing mitochondrial toxicity.³¹ Therefore, the results in the HepG2 glucose assay were removed for further statistical modeling, while HepG2

galactose assay, along with the ratio of the cytotoxicity results for mitochondrial health, were used.

Univariate regression models for hepatotoxic, nephrotoxic, and cardiotoxic drugs

The literature references and the cut-off criteria utilized for physicochemical properties and in vitro assays for statistical analyses are shown in Table 1. The univariate logistic regression models were specified after dichotomizing the individual variables based on published criteria and their association with toxicities. Table 3 includes the odds ratios, confidence intervals, and p-values of the univariate logistic regression models for hepatotoxic (A), nephrotoxic (B), and cardiotoxic drugs (C). In the hepatotoxic model, p-values were less than 0.2 for F_{sp^3} , C_{max} , THLE cell viability assay, glucose/galactose ratio, and RST mitochondrial inhibitory/uncoupling assays. In the nephrotoxic model, p-values were less than 0.2 for physicochemical properties such as hydrogen donor and acceptor count, clogP, TPSA, MW, C_{max} , and RST mitochondrial inhibitory assay. Similarly, for the cardiotoxic model, p-values were less than 0.2 for hydrogen donor count, C_{max} , THLE cell viability assay, HepG2 glucose/galactose cytotoxicity ratio, and RST mitochondrial inhibitory assays. The variables that had p-values less than 0.2 in all three drug groups were further included in the multivariable regression model.

Stepwise multivariable regression model (via backward elimination) for hepatotoxic, nephrotoxic, and cardiotoxic drugs

A stepwise regression model via backward elimination was utilized to choose the statistically significant predictive variables for each drug toxicity category. All the descriptors with p-values less than 0.2 (F_{sp^3} , C_{max} , THLE cell viability assay, glucose/galactose ratio, and RST mitochondrial inhibitory/uncoupling assays) were

included to build a hepatotoxic multivariable model. In the multivariable hepatotoxic predictive model, C_{max} (odds ratio 7.5; 95% CI 3.16-17.79; $p < 0.0001$), and RST mitochondrial inhibitory assay (odds ratio 6.21; 95% CI 1.69-22.79; $p = 0.0059$) were significant predictors (Figure 1A).

Similarly, hydrogen donor and acceptor count, clogP, TPSA, MW, C_{max}, and RST mitochondrial inhibitory assay were included to build the nephrotoxic predictive model. In the multivariable nephrotoxic model, hydrogen acceptor (odds ratio 15.94; 95% CI 2.85-89.00; $p = 0.0016$), TPSA (odds ratio 0.21; 95% CI 0.061-0.74; $p = 0.01$), C_{max} (odds ratio 5.86; 95% CI 2.03-16.9; $p = 0.001$), and RST mitochondrial inhibitory assay (odds ratio 6.43; 95% CI 1.05-39.31; $p = 0.043$) were the significant predictors (Figure 1B).

Finally, hydrogen donor count, C_{max}, THLE cell viability assay, HepG2 glucose/galactose cytotoxicity ratio, and RST mitochondrial inhibitory assays were included to build cardiotoxic predictive model. In the multivariable cardiotoxic predictive model, only the galactose assay (odds ratio 2.77; 95% CI 1.07-7.19; $p = 0.036$) indicated a statistically significant prediction of toxicity (Figure 1C).

Evaluation of predictive model performance via ROC-AUC (Receiving Operating Curves – Area Under Curve) and the Hosmer and Lemeshow goodness-of-fit tests

Assessment of the predictive models was conducted using ROC-AUC, as shown in Figures 2 (A, B, and C). Figure 2A shows the ROC-AUC values of all the predictors individually, along with the combinations of other statistically significant predictors. For the hepatotoxic model, utilizing C_{max}, RST mitochondrial inhibition,

and THLE cytotoxicity assays, the AUC-ROC value was 0.78, indicating a good fit for the predictive model.

Similarly, Figures 2B and 2C show the ROC-AUC values of all the predictors individually along with a combination of other statistically significant predictors for nephrotoxic and cardiotoxic models, respectively. As shown in Figure 2B, for the nephrotoxic predictive model, based on hydrogen acceptor, TPSA, MW, Cmax, and RST mitochondrial inhibition assay, the AUC-ROC value was 0.84, indicating a good predictive model. The cardiotoxic predictive model did not perform as well as hepatotoxic and nephrotoxic models, having a ROC-AUC value of 0.65 (as shown in Figure 2C). For the cardiotoxic predictive model, it can be assumed that this model can predict the cardiotoxic drug only slightly better than a random classifier.

The Hosmer and Lemeshow goodness-of-fit tests were performed to determine if the model for each drug toxicity group fit adequately. The three predictive models fit appropriately ($p = 0.43$ for the hepatotoxic model, $p = 0.84$ for the nephrotoxic model, and $p = 0.21$ for the cardiotoxic model).

Utilization of an ECCS to predict organ toxic drugs

Based on the ECCS, compounds are categorized as Class 1-metabolism as mainly systemic clearance mechanism (high permeability acids/zwitterions with $MW \leq 400$ Da), Class 2-where metabolism is primary clearance mechanism (high permeability bases/neutrals), Class 3A-renal clearance (low permeability acids/zwitterions with $MW \leq 400$ Da), Class 3B-transporter mediated hepatic uptake or renal clearance (low permeability acids/zwitterions with $MW \geq 400$ Da), and Class 4-renal clearance (low permeability bases/neutrals). The study reported that none of the ECCS classes were associated with hepatotoxic or cardiotoxic drugs (as shown in Tables 4, 5A, and 5C). However, ECCS class 2 (odds ratio 0.225; CI 0.095-0.535; $p =$

0.0007) and ECCS class 4 (odds ratio 4.1; CI 1.77-9.52; $p = 0.001$) showed discriminatory value for predictions of nephrotoxic drugs from the drugs with no organ toxicity with lessor or greater odds ratios, respectively (as shown in Table 5B).

1.5 DISCUSSION

Accurate prediction of safety-related severe adverse effects of drug candidates early in the development process is a high priority for pharmaceutical companies. There are various stages of drug development; for example, Exploratory Screen Development (ESD), Screening Designed Synthesis (SDS), Lead Development (LD), Candidate Selection (CS) leading to Candidate Alert Notice (CAN). It is generally believed that utilization of in vitro or in silico derived predictive models at the early stages of discovery improves clinical toxicity prediction. Different pieces of information are available at different stages of drug development. For example, known or calculated physicochemical properties information is available at the ESD or SDS stage. In contrast, various in vitro assays (to assess general cytotoxicity, mitochondrial dysfunction, or bile salt export pump disruption) are usually performed at the LD stage where enough drug product is made for testing after other chemical properties have been refined/optimized. At ESD or SDS stage, where hundreds or thousands of compounds need to be screened, high throughput models described in this study are utilized due to simplicity and low cost. In contrast, at lead development or compound selection stage where a sufficient amount of material and few compounds are nominated for further development, utilization of more expensive human primary or more complex 3D spheroid or organ on a chip model (on the microfluidic platform) are utilized when warranted. Furthermore, during preliminary exploratory toxicity studies (at LD or CS stage) if there is evidence for particular organ toxicity, organ-specific physiologically relevant predictive models are utilized to understand the in vivo animal toxicity translation to human. Therefore, it is essential to understand the impact of different in silico or in vitro assays predictors associated with different organ toxicities in order to implement them at the correct stage of the drug development.

The liver, kidney, and heart have been recognized as three major organ systems that contribute to costly late-stage drug attrition.⁴⁸ Therefore, there has been an increased need to develop various predictive approaches that can identify drug safety liabilities for these organs during the drug design and preclinical stages of the drug development process. For example, various predictive models based on the physicochemical properties of a drug or its effect on in vitro toxicity assays to predict the human hepatotoxicity potential of a drug.^{14, 24} These reported models have limitations as they utilized either physicochemical properties or in vitro assays alone. Furthermore, most of the models have been developed to predict only human clinical hepatotoxicity. Although more recent work has shown the value of integrating physicochemical properties along with in vitro assays of relevance for predicting hepatotoxicity,³⁵ there is a significant gap in the literature for predictive models of nephrotoxicity and cardiotoxicity based on combined approaches that integrate physicochemical properties with in vitro assay results.

In this study, multivariable logistic regression models were developed for predictions of hepatotoxicity, nephrotoxicity, and cardiotoxicity. The present study used basic physicochemical properties (hydrogen bond donor & acceptor count, Fsp³, cLogP, TPSA, MW, and Cmax values), and the in vitro toxicity assay results (THLE cytotoxicity assay, glucose/galactose assays, and RST mitochondrial inhibitory/uncoupling assays) for 215 pharmaceutical drugs. These assays represent large-scale screening assays in use either generally or on an 'ad hoc' basis at the drug design and preclinical stages of the drug development process.

Hepatotoxicity

Our study concluded that, based on the hepatotoxic predictive model, if a drug were to be positive in THLE cell viability assay (IC₅₀ values less than 100 μ M) or caused mitochondrial toxicity via mitochondrial inhibition (IC₅₀ values less than 100 nmoles/mg

of protein) or had clinical C_{max} value of greater than 1 μ M, it was 2, 6.2 or 7.5 times more likely to cause liver injury respectively. Combining these separate descriptors in a single hepatotoxicity model had a ROC-AUC value of 0.78, suggesting that these three descriptors were most influential in separating hepatotoxic drugs from drugs with no known organ toxicity. For hepatotoxicity predictions, using this dataset, cytotoxicity, mitochondrial inhibition, and clinical C_{max} were stronger predictors than the other physicochemical properties analyzed. Systemic exposure, as measured by C_{max}, and followed by evidence of mitochondrial inhibition, were influential descriptors, but are known or testable at different times during the drug development process compared to physicochemical properties. Although cLogP and TPSA values have been used for general toxicity prediction by Hughes et al., they provided a poorer model fit with an AUC-ROC value of only 0.576 (as shown in figure 2A).¹⁰ Leeson reported that low Fsp³ values needed to be combined with high lipophilicity and applied dose values to be useful for predicting “Most DILI” drugs.¹⁴ However, as an individual descriptor, Fsp³ provided a poorer model fit for this dataset with an AUC-ROC value of only 0.573 (as shown in figure 2A) and did not add as much additional value as the combination of C_{max}, THLE cytotoxicity, and mitochondrial inhibitor descriptors. Differences between these two models may be driven by differences in the analysis approach as optimized physicochemical cut-off values for applied dose, cLogP, and Fsp³ values were developed and used by Leeson for individual ionization states based on an extremely larger dataset compared to our study.¹⁴

We also investigated the use of our in-house ECCS classification system for hepatotoxicity prediction. To our knowledge, this is the first study that attempted to use the ECCS class for the prediction of hepatotoxicity. However, unlike BDDCS classification, ECCS classification, based on our dataset, were not able to add value to predict hepatotoxic drugs.¹⁵⁻¹⁷ As stated earlier, ECCS primarily predicts the rate-

determining clearance mechanism, while BDDCS predicts the extent of elimination and solubility. Although BDDCS classification appears more useful for understanding and categorizing different clearance mechanisms for predictions of hepatotoxicity,¹⁵⁻¹⁷ it may not be practical in drug design and selection to avoid the development of BDDCS class 2 drugs, as previously suggested.¹⁹ Many drugs in this category offer the intended therapeutic benefit with a low incidence of clinical hepatotoxicity issues due to low (≤ 100 mg) applied dose (e.g., montelukast, losartan, telmisartan, nifedipine, simvastatin, and pioglitazone).

Nephrotoxicity

Based on the nephrotoxicity predictive model, it was concluded that if a drug had a clinical C_{max} greater than 1 μ M or inhibits mitochondrial respiration at IC₅₀ less than 100 nmoles/mg of protein, or has hydrogen bond acceptor atoms greater than 7, it is 5.8, 6.4 or 15.9, times more likely to cause kidney injury, respectively. Conversely, drugs that had a TPSA ≥ 75 Å² and a MW < 400 Da, were 79% and 71% less likely to cause kidney injury, respectively. The nephrotoxic model had a ROC-AUC value of 0.844, indicating that the descriptors were able to separate nephrotoxic drugs from the no organ toxic drugs. Consequently, for the nephrotoxicity prediction, physicochemical properties had much higher significance based on odds ratios that were either highly correlated with increased (hydrogen bond acceptor ≥ 7) or decreased (TPSA > 75 Å² and a MW < 400 Da) correlation to nephrotoxicity compared with clinical C_{max} and mitochondrial inhibition assays. This finding is consistent with literature that correlates highly toxic compounds with more hydrogen bond acceptor atoms.⁴⁹⁻⁵¹ It is suggested that the compounds with more hydrogen bond acceptor atoms are highly reactive.⁴⁹ Drugs with high hydrogen bond acceptor atom may be more reactive at low concentrations through interactions with enzymes or cellular receptor macromolecules.⁴⁹ To our knowledge, there are no studies that associated high

hydrogen bond acceptor atom with nephrotoxicity. This study found that this physicochemical property exceeded clinical Cmax exposure as a predictor in terms of the magnitude of its odds ratio. There have been previous publications that demonstrate the association of MW(≥ 400 Da), TPSA ($< 75\text{\AA}^2$), and Cmax ($> 1\text{ }\mu\text{M}$) with toxic drugs.^{10, 13, 39, 49, 52} Based on our dataset, the TPSA combined with higher Cmax values showed the importance of predicting kidney injury. Out of all the in vitro assays used here, only RST mitochondrial inhibition assay showed the importance of predicting kidney injury. However, in our opinion, assessing other in vitro approaches may be necessary to better stratify nephrotoxicity risks, given that our first-tier cytotoxicity assays are based on liver-derived cell lines (THLE and HepG2). For better predictions of nephrotoxicity, it may be more important to use primary cultures of renal cells or 3 dimensional (3D) organoids that would have a correct balance of renal uptake and secretion transporters.⁵³ Lin et al. reported that organ-specific toxicity (liver, kidney, and heart) was not better predicted using the corresponding organ-derived immortalized cell lines.³⁰ Factors such as drug accumulation within specific organ cell types, off-target effects, organ-specific drug metabolism, and clearance may need to be assessed since these factors have a significant impact on specific organ toxicity.³⁰

Interestingly, our study concluded that if a drug is in ECCS class 4, where metabolism is primarily through renal clearance (low permeability bases and neutrals), it was four times more likely to cause kidney injury than other ECCS class drugs. On the other hand, ECCS class 2 drugs were 77% less likely to cause kidney injury. Hepatic metabolism is the primary clearance mechanism of ECCS class 2 drugs (high permeability bases and neutrals). Class 4 drugs are more likely to be distributed and accumulate within the kidney, making them more likely to damage proximal tubule kidney cells. Again, due to their primary route of clearance, either kidney or liver, different classes of drugs tend to accumulate in different organs leading to different

organ damage. Our study was successfully able to utilize the ECCS class for differentiating nephrotoxic drugs from drugs with no known organ toxicity.

Cardiotoxicity

Based on the univariate cardiotoxicity predictive model, it was concluded that if a drug is positive in galactose assay (IC_{50} values less than 100 μ M) and has clinical C_{max} values greater than 1 μ M, it was 2.7 and 1.8 times more likely to cause cardiotoxicity, respectively. Similarly, if a drug has hydrogen bond donor atoms less than 5, it was 72% less likely to cause heart injury. The cardiotoxic model had a ROC-AUC value of 0.65, indicating a less robust model fit compared to the hepatotoxicity and nephrotoxicity models, and the cardiotoxic predictive model was less able to separate the cardiotoxic drugs from the no organ toxic drugs. Heart organ injury has both functional (conduction) and structural components. The compounds selected for our analysis were a mixture of structural and functional cardiotoxicants. Clement et al. reported the more holistic approach of integrating electrophysiology, contractility, and cell movement/beating for functional assessment along with various structural assessments for cardiotoxicity.⁵⁴ This holistic approach may hold increased value for predicting cardiotoxic drugs as they are based on more relevant functional parameters of cell health and function.

Important pieces of information become available at different times during the drug discovery, design, and development process. Known or calculated physicochemical properties are determined at the drug design stage such that these descriptors can be utilized in predictive models of safety. Likewise, drug effects in mechanistic in vitro assays can only be determined when drug product is made. While estimates of applied dose and clinical exposure are made close to candidate nomination, these values are validated during Phase 2 clinical studies. It was important for us to determine which descriptors could be used based on the predictive

odds ratio for each organ model and consider when such information would be available. Predictions of hepatotoxicity required information that was available after compound design, making a predictive model hard to implement in that space. Most predictive hepatotoxicity models require the use of clinical dose or systemic exposure (C_{max}) with physicochemical property space predictors to be useful. In advance of this, measurements of mitochondrial inhibition appear to add some value. Both mitochondrial inhibition of oxidative phosphorylation and drug plasma concentration (C_{max}) were two descriptors that were useful in predicting both liver and kidney toxicity. This finding agreed with previous studies by Porceddu et al., who used multiparametric mechanistic endpoints of mitochondrial dysfunction combined with drug plasma concentration to predict DILI with high sensitivity and high positive predictive values.²⁴ Of even more significance is the finding that physicochemical properties could be used for predictions of nephrotoxicity, with either highly correlative positive (hydrogen bond acceptors ≥ 7 , ECCS class 4) or negative (TPSA ≥ 75 Å², MW < 400 Da, ECCS class 2) predictions. Such descriptive elements could be used in the drug design process for compound design and selection, although ECCS class determinations still require drug bulk for passive membrane permeability. None of the drug attributes appeared to help define the cardiotoxic risks of drugs.

1.6 LIMITATION

This data set utilized drugs where in vitro data results are available, which leads to a relatively small number of drugs evaluated in this study. Additionally, this may introduce selection bias since the drugs used in this study may not be random. A small sample size may lead to a large confidence interval in the calculation of the odds ratio, and the lack of statistical power may result a type II error. The whole dataset was used to build models, therefore further studies utilizing these models with additional data will be required. Furthermore, due to the small number of drugs utilized to build predictive models, the findings may not be generalized to all the drugs. The result of this study should only be applied to the physicochemical properties of the drugs evaluated here; the drugs that fall outside of the physicochemical property space evaluated here may hold different results. Finally, the result of this study will not be relevant to biotherapeutic drugs.

To employ logistic regression modeling, drugs were only classified as having one type of toxicity; many drugs in the dataset have been associated with multiple organ toxicities. Additionally, increased risk or association does not imply causation. Further study is necessary to evaluate the predictive nature of these factors using more robust methodologies or in vitro/in vivo mechanistic toxicity studies. Finally, other factors beyond physicochemical properties or in vitro assays can cause toxicity in vivo. For instance, in vitro assays lack metabolic functions, appropriate receptors and transporters, and cellular architecture. Moreover, drug-accumulation within specific organ cell types, off-target effects, organ-specific drug metabolism, and clearance may lead to organ toxicity. Measuring these factors was beyond the scope of this study; however, their inclusion would likely have enhanced the models.

1.7 CONCLUSION

The physicochemical properties of a drug influence various parameters such as absorption, clearance, the volume of distribution, total plasma concentration, and dosing frequency. Measurements of passive membrane permeability along with RST mitochondrial inhibition are useful and relatively affordable compared with in vivo animal toxicity studies. Therefore, we recommend a blended approach of utilizing readily calculated physicochemical properties combined with in vitro toxicity assessments to select molecules with less potential for clinical organ toxicity for the liver and kidney. As suspected, different models are needed for more accurate predictions of hepatic and nephrotoxic drugs. The multivariable analysis showed that drug plasma concentration combined with mitochondrial dysfunction assays was most useful in the prediction of hepatotoxic drugs. However, for nephrotoxic drugs, the physicochemical properties of the drugs such as TPSA and hydrogen acceptor atoms combined with drug plasma concentration, and mitochondrial dysfunction assay were most useful. Mitochondrial dysfunction assay and drug plasma concentration are two descriptors that were useful for predicting organ toxicity for both liver and kidney.

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1.9 TABLES AND FIGURES

Table 1. Literature references and the threshold utilized for the physicochemical properties and the in vitro assays for univariate and multivariable logistic regression modeling.

Physicochemical properties and in vitro assay thresholds utilized for univariate and multivariable logistic regression modeling		Literature reference
Toxic (1)	Not Toxic (0)	
Hydrogen bond donor atoms ≥ 5	Hydrogen bond donor atoms < 5	39
Hydrogen bond acceptor atoms ≥ 7	Hydrogen bond acceptor atoms < 7	39
$Fsp^3 \leq 0.28$	$Fsp^3 > 0.28$	14
$cLogP \geq 3$	$cLogP < 3$	10, 12, 13, 27, 52
$TPSA < 75 \text{ \AA}^2$	$TPSA \geq 75 \text{ \AA}^2$	10, 12, 13, 27, 52
$MW \geq 400 \text{ Da}$	$MW < 400 \text{ Da}$	14, 39
$C_{max} > 1 \text{ }\mu\text{M}$	$C_{max} \leq 1 \text{ }\mu\text{M}$	10, 12, 14, 41, 55
THLE assay $IC_{50} \leq 100 \text{ }\mu\text{M}$	THLE assay $IC_{50} > 100 \text{ }\mu\text{M}$	13, 27
Glucose assay $IC_{50} \leq 100 \text{ }\mu\text{M}$	Glucose assay $IC_{50} > 100 \text{ }\mu\text{M}$	13, 27
Galactose assay $IC_{50} \leq 100 \text{ }\mu\text{M}$	Galactose assay $IC_{50} > 100 \text{ }\mu\text{M}$	13, 27
Glucose/galactose ratio ≥ 2	Glucose/galactose ratio < 2	27
RST Uncoupling assay $IC_{50} \leq 100 \text{ nmol/mg of protein}$	RST Uncoupling assay $IC_{50} > 100 \text{ nmol/mg of protein}$	27
RST Inhibitory assay $IC_{50} \leq 100 \text{ nmol/mg of protein}$	RST Inhibitory assay $IC_{50} > 100 \text{ nmol/mg of protein}$	27

Table 2. Characteristics of physicochemical properties and in vitro assay results of organ toxicity drugs.

	Hepatotoxic (N=70)	Nephrotoxic (N=45)	Cardiotoxic (N=44)	No Organ toxic (N=56)
Independent Predictors	Mean $\pm$ SEM			
Hydrogen donor count	1.942 $\pm$ 0.20	5.844 $\pm$ 0.864*	1.84 $\pm$ 0.22	2.785 $\pm$ 0.458
Hydrogen acceptor count	3.957 $\pm$ 0.31	8.02 $\pm$ 0.85*	3.63 $\pm$ 0.45	3.96 $\pm$ 0.45
Fsp3	0.364 $\pm$ 0.031	0.423 $\pm$ 0.047	0.387 $\pm$ 0.0246	0.40 $\pm$ 0.03
ClogP	2.688 $\pm$ 0.303	0.90 $\pm$ 0.71	3.81 $\pm$ 0.238*	1.72 $\pm$ 0.423
TPSA	80.043 $\pm$ 5.17	166.6 $\pm$ 18.34*	65.12 $\pm$ 6.49	84.72 $\pm$ 11.43
MW	351.6 $\pm$ 16.74	508.5 $\pm$ 47.57*	347.7 $\pm$ 17.54	340.8 $\pm$ 23.93
Cmax	13.43 $\pm$ 2.83*	13.76 $\pm$ 3.34*	5.14 $\pm$ 1.85	7.20 $\pm$ 2.54
THLE cell viability assay	181.9 $\pm$ 14.14*	221.6 $\pm$ 16.32	169.9 $\pm$ 17.63*	230.2 $\pm$ 13.83
Glucose Assay	237.2 $\pm$ 5.44	256.0 $\pm$ 12.78	209.9 $\pm$ 17.37	232.0 $\pm$ 13.85
Galactose Assay	216.5 $\pm$ 12.81	237.6 $\pm$ 14.67	193.2 $\pm$ 18.52	229.0 $\pm$ 16.04
RST Inhibitory Assay	223.6 $\pm$ 13.8*	264.4 $\pm$ 11.52	263.3 $\pm$ 13.11	282.7 $\pm$ 8.45
RST Uncoupler Assay	216.9 $\pm$ 14.91*	266.5 $\pm$ 11.82	244.5 $\pm$ 14.88	257.8 $\pm$ 11.58
*p < 0.05				

Table 3A. Odds of hepatotoxic risk associated with physicochemical properties and in vitro assays.

Univariate Logistic Regression Model				
Hepatotoxic Drugs				
	Independent Variables	Odds Ratio	95% CI	p-value
Physicochemical Properties	Hydrogen donor count	0.58	0.202-1.671	0.3131
	Hydrogen acceptor count	0.926	0.293-2.929	0.8955
	Fsp ³	1.933	0.905-4.129	0.0887*
	cLogP	1.24	0.613-2.51	0.5498
	TPSA	0.658	0.324-1.338	0.2479
	MW	1.516	0.681-3.373	0.3078
	Cmax	5.455	2.529-11.76	<0.0001*
In vitro assays	THLE cell viability assay	2.302	1.035-5.120	0.0409*
	Galactose assay	1.363	0.564-3.294	0.4916
	Glucose/galactose ratio	6.111	0.729-51.233	0.0952*
	RST inhibitory assay	5.957	1.914-18.536	0.0021*
	RST uncoupling assay	1.875	0.817-4.301	0.1378*
*All the variables where p-values are less than 0.2 were included to build the multivariable model				

Table 3B. Odds of nephrotoxic risk associated with physicochemical properties and in vitro assays.

Univariate Logistic Regression Model				
Nephrotoxic Drugs				
	Independent Variables	Odds Ratio	95% CI	p-value
Physicochemical Properties	Hydrogen donor count	4.177	1.658-10.526	0.0024*
	Hydrogen acceptor count	8.712	3.114-24.377	<0.0001*
	Fsp ³	1.66	0.713-3.861	0.2397
	cLogP	0.56	0.246-1.274	0.167*
	TPSA	0.174	0.071-0.430	0.0002*
	MW	3.78	1.611-8.871	0.0023*
	Cmax	4.118	1.784-9.502	0.0009*
In vitro assays	THLE cell viability assay	0.917	0.348-2.419	0.861
	Galactose assay	0.708	0.236-2.123	0.5373
	Glucose/galactose ratio	3.929	0.394-39.127	0.243
	RST inhibitory assay	2.811	0.788-10.029	0.1113*
	RST uncoupling assay	0.754	0.266-2.135	0.5944
*All the variables where p-values are less than 0.2 were included to build the multivariable model				

Table 3C. Odds of cardiotoxic risk associated with physicochemical properties and in vitro assays.

Univariate Logistic Regression Model				
Cardiotoxic Drugs				
	Independent Predictors	Odds Ratio	95% CI	p-value
Physicochemical Properties	Hydrogen donor count	0.249	0.051-1.217	0.0858*
	Hydrogen acceptor count	0.397	0.0076-2.071	0.2728
	Fsp3	0.607	0.231-1.599	0.3127
	ClogP	1.488	0.673-3.29	0.3263
	TPSA	1.494	0.652-3.419	0.3425
	MW	1.387	0.566-3.401	0.4745
	Cmax	1.731	0.751-3.989	0.1979*
In vitro assays	THLE cell viability assay	1.897	0.777-4.628	0.1597*
	Galactose assay	2.629	1.048-6.591	0.0393*
	Glucose/galactose ratio	7.05	0.792-62.733	0.0799*
	RST inhibitory assay	2.459	0.671-9.014	0.1744*
	RST uncoupling assay	1.364	0.528-3.522	0.5217
*All the predictors where p-values are less than 0.2 were included to build the multivariate model				

Table 4. Comparison of hepatotoxic, cardiotoxic, and nephrotoxic drugs with the Extended Clearance Classification System.

Extended Clearance Classification System Properties		Organ toxicity drugs		
ECCS Class	Metabolism Clearance	Hepatotoxic (p-value)	Nephrotoxic (p-value)	Cardiotoxic (p-value)
Class 1A	Systemic Clearance	NS	NS	NS
Class 1B	Systemic Clearance (Transporter mediated uptake)	NS	NS	NS
Class 2	Metabolism clearance	NS	0.0007	NS
Class3A	Renal clearance	NS	NS	NS
Class3B	Transporter mediated hepatic uptake or renal clearance	NS	NS	NS
Class4	Renal clearance	NS	0.001	NS
NS - Not Significant; p > 0.05				

Table 5 Univariate logistic regression model (including odds ratio estimates, 95% CI, and p-values) for hepatotoxic, nephrotoxic, and cardiotoxic drugs using the Extended Clearance Classification System.

	Hepatotoxic drugs		
ECCS Class	Odds Ratio	95% CI	p-value
Class 1	2.46	0.25-24.35	0.44
Class 2	0.93	0.46-1.90	0.84
Class3A	>999.9	<0.001->999.9	0.98
Class3B	>999.9	<0.001->999.9	0.99
Class 4	0.93	0.45-1.92	0.83

	Nephrotoxic drugs		
ECCS Class	Odds Ratio	95% CI	p-value
Class 1	<0.001	<0.001->999.9	0.98
Class 2	0.225	0.095-0.535	0.0007
Class3A	>999.9	<0.001->999.9	0.98
Class3B	<0.001	<0.001->999.9	0.99
Class 4	4.1	1.77-9.52	0.001

	Cardiotoxic drugs		
ECCS Class	Odds Ratio	95% CI	p-value
Class 1	<0.001	<0.001->999.9	0.99
Class 2	1.66	0.72-3.84	0.23
Class3A	NA*	NA*	NA*
Class3B	<0.001	<0.001->999.9	0.99
Class 4	0.70	0.3-1.63	0.41

*NA- Not applicable

Figure 1A: Adjusted odds ratio (including 95% Confidence Interval (CI)) of hepatotoxic risk associated with physicochemical properties and in vitro assays

Figure 1B: Adjusted odds ratio (including 95% CI) of nephrotoxic risk associated with physicochemical properties and in vitro

Figure 1C: Adjusted odds ratio (including 95% CI) of cardiotoxic risk associated with physicochemical properties and in vitro assays

Figure 2A: Hepatotoxic model performance evaluation via ROC-AUC, a plot of sensitivity Vs. 1-specificity.

Figure 2B: Nephrotoxic model performance evaluation via ROC-AUC, a plot of sensitivity Vs. 1-specificity.

Figure 2C: Cardiotoxic model performance evaluation via ROC-AUC, a plot of sensitivity Vs. 1-specificity.

MANUSCRIPT II

**Title: Hepatotoxicity reports in the FDA adverse event reporting
database: a comparison of drugs that cause injury via mitochondrial or
other mechanisms**

Payal Rana^{*a, b}, Michael D. Aleo, Xuerong Wen^b, Fatemeh Akhlaghi^b and

Stephen Kogut^b

*^aDrug Safety Research & Development, Pfizer, Eastern Point Road, Groton, CT
06340, USA*

^bUniversity of Rhode Island, College of Pharmacy, Kingston, RI 02881, USA

*Corresponding author: Address: Payal Rana, Drug Safety R&D, Eastern Point Road,
Groton, CT 06340, USA; email: payal.m.rana@pfizer.com

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2.1 ABSTRACT

Drug-induced liver injury (DILI) is a leading reason for preclinical safety attrition and post-market drug withdrawals. Drug-induced mitochondrial toxicity has been shown to play an essential role in various forms of DILI, especially in idiosyncratic liver injury. This study examined liver injury reports submitted to the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) for drugs associated with hepatotoxicity via mitochondrial mechanisms compared with non-mitochondrial mechanisms of toxicity. The frequency of hepatotoxicity was determined at a group level and individual drug level. A Reporting Odds Ratio (ROR) was calculated as the measure of effect. Between the two DILI groups, reports for DILI involving mitochondrial mechanisms of toxicity had a 1.43 (95% CI 1.42-1.45; $p < 0.0001$) times higher odds compared to drugs associated with non-mitochondrial mechanisms of toxicity. Antineoplastic, antiviral, analgesic, antibiotic, and antimycobacterial drugs were the top 5 drug classes with the highest ROR values. Although the top 20 drugs with the highest ROR values included drugs with both mitochondrial and non-mitochondrial injury mechanisms, the top 4 drugs (ROR values >18 : benzbromarone, troglitazone, isoniazid, rifampin) were associated with mitochondrial mechanisms of toxicity. The major demographic influence for DILI risk was also examined. There was a higher mean patient age among reports for drugs that were associated with mitochondrial mechanisms of toxicity (56.1 ± 18.33 (SD)) compared to non-mitochondrial mechanisms (48 ± 19.53 (SD)) ($p < 0.0001$), suggesting that age may play a role in susceptibility to DILI via mitochondrial mechanisms of toxicity. Univariate logistic regression analysis showed that reports of liver injury were 2.2 (odds ratio: 2.2, 95% CI 2.12-2.26) times more likely to be associated with older patient age, as compared with reports involving patients less than 65 years of age. Compared to

males, female patients were 37% less likely (odds ratio: 0.63, 95% CI 0.61-0.64) to be subjects of liver injury reports for drugs associated with mitochondrial toxicity mechanisms. Given the higher proportion of severe liver injury reports among drugs associated with mitochondrial mechanisms of toxicity, it is essential to understand if a drug causes mitochondrial toxicity during preclinical drug development when drug design alternatives, more clinically relevant animal models, and better clinical biomarkers may provide a better translation of drug-induced mitochondrial toxicity risk assessment from animals to humans. Our findings from this study align with mitochondrial mechanisms of toxicity being an important cause of DILI, and this should be further investigated in real-world studies with robust designs.

2.2 INTRODUCTION

Severe drug-induced liver injury (DILI) is a rare, potentially life-threatening adverse event with an incidence of 10-15 cases per 10,000 to 100,000 patients per year.¹⁻⁴ The clinical profile of DILI is challenging to diagnose as it can mimic almost any type of liver disease^{5, 6} and mild, asymptomatic transaminase elevations may mimic those caused by diet.⁷ Prediction of liver injury remains a challenge for the pharmaceutical industry, regulators, and clinicians.⁵ DILI symptoms range from non-specific mild elevations in liver enzymes (aminotransferases) to severe liver illnesses such as cirrhosis or acute hepatitis^{5, 6} and there is no specific biomarker that links drug exposure as the contributing cause of liver injury.⁸

There are two types of DILI: intrinsic (i.e., dose-dependent) and idiosyncratic (i.e., dose-independent).⁶ A well-known example of intrinsic DILI is acetaminophen-induced liver injury, as it is dose-dependent, the onset is hours to days, and predictable.⁹ On the other hand, idiosyncratic DILI is not dose-dependent, occurs in a small portion of drug-exposed individuals, its onset is days to weeks, and unpredictable.⁹ Idiosyncratic DILI is highly dependent on environmental and host factors that alter the susceptibility of individual patient responses to the drug. Hamilton et al. suggested that DILI is the convergence of three influencing risk factors: host factors, environmental factors, and drug-specific factors.¹⁰ Host-related risk factors include genetics, ethnicity, gender, comorbidities, alterations in drug transport, drug clearance capabilities, age, and mitochondrial function variability.¹⁰ Environmental risk factors include lifestyle, viral co-infection, co-prescriptions, diet, and alcohol consumption.¹⁰ Finally, drug-specific risk factors include the relationship of applied dose (exposure) and chemical structure with reactive metabolite formation, mitochondrial dysfunction, and lipophilicity.¹⁰⁻¹² Therefore, the mechanism of DILI is a highly complex process that varies from patient to patient and is influenced by host,

environmental, and drug-specific factors that influence the liver's ability to adapt and recover from an injury caused by a drug.⁸

In recent years, drug-induced mitochondrial toxicity has been shown to play an essential role in intrinsic and idiosyncratic DILI. Many medications from different drug classes, such as antidiabetic, antilipidemic, antiviral, antibiotic, anti-inflammatory, and antipsychotic agents have toxicities mediated by mitochondrial mechanisms, which may contribute to DILI.¹³ Mitochondrial toxicants affect mitochondrial homeostasis by numerous mechanisms such as oxidative stress, inhibition or uncoupling of respiratory complexes of electron transport chain, impairment of mitochondrial replication or promoting mitochondrial DNA damage.¹⁴ Drug-induced mitochondrial toxicity is difficult to be detected in standard preclinical animal testing models and requires specific studies to examine disruptions in liver energy status.¹⁵ Only recently, there has been the development of clinical biomarkers specific for mitochondrial dysfunction in disease¹⁶ and DILI¹⁷ beyond changes in blood lactate. With these inadequacies, a drug candidate can enter human clinical trials only to fail for evidence of mitochondrial toxicity.^{18, 19} Examples of non-mitochondrial toxicity mechanisms that drive DILI are a generation of reactive metabolites, activation of cell death pathways, activation of innate or adaptive immune response pathways, or disruption of cellular homeostasis.²⁰ This study evaluates the frequency of reports of hepatotoxicity injury in drugs that cause DILI in mitochondrial mechanisms compared to the non-mitochondrial mechanisms.

Patient demographics influence risk or susceptibility for DILI. Boelsterli and Lim (2007) indicated that older age and female gender were important susceptibility factors for DILI; however, the reasons were still unknown.²¹ There are no clinical studies that link the sensitivity of the female gender to DILI caused by mitochondrial dysfunction. Amacher et al. indicated that women are more susceptible to DILI than men.²² Several hypotheses were proposed to explain gender differences in susceptibility, including

pharmacokinetic or pharmacodynamic differences, interactions of sex hormones with signaling molecules, and a difference in immune system responses.²² Similarly, it is believed that older adults are more susceptible to DILI caused by mitochondrial dysfunction. The review published by Will et al.¹³ indicated that the most commonly used prescription and over-the-counter (OTC) medications for geriatric patients, such as antilipidemic, pain, and heartburn medications, had published reports of toxicities linked to mitochondrial dysfunction.¹³ As the United States' elderly population is growing rapidly, identifying and addressing risk factors of DILI, where mitochondrial dysfunction may play a substantial role in adverse events, will be beneficial to this vulnerable patient population. Therefore, in this study, we evaluated the patient age and gender associated with DILI reports (measured by reporting odds ratio) for hepatotoxicants with mitochondrial and non-mitochondrial injury mechanisms.

Given that mitochondrial dysfunction is a common characteristic of drugs that cause liver injury, a better understanding of the association between the probability of liver injury induced by drugs that are mitochondrial toxicants and the influence of patient's age and gender would be beneficial for clinicians and drug developers. If a drug is associated with mitochondrial mechanisms of liver injury, clinicians could incorporate mitochondrial injury-specific biomarkers into clinical trials.²³⁻²⁵ Additionally, the development of clinically relevant animal models or study designs may provide drug-induced mitochondrial toxicity risk translation from animals to humans.²⁴

This study investigated liver injury reports submitted to the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) and compared the frequency of reports between drugs that can cause hepatotoxicity via mitochondrial mechanisms and those without mitochondrial mechanisms of toxicity. Additionally, we determined if there were age and gender differences associated with DILI reports involving drugs with or without mitochondrial toxicities.

2.3 METHODS

Study design:

Data Source:

The United States Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) database is the largest national repository of spontaneous drug event reports, having accumulated over 28 million adverse events reports. Healthcare professionals, patients, manufacturers, and lawyers can submit potential drug-induced adverse events of small and large therapeutic (biologics) classes and medical devices to the FAERS database. The FAERS database has a vital role in post-market drug surveillance in terms of detection and characterization of drug and device-related adverse events.

We extracted adverse event reports from the FAERS database for the timeframe from January 1998 to May 2019. In this study, the reports included severe adverse events, such as hospitalization, disability, or death. The types of reports were classified by FAERS as direct, expedited, or periodic. Direct reports were submitted to FDA from consumer or health care professionals; whereas, expedited reports were sent from the manufacturer within 15 days of severe adverse events occurrence not included in the product label.²⁶ Adverse drug event reports considered periodic were submitted from manufacturers, included in the label, and sent to the FDA quarterly or annually.²⁶ The main selection criterion was “primary suspect” drugs. “Secondary suspect” drugs were excluded because of the greater uncertainty of the association between the drug and the reported adverse events. FAERS reports were coded using the MedDRA (Medical Dictionary for Regulatory Activities) terms for DILI.²⁷ Although DILI has complex clinical symptoms, there has been documentation for the utilization

of the FAERS database to investigate emerging DILI adverse events for newly marketed drugs.⁸

Inclusion/exclusion criteria:

Drugs that cause liver injury have been annotated using the United States National Center for Toxicological Research Liver Toxicity Knowledge Base (NCTR-LTKB), which utilizes hepatotoxic descriptions from the FDA-approved drug labeling regulatory documents as well as evaluating causality evidence in the literature.²⁸ This database was created by the FDA to help clinicians, toxicologists, and researchers access information on DILI annotation of various drugs.^{28, 29} NCTR-LTKB serves as a centralized source to study the mechanism of DILI and the development or validation of emerging biomarkers and predictive models.²⁹ This is the largest publicly available annotated DILI dataset containing three groups based on their potential to cause liver toxicity (Most DILI concern-(192 drugs), Lesser DILI concern-(278 drugs), and No-DILI concern (312 drugs)) with confirmed causal evidence connecting a drug to liver injury.²⁸ The FAERS database uses FDA drug labeling information for the classification of drugs according to their potential to cause DILI. This study utilized drugs with "most-DILI concern," which were defined based on hepatotoxicity resulting in market withdrawal (in US and ex-US), black box warning, or high severity of liver injury noted as part of the warning and precautions label.²⁸⁻³⁰ Therefore, both mitochondrial and non-mitochondrial mechanisms of toxicity groups are associated with severe hepatic injury.

The study drugs represented various drug classes such as analgesic, anti-inflammatory, antidepressant, antibiotic, antidiabetic, and antineoplastic agents. Most of these drugs had been withdrawn, have boxed warnings, or have warnings and precautions for liver injury in their prescribing labels. The details of DILI severity categories based on the DILI description are included in the drug labeling: severity

level 1; steatosis, level 2; cholestasis and steatohepatitis, level 3; liver aminotransferases increase, level 4; hyperbilirubinemia, level 5; jaundice, level 6; liver necrosis, level 7; acute liver failure, and level 8; hepatotoxicity.²⁸ Examples of withdrawn drugs include bromfenac, chlorzoxazone, troglitazone, and trovafloxacin, which have been assigned a severity level of 8, suggesting evidence of fatal hepatotoxicity. Drugs such as bosentan, danazol, ketoconazole, nefazodone, tolcapone, and valproic acid have box warning in their product labeling and have severity categories ranging from 3 to 8.

Classification of Drugs as Mitochondrial Toxicants:

Drugs with mitochondrial mechanisms of toxicity were defined by literature evidence of mitochondrial injury mechanisms (yes or no) of in vitro (e.g., cellular production of reactive oxygen species via oxidative stress, inhibition or uncoupling of respiratory complexes of electron transport chain, induction of mitochondrial membrane permeability transition pore, or mitochondrial DNA damage) or in vivo mitochondrial toxicity from animal studies. Our classification was based on the parent drug-induced toxicity and not the metabolite. Possible drug effects on mitochondrial biogenesis or respiratory capacity were not considered. Drugs with the non-mitochondrial mechanisms of toxicity were defined by literature evidence of the absence of mitochondrial injury mechanisms or lack of evidence. It is important to note that 8.2% of drugs had no literature evidence of the type of toxicity mechanism, meaning it could be a mitochondrial or non-mitochondrial mechanism. Furthermore, the non-mitochondrial mechanisms of toxicity drugs are not proven to have non-mitochondrial mechanisms. For these drugs, there is simply no evidence of mitochondrial mechanisms of toxicity information that is publicly available yet.

Outcome:

We determined the number of reports for hepatotoxicity at a group level and an individual drug level using the Reporting Odds Ratio (ROR). As shown in Table 1, we calculated total hepatotoxicity and all other adverse events for both the DILI groups. For ROR calculations, numerators are derived by multiplying the hepatotoxicity reports for mitochondrial mechanisms of toxicity drug group with all other adverse event reports of non-mitochondrial mechanisms of toxicity per drug group. The denominator is calculated by multiplying hepatotoxicity adverse event reports of non-mitochondrial mechanisms of toxicity with all the adverse events reported for mitochondrial mechanisms of toxicity per drug group.³¹ Therefore, the ROR for drugs associated with mitochondrial mechanism of toxicity was 1.43 ($\text{ROR} = (40,343 \times 1,342,486) / (586,989 \times 64,358) = 1.43$).

We also examined the RORs at the individual drug level, as shown in Table 2. A case (hepatotoxicity reports) or non-case (all other adverse event reports) disproportionality approach was utilized by creating a two-by-two contingency table, as demonstrated below using acetaminophen as an example³¹ During this timeframe, a total of 383,540 hepatotoxicity reports and a total of 27,852,908 adverse event counts of any drug type were collected. For ROR calculations, numerators are derived by multiplying the hepatotoxicity reports for a drug of interest with all other adverse events reports. The denominator is calculated by multiplying hepatotoxicity adverse event reports of all other drugs (excluding acetaminophen) with all the adverse events reported with a drug of interest³¹ ($\text{ROR for Acetaminophen} = (8,509 \times 27,852,908) / (51,732 \times 383,540) = 11.94$). Within the timeframe, a ROR higher than 1 for a drug indicates a higher proportion of severe liver injury reports for a drug of interest than all the other drug reports in the database. In this case, acetaminophen was associated

with proportionally more reports for serious liver adverse events than other drugs in the database.

Association of age, gender, and other factors in two groups of DILI (mitochondrial and non-mitochondrial mechanisms of toxicity)

Mitochondrial function declines with aging;¹³ therefore, we evaluated if reports of severe DILI were disproportionately associated with older patient age, indicating potential susceptibility to DILI from mitochondrial mechanisms of toxicity. Furthermore, since gender may play an important role in the sensitivity of DILI, we also evaluated the frequency of reports according to patient gender. The mean and standard deviation (SD) of patient age were calculated and compared between DILI reports caused by drugs associated with mitochondrial mechanisms of toxicity and DILI reports associated with non-mitochondrial mechanisms. Patient age was dichotomized into ≤ 65 years or > 65 years for comparison. Other factors, including drug severity class, patient weight, report type, and label section, were examined in a descriptive analysis.

Statistical Analysis:

Descriptive statistics were used to compare the gender and age of reports for the DILI drug groups associated with mitochondrial mechanisms of toxicity and associated with non-mitochondrial mechanisms. The statistical significance of differences in categorical variables such as age group, DILI severity, drug label, and report type between two categories of DILI drugs was examined using the chi-square test. Whereas, differences in continuous variables such as mean patient age between the two categories of hepatotoxic drugs were compared with the two-tailed student's t-test for independent samples. The unadjusted association of age and gender with mitochondrial mechanisms of toxicity DILI group (against non-mitochondrial mechanisms of toxicity DILI group) was determined using univariate logistic regression

analysis. ROR calculations were carried out using a two-by-two contingency table using OpenEpi (version 3.01; Centers for Disease Control and Prevention), which calculates 95% CI and p-values via Taylor series.³² Chi-square tests were performed with SAS version 9.4 (SAS Institute, Cary, NC), and two-sided t-tests were performed at the 0.05 significance level via GraphPad Prism version 8 (La Jolla, CA). All statistical tests were two-sided with a significance level at 0.05.

2.4 RESULTS

We included 192 drugs classified as having the highest DILI risk (“Most-DILI concern”) via the NCTR-LTKB database. Out of these 192 drugs, 134 drugs had searchable FAERS reports, while the remaining 58 drugs were either withdrawn before our study period or were withdrawn from the European market before US approval. Therefore, the final data set contained 134 drugs, which were categorized as 56 drugs causing hepatotoxicity via mitochondrial mechanisms, and 78 drugs were classified as causing hepatotoxicity via non-mitochondrial mechanisms.

Table 3 indicates the characteristics of the event reports included in the study. A total of 104,701 adverse event reports were extracted from FAERS for the period spanning January 1998 to May 2019. Of these, 40,343 (38.5%) reports of hepatotoxicity were for drugs that were associated with mitochondrial mechanisms of toxicity, whereas 64,358 (61.5%) reports of hepatotoxicity were for drugs associated with non-mitochondrial mechanisms of toxicity. Furthermore, drugs were categorized based on the NCTR-LTKB severity classification. There was a statistically significant difference in DILI severity ($p < 0.0001$) between the two groups of DILI drugs (mitochondrial mechanisms compared to non-mitochondrial mechanisms). There was a 5.5 percentage point difference in reports for more severe DILI (liver failure/hepatotoxicity) for drugs associated with mitochondrial mechanisms of toxicity compared to non-mitochondrial mechanisms (76.3% compared to 70.8%, respectively, $p < 0.0001$). As shown in Table 3, the FAERS reports were classified based on the drug label section for liver injury; there was a statistically significant difference in drug labels ($p < 0.0001$) between the two groups of DILI drugs (mitochondrial mechanisms compared to non-mitochondrial mechanisms). Additionally, 24.6% of mitochondrial mechanisms of toxicity drugs had a box warning label as compared to 19.8% of non-mitochondrial mechanisms of toxicity drugs, and 64.9% of mitochondrial mechanisms

of toxicity drugs had warning and precautions label as compared to 79.1% of non-mitochondrial mechanisms of toxicity drugs. For drugs withdrawn due to hepatotoxicity, there were high numbers of reports ($n = 4227$, 10.5%) for drugs that are associated with mitochondrial toxicity mechanisms, compared to a lower number of reports ($n = 747$, 1.2%) for drugs with non-mitochondrial mechanisms of toxicity ($p < 0.0001$). Over 88% of reports were expedited, while the rest of the reports were either direct or periodic. In summary, there was a statistically significant difference between drug severity classification, label, and liver injury severity according to the drug's ability to cause toxicity through mitochondrial mechanisms.

We also examined patient bodyweight, but 79%-81% of the reports did not have this information documented. Among the 19%-21% of reports where the bodyweight data was present, the average difference between the two groups of DILI drugs (mitochondrial compared to non-mitochondrial mechanisms) was only 1.6 kg (68.6 ± 20.8 compared to 70.2 ± 23.4 ; $p < 0.0001$). In this case, the small p-value may be because the large sample size overpowered the comparison. As large numbers of the reports were missing bodyweight, further analysis was not performed. As shown in Table 3, a higher percentage of males were the subjects of hepatotoxicity reports via mitochondrial mechanisms compared to the subjects of reports involving hepatotoxicity via the non-mitochondrial mechanisms (49.1% compared to 37.8%, $p < 0.0001$). About 7% to 8.3% of reports were missing information about the patient's gender.

Table 3 presents the difference in the mean and distribution of age among the two groups. The patient's age was recorded in more than 71% of the reports from both the groups. As shown in Table 3, there was a statistically significant difference ($p < 0.0001$) between the mean age of patients with hepatotoxicity in drugs that are associated with mitochondrial mechanisms (56.1 ± 18.33 (SD)) compared to non-

mitochondrial mechanisms of toxicity (48 ± 19.53 (SD)). In other words, reports involving drugs associated with mitochondrial mechanisms of hepatic toxicity displayed a higher mean age than reports for drugs associated with non-mitochondrial mechanisms of hepatic toxicity.

Figure 2 indicates the ROR values of drugs associated with mitochondrial mechanisms of toxicity; benzbromarone, troglitazone, isoniazid, rifampin, and nimesulide had the highest ROR values in the group. Figure 3 indicates the ROR values of drugs associated with non-mitochondrial mechanisms of toxicity; telithromycin, gemtuzumab ozogamicin, mexiletine, dactomycin, and disulfiram had the highest ROR values in the group. Table 4 indicates the top 20 drugs with the highest ROR values in both groups of hepatotoxicants. The top 20 drugs with the highest ROR values included drugs with either mitochondrial or non-mitochondrial injury mechanisms. The top 4 drugs, which had ROR values higher than 18 (benzbromarone, troglitazone, isoniazid, rifampin), were associated with mitochondrial mechanisms of toxicity. Furthermore, the top two drugs, benzbromarone, and troglitazone were withdrawn from the market.

Table 5 indicates the RORs between the two groups of drugs that caused liver injury via mitochondrial compared to non-mitochondrial mechanisms. Between the two DILI groups, reports for DILI involving mitochondrial mechanisms of toxicity had a 1.43 (95% CI 1.42-1.45; $p < 0.0001$) times higher odds compared to drugs associated with non-mitochondrial mechanisms of toxicity. The univariate logistic regression model was used after dichotomizing age and gender. Table 6 indicates a statistically significant risk association of age or gender with hepatotoxic drugs with mitochondrial toxicity mechanisms. Reports of liver injury were 2.2 (odds ratio: 2.2, 95% CI 2.12-2.26) times more likely to be associated with older patient age, as compared with reports involving patients under 65 years of age. On the other hand, female patients

were 37% less likely to be subjects of liver injury reports for drugs associated with mitochondrial mechanisms of toxicity compared to males (Odds Ratio 0.63, 95% CI 0.61-0.64). Tables 7 to 13 contain DILI reports, all adverse event reports, ROR, and 95% Confidence Interval (CI) for all the drugs evaluated in the study.

Figure 4 indicates the totality of all ROR scores of DILI drugs with mitochondrial or non-mitochondrial mechanisms of toxicity. Drugs from the antineoplastic, antiviral, analgesic, antibiotic, and antimycobacterial classes were the top 5 drugs classes associated with higher ROR scores. Drugs from the antidiabetic, antiretroviral, anti-inflammatory, anti-Parkinson, vasoactive, neuroprotective, and antihyperlipidemic drug classes (pointed in brown arrow) were primarily associated with mitochondrial mechanisms. Alternatively, leukotriene pathway modulators, alcohol antagonists, CNS stimulants, and platelet inhibitor drug classes (pointed in red arrow) were the drugs with non-mitochondrial mechanisms having higher RORs. Figures 5 and 6 categorize these two groups of drugs based on the drug label section and severity class. We did not observe any notable trend between mitochondrial and non-mitochondrial mechanisms and drug label section, or severity class.

2.5 DISCUSSION

Prediction and characterization for DILI during preclinical drug development and post-approval remains a challenge for the pharmaceutical industry, toxicologists, clinicians, physicians, health authorities, and regulators.⁵ Characterizing DILI has been a challenge due to its unpredictability, lack of accurate biomarkers, poorly defined pathogenesis, and its potential to cause fatal liver failure.⁵ In the past two decades, drug-induced mitochondrial dysfunction has been established as an important contributing mechanism associated with liver, muscle, heart, kidney, and central nervous system toxicity.¹³ Mitochondrial dysfunction is one of the reasons known to cause muscle toxicity by HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitor (cerivastatin), cardiovascular toxicity by anthracyclines (daunorubicin, doxorubicin, idarubicin), and DILI by an antidepressant (nefazodone), antibiotics (isoniazid, ketoconazole(oral)), and anxiolytic (panadiplon) drugs.^{19, 33-36}

We calculated the ROR for reports of severe hepatotoxicity adverse events among drugs with the highest risk for DILI, for drugs having mitochondrial or non-mitochondrial mechanisms of toxicity. Brinker et al. indicated that various measures of disproportionate reporting of adverse events such as Proportional Reporting Ratio (PRR), Multi-item Gamma Poisson Shrinker (MGPS), and the Bayesian Confidence Propagation Neural Network (BCPNN) had been used in analyses of surveillance databases.⁸ Each of these methods may have different strengths and limitations and may lead to different sensitivity and specificity for a drug's risk reporting.⁸ Various health regulatory authorities use different statistical measures for reporting. For example, the European Medicines Agency (EMA) uses PRR; FDA and UK's Medicines and Healthcare products Regulatory Agency (MHRA) uses MGPS. Whereas, the World Health Organization (WHO) has utilized the BCPNN method for reporting.⁸ These reporting measures have been used to generate hypotheses and do not infer

adverse event-causal associations. It has been suggested that there is not one single measure of effect that is superior to the others.^{8, 37, 38} Our study utilized reporting odds ratios to characterize the frequency of liver injury reports as it is a straightforward and frequently used measure for the analysis of FAERS data.^{31, 39-43}

The review published by Will et al.¹³ indicated that the most commonly used prescriptions and over the counter medications for geriatric patients had published reports of various toxicities linked to mitochondrial dysfunction.¹³ Our study reported that reports for DILI involving mitochondrial mechanisms of toxicity had a 1.43 (95% CI 1.42-1.45; $p < 0.0001$) times higher odds compared to drugs associated with non-mitochondrial mechanisms of toxicity. The ROR scores with the highest risk of liver injury based on mitochondrial or non-mitochondrial mechanisms were highest for drugs from the antineoplastic, antiviral, analgesics, antibiotics, and antimycobacterial classes. This finding agreed with the published literature. Sonawane et al. indicated that antineoplastic, analgesics, and antibiotics were among the top 10 drugs that reported severe adverse events in the FAERS database from 2006 to 2014.²⁶ Additionally, our study observed that over 88% of adverse reports were expedited, while the rest of the reports were either direct or periodic in both drug categories. This observation also agreed with the published literature. Sonawane et al. also reported that expedited reports were the most common and over 72% of all serious adverse events with available data on the report type.²⁶

Antidiabetic, antiretroviral, anti-inflammatory, anti-Parkinson, vasoactive, neuroprotective, antihyperlipidemic drug classes were primarily associated with mitochondrial toxicity mechanisms. In recent years, an impaired mitochondrial function has been documented as one of the critical factors in inflammation, sarcopenia, metabolic (obesity, type 2 diabetes, non-alcoholic fatty liver disease), and neurodegenerative diseases (Parkinson's, Alzheimer's, Huntington's diseases).^{13, 44-46}

Patients with reduced mitochondrial function occurring as a manifestation of their underlying disease state may be more vulnerable to drugs that cause toxicity via mitochondrial mechanisms. Alternatively, antiadrenergic, leukotriene pathway modulators, alcohol antagonist, CNS stimulants, and platelet inhibitors were drug classes that were primarily associated with non-mitochondrial toxicity mechanisms.

We identified statistically significant differences ($p < 0.0001$) in drug severity classification, label section for liver injury, and report type between these two mechanisms for DILI. For drugs withdrawn for liver injury, there were a higher number of hepatotoxicity reports (10.5%) associated with mitochondrial than non-mitochondrial mechanisms (1.2%). Dykens and Will (2007) noted that 38 marketed drugs withdrew from the market between 1994 and 2006. Among these, for cerivastatin, nefazodone, troglitazone, and tolcapone, there was substantial evidence of mitochondrial-induced organ toxicity.³⁴ Therefore, our observations agreed with reports in the medical literature of drug-induced mitochondrial dysfunction playing an important role in drug withdrawal. Furthermore, Boelsterli and Lim (2007) suggested that several drugs, such as amiodarone, dantrolene, diclofenac, isoniazid, lamivudine, leflunomide, mefenamic acid, nimesulide, perhexiline, simvastatin, stavudine, sulindac, tolcapone, troglitazone, trovafloxacin, and valproic acid, are associated with idiosyncratic DILI with a clear link to mitochondrial toxicity.²¹ Many of these drugs reported a relatively higher ROR in our study.

Our study reported an older mean patient age (56.1 ± 18.33 (SD)) associated with reports for drugs that cause DILI via mitochondrial mechanisms compared to mean age (48 ± 19.53 (SD)) associated with reports for drugs that cause injury via non-mitochondrial mechanisms ($p < 0.0001$). This was further substantiated in a univariate logistic regression analysis where reports of liver injury were 2.2 (odds ratio: 2.2, 95% CI 2.12-2.26) times more likely to be associated with older patient age, as

compared with reports involving patient ages under 65 years. This finding is consistent with physiological information indicating age as a risk factor for both mitochondrial DNA abnormality and increased oxidative stress-related injury.⁴⁵ There is evidence that mitochondrial function declines with age, including the role of mitochondrial DNA mutation, increased production of reactive oxygen species, and the dysfunction in oxidative phosphorylation pathways.⁴⁴ The hallmark of mitochondrial aging includes a decreased mitochondrial number, reduced mitochondrial function, and individual electron transport chain activities.¹³ Mitochondrial function deteriorates progressively with age. Therefore, older age populations may be more vulnerable to hepatotoxic drugs associated with mitochondrial mechanisms of toxicity.

Our study indicated that female patients were 37% less likely to report liver injury adverse events for drugs associated with mitochondrial mechanisms of toxicity compared to males. There are conflicting reports associating male gender as a susceptibility factor for DILI, and a clear link for this association is absent in the literature.^{21, 47} Several articles allude to the potential involvement of a reactive metabolite, and differences in pharmacokinetics, pharmacodynamics, sex hormones, and immune system response between males and females.^{21, 22}

2.6 CONCLUSION

Mitochondria play an important part in DILI, including idiosyncratic liver injury. There have been various proposed mechanisms for mitochondrial involvement in DILI.⁴⁵ There is a gap in the literature describing the differences in clinical outcomes for patients who experienced DILI from mitochondrial mechanisms of toxicity as compared with non-mitochondrial mechanisms of toxicity drugs. There are limitations in detecting drugs that have mitochondrial liability in the drug development phase of the discovery. For the most part, drug-induced mitochondrial toxicity does not reveal itself in animal models due to the young age, lack of genetic divergence, health status, and lack of concomitant drug exposure.¹³ Therefore, drug-induced mitochondrial toxicity is often idiosyncratic, meaning it is not predictable until a large population is exposed.^{21, 46} Based on this study, we provide evidence of a higher proportion of reports of severe liver injury adverse events among drugs associated with mitochondrial mechanisms of toxicity as compared with non-mitochondrial mechanisms of toxicity. Furthermore, we found that reports of liver injury were 2.2 (odds ratio: 2.2, 95% CI 2.12-2.26) times more likely to be associated with older patient age, as compared with reports involving patients ages under 65 years. This finding aligns with the theory that age is a susceptibility factor in liver injury via the mitochondrial mechanisms of toxicity.

2.7 LIMITATION

The FAERS database describes adverse event reports but does not include information about the number of patients treated with a drug. Therefore, incidence rates, prevalence rates, and causal relationships between drugs and safety adverse events cannot be determined for drugs according to mitochondrial or non-mitochondrial mechanisms of toxicity. For example, the population incidence of DILI may be higher for drugs associated with non-mitochondrial mechanisms than mitochondrial mechanisms of toxicity. Nevertheless, researchers and health authorities have used the FAERS database for adverse event signal identification, developing ideas, and hypothesis generation despite this limitation. The hypothesis and ideas generated using this database could serve as a foundation for more robust study designs, and for in vitro or in vivo studies investigating the causal relationship of a drug with liver injury. The FAERS database provides a suitable source to evaluate the volume and characteristics of adverse event reports for marketed medications. Furthermore, factors such as age, gender, weight, drug severity class, and label section of FAERS reports can provide valuable insights to health authorities during the post-market surveillance of marketed medications.

The FAERS database is a spontaneous reporting system with limitations when used for drug safety research, including the potential for under or over-reporting events, duplicate reports, influence of media, and uncertainty of reported events.^{8, 48} For example, troglitazone received significant media attention due to a class-action lawsuit which called attention to its DILI risk. Therefore, troglitazone may have a higher number of hepatotoxicity reports than some drugs that did not receive media attention. Moreover, the FAERS database could be associated with the "Weber effect," where adverse event reports are higher in initial marketing stages following a gradual decline.⁸

Patients with an underlying condition such as obesity may be more vulnerable to drugs that cause toxicity via mitochondrial mechanisms; thus, we attempted to include patient weight in our study. However, about 79-81% of the reports missed the bodyweight information; therefore, the effect of patient weight was not examined. The study may also have several unmeasured confounding factors as patient comorbidities, pre-existing liver disease, and concomitant drug use is not captured in FAERS reports. Additionally, the findings regarding age and gender are unadjusted; therefore, it should be used merely for hypothesis generation. Moreover, gender bias may be due to disease demographics. Some of the DILI drugs with mitochondrial toxicity mechanisms are prescribed for diseases with a higher male predisposition. For example, benzbromarone is prescribed for gout, which has six times higher occurrence in males.⁴⁹ Similarly, isoniazid and rifampin are prescribed for the treatment of tuberculosis, which has two times higher occurrence in males.⁵⁰

ROR depends on the reporting rates of liver injury adverse events and all other adverse events reports in compared drug classes. DILI drugs associated with non-mitochondrial mechanisms of toxicity have a significantly higher number of non-hepatic adverse events reports. Therefore, we are not sure if larger ROR values are due to the higher reporting of hepatotoxicity in the drugs with mitochondrial mechanisms of toxicity, or higher reporting of non-hepatic adverse events reported for the drugs with non-mitochondrial mechanisms of toxicity.

For this analysis, we utilized ROR, which is a disproportionality measurement of spontaneous reports and not a method to measure drug-related risks quantitatively. Regulatory actions in response to safety concerns related to age and gender using the FAERS database must be determined via individual cases to determine causality. Despite these database limitations, we were able to show that drugs that cause hepatotoxicity via mitochondrial mechanisms were associated with a higher proportion

of adverse event reports than drugs having non-mitochondrial mechanisms of toxicity. Additionally, age may play a role in susceptibility to DILI via mitochondrial mechanisms of toxicity. Our findings from this study align with mitochondrial mechanisms of toxicity being an important cause of DILI, and this should be further investigated in real-world studies with robust designs.

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2.9 TABLES AND FIGURES

Table 1. Reporting odds ratio estimates for DILI drug groups.

Reporting odds ratio estimates for DILI drug groups			
FAERS Reports (N)			
DILI groups	Hepatotoxicity	All other Adverse Events	Total
Drugs associated with mitochondrial mechanisms of toxicity	40,343	586,989	627,332
Drugs associated with non-mitochondrial mechanisms of toxicity	64,358	1,342,486	1,406,844
Total	104,701	1,929,475	2,034,176

Table 2. Example reporting odds ratio estimate for an individual drug: acetaminophen.

Reporting odds ratio estimates for an individual drug			
FAERS Reports (N)			
	Hepatotoxicity	All other Adverse Events	Total
Acetaminophen	8,509	51,732	60,241
All other drugs of any type	383,540	27,852,908	28,236,448
Total	392,049	27,904,640	28,296,689

Table 3. Characteristics of patients and hepatotoxic drugs associated with mitochondrial mechanisms of toxicity compared to non-mitochondrial mechanisms.

Characteristics of the study drugs		
Characteristics	Hepatotoxicity via Mitochondrial mechanism (56 drugs)	Hepatotoxicity via Non-Mitochondrial mechanism (78 drugs)
FAERS Report Counts (N)	40,343 (38.5%)	64,358 (61.5%)
Reports based on NKTR drug severity classification		
3 - Liver aminotransferases increase	0 (0%)	3,048 (4.7%)
4 - Hyperbilirubinemia	1,958 (4.9%)	2,292 (3.6%)
5 - Jaundice	7,526 (18.7%)	13,392 (20.8)
6 - Liver necrosis	0 (0%)	35 (0.05%)
7 -Acute liver failure	4,581 (11.3%)	17,207 (26.7%)
8 - Fatal hepatotoxicity	26,278 (65%)	28,384 (44.1)
Reports combined based on less and severe DILI		
Less severe injury	9484 (23.5%)	18,767 (29.2%)
Liver failure/hepatotoxicity	30,859 (76.5%)	45,591 (70.8%)
Reports based on drug label section		
Warning & precautions	26,177 (64.9%)	50,898 (79.1%)
Box warning	9,939 (24.6%)	12,713 (19.8%)
Withdrawn	4227 (10.5%)	747 (1.2%)
Report type		
Direct	1,992 (4.9%)	2,393 (3.7%)
Expedited	35,569 (88.2%)	57,119 (88.8%)
Periodic	2,782 (6.9%)	4,846 (7.5%)
Patient Characteristics		
Weight (Kg)		
FAERS Report Counts (N)	7666 (19%)	13532 (21%)
Weight Missing	32677 (81%)	50826 (79%)
Mean ± Stdev	68.6 ± 20.8	70.2 ± 23.4
Gender		
Male	19,818 (49.1%)	24,353 (37.8%)
Female	17,711 (43.9%)	34,690 (53.9%)
Gender Missing	2,814 (7%)	5315 (8.3%)
Age (Years)		
FAERS Report Counts (N)	30,324 (75.2%)	46310 (71.9%)

Age missing	10019 (24.8%)	18046 (28%)
Mean $\pm$ Stdev	56.1 $\pm$ 18.33	48 $\pm$ 19.53
A statistical difference between two DILI groups across categorical variables was performed using a chi-square test. Comparisons of continuous variables were performed using t-tests; P values were < 0.0001 for all the variables (P<0.05 was considered significant)		

Table 4. Top 20 drugs with the highest reporting odds ratio in both DILI groups.

Drug Class	Drug Name	Severity Class	Label Section	Mitochondrial Toxicity	ROR
Antigout agent	Benzbromarone	8	Withdrawn	Yes	36.31
Antidiabetic agent	Troglitazone	8	Withdrawn	Yes	31.02
Antimycobacterials	Isoniazid	8	Box warning	Yes	20.79
Antimycobacterials	Rifampin	8	Warnings and precautions	Yes	18.64
Antibiotics	Telithromycin	8	Warnings and precautions	No	18.33
Antineoplastics	Gemtuzumab ozogamicin	8	Box warning	No	18.08
Antiarrhythmics	Mexiletine	3	Box warning	No	17.8
Antineoplastics	Dactinomycin	8	Warnings and precautions	No	17.25
Anti-inflammatory agent	Nimesulide	8	Withdrawn	Yes	15.07
Antialcoholics	Disulfiram	8	Warnings and precautions	No	14.82
Antivirals	Didanosine	8	Warnings and precautions	Yes	14.38
Stimulants; Central Nervous System	Pemoline	8	Withdrawn	No	14.24
Platelet inhibitors	Ticlopidine	4	Warnings and precautions	No	13.51
Antibiotics	Trovafloxacin mesylate	8	Withdrawn	Yes	13.48
Antithyroid agents	Propylthiouracil	8	Box warning	No	13.33
NSAID	Bromfenac	8	Withdrawn	No	13.01
Antiretroviral drugs	Stavudine	8	Box warning	Yes	12.83
Hormone modifiers	Danazol	8	Box warning	Yes	12.82
Antiparkinson agents	Tolcapone	8	Box warning	Yes	12.25
Antivirals	Tipranavir	8	Box warning	No	12.04

Table 5. Reporting odds ratio estimate for hepatotoxic drugs associated with mitochondrial mechanisms of toxicity compared to non-mitochondrial mechanisms.

DILI group	Odds ratio	95% CI	p-value
Drugs associated with mitochondrial mechanisms of toxicity	1.43	1.42-1.45	<0.0001

Table 6. Unadjusted association of age and gender with hepatotoxic drugs with mitochondrial toxicity mechanisms as compared with non-mitochondrial mechanisms, using a univariate logistic regression model.

Independent variable	Odds ratio	95% CI	p-value
Age			
<65 years		Reference	
>65 years	2.2	2.12-2.26	<0.0001
Gender			
Male		Reference	
Female	0.63	0.61-0.64	<0.0001

Table 7. Adverse-event (AE) report counts and risk estimates of DILI for analgesics, anti-adrenergic, anti-alcoholic, and antiarrhythmic medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Analgesics	Chlorzoxazone	Yes ⁵⁰	30	229	9.514	6.502, 13.92 ¹
Analgesics	Diclofenac	Yes ^{51, 52}	350	5647	4.501	4.04, 5.014 ¹
Analgesics	Diflunisal	Yes ^{52, 53}	2	60	2.421	0.5917, 9.902
Analgesics	Etodolac	Yes ⁵⁴	105	1823	4.183	3.436, 5.092 ¹
Analgesics	Sulindac	Yes ⁵⁵	52	434	8.701	6.526, 11.6 ¹
Analgesics	Indomethacin	Yes ⁵⁶	126	2663	3.436	2.874, 4.108 ¹
Analgesics	Acetaminophen	No ⁵⁷	8509	51732	11.94	11.67, 12.22 ¹
Analgesics	Chlormezanone	No ⁵⁸	6	135	3.228	1.425, 7.311 ¹
Analgesics	Fenoprofen	No ⁵⁹	8	50	11.62	5.509, 24.51 ¹
Analgesics	Mefenamic acid	No ¹¹	50	391	9.287	6.918, 12.47 ¹
Analgesics	Milnacipran	No*	50	2795	1.299	0.9822, 1.718
Antiadrenergic	Labetalol	No ⁵²	47	701	4.869	3.624, 6.542 ¹
Antiadrenergic	Methyldopa	No ⁶⁰	116	718	11.73	9.643, 14.28 ¹
Alcohol antagonist	Disulfiram	No ⁶¹	100	490	14.82	11.95, 18.38 ¹
Antiarrhythmics	Amiodarone	Yes ⁵⁷	1590	18220	6.337	6.02, 6.671 ¹
Antiarrhythmics	Dronedarone	Yes ⁶²	444	5433	5.935	5.387, 6.538 ¹
Antiarrhythmics	Mexiletine	No*	125	510	17.8	14.64, 21.65 ¹

*No literature evidence; ¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Table 8. Adverse-event report counts and risk estimates of DILI for antibiotics, anticonvulsants, antidepressants, and antidiabetic medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Antibiotics	Ciprofloxacin	Yes ⁶³	1299	28830	3.272	3.095, 3.459 ¹
Antibiotics	Clarithromycin	Yes ⁶⁴	1225	18499	4.809	4.538, 5.096 ¹
Antibiotics	Nitrofurantoin	Yes ^{52, 57}	434	3044	10.35	9.363, 11.45 ¹
Antibiotics	Trovaflaxacin mesylate	Yes ⁶⁵	1168	6291	13.48	12.67, 14.35 ¹
Antibiotics	Minocycline	No*	604	4973	8.82	8.105, 9.598 ¹
Antibiotics	Telithromycin	No ⁶⁶	1049	4157	18.33	17.12, 19.61 ¹
Anticonvulsants	Carbamazepine	Yes ^{67, 68}	1606	22379	5.212	4.954, 5.483 ¹
Anticonvulsants	Phenytoin	Yes ^{67, 68}	547	15593	2.548	2.339, 2.774 ¹
Anticonvulsants	Valproic acid	Yes ^{68, 69}	945	11096	6.185	5.787, 6.61 ¹
Anticonvulsants	Divalproex	No*	623	14095	3.21	2.962, 3.478 ¹
Anticonvulsants	Felbamate	No ⁷⁰	18	364	3.591	2.237, 5.764 ¹
Anticonvulsants	Fosphenytoin	No ⁷¹	68	981	5.034	3.937, 6.436 ¹
Anticonvulsants	Lamotrigine	No ⁵²	1849	45375	2.959	2.824, 3.1 ¹
Antidepressant	Clomipramine	Yes ⁷²	64	1500	3.098	2.413, 3.979 ¹
Antidepressant	Nefazodone	Yes ¹⁴	608	4707	9.38	8.62, 10.21 ¹
Antidepressant	Duloxetine	No ⁷³	2162	50955	3.081	2.951, 3.217 ¹
Antidepressants	Nortriptyline	Yes ⁷⁴	6	1162	0.375	0.1681, 0.8363 ¹
Antidiabetic agent	Acarbose	Yes ⁷⁵	224	1505	10.81	9.393, 12.44 ¹
Antidiabetic agent	Troglitazone	Yes ⁷⁶	3047	7133	31.02	29.73, 32.37 ¹

*No literature evidence; ¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Table 9. Adverse-event report counts and risk estimates of DILI for antifungals, antigout, antihelminthic, antihyperlipidemic, antiinfectives, anti-inflammatory, and antimycobacterial medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Antifungals	Itraconazole	Yes ⁷⁷	566	7403	5.552	5.097, 6.048 ¹
Antifungals	Ketoconazole	Yes ^{11, 78}	233	4364	3.877	3.398, 4.424 ¹
Antifungals	Fluconazole	No ⁷⁸	920	12475	5.356	5.008, 5.727 ¹
Antifungals	Griseofulvin, Microcrystalline	No ⁷⁹	26	203	9.301	6.184, 13.99 ¹
Antifungals	Micafungin	No ⁸⁰	213	1765	8.764	7.602, 10.1 ¹
Antifungals	Terbinafine	No ⁸¹	1330	12101	7.982	7.542, 8.447 ¹
Antifungals	Voriconazole	No ⁸²	780	9093	6.229	5.79, 6.702 ¹
Antigout agent	Benzbromarone	Yes ⁵²	1	2	36.31	3.293, 400.4 ¹
Antigout agent	Allopurinol	No ⁸³	626	5353	8.493	7.817, 9.226 ¹
Antigout agent	Febuxostat	No ⁸⁴	161	3910	2.99	2.554, 3.501 ¹
Anthelmintics	Albendazole	Yes ⁸⁵	108	879	8.923	7.306, 10.9 ¹
Anthelmintics	Thiabendazole	No ⁸⁶	5	45	8.069	3.203, 20.33 ¹
Antihyperlipidemic	Gemfibrozil	Yes ^{52, 76}	267	2835	6.839	6.033, 7.754 ¹
Antihyperlipidemic	Atorvastatin calcium	Yes ⁵⁷	5522	89525	4.479	4.358, 4.604 ¹
Antihyperlipidemic	Niacin	Yes ⁸⁷	219	47451	0.3352	0.2935, 0.3828 ¹
Antiinfectives	Erythromycin	No ⁸⁸	154	3753	2.98	2.536, 3.501 ¹
Antiinfectives	Levofloxacin	No ⁸⁹	1117	37160	2.183	2.057, 2.317 ¹
Anti-inflammatory agent	Nimesulide	Yes ^{52, 57}	11	53	15.07	7.873, 28.85 ¹
Antimycobacterials	Isoniazid	Yes ⁵²	678	2368	20.79	19.09, 22.65 ¹
Antimycobacterials	Rifampin	Yes ²⁴	1291	5029	18.64	17.54, 19.82 ¹
Antimycobacterials	Ethambutol	No ⁹⁰	117	847	10.03	8.268, 12.17 ¹

¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Table 10. Adverse-event report counts and risk estimates of DILI for antineoplastics medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Antineoplastics	Asparaginase	Yes ⁹¹	145	1993	5.283	4.464, 6.254 ¹
Antineoplastics	Bicalutamide	Yes ⁵²	371	4033	6.68	6.006, 7.43 ¹
Antineoplastics	Erlotinib	Yes ⁵⁷	915	42889	1.549	1.451, 1.654 ¹
Antineoplastics	Flutamide	Yes ^{44, 52}	125	807	11.25	9.317, 13.58 ¹
Antineoplastics	Nilutamide	Yes ⁷⁶	24	259	6.729	4.429, 10.22 ¹
Antineoplastics	Oxaliplatin	Yes ⁹²	1297	19069	4.939	4.669, 5.226 ¹
Antineoplastics	Pazopanib	Yes ⁹³	1004	19660	3.709	3.48, 3.952 ¹
Antineoplastics	Sorafenib	Yes ¹¹	3815	24782	11.18	10.8, 11.57 ¹
Antineoplastics	Tamoxifen	Yes ^{52, 76, 94}	257	6330	2.948	2.603, 3.34 ¹
Antineoplastics	Bexarotene	No ⁹⁵	14	575	1.768	1.041, 3.004 ¹
Antineoplastics	Bortezomib	No ⁹⁶	771	24288	2.305	2.146, 2.477 ¹
Antineoplastics	Busulfan	No ⁵²	625	3783	12	11.02, 13.06 ¹
Antineoplastics	Cytarabine	No ⁹⁷	575	7603	5.492	5.045, 5.978 ¹
Antineoplastics	Dacarbazine	No ⁹⁸	35	821	3.096	2.207, 4.342 ¹
Antineoplastics	Dactinomycin	No ⁹⁹	200	842	17.25	14.78, 20.13 ¹
Antineoplastics	Estramustine	No ¹⁰⁰	54	719	5.454	4.136, 7.192 ¹
Antineoplastics	Exemestane	No ¹⁰¹	312	6728	3.368	3.006, 3.773 ¹
Antineoplastics	Gefitinib	No ¹⁰²	798	8074	7.178	6.674, 7.719 ¹
Antineoplastics	Gemcitabine	No ¹⁰³	1738	25409	4.967	4.731, 5.215 ¹
Antineoplastics	Gemtuzumab ozogamicin	No ¹⁰⁴	1067	4286	18.08	16.91, 19.33 ¹
Antineoplastics	Hydroxyurea	No ⁵²	129	3109	3.013	2.527, 3.594 ¹
Antineoplastics	Imatinib	No ¹¹	1891	47575	2.887	2.757, 3.023 ¹
Antineoplastics	Interferon alfa-2a,	No ¹⁰⁵	32	322	7.217	5.019, 10.38 ¹
Antineoplastics	Lapatinib ditosylate	No ¹¹	660	14473	3.312	3.063, 3.581 ¹

Antineoplastics	Mercaptopurine	No ⁵²	278	2051	9.843	8.684, 11.16 ¹
Antineoplastics	Pentostatin	No*	43	403	7.749	5.658, 10.61 ¹
Antineoplastics	Sunitinib	No ¹⁰⁶	2119	42918	3.586	3.432, 3.746 ¹

*No literature evidence; ¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Table 11. Adverse-event report counts and risk estimates of DILI for antiparkinson, antipsoriatics, antipsychotics, antiretroviral, antithyroid, and antiviral medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Antiparkinson agents	Tolcapone	Yes ⁷⁶	179	1061	12.25	10.46, 14.35 ¹
Antipsoriatics	Acitretin	No ¹⁰⁷	167	2754	4.404	3.767, 5.148 ¹
Antipsychotics	Clozapine	Yes ¹⁴	1453	67935	1.553	1.474, 1.636 ¹
Antiretroviral drugs	Nevirapine	Yes ¹⁰⁸	1596	9761	11.87	11.26, 12.52 ¹
Antiretroviral drugs	Stavudine	Yes ¹⁰⁹	774	4382	12.83	11.88, 13.85 ¹
Antiretroviral drugs	Zidovudine	Yes ¹⁰⁹	468	4788	7.098	6.455, 7.805 ¹
Antithyroid agents	Methimazole	Yes ¹¹⁰	170	1382	8.933	7.617, 10.48 ¹
Antithyroid agents	Propylthiouracil	No ¹¹¹	121	659	13.33	10.98, 16.19 ¹
Antiviral	Abacavir Sulfate	No ¹¹²	812	6685	8.82	8.20, 9.48 ¹
Antivirals	Didanosine	Yes ¹¹³	557	2812	14.38	13.13, 15.75 ¹
Antivirals	Efavirenz	Yes ¹¹⁴	760	7903	6.984	6.482, 7.524 ¹
Antivirals	Ritonavir	Yes ⁵⁷	880	9262	6.9	6.438, 7.394 ¹
Antivirals	Peginterferon alfa-2b	Yes ¹¹⁵	394	21440	1.335	1.208, 1.474 ¹
Antivirals	Darunavir ethanolate	No ¹¹⁶	418	4238	7.163	6.478, 7.92 ¹
Antivirals	Etravirine	No ¹¹⁷	145	1249	8.431	7.099, 10.01 ¹
Antivirals	Interferon alfacon-1	No*	92	1273	5.248	4.247, 6.485 ¹
Antivirals	Maraviroc	No ¹¹⁸	97	1567	4.495	3.662, 5.519 ¹
Antivirals	Raltegravir potassium	No ¹¹⁶	414	6012	5.001	4.527, 5.525 ¹
Antivirals	Tipranavir	No ¹¹⁹	118	712	12.04	9.905, 14.62 ¹

*No literature evidence; ¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Table 12. Adverse-event report counts and risk estimates of DILI for biologics, and disease-modifying antirheumatic medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Biologics	Natalizumab	No*	1804	177402	0.7385	0.7049, 0.7736 ¹
Carbonic anhydrase inhibitor	Acetazolamide	Yes ^{68, 69}	26	768	2.459	1.663, 3.634 ¹
Chelators	Deferasirox	No ¹²⁰	1933	27136	5.173	4.939, 5.418 ¹
Retinoids	Isotretinoin	No*	1949	58079	2.437	2.329, 2.55 ¹
Disease-modifying antirheumatic drugs	Azathioprine	Yes ¹²¹	549	4372	9.119	8.344, 9.966 ¹
Disease-modifying antirheumatic drugs	Leflunomide	Yes ¹²²	1682	13795	8.855	8.417, 9.315 ¹
Disease-modifying antirheumatic drugs	Infliximab	No*	4260	146690	2.109	2.045, 2.175 ¹
Disease-modifying antirheumatic drugs	Methotrexate	No ¹²³	2208	31526	5.086	4.871, 5.311 ¹
Disease-modifying antirheumatic drugs	Sulfasalazine	No ¹²⁴	781	7658	7.406	6.88, 7.973 ¹

*No literature evidence; ¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Table 13. Adverse-event report counts and risk estimates of DILI for diuretics, gastrointestinal, hematinics, hormone modifier, immunomodulators, leucoxene antagonists, musculoskeletal, neuroprotective, NSAID, CNS stimulants, and vasoactive medications.

Drug Class	Drug Name	Mitochondrial Toxicity & Reference	DILI reports	All AE reports	ROR	95% CI
Diuretics	Tolvaptan	No ¹²⁵	726	4730	11.15	10.31, 12.05 ¹
Endothelin receptor antagonist	Bosentan	No ¹¹	2125	41452	3.723	3.564, 3.889 ¹
Gastrointestinal	Orlistat	No ¹²⁶	1335	31799	3.049	2.886, 3.221 ¹
Hematinics	Eltrombopag olamine	No ¹²⁷	596	10450	4.142	3.813, 4.498 ¹
Hormone modifiers	Danazol	Yes ⁵²	45	255	12.82	9.335, 17.59 ¹
Hormone modifiers	Oxandrolone	Yes ¹²⁸	12	135	6.455	3.577, 11.65 ¹
Hormone modifiers	Nandrolone decanoate	No ¹²⁹	15	138	7.894	4.633, 13.45 ¹
Hormone modifiers	Oxymetholone	No ¹³⁰	17	122	10.12	6.093, 16.81 ¹
Hormone modifiers	Testosterone	No ¹³¹	175	30408	0.4179	0.3602, 0.4849 ¹
Immunomodulators	Interferon-beta-1a	No*	5343	185985	2.086	2.03, 2.144 ¹
Immunomodulators	Interferon beta-1b, recombinant	No*	2018	31442	4.661	4.455, 4.876 ¹
Immunosuppressives	Cyclosporine	No ¹³²	2344	39804	4.277	4.102, 4.459 ¹
Leukotriene pathway modulators	Zafirlukast	No*	151	1224	8.959	7.565, 10.61 ¹
Leukotriene pathway modulators	Zileuton	No ¹³³	28	323	6.295	4.279, 9.262 ¹
Musculoskeletal agents	Dantrolene	No ¹³⁴	66	570	8.409	6.517, 10.85 ¹
Musculoskeletal agents	Tizanidine	No*	133	2125	4.545	3.815, 5.416 ¹
Neuroprotective	Riluzole	Yes ¹³⁵	75	935	5.825	4.604, 7.37 ¹
NSAID	Bromfenac	No ¹³⁶	670	3739	13.01	11.99, 14.13 ¹
Platelet inhibitors	Ticlopidine	No ¹³⁷	532	2860	13.51	12.31, 14.82 ¹
Selective norepinephrine reuptake inhibitor	Atomoxetine	No ⁵²	520	22207	1.7	1.559, 1.855 ¹
Stimulants; Central Nervous System	Pemoline	No ¹³⁸	71	362	14.24	11.04, 18.37 ¹

Vasoactive agents	Papaverine	Yes ¹³⁹	2	24	6.052	1.43, 25.6 ¹
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*No literature evidence; ¹ p < 0.05 and 95% confidence limits testing exclusion of 0 or 1, as indicated

Figure 1. Reporting odds ratios for hepatotoxic drugs having mitochondrial mechanisms of DILI.

Figure 2. Reporting odds ratios for hepatotoxic drugs having non-mitochondrial mechanisms of DILI.

Figure 3. Sum of all ROR of “most-DILI concern” drugs associated with mitochondrial and non-mitochondrial mechanisms of toxicity per therapeutic class.

Figure 4. Categorization based on liver injury drug label for “most-DILI concern” drugs based on their association with mitochondrial and non-mitochondrial mechanisms of toxicity.

Figure 5. Categorization based on liver injury severity class for “most-DILI concern” drugs based on their association with mitochondrial and non-mitochondrial mechanisms of toxicity.

MANUSCRIPT III

Title: Liver injury among patients newly treated with atypical antipsychotic medications

Payal Rana^{a, b}, Xuerong Wen^b, Michael D Aleo^a Fatemeh Akhlaghi^b, and Stephen
Kogut^b

^aDrug Safety Research & Development, Pfizer, Eastern Point Road, Groton, CT
06340, USA

^b University of Rhode Island, College of Pharmacy, Kingston, RI 02881, USA

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3.1 ABSTRACT

Background

Compared to typical antipsychotics, atypical antipsychotic (AAP) drugs have improved safety profiles and rarely cause liver injury. However, no retrospective cohort studies have evaluated the short-term risk of liver injury associated with specific AAPs using an administrative database in the United States.

Goal

This study aimed to identify significant risk factors of liver injury in patients taking olanzapine, quetiapine, risperidone, or ziprasidone compared to aripiprazole. First, we determined the cumulative incidence rate of liver injury for each AAP among newly treated patients. Among these patients, we also investigated the association between liver injury and type of AAP, controlling for various risk factors (age, gender, and comorbidities)

Methods

We conducted a retrospective cohort study using an administrative claims database to identify adults prescribed AAPs from 2010-2015. The new AAP user cohort was selected based on a 12-months pre-index period with no AAP use, followed by a 2-months AAP exposure period. ICD9 diagnosis codes were analyzed to identify liver injuries (as a composite outcome), including liver enzyme elevation, jaundice, hepatitis, cholestasis, cirrhosis, liver necrosis, or acute liver failure occurring during the ten months follow-up period. The demographic and clinical characteristics of patients prescribed AAP medications and liver injury outcome groups were examined and adjusted for covariates. The cumulative incidence rates of liver injury among patients taking AAP in outpatient and inpatient settings were calculated. Univariate and multivariate logistic regression analyses were utilized (including odds ratio, 95% confidence interval, and p-values) to identify risk factors associated with liver injury.

Results

Overall, the cumulative incidence rate of liver injury in various AAP therapies ranged from 15.2 to 25.1 per 1000 persons per year. There was no significant association between exposure to any of the AAP medications with the composite outcome. As over 90% of the patients over the age of 76 were prescribed dosages of AAPs that were below the daily recommended doses, a sensitivity analysis was conducted that included only patients 75 years and younger. This analysis showed that quetiapine was associated with 22% (adjusted Odds Ratio (aOR) 1.22, CI 1.04-1.44, $p = 0.02$) increased risk of liver injury as compared with aripiprazole. Having 1 to 3 comorbidities (aOR 1.27, CI 1.09-1.48, $p = 0.003$) or 4 to 10 comorbidities (aOR 1.57, CI 1.22-2.02, $p = 0.0004$) was associated with 27% or 57% increased risk of liver injury as compared with no comorbidities, respectively. Comorbidities such as documented alcohol abuse (aOR 1.63, CI 1.27-2.08, $p < 0.0001$), hypertension (aOR 1.46, CI 1.23-1.73, $p < 0.0001$), obesity (aOR 1.36, CI 1.12-1.65, $p = 0.002$), and hyperlipidemia (aOR 1.31, CI 1.1-1.54, $p = 0.0012$) were associated with increased risk of liver injury. Anxiety diagnosis (aOR 1.2, CI 1.03-1.38, $p = 0.021$) was associated with a 20% increased risk of liver injury.

Conclusion

This retrospective cohort study determined the cumulative incidence rate of liver injury as 15.2 to 25.1 per 1000 persons per year among patients newly treated with AAP medications. Compared to aripiprazole, quetiapine was associated with a 22% increased risk of liver injury during the first months of treatment in patients 75 years and younger. Various comorbidities, including alcohol abuse, hypertension, obesity, and hyperlipidemia, were also associated with higher odds of liver injury.

3.2 INTRODUCTION

Drug-induced liver injury (DILI) is one of the leading reasons for clinical candidate termination during drug discovery and development. Similarly, many drugs have received a black-box warning or were withdrawn from the market due to DILI.^{1, 2} It is reported that DILI is the 4th leading cause of liver disease leading to liver failure.¹ Many drug classes are associated with DILI, including nonsteroidal anti-inflammatory drugs (NSAID), antiepileptic, anti-lipidemic, anti-infective, oncology, immunosuppressive, and psychotherapeutic drugs.^{3, 4} There has been literature evidence of liver injury for psychotherapeutic drug class.⁵ Antipsychotics are broadly categorized into two types: typical or atypical. Antipsychotic drugs, which are mainly prescribed for psychotic disorders, including schizophrenia, psychosis, depression, insomnia, aggression, anxiety, and bipolar disorder^{6, 7} have been implicated with clinical hepatotoxicity.⁵

Atypical antipsychotics (AAP) have been associated with a variety of adverse side effects including weight gain (obesity), metabolic diseases, and nonalcoholic fatty liver disease.^{8, 9} Patients treated with antipsychotic drugs such as chlorpromazine and clozapine have increased risk of mild to severe liver injuries.^{10, 11} Derby et al. (1993) conducted a cohort study of over 10,000 users of chlorpromazine in the United Kingdom (UK).¹² They reported that chlorpromazine was associated with DILI with a frequency of 1.3/1000 users (95% CI; 0.8-2.2).¹² More recently, De Abajo et al. (2004) performed a population-based study in the United Kingdom of over 1.6 million subjects from 1994 to 1999; they reported that chlorpromazine had the strongest association with liver enzyme elevations, with an adjusted odds ratio (aOR) of 416 (95% CI; 45-3840).¹¹ Furthermore, several case-control studies reported an association of antipsychotics with liver injury.^{10, 13} It is believed that, compared to typical antipsychotics, atypical antipsychotics (AAP) drugs have improved safety profiles and

only rarely cause liver injury.⁵ However, despite their potentially safer profile, asymptomatic increases in transaminase liver enzymes and bilirubin have been reported.⁵ When occurring, elevated liver enzymes typically appear after six weeks of AAP treatment, and levels tend to disappear with drug withdrawal.^{5, 14} There are also reports of AAP inducing liver function abnormalities in the clinical setting.⁶ Hence, clinical guidelines for liver enzymes measurement during, and initiation of the AAP drug therapy have been put in place.⁵

To our knowledge, there are no retrospective cohort studies that evaluated clinical liver injury risk of AAP during the initial treatment months using a geographically diverse US database. This study examined the liver injury risk of various AAP medications and liver injury risk factors among newly treated patients. Strong associations between liver injury and particular AAP types as identified in real-world research such as this study, might help prescribers select appropriate AAP medications for patients at higher risk. These study results may help clinicians understand liver injury risks in balance with drug therapy, improve therapy adherence, and monitor for liver injury. Furthermore, additional information about liver injury risk among AAPs is needed to guide therapeutic monitoring protocols and more readily identify medication-related causes.

This study aimed to identify significant risk factors of liver injury in patients taking olanzapine, quetiapine, risperidone, and ziprasidone compared to aripiprazole. First, we calculated the cumulative incidence rate of liver injury among patients newly treated with AAP medications. Then we estimated the risk of liver injury among patients newly treated with AAPs in a multivariable framework that controlled for various risk factors (age, sex, and comorbidities), using a retrospective cohort study based on a nationwide insurance claims database.

3.3 METHODS

Study design:

Data Source:

The Optum Clinformatics Data Mart (OptumInsight, Eden Prairie, MN) is an administrative health claims database from a large national insurer. Our study analyzed claims from January 1, 2010, to December 31, 2015. For the study, the Optum database containing de-identified data on age, geographic location, drugs, inpatient and outpatient services, medical services, eligibility, and pharmacy claims were used; Institutional Review Board (IRB) approval number was IRB1920-030. The data contain information on member eligibility, medical claims, facility detail, prescription claims, inpatient confinement, provider, and death date. For this study, lab results and death data were not utilized. The wide geographic representation and size of the database serve as a valuable tool to examine liver injury risk in real-world settings. Inpatient and outpatient medical claims included diagnosis codes based on the International Classification of Diseases 9th (ICD9). Clinical procedures (e.g., laboratory testing) are recorded using the Current Procedural Terminology (CPT) codes

Cohort selection:

Drugs under the psychotherapeutic medication class of American Hospital Formulation Service (AHFS – 28:16 (psychotherapeutic agents)) were used for cohort selection. AHFS – 28:16.08.04 (Atypical Antipsychotics), a pharmacologic therapeutic classification, for AAP were used for the study cohort. The cohort included patients who were newly treated with an AAP medication between January 1, 2011, to March 1, 2015. The index date was considered the date of the first AAP drug pharmacy claim

following a pre-index period (spanning January 1, 2010, to January 1, 2014) of 12-months without antipsychotic therapy.

Figures 1 and 2 indicate the schematic diagram of the study design and the final cohort selection exclusion criteria, respectively. Patients were excluded from the study if they meet any of the following criteria: 1) were prescribed any typical or atypical antipsychotic medications in the pre-index period, 2) had less than 12-months of continuous enrollment before and following the index period, or were not continuously eligible for health and pharmacy benefits during the entire pre-index or exposure periods, 3) were less than 18 years of age as of the index date, 4) had a liver injury or HIV diagnosis at any time during their pre-index period or in the exposure period (60 days after the index date), 5) did not have at least two prescription claims for AAP during the exposure period, 6) were dispensed chronic concomitant drugs that were highly associated with liver injury based on LiverTox database.

Bjornsson (2016) published various drug classes that had been implicated with DILI. The article categorized drugs from the publicly available LiverTox database (<http://livertox.nlm.nih.gov>) into different groups based on their likelihood of causing hepatotoxicity.¹⁵ Category A was DILI drugs as having more than 100 published case reports of hepatotoxicity.¹⁵ In this study, we excluded patients prescribed chronic concomitant medicines that had a high likelihood of causing liver injury. If the patients were prescribed one or more category A DILI drugs with an increased liver injury association, they were excluded from the study. The patients prescribed concomitant drugs allopurinol, amiodarone, azathioprine, carbamazepine, dantrolene, disulfiram, flutamide, hydralazine, isoniazid, ketoconazole, methotrexate, methyldopa, minocycline, phenytoin, propylthiouracil, quinidine, rifampin, sulfasalazine, ticlopidine, valproate or valproic acid were excluded from the study.¹⁵

Exposure Definition:

AAP medication users were defined as having received at least two prescription claims for any quantity and duration during the exposure period. They were monitored for liver injury diagnosis for the subsequent ten months following the exposure period. The exposure period ended on the 60th day from the index day.

Selection of control group:

The most frequently prescribed AAPs prescribed were olanzapine, quetiapine, risperidone, ziprasidone, and aripiprazole. A review published by Telles-Correia (2017) examined psychotherapeutics and liver injury, they did not describe any liver injury reports for aripiprazole.⁵ Therefore, aripiprazole was used as a reference. On the other hand, olanzapine, ziprasidone, risperidone, and quetiapine had reports of liver enzyme elevations or other liver injuries.⁵

Covariates:

A variety of demographic and clinical characteristics were examined, including patient age, gender, health plan type, geographic location, comorbidities, and disease burden (Elixhauser comorbidity index)^{16, 17} associated with the indexed prescription during the pre-index period. Patients taking AAP may have several comorbidities; therefore, the Elixhauser comorbidity measures could help with risk assessment of safety data. The Elixhauser index utilizes 31 conditions using predictors of mortality in patients with cardiac, gastrointestinal, hepatobiliary, oncologic, hypertension, obesity, weight loss, and psychiatric conditions.¹⁸

The clinical practice guidelines from the European Association for the Study of Liver (EASL) indicated that older age, female gender, alcohol intake, and comorbidities (metabolic disease, chronic hepatitis) might be risk factors for DILI.¹⁹ Based on the evidence supported by several published articles, the study investigated various comorbidities such as hypertension, type 2 diabetes, obesity, alcohol abuse,

hyperlipidemia, renal disease, and cardiovascular disease associated with liver injury among patients newly treated with AAP medications.²⁰⁻²³

The study included new users of AAP drug therapy only. Therefore, they are expected to be initiated on lower doses of AAP medications. New patients are usually prescribed low dose therapy and then gradually increase the dose based on the effectiveness and side effects. Given that the AAP drug class has diverse physicochemical drug properties, different drug concentrations in the human plasma level (Cmax) are needed to achieve optimum drug effects.²⁴ Various adult daily doses such as 10-30 mg for olanzapine, 300-800 mg for quetiapine, 2-6 mg for risperidone, 10-30 mg for paliperidone, 10-30 mg for aripiprazole, and 80-160 mg for ziprasidone are recommended.²⁵

Although AAP medications were primarily approved for schizophrenia, these drugs are also prescribed for other neuropsychiatric disorders such as bipolar disorder, psychosis, depression, anxiety, insomnia, and aggression.⁷ ICD9 codes (as shown in Table 1) were used to determine the diagnosis for AAP medications prescription for the cohort (14 days prior and following the index day). These diagnoses were examined as independent variables.

Outcome Assessment:

ICD9 diagnosis codes were utilized to identify liver injuries including liver enzyme elevation, jaundice, hepatitis, cholestasis, cirrhosis, liver necrosis, or acute liver failure occurring during the ten months follow up period; which spanned from March 1, 2011, to December 31, 2015. If a patient was identified with any of the ICD9 codes from Table 1, they were considered positive for the liver injury outcome. There was one composite outcome score (yes or no) for liver injury outcome. There are published studies for the administrative claim database utilized these diagnosis codes to identify liver injury with high positive predictive values.^{26,27} Jinjuvadia et al. reported

using these ICD9 codes with 53%-98% sensitivity and 59-98% accuracy.¹⁷ Bui et al. reported positive predictive values from 48-84% for liver injury diagnosis codes.²⁸

Sensitivity Analyses:

Sensitivity analysis to include patient's population prescribed therapeutic doses

Patients aged 76 and older are more likely to be prescribed lower doses, and use AAPs on an as-needed basis, for example when used for off-label indications such as agitation or insomnia. To investigate this issue, we examined the AAP doses prescribed among patients ages 76 and older and found that a majority received substantially lower doses than described in the FDA label. Therefore, this population was excluded from the study cohort in a sensitivity analysis that examined the risk of liver injury among patients who were generally prescribed AAPs at usual recommended doses.

Sensitivity analysis – Liver injury models based on liver enzyme exclusion and most severe liver injury

Additional to the composite liver injury outcome, we also conducted analyses to include only more severe forms of liver injury as the outcome, e.g., cases who had jaundice or worse (excluding liver enzyme elevations) and the outcome of most severe liver injury cases, including liver necrosis and acute liver failure.

Statistical Analysis:

Baseline demographic characteristics between groups were evaluated. In this analysis, the differences of categorical variables, various demographic and clinical characteristics, between different AAP users were compared using a chi-square test. A comparison of a continuous variable, such as age, was performed using a student's t-test for independent samples. In this study, the cumulative incidence rate was calculated by dividing the number of new cases of liver injury events in the population over 10-months by the total number of AAP users at risk of developing a liver injury.

The study utilized a stepwise logistic regression model via backward elimination to choose the statistically significant independent variables associated with liver injury. The study used univariate and multivariable analysis. The univariate analysis evaluated the unadjusted association between the liver injury outcome and a single variable. In contrast, the multivariable analysis assessed the adjusted association between liver injury outcomes with several variables simultaneously.^{29, 30} Before the multivariable analysis, collinearity assessment of independent variables was performed using the variation inflation factor and condition index. Collinearity assessment determined that there were no variables that were highly correlated (variation inflation factor greater than 5 or conditional index greater than 30).

Model development started with a univariate logistic regression analysis (based on liver injury outcome: no or yes) and reported odds ratios, 95% CI, and p-values. The variables with less than 0.1 p-values were further included in the multivariable logistic regression analysis. The AAP drug type, age, and gender were included in the multivariable model throughout. For the rest of the covariates, a stepwise multivariable logistic regression model via backward elimination was utilized to choose the most statistically significant variables after removing insignificant variables with p-value ≥ 0.05 .^{29, 31}

The Hosmer and Lemeshow goodness-of-fit test was used to determine the performance of the model in this study. If the p-value is higher than 0.05, the model fits appropriately with no significant difference between observed and predicted values.³⁰ The Area Under Curve – Receiving Operating Curve (AUC-ROC) method was used to identify the optimal model that predicts the liver injury outcome. AUC-ROC is a plot of sensitivity vs. 1-specificity.^{30, 32, 33} SAS version 9.4 (SAS Institute, Cary, NC) was used to perform all the analysis, and all statistical tests were two-sided with the 0.05 significance level.

3.4 RESULTS

Study cohort

As shown in Figure 2, we selected 669,972 patients prescribed AAP medications from January 1, 2010 to December 31, 2015. We excluded 40,262 patients who were prescribed AAP medications during the pre-index period (12-months before the index date), 519,863 patients had less than 12-months of continuous enrollment prior to or following the index period, 11,157 patients were younger than 18 years of age, and 5,810 patients had a liver injury or HIV diagnosis during the pre-index or exposure period. As described in the cohort selection section, we excluded 33,465 patients who were also prescribed chronic concomitant medications implicated in DILI during the pre-index or exposure period. Therefore, after applying all exclusion criteria, the final cohort contains 44,414 patients who were dispensed the most frequently used AAP medications, including quetiapine (41.4%), aripiprazole (27.5%), risperidone (20.5%), olanzapine (8.6%), and ziprasidone (2%).

Demographic and clinical characteristics of the study population

A detailed description of demographic and clinical characteristics of the study population is presented in Table 2 (A, B, C). Overall, more than 36% of patients were between the ages of 41 and 65, followed by 31.3% of patients over 76. A large proportion of the study population (66.1%) were female. Most of the patients (40.3%) enrolled to a health maintenance organization plan, followed by 34.7% of the patients who had a point of service plan. There were similar proportions of patients with commercial (52.8%) and Medicare (47.2%) insurance type. Most of the patients were in the south (43.3%) and the west (28.6%) geographic region. About 79% of patients had diagnoses other than schizophrenia, psychosis, major depressive, bipolar disorder, or anxiety. The top comorbidities in the study population were hypertension (46%), hyperlipidemia (36.4%), cardiovascular disease (26%), and type 2 diabetes

(18.7%). Approximately 45% and 9% of the patients had Elixhauser comorbidity scores of 1-3 and 4-10, respectively, indicating that over half of the population consisted of patients with at least one comorbidity.

Liver injury incidence rate for AAP medications

Table 3 indicates the cumulative incidence rate of liver injury in patients taking AAP medications, combining diagnoses from outpatient and inpatient settings. Overall, the cumulative incidence rate per 1000 persons over 10 months was 15.3. The cumulative incidence rate of various AAP therapies ranged from 12.7 to 20.9. Based on the cohort selected, ziprasidone had the highest incidence rate of 20.9; alternatively, risperidone had the lowest incidence rate of 12.7 per 1000 persons over 10 months. Liver injury diagnosis was further characterized by the severity of the injury. Overall, cholestasis or cirrhosis liver injury was the most frequent type of liver injury reported, representing 44.8% of all types of liver injuries documented during the follow-up period. On the other hand, liver necrosis was least frequently reported (3.4%). Similar trends were observed for particular AAP types, where cholestasis or cirrhosis represented 39.6% to 46.2% of documentations of liver injury, while liver enzyme elevations ranged from 24.5% to 34.1% of documented liver injuries. Acute liver failure, the most severe form of liver injury, represented 24.2% to 31.2% of documented liver injuries. It is important to note here that a patient can report more than one form of liver injury during the period observed.

Liver injury outcomes using univariate logistic regression modeling

The univariate logistic regression models were used after dichotomizing the individual demographic variables such as age, gender, type of health plan and insurance, and geographic region. Moreover, the comorbidities were selected based on published research on the association of these factors with liver injury risk. The collinearity assessment did not identify any independent variables that were highly

collinear; therefore, all the variables were further included in univariate and multivariable logistic regression analyses.

Table 4 includes the unadjusted odds ratios, number of patients with or without liver injury diagnosis, 95% confidence intervals, and p-values of the univariate regression models for liver injury outcomes. Overall, there were low numbers of cases (1025; 2.3%) of patients with liver injury diagnosis as compared with the number of patients without liver injury (43,389; 97.7%). Based on the model, p-values were less than 0.1 for age, type of health plan and insurance plan, geographic region, various diagnosis near index date, patient comorbidities, and Elixhauser comorbidity index. Although p-values were greater than 0.1 for various AAP medications and gender, they were included in the multivariable model given the importance of these variables to the analysis.

Liver injury outcomes using multivariable logistic regression modeling

To determine the significant predictive variables associated with liver injury outcome risk, a multivariable logistic regression model was applied and fitted with independent variables, including AAP medication type, age, gender, diagnosis, comorbidities, and Elixhauser's comorbidity index. Health plan type, insurance type, and geographic regions were excluded from the model as they did not show significant associations during the multivariable model building process ($p > 0.05$). Similarly, schizophrenia/psychosis diagnosis, other diagnoses near index date, type 2 diabetes comorbidity, and renal disease comorbidities were also removed due to their lack of statistical significance during model building ($p > 0.05$). As shown in figure 3 and Table 5, having 1 to 3 comorbidities (aOR 1.36, CI 1.17-1.56, $p < 0.0001$) or 4 to 10 comorbidities (aOR 1.59, CI 1.27-1.97, $p < 0.0001$) were associated with 36%, and 59% increased risk of liver injury, respectively. Similarly, comorbidities such as alcohol abuse (aOR 1.57, CI 1.23-2.02, $p = 0.0003$), hypertension (aOR 1.51, CI 1.29-1.77,

$p < 0.0001$), obesity (aOR 1.35, CI 1.12-1.61, $p = 0.002$), hyperlipidemia (aOR 1.27, CI 1.1-1.47, $p = 0.0013$), and cardiovascular diseases (aOR 1.26, CI 1.08-1.47, $p = 0.0027$) were associated with increased risk of liver injury. Major depressive or bipolar disorder diagnosis (aOR 1.17, CI 1.01-1.35, $p = 0.032$) or anxiety diagnosis (aOR 1.2, CI 1.03-1.37, $p = 0.022$) was associated with 16%, and 20% increased risk of liver injury. Patients aged 76 or older (aOR 0.45, CI 0.35-0.58, $p < 0.0001$) were associated with a 55% decreased risk of liver injury. AAP therapy and gender were included in the multivariable logistic regression model for liver injury risk; however, both variables did not significantly associate with the outcome measured. The Hosmer and Lemeshow goodness-of-fit test was performed to determine if the liver injury association model fit adequately; the model fit appropriately with a p -value of 0.09. Furthermore, the predictive model's assessment was also conducted using ROC-AUC; the ROC-AUC value was 0.66, indicating a good predictive model.

Daily dose distribution of AAP medications in patients ages 76 and older

Table 6 indicates the daily dose distribution of AAP medications for patients ages 76 or older. For aripiprazole, daily recommended doses are between 10 to 30 mg, over 90% of the ≥ 76 age patients were prescribed below the daily recommended doses (i.e., 2 and 5 mg doses). The recommended dose of olanzapine is between 10 to 20 mg daily, approximately 93% of patients age ≥ 76 were prescribed below the daily recommended doses (i.e., 2.5 and 5 mg doses). The recommended dose of risperidone is between 2 to 8 mg daily; over 99% of these older patients were prescribed below the daily recommended doses (i.e., 0.25, 0.5, and 1 mg doses). The recommended dose of ziprasidone is between 40 to 80 mg daily; over 95% of these older patients were prescribed below or at the lower end of the daily recommended doses (i.e., 20 and 40 mg doses). Finally, the recommended dose of quetiapine is

between 300 to 800 mg daily, over 97% of these older patients were prescribed below the daily recommended doses (i.e., 25 and 50 mg doses).

Sensitivity analysis – Predictive variables for liver injury outcomes in patients 75 and younger

As indicated above, >90% of the patients over the age of 76 were prescribed below the daily recommended AAP drug doses. Therefore, in a sensitivity analysis, we included only patients age 75 or younger using the same multivariable logistic regression modeling approach as previously described. As shown in Table 7, in this model quetiapine was associated with a 22% (aOR 1.22, CI 1.04-1.44, $p = 0.02$) increased risk of liver injury in 75 and younger patients as compared with aripiprazole use. Patients having 1 to 3 comorbidities (aOR 1.27, CI 1.09-1.48, $p = 0.003$) or 4 to 10 comorbidities (aOR 1.57, CI 1.22-2.02, $p = 0.0004$) were associated with 27% and 57% increased risk of liver injury, respectively. Similarly, comorbidities such as alcohol abuse (aOR 1.63, CI 1.27-2.08, $p < 0.0001$), hypertension (aOR 1.46, CI 1.23-1.73, $p < 0.0001$), obesity (aOR 1.36, CI 1.12-1.65, $p = 0.002$), and hyperlipidemia (aOR 1.31, CI 1.1-1.54, $p = 0.0012$) were associated with increased risk of liver injury. Anxiety diagnosis (aOR 1.2, CI 1.03-1.38, $p = 0.021$) was associated with a 20% increased risk of liver injury. Based on the Hosmer and Lemeshow goodness-of-fit test, the p -value was 0.17, and the AUC-ROC value was 0.64.

Sensitivity analysis – More restricted definitions of the liver injury outcome in patients 75 and younger

In addition to the composite liver injury outcome, we also built separate models based on liver injury severity. Table 8 indicates the multivariable model for composite liver injury outcomes, excluding elevated enzymes, for the population of patients age 75 and younger. Similar to the composite liver injury outcome model, quetiapine was associated with a 22% (aOR 1.22, CI 1.01-1.46, $p = 0.036$) increased risk of liver injury.

Having 1 to 3 comorbidities (aOR 1.32, CI 1.1-1.71, $p = 0.002$) or 4 to 10 comorbidities (aOR 1.78, CI 1.35-2.33, $p = <0.0001$) were associated with 32% and 78% increased risk of liver injury. Similarly, comorbidities such as alcohol abuse (aOR 1.76, CI 1.35-2.3, $p <0.0001$), renal disease (aOR 1.37, CI 1.03-1.83, $p = 0.03$), hypertension (aOR 1.57, CI 1.31-1.89, $p <0.0001$), obesity (aOR 1.38, CI 1.11-1.71, $p = 0.003$), and hyperlipidemia (aOR 1.29, CI 1.08-1.54, $p = 0.005$) were associated with increased risk of liver injury. Major depressive or bipolar disorder diagnosis (aOR 1.26, CI 1.06-1.48, $p = 0.007$) was associated with a 26% increased risk of liver injury. Based on the Hosmer and Lemeshow goodness-of-fit test, the p -value was 0.3, and the AUC-ROC value was 0.65. We also examined the most severe liver injury outcomes, including liver necrosis and acute liver failure for patients ages 75 and younger. As shown in Table 9, we did not observe any significant association of any particular AAPs with the outcomes measured.

3.5 DISCUSSION

Drug-induced liver injury is rare but can result in life-threatening liver failure, potentially leading to death. DILI was first described over 70 years ago.³⁴ Since then, it has become a major concern for clinicians, the pharmaceutical industry, and health authorities as more than 1000 drugs have been associated with liver injury.³⁴ Many drug classes are associated with DILI; the antipsychotic drug class is one of them. Typical antipsychotics were first discovered in the 1950s; whereas, atypical antipsychotics were introduced in the 1990s.³⁵ Current studies have reported that there has been increased use of AAPs in recent years.³⁶ It is generally believed that, compared to typical antipsychotics, atypical antipsychotics have less adverse side effects.^{5, 35} Also, AAPs have better molecular toxicity profile; they are not associated with mitochondrial mechanisms of toxicity. However, there have been reports of liver enzymes and bilirubin elevation along with liver function abnormalities in clinical setting.^{5, 6} Moreover, there is a gap in the literature evaluating the incidence and the risk association for AAP in the real-world.

Based on the cohort selected in the study, we report that quetiapine (41.4%) was the most frequently prescribed AAP. Our reported prescription rate agreed with a study that examined the international trends in antipsychotic use.³⁶ The study reported that in 2014, quetiapine was the most frequently used AAP in all age groups in most of the countries studied.³⁶ Our study cohort was predominantly (>66%) female, and over 31% of the cohort was older than 76 years. This finding was consistent with a published report that the prevalence of antipsychotic use has increased by 15% in people aged 65 and older.³⁶ We reported that 79% of our cohort patients had diagnoses other than schizophrenia, psychosis, major depressive, bipolar disorder, or anxiety. This finding agreed with the published study by Carton et al., who reported that up to 93% of antipsychotics could be prescribed for off-label use.³⁷

Based on published literature, AAP-induced severe liver toxicity is rare. Telles-Correia (2017) published a review of psychotherapeutics and liver injury, where the authors did not report any liver injury reports for aripiprazole.⁵ Olanzapine, risperidone, and quetiapine had reports of liver enzyme elevations.⁵ Olanzapine and risperidone had reports of liver injury via immune mechanisms causing hepatocellular and cholestatic lesions.⁵ We reported the cumulative incidence rate of 15.3 per 1000 persons over 10 months for AAP medications. Based on the cohort selected, ziprasidone had the highest incidence rate of 20.9; alternatively, risperidone had the lowest incidence rate of 12.7 per 1000 persons over 10 months. However, these are rates of liver injury of patients who were prescribed AAPs; yet we do not know if the liver injury was caused by the AAP.

To our knowledge, there were no studies that evaluated the cumulative incidence rate of liver injury using the claims database. Liver injury diagnosis was further characterized by the severity of the injury. Of all the liver injuries reported, cholestasis or cirrhosis was reported most frequently at the 44.8% of all types reported, followed by acute liver failure which represented 28.4% of documented liver injuries. Our study reported liver enzyme elevation represented 24.5% to 34.1% of liver injury events. There are conflicting reports for liver injuries enzyme elevation (alanine transaminase, aspartate transaminase, and lactate dehydrogenase) with AAP therapy. Some reports indicated that liver enzymes' elevation is infrequent or low with AAP agents,^{38, 39} whereas some studies have reported increases in transaminase liver enzymes and bilirubin with AAP therapy.⁵

We evaluated AAP therapy in the multivariable logistic regression model for liver injury risk; however, it did not significantly associate with the liver injury outcome. We believed that it is due to the inclusion of patients 76 ages and older. We demonstrated that over 90% of the patients over the age of 76 were prescribed below

the daily recommended doses for all the AAP medications. In other words, older patients in the nursing home facility may be prescribed lower strength AAP medications for off-label use of minor symptoms such as agitation or insomnia, and likely use the AAP on an as-needed basis (i.e. sporadically). Several studies have reported that off-label AAP medications use expected in about ~65% of elderly patients (≥ 65 years) from 42 states Medicaid programs;⁴⁰ additionally, 83% of Medicare claims for AAP were associated with off-label conditions and 88% with dementia.⁴¹ Moreover, older age patients will mostly be in a nursing home setting, which may be closely monitored for liver enzyme elevation. They are more prone to stop AAP drug therapy if they show an elevation in liver enzymes. Therefore, we only included patients 75 and younger in a sensitivity analysis using multivariable logistic regression modeling. After excluding older age patients (≥ 76 years), quetiapine was associated with a 22% increased risk of liver injury. Similarly, the sensitivity analysis for composite liver injury outcomes, excluding elevated enzymes for patients 75 and younger, was performed. We found that quetiapine AAP medication was associated with a 22% increased risk of liver injury and excluding liver enzyme elevation provided the same risk association of quetiapine with liver injury. Liver enzyme elevations could be due to a variety of reasons and could sometimes be transient. Elevation in liver enzymes does not always progress to liver injury outcomes. Vatsalya et al. investigated quetiapine-induced liver injury (received 12-weeks of therapy) in heavy alcohol drinking 218 patients.⁴² They concluded that quetiapine did not cause liver injury (measured by liver enzymes) in patients 18-65 years of age with pre-existing liver injury.⁴² Furthermore, liver injury incidence and severity were low in quetiapine-treated patients.⁴² Quetiapine is associated with mild liver enzyme elevations. However, several case-control studies reported patients developing acute liver injury or hepatic failure after receiving quetiapine therapy.⁴³⁻⁴⁶ Therefore, the evidence of the association of quetiapine-

induced liver injury is reported in several case-control studies but lacks more extensive studies. The published reports suggest that quetiapine-induced hepatotoxicity can be idiosyncratic, and the mechanism is unknown.⁴⁶ Based on sensitivity analysis, we did not observe any significant association of any AAPs with the severe liver injury outcomes, including liver necrosis and acute liver failure. We believe that the 10-months of follow-up period may not be sufficient enough to observe severe liver injury symptoms.

We investigated comorbidities such as hypertension, type 2 diabetes, obesity, alcohol abuse, hyperlipidemia, renal disease, and cardiovascular disease associated with a liver injury among patients newly treated with AAP medications.²⁰⁻²³ The clinical practice guidelines from the European Association for the Study of Liver (EASL) indicated that older age, female gender, alcohol intake, and comorbidities (metabolic disease, chronic hepatitis) might be risk factors for DILI.¹⁹ Quetiapine was associated with increased risk of DILI (for patients 75 and younger) for comorbidities; having 1 to 3 comorbidities or 4 to 10 comorbidities were associated with 27% and 57% increased risk of liver injury. We further reported that comorbidities such as alcohol abuse (63%), hypertension (46%), obesity (36%), and hyperlipidemia (31%) were associated with increased risk of liver injury. Hayes et al. performed a population-based cohort study using UK health records from 1995 to 2012 in bipolar disorder patients.⁴⁷ In the study, for ~1.5 years of drug treatment duration, there were 67% increased rates of 15% weight gain in 1376 patients taking quetiapine (hazard ratio (HR) 1.67, 95% CI 1.24-2.20, $p < 0.001$).⁴⁷ Hayes et al. did not observe a statistically significant difference in hepatotoxicity in quetiapine treatment than lithium.⁴⁷ In contrast, compared to lithium, quetiapine was associated with lower risk of kidney and endocrine adverse events (kidney disease HR 0.62, 95% CI 0.47–0.80, $p < 0.001$; hypercalcemia HR 0.23, 95% CI 0.07–0.73, $p = 0.013$).⁴⁷

Ucok et al. indicated that AAP medication users are associated with a higher risk of weight gain, new-onset of diabetes mellitus, hyperlipidemia, prolongation of the QTc interval of the electrocardiogram, myocarditis, sexual dysfunction, extrapyramidal side effects, and new-onset of cataracts.²⁰ Meyer and Koro (2004) published a review indicating that quetiapine (along with olanzapine and clozapine) has been associated with a higher risk of hyperlipidemia.⁴⁸ Atmaca et al. showed that quetiapine was associated with increased triglyceride levels in a 6-week prospective study of schizophrenia patients (11.64 ± 4.56 mg/dl, $p < 0.05$).⁴⁹ Centorrino et al. indicated that patients taking psychotropic medications had a higher prevalence of metabolic comorbidities than the general population (37% compared to 30%; $p < 0.0001$).²¹

3.6 CONCLUSION

This retrospective cohort study determined the cumulative incidence rate of liver injury 15.2 to 25.1 per 1000 persons per year among patients newly treated with AAP medications. Compared to aripiprazole, quetiapine was associated with a 22% increased risk of liver injury during the first months of treatment in patients 75 years and younger. We propose further prospective real-world studies to substantiate these findings. We propose that patients on quetiapine AAP should be monitored for liver enzyme elevations as an early sign of liver injury. Finally, patients with various comorbidities such as alcohol abuse, hypertension, obesity, and hyperlipidemia, should be monitored closely for liver injury when they are prescribed AAPs.

3.7 LIMITATION

The study used an administrative claims database where the data was collected mainly for claim purposes; thus, billing and coding errors may be present. Studies utilizing claims database could be relatively inexpensive; however, there is no control over the types of data collected. Therefore, there may be insufficient or inadequate information on exposures, outcomes, and confounders. Observational retrospective cohort studies such as ours are a less robust design than randomized control trials due to potential inherent biases; therefore, a causality of scientific mechanism-based hypothesis for an AAP-induced liver injury was not possible. Additionally, increased risk or association does not imply causation. Further study(is) may be necessary to evaluate these factors' predictive nature using more robust methodologies and in vitro/in vivo mechanistic toxicity studies. Medical claims databases do not contain information on potential risk factors such as illicit drug use, over-the-counter drug use, and smoking; these additional potential risk factors remain unknown.⁵⁰ Finally, using the claims database allowed drug use assessment based mainly on pharmacy claims; the real use patterns of medication use was unknown.

The study contains fixed exposure and follow up period as we planned to evaluate AAP treatment during initial stages where doses are low. We intended to examine the differential risk of liver injury during the treatment initiation phase and not over a longer time. This short-term analysis is adequate as there is no time between exposure and outcome; it will enable us to evaluate the temporal relationship between exposure and outcome. However, fixed exposure and follow-up period could cause selection bias. Moreover, it is possible that a patient could have a liver injury after one or two months of follow up period and could discontinue the treatment. If patients switched to different AAP within the 60-days exposure period, they were excluded from the study. However, if a patient switched to different antipsychotic drugs outside 60-

days of the exposure period, their outcome was attributed to the AAP initially prescribed. The study assessed the cumulative incidence rate of liver injury between AAP among new users with various comorbidities. Therefore, the underlined comorbidities influencing liver injury in the new AAP users cannot be ruled out. From the Optum database, liver function tests were not available; hence, quantitative elevation in hepatic enzyme levels was not investigated. This study only assessed short term outcomes (10-months); therefore, the long-term prognosis of drug-induced hepatotoxicity and chronic hepatopathy were not evaluated. This information could add value to the study, as in many cases, short-term liver enzyme elevations could be reversed and may not necessarily result in long-term liver damage. Moreover, there are limitations of using only ICD9 codes to identify adverse events such as liver injury, which have poor sensitivity and may lead to underreporting of liver injuries.²⁶ Junjuvadia et al. (2007) recommended combining a text search of the dictated medical record with ICD9 codes to identify liver injury adverse events in an administrative claims database.

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3.9 TABLES AND FIGURES

Table 1. Literature references and diagnosis terms for the ICD9 codes used in the study.

Literature Reference	Diagnosis Term	ICD9 codes
Jinjuvadia et al. ²⁶ ; Udo et al. ⁵¹	Jaundice	277.4
Forns et al. ⁵²	Nonspecific elevation of transaminase or lactate dehydrogenase	790.4
Jinjuvadia et al. ²⁶ ; Lo Re et al. ²⁷ Udo et al. ⁵¹ ; Bui et al. ²⁸ Forns et al. ⁵²	Acute and subacute necrosis of liver	570.x
Jinjuvadia et al. ²⁶ ; Lo Re et al. ²⁷ Udo et al. ⁵¹	Liver failure, not otherwise specified	572.8
Schmidt et al. ⁵³ ; Kramer et al. ⁵⁴ ; Liu et al. ⁵⁵	Cirrhosis	571.2, 571.5, 571.6
Jinjuvadia et al. ²⁶ ; Udo et al. ⁵¹ Lo Re et al. ²⁷ ; Bui et al. ²⁸ ; Forns et al. ⁵²	Jaundice, hepatocellular or Hepatitis	573.3
Jinjuvadia et al. ²⁶	Cholestasis	576.8
Jinjuvadia et al. ²⁶ ; Bui et al. ²⁸	Jaundice alone	782.4
Lo Re et al. ²⁷ ; Udo et al. ⁵¹ Bui et al. ²⁸ ; Forns et al. ⁵²	Acute liver failure, hepatic coma, hepatitis	572.2, 572.4, 573.8, and V42.7
Udo et al. ⁵¹	Unspecified disorder of the liver	573.9
Dietz et al. ⁵⁶	HIV diagnosis	042,079.53, 795.71, V08
Meller et al. ⁵⁷ ; Lau et al. ⁵⁸	Obesity	V85.xx, 278
Klompas et al. ⁵⁹ ; Hux et al. ⁶⁰ ; Wang et al. ⁶¹	Diabetes mellitus (type 2)	250.x0, 250.x2
Franklin et al. ⁶² ; Wang et al. ⁶¹ ; Lambert et al. ²³	Hyperlipidemia	272.0-272.4
Wang et al. ⁶¹	Hypertension	401.xx-405.xx
Du Cheyron et al. ⁶³ ; Moreau et al. ⁶⁴ ; Liu et al. ⁵⁵ ; Wang et al. ⁶¹	Renal disease	403.xx, 404.xx, 580.xx – 588.xx
Wang et al. ⁶¹	Cardiovascular disease	420.xx-429.xx; 433.xx-434.xx
Issak et al. ⁶⁵	Alcohol abuse	305.0
Cloutier et al. ⁶⁶ ; Lambert et al. ²³ ; Litz et al. ⁶⁷	Schizophrenia	295.00-295.99
Gardner et al. ⁶⁸ ; Litz et al. ⁶⁷	Major depressive disorder/bipolar disorder (MDDBP)	296.00-296.09, 296.10-296.19, 296.40-296.80, 296.20-296.39
Litz et al. ⁶⁷	Anxiety disorder	300.00-300.99, 309.00-309.99
Litz et al. ⁶⁷	Personality disorder	301.00-301.99, 301.20-301.99
Litz et al. ⁶⁷	Other psychosis	297.00-299.99

Table 2A. Demographic and clinical characteristics of patients prescribed atypical antipsychotic drugs.

Demographic and clinical characteristics of patients prescribed atypical antipsychotic drugs						
	Aripiprazole (N=12214; 27.5%)	Olanzapine (N=3833; 8.6%)	Quetiapine (N=18404; 41.4%)	Risperidone (N=9062; 20.4%)	Ziprasidone (N=901; 2%)	Total (N=444 14; 100%)
Age (Years)						
Mean (SD)	49.15 (16.4)	63.72 (19.6)	63.54 (20.4)	65.9 (20.4)	50.3 (17.88)	
18-40 (reference)	3577 (29.3%)	555 (14.5%)	3964 (16.1%)	1303 (14.4%)	277 (30.7%)	8676 (19.5%)
41-65	6789 (55.4%)	1233 (32.2%)	5621 (30.5%)	2309 (25.5%)	438 (48.6%)	16370 (36.7%)
66-75	1123 (9.2%)	619 (16.2%)	2410 (13.1%)	1206 (13.3%)	89 (9.9%)	5447 (12.3%)
≥76	745 (6.1%)	1426 (37.2%)	6409 (40.3%)	4244 (46.8%)	97 (10.8%)	13921 (31.3%)
Gender						
Female (reference)	8692 (71.2%)	2449 (63.9%)	11694 (63.5%)	5885 (64.9%)	629 (69.8%)	29349 (66.1%)
Male	3522 (28.8%)	1384 (36.1%)	6710 (36.5%)	3177 (35.1%)	272 (30.2%)	15065 (33.9%)
Health plan						
HMO (reference)	2783 (22.8%)	1921 (50.1%)	8351 (45.4%)	4575 (50.5%)	256 (28.4%)	17886 (40.3%)
EPO	1248 (10.2%)	188 (4.9%)	942 (5.1%)	377 (4.2%)	74 (8.2%)	2829 (6.4%)
IND	116 (1%)	113 (3%)	627 (3.4%)	320 (3.5%)	5 (0.5%)	1181 (2.7%)
PPO	751 (6.2%)	239 (6.2%)	1321 (7.2%)	647 (7.1%)	54 (6%)	3012 (6.8%)
POS	6801 (55.7%)	1033 (27%)	5161 (28%)	2013 (22.2%)	419 (46.5%)	15427 (34.7%)
Other	515 (4.2%)	339 (8.8%)	2002 (11%)	1130 (12.5%)	93 (10.3%)	4092 (9.1%)
HMO, Health Maintenance Organization; EPO, Exclusive Provider Organization; PPO, Preferred Provider Organization; POS, Point of Service; Bold values represent p-value less than 0.05 exposed vs. non-exposed (Statistical difference between different AAP users and various demographic and clinical characteristics groups on a categorical variable were performed using a chi-square test, and a comparison of all the continuous variables were performed using t-tests)						

Table 2B. Demographic and clinical characteristics of patients prescribed atypical antipsychotic drugs.

Demographic and clinical characteristics of patients prescribed atypical antipsychotic drugs						
	Aripiprazole (N=12214; 27.5%)	Olanzapine (N=3833; 8.6%)	Quetiapine (N=18404; 41.4%)	Risperidone (N=9062; 20.4%)	Ziprasidone (N=901; 2%)	Total (N=44414; 100%)
Type of insurance						
Commercial (reference)	9745 (79.8%)	1620 (42.3%)	8240 (44.8%)	3281 (36.2%)	582 (64.6%)	23468 (52.8%)
Medicare	2469 (20.2%)	2213 (57.7%)	10164 (55.2%)	5781 (63.8%)	319 (35.4%)	20946 (47.2%)
Geographic region						
Northeast (reference)	1094 (9%)	267 (7%)	1545 (8.4%)	742 (8.2%)	48 (5.3%)	3696 (8.3%)
Midwest	2683 (22%)	769 (20%)	3400 (18.5%)	1739 (19.2%)	191 (21.2%)	8782 (19.8%)
West	2491 (20.4%)	1398 (36.5%)	2981 (15.7%)	2981 (32.9%)	163 (18.1%)	12718 (28.6%)
South	5946 (48.7%)	1399 (36.5%)	5685 (31%)	3600 (39.7%)	499 (55.4%)	19218 (43.3%)
Diagnosis near index date						
Schizophrenia/Psychosis	714 (6%)	598 (15.6%)	1768 (9.6%)	1447 (16%)	174 (19.3%)	4701 (10.6%)
Major depressive/bipolar disorder	4106 (33.6%)	765 (20%)	3006 (16.3%)	1709 (18.9%)	344 (38.2%)	9930 (22.4%)
Anxiety	3207 (26.3%)	721 (18.8%)	3607 (19.6%)	1611 (17.8%)	220 (24.4%)	9366 (21.1%)
Others	9007 (73.7%)	3112 (81.2%)	14797 (80.4%)	7451 (82.2%)	681 (75.5%)	35048 (78.9%)
Bold values represent p-value less than 0.05 exposed vs. non-exposed (Statistical difference between different AAP users and various demographic and clinical characteristics groups on a categorical variable were performed using a chi-square test, and a comparison of all the continuous variables were performed using t-tests)						

Table 2C. Demographic and clinical characteristics of patients prescribed atypical antipsychotic drugs.

Demographic and clinical characteristics of patients prescribed atypical antipsychotic drugs						
	Aripiprazole (N=12214; 27.5%)	Olanzapine (N=3833; 8.6%)	Quetiapine (N=18404; 41.4%)	Risperidone (N=9062; 20.4%)	Ziprasidone (N=901; 2%)	Total (N=44414; 100%)
Patients with comorbidities						
Obesity	1319 (11%)	228 (6%)	1233 (6.7%)	596 (6.6%)	127 (14.1%)	3513 (7.9%)
Diabetes mellitus (type 2)	1780 (14.6%)	727 (19%)	3532 (19.2%)	2065 (22.8%)	208 (23.1%)	8312 (18.7%)
Hyperlipidemia	3987 (32.6%)	1407 (36.7%)	6891 (37.4%)	3569 (39.4%)	327 (36.3%)	16181 (36.4%)
Renal disease	498 (4.1%)	391 (10.2%)	1774 (9.6%)	1089 (12%)	53 (6%)	3805 (8.6%)
Hypertension	4449 (36.4%)	1882 (49.1%)	9205 (50.0%)	4850 (53.5%)	404 (44.8%)	20790 (46.8%)
Cardiovascular disease	1897 (15.5%)	1099 (28.7%)	5413 (29.4%)	2910 (32.1%)	207 (23%)	11526 (26%)
Alcohol abuse	475 (4%)	148 (4%)	786 (4.3%)	340 (4%)	60 (6.6%)	1809 (4.1%)
Elixhauser comorbidity index						
0	5676 (46.5%)	1799 (46.9%)	8493 (46.2%)	4143 (45.7%)	360 (40%)	20471 (46.1%)
1-3	5795 (47.5%)	1666 (43.5%)	8098 (44%)	3935 (43.4%)	457 (50.7%)	19951 (44.9%)
4-10	743 (6.1%)	368 (9.6%)	1813 (9.9%)	984 (10.9%)	84 (9.3%)	3992 (9%)
Bold values represent p-value less than 0.05 exposed vs. non-exposed (Statistical difference between different AAP users and various demographic and clinical characteristics groups on a categorical variable were performed using a chi-square test, and a comparison of all the continuous variables were performed using t-tests)						

Table 3. Cumulative incidence of liver injury rate in patients prescribed atypical antipsychotic drugs.

	Aripiprazole	Olanzapine	Quetiapine	Risperidone	Ziprasidone	Total
Number of diagnosis reported	18043	6483	30647	15191	1484	71848
liver injury diagnoses (No)	17722 (98.2%)	6392 (98.6%)	30186 (98.5%)	14998 (98.7%)	1453 (97.9%)	70751 (98.5%)
Liver injury diagnosis (Yes)	321 (1.78%)	91 (1.4%)	461 (1.5%)	193 (1.3%)	31 (2.1%)	1097 (1.5%)
Liver enzyme elevation only	87 (27.1%)	31 (34.1%)	113 (24.5%)	49 (25.4%)	10 (32.3%)	290 (26.4%)
Jaundice or hepatitis	70 (21.8%)	15 (16.5%)	107 (23.2%)	35 (18.1%)	6 (19.4%)	233 (21.2%)
Cholestasis or cirrhosis	141 (43.9%)	36 (39.6%)	213 (46.2%)	89 (46.1%)	13 (41.9%)	492 (44.8%)
Liver necrosis	12 (3.7%)	5 (5.5%)	10 (2.2%)	7 (3.6%)	3 (9.7%)	37 (3.4%)
Acute liver failure	100 (31.2%)	22 (24.2%)	130 (28.2%)	51 (26.4%)	9 (29%)	312 (28.4%)
Cumulative incidence rate (per 1000 person over 10 months)	17.80	14.03	15.00	12.70	20.90	15.30

Table 4. Number of patients with or without liver injury diagnosis, unadjusted odds ratio, 95% confidence interval, and p-values of independent variables for liver injury outcome using univariate logistic regression modeling.

Independent variables	No liver injury	Liver injury	Odds ratio estimates	Confidence Intervals	*p-value
Total	43389 (97.7%)	1025 (2.3%)			
AAP medications					
Aripiprazole	11914 (27.5%)	300 (29.3%)	Reference		0.125
Olanzapine	3750 (8.6%)	83 (8.1%)	0.88	0.69-1.12	
Quetiapine	17971 (41.4%)	433 (42.4%)	0.96	0.82-1.11	
Risperidone	8880 (20.5%)	182 (17.8%)	0.81	0.68-0.98	
Ziprasidone	874 (2%)	27 (2.6%)	1.22	0.82-1.83	
Age (Years)					
18-40	8504 (19.6%)	172 (16.8%)	Reference		<0.0001*
41-65	15863 (36.6%)	508 (49.6%)	1.58	1.33-1.89	
66-75	5287 (12.2%)	160 (15.6%)	1.5	1.2-1.9	
≥76	13736 (31.7%)	185 (18.1%)	0.66	0.54-0.82	
Gender					
Female	28695 (66.1%)	654 (63.8%)	Reference		0.12
Male	14695 (33.9%)	371 (36.2%)	1.11	0.97-1.26	
Type of health plan					
HMO	17529 (40.4%)	357 (34.8%)	Reference		0.0005*
EPO	2758 (6.4%)	71 (6.9%)	1.26	0.98-1.64	
IND	1159 (2.7%)	22 (2.2%)	0.93	0.6-1.44	
PPO	2916 (6.7%)	96 (9.4%)	1.62	1.29-2.03	
POS	15041 (34.7%)	386 (37.7%)	1.26	1.09-1.46	
Other	3986 (9.2%)	93 (9.1%)	1.15	0.9-1.44	
Type of Insurance					
Commercial	22885 (52.7%)	583 (56.9%)	Reference		0.0088*

Medicare	20504 (47.3%)	442 (43.1%)	0.85	0.75-0.96	
Geographic region					
Northeast	3593 (8.3%)	103 (10.1%)	Reference		<0.0001*
Midwest	8581 (19.8%)	201 (19.6%)	0.82	0.64-1.04	
West	12485 (28.8%)	233 (22.7%)	0.65	0.52-0.82	
South	18730 (43.2%)	488 (47.6%)	0.91	0.73-1.13	
Diagnosis near index date (yes)					
Schizophrenia/Psy chosis	4543 (10.5%)	158 (15.4%)	1.56	1.31-1.85	<0.0001*
Major depressive/bipolar disorder	9611 (22.2%)	319 (31.1%)	1.59	1.39-1.82	<0.0001*
Anxiety	9069 (20.9%)	297 (29%)	1.54	1.35-1.77	<0.0001*
Others/diagnosis not documented	34320 (79.1%)	728 (71%)	0.65	0.57-0.74	<0.0001*
Patients comorbidities (yes)					
Obesity	3362 (7.8%)	151 (14.7%)	2.06	1.73-2.45	<0.0001*
Diabetes mellitus (type 2)	8037 (18.5%)	275 (26.8%)	1.61	1.4-1.86	<0.0001*
Hyperlipidemia	15672 (36.1%)	509 (49.6%)	1.75	1.54-1.98	<0.0001*
Renal disease	3692 (8.5%)	113 (11%)	1.33	1.09-1.63	0.0045*
Hypertension	20162 (46.5%)	628 (61.3%)	1.82	1.61-2.07	<0.0001*
Cardiovascular disease	11182 (25.7%)	344 (33.6%)	1.46	1.28-1.66	<0.0001*
Alcohol abuse	1728 (4%)	81 (7.9%)	2.07	1.64-2.61	<0.0001*
Elixhauser comorbidity index					
0	20133 (46.4%)	338 (33%)	Reference		<0.0001*
1-3	19402 (44.7%)	549 (53.6%)	1.69	1.47-1.93	
4-10	3854 (8.9%)	138 (13.5%)	2.13	1.75-2.61	
*All the variables where p-values are less than 0.1 were included to build a multivariable model					

Table 5. Adjusted odds ratio, 95% confidence interval, and p-values of independent variables for liver injury outcome using a multivariable logistic regression model.

Multivariable logistic regression analysis of independent variables for liver injury outcome				
	Independent variables	Odds ratio	95% CI	p-value
AAP therapy	Olanzapine	1.05	0.82-1.35	0.7
	Quetiapine	1.16	0.99-1.36	0.06
	Risperidone	1.01	0.83-1.23	0.92
	Ziprasidone	1.13	0.76-1.69	0.55
Gender	Male	1.03	0.9-1.18	0.64
Age	Age 41-65	1.15	0.95-1.39	0.14
	Age 66-75	0.92	0.72-1.18	0.52
	Age ≥76	0.45	0.35-0.58	<0.0001
Diagnosis	Major depressive/bipolar disorder	1.17	1.01-1.35	0.032
	Anxiety	1.19	1.03-1.37	0.022
Comorbidity	Obesity	1.35	1.12-1.61	0.002
	Hyperlipidemia	1.27	1.1-1.47	0.0013
	Hypertension	1.51	1.29-1.77	<0.0001
	Cardiovascular disease	1.26	1.08-1.47	0.0027
	Alcohol abuse	1.57	1.23-2.0	0.0003
Elixhauser comorbidity index	1 to 3	1.36	1.17-1.56	<0.0001
	4 to10	1.59	1.27-1.98	<0.0001
<p>Bold values indicate statistically significant (p-value less than 0.05) variables. Reference categories for the variable were: Elixhauser score of zero, no comorbidity, no diagnosis, age group 18-40, female gender, and aripiprazole AAP therapy. Hosmer and Lemeshow goodness-of-fit test p-value was 0.09. ROC-AUC value was 0.66.</p>				

Table 6. Dose distribution of atypical antipsychotic drugs in patients ages 76 and older.

Aripiprazole (N=1475; 5.6%); Daily recommended dose - 10-30 mg		Olanzapine (N=2192; 8.4%); Daily recommended dose - 10-20 mg	
Doses	Patients prescribed	Doses	Patients prescribed
2 mg	927 (62.9%)	2.5 mg	1349(61.5%)
5 mg	413 (28%)	5 mg	686 (31%)
10 mg	62 (4.2%)	7.5 mg	13 (0.6%)
15 mg	23 (1.6%)	10 mg	130 (5.9%)
20 mg	5 (0.34%)	15 mg	9(0.4%)
30 mg	24 (1.63%)	20 mg	5 (0.23%)
Risperidone (N=8574; 32.7%); Daily recommended dose - 2-8 mg		Ziprasidone (N=356; 1.4%); Daily recommended dose - 40-80 mg twice	
Doses	Patients prescribed	Doses	Patients prescribed
0.25 mg	3851 (44.9%)	20 mg	206 (57.9%)
0.5 mg	3656 (42.6%)	40 mg	131 (36.8%)
1 mg	854 (10%)	60 mg	4 (1.2%)
3 mg	11 (0.13%)	80 mg	15 (4.2%)
Quetiapine (N=13589; 51.9%); Daily recommended dose - 300-400 mg			
Doses	Patients prescribed		
25 mg	11391 (84.4%)		
50 mg	1898 (14%)		
100 mg	191 (1.4%)		
150 mg	114(0.8%)		
200 mg	101 (0.74%)		
300 mg	23 (0.17%)		
Bold values indicate the highest % of prescribed doses.			

Table 7. Sensitivity analysis - adjusted odds ratio, 95% confidence interval, and p-values of independent variables for liver injury outcome for patients 75 and under.

Multivariable logistic regression analysis of independent variables for liver injury outcome for patients 75 and younger				
	Independent variables	Odds ratio	95% CI	p-value
AAP therapy	Olanzapine	1.12	0.85-1.47	0.42
	Quetiapine	1.22	1.04-1.44	0.02
	Risperidone	1.06	0.85-1.31	0.6
	Ziprasidone	1.25	0.84-1.88	0.27
Gender	Male	1.05	0.91-1.22	0.48
Age	Age 41-65	1.18	0.97-1.43	0.09
	Age 66-75	0.95	0.74-1.23	0.7
Diagnosis	Major depressive/bipolar disorder	1.16	1.0-1.35	0.05
	Anxiety	1.17	1.0-1.36	0.05
Comorbidity	Obesity	1.36	1.12-1.65	0.002
	Hyperlipidemia	1.31	1.11-1.54	0.0012
	Hypertension	1.46	1.23-1.73	<0.0001
	Alcohol abuse	1.63	1.27-2.08	<0.0001
Elixhauser comorbidity index	1 to 3	1.27	1.09-1.48	0.003
	4 to10	1.57	1.22-2.02	0.0004
Bold values indicate statistically significant (p-value less than 0.05) variables. Reference categories for the variable were: Elixhauser score of zero, no comorbidity, no diagnosis, age group 18-40, female gender, and aripiprazole AAP therapy. Hosmer and Lemeshow goodness-of-fit test p-value was 0.17. ROC-AUC value was 0.64.				

Table 8. Sensitivity analysis - adjusted odds ratio, 95% confidence interval, and p-values of independent variables for composite liver injury outcome (excluding liver enzyme elevation) for patients 75 and under.

Multivariable logistic regression analysis of independent variables for composite liver injury outcome excluding elevated enzymes for patients 75 and younger				
	Independent variables	Odds ratio	95% CI	p-value
AAP therapy	Olanzapine	0.94	0.68-1.29	0.69
	Quetiapine	1.22	1.01-1.46	0.036
	Risperidone	1.02	0.8-1.29	0.89
	Ziprasidone	1.14	0.7-1.81	0.59
Gender	Male	0.99	0.84-1.16	0.87
Diagnosis	Major depressive/bipolar disorder	1.26	1.06-1.48	0.007
Comorbidity	Obesity	1.38	1.11-1.71	0.003
	Hyperlipidemia	1.29	1.08-1.54	0.005
	Hypertension	1.57	1.31-1.89	<0.0001
	Renal disease	1.37	1.03-1.82	0.03
	Alcohol abuse	1.76	1.35-2.3	<0.0001
Elixhauser comorbidity index	1 to 3	1.32	1.1-1.71	0.002
	4 to 10	1.78	1.35-2.33	<0.0001
Bold values indicate statistically significant (p-value less than 0.05) variables. Reference categories for the variable were: Elixhauser score of zero, no comorbidity, no diagnosis, age group 18-40, female gender, and aripiprazole AAP therapy. Hosmer and Lemeshow goodness-of-fit test p-value was 0.3. ROC-AUC value was 0.65.				

Table 9. Sensitivity analysis - adjusted odds ratio, 95% confidence interval, and p-values of independent variables for severe liver injury outcome including liver necrosis and acute liver failure for patients 75 and under.

Multivariable logistic regression analysis of independent variables for severe liver injury outcome including liver necrosis and acute liver failure for patients 75 and younger				
	Independent variables	Odds ratio	95% CI	p-value
AAP therapy	Olanzapine	0.87	0.52-1.45	0.58
	Quetiapine	1.06	0.78-1.43	0.71
	Risperidone	0.77	0.51-1.16	0.21
	Ziprasidone	1.48	0.76-2.87	0.24
Gender	Male	0.92	0.71-1.2	0.54
Insurance	Medicare	1.53	1.1-2.1	0.01
Region	Midwest	0.56	0.36-0.86	0.008
	West	0.52	0.33-0.81	0.004
	South	0.58	0.4-0.84	0.004
Diagnosis	Major depressive/bipolar disorder	1.39	1.06-1.8	0.017
Comorbidity	Hyperlipidemia	1.41	1.06-1.87	0.017
	Renal disease	1.68	1.08-2.6	0.02
	Cardiovascular disease	1.39	1.02-1.89	0.04
<p>Bold values indicate statistically significant (p-value less than 0.05) variables. Reference categories for the variable were: no comorbidity, no diagnosis, commercial insurance, northeast region, female gender, and aripiprazole AAP therapy. Hosmer and Lemeshow goodness-of-fit test p-value was 0.8. ROC-AUC value was 0.65.</p>				

Figure 1. Schematic diagram of the study design.

Figure 2. Inclusion and exclusion criteria for cohort selection.

Figure 3. Adjusted odds of liver injury associated with AAP use and other risk factors

*P-value was greater than 0.05. Reference categories for the variable were: AAPs were compared with aripiprazole as reference group; Elixhauser score of zero, no comorbidity, no diagnosis, age group 18-40, female gender, and aripiprazole AAP therapy. Hosmer and Lemeshow goodness-of-fit test p-value was 0.09. ROC-AUC value was 0.66.