Published in final edited form as:

Alcohol Clin Exp Res. 2019 April ; 43(4): 593–596. doi:10.1111/acer.13967.

Introduction to the Virtual Issue “Translational Studies in AUD: Liver Disease”

Irina A. Kirpich, PhD, MPH^{1,2,3,4} and Craig J. McClain, MD^{1,2,3,4,5}

¹Division of Gastroenterology, Hepatology, and Nutrition, Department of Medicine, University of Louisville School of Medicine, Louisville, Kentucky, USA

²Department of Pharmacology and Toxicology, University of Louisville School of Medicine, Louisville, Kentucky, USA

³Hepatobiology and Toxicology Program, University of Louisville, Louisville, Kentucky, USA

⁴University of Louisville Alcohol Research Center, University of Louisville, Louisville, Kentucky, USA

⁵Robley Rex Veterans Medical Center, Louisville, Kentucky, USA

INTRODUCTION

Recent high visibility publications raised the overall awareness of the importance of alcohol use disorders (AUD) and alcohol-associated liver disease as major health problems in the United States and worldwide. While this is not earth-shattering news to the alcohol-research community, it markedly enhances the level of public awareness of these important issues. An article in the *Lancet* involving a very large number of centers and authors generated estimates of alcohol use and alcohol-contributable deaths and disability in life years for 195 locations between 1990–2016 (Collaborators, 2018). The take-home message is that, no matter whether you live in a highly-developed or an under-developed country, AUDs and alcohol-induced organ injury, such as liver disease, exert important health impacts. Several other high-visibility articles point out the increasing mortality due to cirrhosis and liver cancer in the United States, with alcohol playing a critical role (Kim et al., 2018; Tapper and Parikh, 2018). These articles indicate that deaths from hepatitis C are now beginning to trend downward due to new direct-acting agents, while deaths from alcohol-associated liver disease are on the rise. This virtual issue of *Alcoholism: Clinical and Experimental Research (ACER)* focuses on the interactions of AUD and alcohol-associated liver disease. We cite important recent articles from *ACER* and a few selected companion papers from other journals that evaluate early diagnosis and mechanisms of alcohol-associated liver disease (Kirpich et al., 2017); (Samala et al., 2018), the role of alcohol withdrawal on the gut

Corresponding author: Irina A. Kirpich, PhD, MPH, Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition University of Louisville, 505 Hancock St, Louisville, KY 40202, Tel.: 502-852-7653; Fax: 502-852-8927, i0kirp01@louisville.edu.

AUTHORS CONTRIBUTIONS

IAK and CJM worked equally together to conceptualize, organize, and write this commentary.

CONFLICT OF INTEREST

Both authors declare no conflicts of interest.

permeability and endotoxemia (Donnadieu-Rigole et al., 2018; Kirpich et al., 2017), the impact of continued drinking on the gut-liver axis in alcoholic liver disease (ALD) (Bajaj et al., 2017), and methods of assessing drinking status in liver transplant recipients (Donnadieu-Rigole et al., 2017; Fleming et al., 2017).

EARLY DIAGNOSIS AND MECHANISMS OF ALCOHOL-ASSOCIATED LIVER DISEASE

Alcohol use disorders (AUD), characterized by alcohol dependence and abuse, can be associated with multi-organ pathology, including alcoholic liver disease (ALD). Early diagnosis of the alcohol-associated liver damage is important in order to reinforce the need for abstinence and to initiate drug/nutritional/ therapy. In a study investigating a cohort of 48 otherwise healthy participants with AUD, but no clinical signs of ALD (34 males (M)/14 females (F)), an increase in plasma alanine aminotransferase levels (ALT, normal range < 40 U/L) as a marker of liver injury, was identified in a subset of patients (27M/6F) (Kirpich et al., 2017). High ALT levels in these AUD patients were positively correlated with elevated levels of Keratin 18 (K18) M65 ($r = 0.473$, $p = 0.005$), and K18 M30 ($r = 0.357$, $p = 0.041$). Keratin 18 is a type 1 cytokeratin found in epithelial cells in two forms - full length (M65) and caspase cleaved (M30), and serum K18 levels have been postulated to be a good biomarker for hepatocyte death in alcoholic hepatitis (AH) (Woolbright et al., 2017). Moreover, the K18 M65:M30 ratio provides an estimate of the type of cell death (necrotic vs apoptotic) (Kramer et al., 2004). In this study (Kirpich et al., 2017), the K18 M65:M30 ratio was slightly greater than 2 in patients with liver injury (based on elevated ALT levels), and was significantly higher than in subjects without liver damage, suggesting a necrosis was the predominant type of cell death in these patients. Of interest, there was relatively slow improvement in both K18 M65 and K18 M30, and levels of both the full length and caspase cleaved fragments were still significantly elevated after 8 days of abstinence. A serum K18 level >2000 IU has been suggested to be diagnostic of AH on liver biopsy (Bissonnette et al., 2017). The AUD patients with elevated ALT levels frequently had admission K18 M65 levels 1000 IU (and 3 patients had levels >2000 IU), indicating prominent hepatocyte death. Importantly, the patients with AUD were consuming approximately 15 drinks a day, and those with early liver injury actually had slightly lower consumption than those without (14.3 ± 0.9 vs 16.8 ± 1.9 drinks a day). This amount of alcohol intake is similar to what we have observed in patients admitted with severe AH (unpublished data). Thus, levels of drinking, *per se*, do not appear to distinguish patients with AUD having no liver injury from those with early liver injury or those with severe AH.

Another recently published paper in *ACER* demonstrated that patients with well characterized AH (MELD 23 ± 7.2) consumed significantly less alcohol compared to the heavy drinking control group with no prior history of ALD (e.g., average drinks per drinking day 11.1 ± 7.7 vs 17.2 ± 13.2 , $P = 0.0001$; or average grams of alcohol consumed per day 152.7 ± 109.1 vs 232.5 ± 192.9 , $P = 0.0005$; (Samala et al., 2018). These levels of alcohol consumption are very similar to those observed in our heavy drinking and AH subjects, noted previously. In the study by Samala, et al., the average AST and ALT levels in the control heavy drinking AUD patients were < 30 IU and K18 determinations were not

performed. The authors speculated that there is “a threshold effect of alcohol consumption, beyond which variations in alcohol consumption may not predict risk of AH”. Moreover, other than the quantity of alcohol consumed, factors such as age, sex, and body mass index (BMI) may predispose heavy drinking individuals to develop severe ALD and/or AH (Liangpunsakul et al., 2016). Interestingly, the prevalence of posttraumatic stress disorders in heavy drinkers with AH was 34% and was not different from heavy drinking controls without liver disease (34%).

ROLE OF ALCOHOL WITHDRAWAL ON GUT PERMEABILITY AND ENDOTOXEMIA

Numerous clinical and experimental studies have shown that alcohol consumption (chronic and acute) impairs intestinal barrier integrity resulting in increased endotoxemia, a critical factor contributing to alcohol-mediated liver disease (Szabo, 2015). Two studies recently published in *ACER* (Donnadieu-Rigole et al., 2018; Kirpich et al., 2017) evaluated effects of alcohol withdrawal on gut permeability and endotoxemia in patients with AUD/ALD. Patients with mild ALD demonstrated a positive correlation between ALT (a marker of liver injury) and plasma LPS (a marker of endotoxemia) at admission to an alcohol treatment program (Kirpich et al., 2017). Elevated LPS levels significantly decreased by day 8, and persisted at those levels at day 15 of abstinence. Interestingly, while LPS levels were reduced in both sexes by the day 8 compared to initial values, a statistically significant drop was noted only in males. Another study (Donnadieu-Rigole et al., 2018) evaluated several other indirect markers of endotoxemia and gut permeability in patients with AUD, including plasma LBP, sCD14, I-FABP, and 16S rDNA. Compared to healthy controls, these markers were significantly higher in AUD patients hospitalized for alcohol withdrawal. Six weeks of abstinence resulted in significantly decreased plasma LBP and sCD14. Notably, this study provided some evidence that obesity may exacerbate alcohol-mediated intestinal barrier dysfunction and negatively impact its recovery. Thus, AUD patients with a high BMI had higher levels of plasma zonulin (an indirect marker of intestinal tight junction integrity) before alcohol withdrawal and a greater decrease following 6 weeks of abstinence than AUD patients with a normal BMI.

THE IMPACT OF CONTINUED DRINKING ON GUT-LIVER AXIS

The impact of continued drinking on the gut-liver axis in ALD, specifically in patients with alcoholic cirrhosis, was investigated in the study by Bajaj, et al. (Bajaj et al., 2017). This study comprehensively evaluated intestinal inflammation and barrier integrity, microbiota alterations and microbial functionality (e.g., bile acid and metabolomic changes) in the duodenum, terminal ileum, and the colon. Compared to healthy controls and abstinent cirrhotic individuals, the actively drinking cirrhotic patients had higher endotoxemia and intestinal inflammation with no changes between groups in the intestinal anti-microbial peptides. In agreement with previously published studies, stool microbiota composition was significantly different between controls and actively drinking cirrhotic patients, and more interestingly, between abstinent and actively drinking cirrhotic patients. Importantly, the authors found that actively drinking cirrhotic patients had significant dysbiosis not only in

stool, but also in the colonic, terminal ileum, and duodenal mucosa. This dysbiosis was characterized by a reduction in the potentially beneficial taxa (e.g., *Lachnospiraceae* and *Ruminococcaceae*) in the lower intestinal mucosa and increased taxa belonging to *Pasteurellaceae* in the duodenum compared to healthy controls and abstinent cirrhotic patients. In addition, the authors demonstrated that actively drinking cirrhotic patients had significantly lower stool metabolites, including amino acids, bioenergetic and pyrimidine intermediates. Collectively, these data suggest that continued alcohol consumption, rather than underlying liver damage, was a driving force in alterations of the gut microbiota composition and function.

METHODS OF ASSESSING DRINKING STATUS IN ALCOHOL-DEPENDENT LIVER TRANSPLANT RECIPIENTS

Many different healthcare providers and employers are interested in whether subjects are drinking alcohol, and, if so, how much. Thus, there is a need to assess a range of patients from those seen in clinic for ALD or in transplant clinic (being evaluated for liver transplantation) to airline pilots. Several recent articles in *ACER* deal with this issue in various patient populations, including two recent articles assessing patients following liver transplantation. The first study was a prospective single-site study evaluating alcohol relapse after liver transplantation; 141 patients were consecutively evaluated in a liver transplant program in France (Donnadieu-Rigole et al., 2017). Patients were seen by their hepatologist and by an addiction specialist; patients completed the Alcohol Use Disorders Identification Test Consumption (AUDIT-C), a widely used screening instrument for hazardous and harmful alcohol consumption. Following the test, the patients were classified into one of four alcohol consumption categories based on frequency and quantity of alcohol drinks per week (abstinence 0; occasional <2; regular 3–5; and excessive >6). The hepatologist also classified the patients into one of four categories evaluating alcohol use by another method (abstinence 0 drinks; occasional <4 glasses on a single occasion and not regular; regular <14 glasses per week for women, <21 glasses per week for men, and <4 glasses on a single occasion; and excessive >14 units per week for women and > 21 units per week for men or > 4 drinks on a single occasion). The addiction specialist determined the patient's drinking status by collecting alcohol consumption history during the last month using the timeline follow-back method. Thereafter, the patient's alcohol consumption as assessed by three different methods was compared. Alcohol consumption was identified by hepatologists in 21.9%, by AUDIT-C in 36.8%, and by addiction specialists in 41.1% of subjects. Hepatologists identified only 2/141 patients as excessive drinkers, the AUDIT-C identified 3/141, and the addiction specialists identified 10/141. Interestingly, traditionally-used liver tests, such as AST, seemed to be of no benefit in evaluating levels of drinking, and alcohol consumption did not correlate with markers of liver injury. Thus, while the relatively large number of subjects were consuming some alcohol, only a small number demonstrated what was considered to be "excessive" alcohol consumption. Of some concern, there was no correlation between standard liver tests and alcohol abuse. Indeed, measuring alcohol intake is a complicated challenge, and this study demonstrated that using a combination of several methods may improve the sensitivity and specificity of identifying drinking behavior and drinking status.

The second study prospectively evaluated 213 liver transplant recipients from two U.S. liver transplant centers (Fleming et al., 2017). Of those subjects, 70.9% (151/213) had a history of alcohol dependence prior to transplantation and 29.1% (62/213) served as non-alcohol-dependent controls. Alcohol use for the past 30 days was assessed using the traditional calendar time line follow-back method. Alcohol intake was assessed biochemically using phosphatidylethanol (PEth) with a cutoff of >8 ng/ml. Blood samples were collected at baseline and at 6- and 12-months of follow up. The results of self-reported drinking showed that 14.5% (9/62) of the controls and 8.6% (13/151) of the alcohol-dependent subjects reported any alcohol use at baseline. Of the 213 subjects, only six reported drinking more than six drinks in the past 30 days. In the control group, 13/62 subjects were positive for PEth, and of these, only three had reported recent alcohol use. In the alcohol-dependent group, 44/151 subjects were positive, with only eight of these admitting to alcohol use in the past 30 days. In both groups, those who self-reported drinking had higher PEth levels. The PEth assay is reported to be 100% specific and to detect more than 90% of moderate to heavy drinkers. Using this assay, this study detected a large number of “clandestine” drinkers. Why these subjects did not “self report” is unclear. No information on liver enzyme abnormalities or organ injury was included in this study. Importantly, both of these studies show relatively high rates of alcohol consumption post-liver transplantation. The PEth test appears to be a sensitive, objective marker of drinking, and it uncovered clandestine drinking.

In conclusion, these articles improve our understanding of AUD and ALD, and point out gaps in our knowledge. It is apparent from several of these studies that some subjects drink incredibly heavily but develop no liver disease. Others drink the same amount and develop severe AH. Determining what factors, other than heavy alcohol consumption, lead to liver injury and AH will be important, as will biomarkers of hepatocyte death. Moreover, patients with liver disease who continue to drink vs. those who abstain have different stool metabolomics profiles (Bajaj et al., 2017), which may prove to be valuable biomarkers in the future. Lastly, depending on criteria used, it appears that many patients consume alcohol following liver transplantation. We need better methods/biomarkers, such as PEth, to quantify drinking, a better understanding concerning how much drinking post-transplant causes liver injury, and better biomarkers of liver injury/liver cell death. This will become especially important as we transplant more patients with ALD, including those with acute severe AH.

ACKNOWLEDGMENTS

The authors thank Ms. Marion McClain for proofreading the manuscript.

Funding: This work was supported by several grants from the National Institute of Health (NIH) including R01 AA024102-01A1 (IAK), U01AA022489 (CJM), 1U01AA021901-01 (CJM), 1U01AA021893-01 (CJM), R01 AA023681 (CJM), and the VA (CJM). Research reported in this publication was also supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health under grant number P20GM113226 (CJM), and by the National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health under Award Number P50AA024337 (CJM).

REFERENCES

- Bajaj JS, Kakiyama G, Zhao D, Takei H, Fagan A, Hylemon P, Zhou H, Pandak WM, Nittono H, Fiehn O, Salzman N, Holtz M, Simpson P, Gavis EA, Heuman DM, Liu R, Kang DJ, Sikaroodi M, Gillevet PM (2017) Continued Alcohol Misuse in Human Cirrhosis is Associated with an Impaired Gut-Liver Axis. *Alcohol Clin Exp Res* 41: 1857–65. [PubMed: 28925102]
- Bissonnette J, Altamirano J, Devue C, Roux O, Payance A, Lebrec D, Bedossa P, Valla D, Durand F, Ait-Oufella H, Sancho-Bru P, Caballeria J, Gines P, Boulanger CM, Bataller R, Rautou PE (2017) A prospective study of the utility of plasma biomarkers to diagnose alcoholic hepatitis. *Hepatology* 66: 555–63. [PubMed: 28120471]
- Collaborators GBDA (2018) Alcohol use and burden for 195 countries and territories, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet* 392: 1015–35. [PubMed: 30146330]
- Donnadieu-Rigole H, Olive L, Nalpas B, Winter A, Ursic-Bedoya J, Faure S, Pageaux GP, Perney P (2017) Follow-Up of Alcohol Consumption After Liver Transplantation: Interest of an Addiction Team? *Alcohol Clin Exp Res* 41: 165–70. [PubMed: 27936489]
- Donnadieu-Rigole H, Pansu N, Mura T, Pelletier S, Alarcon R, Gamon L, Perney P, Apparailly F, Lavigne JP, Donyach-Remy C (2018) Beneficial Effect of Alcohol Withdrawal on Gut Permeability and Microbial Translocation in Patients with Alcohol Use Disorder. *Alcohol Clin Exp Res* 42: 32–40. [PubMed: 29030980]
- Fleming MF, Smith MJ, Oslakovic E, Lucey MR, Vue JX, Al-Saden P, Levitsky J (2017) Phosphatidylethanol Detects Moderate-to-Heavy Alcohol Use in Liver Transplant Recipients. *Alcohol Clin Exp Res* 41: 857–62. [PubMed: 28196282]
- Kim D, Li AA, Gadiparthi C, Khan MA, Cholaneril G, Glenn JS, Ahmed A (2018) Changing Trends in Etiology-Based Annual Mortality From Chronic Liver Disease, From 2007 Through 2016. *Gastroenterology* 155: 1154–63 e3. [PubMed: 30009816]
- Kirpich IA, McClain CJ, Vatsalya V, Schwandt M, Phillips M, Falkner KC, Zhang L, Harwell C, George DT, Umhau JC (2017) Liver Injury and Endotoxemia in Male and Female Alcohol-Dependent Individuals Admitted to an Alcohol Treatment Program. *Alcohol Clin Exp Res* 41: 747–57. [PubMed: 28166367]
- Kramer G, Erdal H, Mertens HJ, Nap M, Mauermann J, Steiner G, Marberger M, Biven K, Shoshan MC, Linder S (2004) Differentiation between cell death modes using measurements of different soluble forms of extracellular cytokeratin 18. *Cancer research* 64: 1751–6. [PubMed: 14996736]
- Liangpunsakul S, Puri P, Shah VH, Kamath P, Sanyal A, Urban T, Ren X, Katz B, Radaeva S, Chalasani N, Crabb DW, Translational R, Evolving Alcoholic Hepatitis Treatment, C (2016) Effects of Age, Sex, Body Weight, and Quantity of Alcohol Consumption on Occurrence and Severity of Alcoholic Hepatitis. *Clin Gastroenterol Hepatol* 14: 1831–38 e3. [PubMed: 27320325]
- Samala N, Lourens SG, Shah VH, Kamath PS, Sanyal AJ, Crabb DW, Tang Q, Radaeva S, Liangpunsakul S, Chalasani N (2018) Posttraumatic Stress Disorder in Patients with Heavy Alcohol Consumption and Alcoholic Hepatitis. *Alcohol Clin Exp Res* 42: 1933–38. [PubMed: 30080255]
- Szabo G (2015) Gut-liver axis in alcoholic liver disease. *Gastroenterology* 148: 30–6. [PubMed: 25447847]
- Tapper EB and Parikh ND (2018) Mortality due to cirrhosis and liver cancer in the United States, 1999–2016: observational study. *BMJ* 362: k2817. [PubMed: 30021785]
- Woolbright BL, Bridges BW, Dunn W, Olson JC, Weinman SA, Jaeschke H (2017) Cell Death and Prognosis of Mortality in Alcoholic Hepatitis Patients Using Plasma Keratin-18. *Gene Expr* 17: 301–12. [PubMed: 28770701]