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Prognostic Value of the ^{13}C-Methacetin Breath Test in Adults with Acute Liver Failure and Non-Acetaminophen Acute Liver Injury

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Abbreviations

ALF Acute liver failure

ALFSG Acute Liver Failure Study Group

ALI Acute liver injury

ALT alanine aminotransferase

APAP Acetaminophen

AUROC Area under the receiver operating characteristic curve

cPDR20 Cumulative percent dose recovery of methacetin at 20 minutes

CYP1A2 Cytochrome P-450 IA2

DILI Drug-induced liver injury

HE Hepatic encephalopathy

ICU Intensive care unit

INR International Normalized Ratio of the prothrombin time

KCC King's college criteria

LT Liver transplantation

MBT Methacetin breath test

MELD Model for End-stage Liver Disease

NAC N-acetylcysteine

PDR Percent dose recovery

SAE Serious adverse event

SD Standard deviation

TFS Transplant-free survival

ULN Upper limit of normal

Words = 4127

Abstract

The ^{13}C-Methacetin breath test (MBT) is a non-invasive, quantitative hepatic metabolic function test. The aim of this prospective, multicenter study was to determine the utility of initial and serial ^{13}C- MBT in predicting 21 day outcomes in adults with acute liver failure (ALF) and non-acetaminophen acute liver injury (ALI). **Methods:** The ^{13}C-MBT Breath ID device (Exalenz Biosciences, Ltd.) provided the percent dose recovery (PDR) throughout 60 minutes after administration of ^{13}C methacetin solution as the change in exhaled $^{13}CO_2$/ $^{12}CO_2$ compared to pre-ingestion ratio on study days 1, 2, 3, 5, and 7. Results were correlated with 21-day transplant-free survival and other prognostic indices.

Results: 280 subjects were screened for enrollment between May 2016 and August 2019. Median age of the 62 enrolled patients with adequate data was 43 years, 79% were Caucasian and 76% had ALF with the remaining 24% having ALI. The mean PDR peak on Day 1 or 2 was significantly lower in non-survivors compared to transplant-free survivors (2.3 %/hr vs 9.1 %/hr, $p < 0.0001$). In addition, serial PDR peaks were consistently lower in non-survivors vs survivors ($p < 0.0001$). The AUROC of the ^{13}C-MBT in the combined cohort was 0.88 (95% CI: 0.79-0.97) and higher than that provided by King's College (AUROC= 0.70) and MELD scores (AUROC=0.83). The ^{13}C-MBT was well tolerated with only 2 gastrointestinal adverse events reported.

Conclusions: The ^{13}C-MBT is a promising tool to estimate the likelihood of hepatic recovery in ALF and ALI patients. Use of the PDR peak data from the ^{13}C-MBT point of care test may assist with medical decision making and help avoid unnecessary transplantation in critically ill ALF and ALI patients.

Word count= 274

Introduction

Acute liver failure (ALF) is a rare but potentially devastating syndrome defined by the sudden onset of coagulopathy (i.e. an international normalized ratio (INR) of $\geq$ 1.5) and hepatic encephalopathy in a patient without known pre-existing liver disease (1). Although a multitude of disease processes can lead to severe acute liver injury (ALI) and ALF, there are only ~ 3,000 cases of ALF each year in the United States (2). Recent studies have demonstrated the importance of ALF etiology in distinguishing favorable short term prognosis with a 50-70% transplant free survival (TFS) (hepatitis A, acetaminophen (APAP) overdose, and ischemia) from conditions with an unfavorable prognosis (idiosyncratic drug induced liver injury (DILI), autoimmune, hepatitis B), with a 10 to 30% TFS (2, 3). Although emergency liver transplantation (LT) is associated with excellent 1-year survival, the ongoing shortage of donor organs preclude many ALF patients from undergoing life-saving LT. Conversely, reliable identification of patients destined to recover with their native liver is essential to avoid unnecessary LT. Therefore, an accurate and reliable bedside test to identify ALF patients who will recover with supportive care from those who will die without LT remains a critical unmet need. Although many studies have attempted to identify features of ALF which predict outcome, all suffer from relatively poor diagnostic accuracy (3).

The Acute Liver Failure Study Group (ALFSG) is a consortium of North American academic medical centers that has prospectively studied the etiologies and outcomes of adults with ALF since 1998 (4). In addition, a prospective registry of adults with severe ALI, defined by an INR $\geq$ 2.0, serum alanine aminotransferase (ALT) $\geq$ 10 x upper limit of normal (ULN), and bilirubin $\geq$ 3 mg/dl, was established in 2010 (5). Over the years, the ALFSG has conducted clinical trials in an effort to improve TFS in both ALF and ALI patients (6,7). The ^{13}C-Methacetin breath test (^{13}C-MBT) is a semi-quantitative, non-invasive hepatic metabolic function test that assays the biotransformation of 75 mg of ^{13}C-labelled methacetin by hepatic cytochrome-P450 1A2 (CYP1A2) to $^{13}CO_2$ and acetaminophen (8). The ^{13}C-MBT has been shown to be more accurate than the Model for End-stage Liver Disease (MELD) score in predicting decompensation and survival in patients with cirrhosis undergoing LT evaluation (9). The ^{13}C-MBT may also be useful in patients with severe acute liver disease but the number of patients studied has been limited (10). The aim of the current study is to demonstrate the potential utility of the initial

and serial ^{13}C-MBT measurements as prognostic tools in adult subjects with ALF and severe non-APAP ALI who were followed until death, LT, or discharge from the hospital 21 days after enrollment.

METHODS

Objective- The predefined primary study outcome was to assess the relationship between the day 1 (initial) percent dose recovery (PDR) rate, known as PDR peak, which reflects the maximum rate of metabolism of ^{13}C-methacetin measured as the change in $^{13}CO_2/\ ^{12}CO_2$ ratio after ingestion of a a 75 mg dose of ^{13}C-methacetin normalized using the patient's height and weight, and TFS at Day 21 in eligible adult patients with ALF and non-APAP ALI. Our research hypothesis was that the initial PDR peak values would be significantly higher in patients who survived compared to those who died or underwent LT by Day 21. The secondary outcomes in this study were to determine the optimal cut point for the PDR peak which best distinguishes between outcomes (TFS *vs.* death/LT). Other exploratory secondary outcomes included the change in ^{13}C-MBT measurements over a maximum of 7 days from enrollment and to assess the relationship between single time points of ^{13}C-MBT measurements and outcome. Lastly, we set out to compare the PDR peak as a prognostic tool for predicting Day 21 TFS in conjunction with other clinical parameters such as etiology, laboratory measurements, and other published prognostic indices.

Study population- The study protocol was approved by local institutional review boards (IRBs) of participating sites. An Investigational Device Exemption (IDE) application with the US Food and Drug Administration was approved (IDE #G150226) in November 2015. Written informed consent to participate was obtained from the patient or a legally authorized representative before enrollment per local regulations. The study was initiated in May, 2016 in ALF patients age >18-70 years with any degree of encephalopathy and an INR $\geq$1.5 caused by an illness of <24 weeks duration. Initially, exclusion criteria included consumption of a multitude of medications thought to potentially interfere with the metabolism of ^{13}C-methacetin (**See supplementary Materials- Table 1**) (9). After further consideration of these exclusions, the eligibility criteria were modified in March, 2018 to increase the upper age range to 80 years and allow for use of famotidine or acyclovir within 48 hours of enrollment as well as use of statins in the 30 days prior to enrollment. Eligibility criteria were also widened to include patients with severe non-APAP ALI since these patients have been shown to have a 41% risk of progressing to ALF or dying within 21 days, with a similar proportion dying or undergoing LT as those with ALF (5). In contrast,

patients with APAP-ALI remained excluded due to very low rates of progression to ALF (4%). These changes in eligibility criteria were approved by all IRBs and regulatory authorities.

Study Design- Testing involved the administration of a ready to use solution of a 75 mg dose of ^{13}C-methacetin mixed into 150 ml of water followed by retrieval of expired $^{13}CO_2$ and $^{12}CO_2$ over a period of one hour after substrate administration (9). A maximum of 5 tests were administered on study days 1, 2, 3, 5 and 7. Subjects were required to have been fasting from solid food for a minimum of 6 hours, and nasoenteric tube feeding had to be held for a minimum of 4 hours, prior to substrate administration. In addition, other medications could not be given in the hour prior to test substrate administration. The solution of ^{13}C-methacetin was swallowed by non-intubated patients who were upright and cooperative; substrate was otherwise administered *via* nasoenteric tube to other participants. Exhaled breath was collected at the bedside for a total of 75 minutes, including up to 15 minutes for baseline determination and 60 minutes of $^{13}CO_{2/}{}^{12}CO_2$ ratio collection following administration of ^{13}C-methacetin using a nasal cannula for conscious patients or a ventilator hose adaptor for the endotracheal tube of intubated patients connected to the BreathID MCS device (Exalenz Biosciences Ltd., Modiin, Israel). During the breath collection period, all other ICU care including continuous renal replacement, intravenous medications, and other treatments were continued. Investigators were blinded to the MBT results to avoid bias in patient management. Calculation of the PDR peak, cumulative PDR at 20 minutes (cPDR20) and other MBT parameters were performed at the data coordinating center (DCC) independent of the sponsor and site investigators. The investigational substrate was stored locally at room temperature.

Previous studies have demonstrated that the inter-test coefficient of variation of the ^{13}C-MBT in 53 healthy volunteers and liver disease patients is 13.2 % (95% CI: 11.1-15.2%) for the PDR peak and the coefficient of variation is 23.9% (95% CI: 20.3%-27.4%) for the cPDR20 (10, 11).

Safety assessments- All serious adverse events (SAEs) were collected from Day 1 through Day 21 of the study and graded per standard protocol; relatedness to the test device was determined by the local investigator. An independent medical safety monitor reviewed each SAE in real-time for expectedness and relatedness. All non-serious adverse events were collected from Day 1 to Day 7. An independent Data and Safety Monitoring Board (DSMB) reviewed aggregate data throughout the study period. Since the primary product of the demethylation of methacetin is acetaminophen, serum APAP-cysteine adducts testing was performed in the first 20 study participants prior and after each ^{13}C-MBT test administration. Serum APAP-cysteine adducts were measured in the research lab of Dr. Laura James at

the University of Arkansas using a previously described HPLC method (12). The detection of a serum APAP- cysteine adduct in non-APAP-induced ALI/ALF patients was considered significant if a value of >1.0 nmol/L was detected. In subjects with presumed APAP hepatotoxicity, an increase of serum APAP-cysteine adduct levels above their initial value was assessed in conjunction with serum liver biochemistries. An independent group of experienced clinical investigators reviewed the adduct data as they became available to ensure that the small dose of APAP per test administration would not result in additional liver injury. After reviewing the adduct test results from 20 consecutively evaluable patients where no values > 1.0 nmol/L were observed, the independent group concluded that there were no safety concerns and further testing for APAP-cysteine adducts was halted.

Sample size- Up to 200 evaluable patients meeting eligibility criteria were to be consecutively enrolled at the 11 sites of the ALFSG participating in this study. An evaluable patient is one who completed one or more ^{13}C-MBT tests measured for a minimum of 30 minutes after ingestion of ^{13}C-methacetin.

Statistical analysis- A futility analysis was planned after 100 consecutive enrollments to consider stopping the study early if there were no signal of a difference in the initial PDR peak distribution between those who reached 21-day TFS and those who died or underwent LT by day 21. The final analysis was based on a comparison of the initial mean PDR peak between the TFS and non-TFS outcome groups using a two-sided significance level of 0.10. Planned exploratory analyses for hypothesis generation included subgroup analyses, examination of the cumulative PDR at 20 minutes of testing (cPDR20), and the comparison of the initial PDR peak to the currently available ALFSG Prognostic Index for predicting 21-day TFS. All statistical analyses are two-sided tests and conducted using SAS V9.3 or higher (SAS Institute, Inc., Cary, NC).

RESULTS

Study population- Between May, 2016 and August 2019, 280 patients were screened for eligibility and 76 were enrolled into the study (**Figure 1**). A total of 9 subjects never received an ^{13}C-MBT due to death (1), LT (2), withdrawal of consent (3), and device malfunction/pharmacy delay (3). In addition, 3 subjects had an eligibility violation of APAP-ALI, and 2 subjects had non-evaluable data (<30 minutes duration of the ^{13}C-MBT), leaving a total of 62 subjects with adequate ^{13}C-MBT data for analysis

Demographics of the study population are depicted in **Table 1**. The median age of the 62 subjects was 43 years (range: 18 to 73), 61% were female, 79% were Caucasian, 76% had ALF due to a multitude of etiologies and 24% had non-APAP ALI. At enrollment into the study, 42% had grade 3 or 4 hepatic encephalopathy with a median bilirubin of 7.2 mg/dl (range: 0.6 to 36) and a median INR of 2.7 (range: 1.4 to 10.7). In addition, 34% of the study population were intubated and 21% were receiving renal replacement therapy.

PDR peak and clinical outcome- The primary outcome assessment was the comparison of the Day 1 PDR peak between those that had 21-day TFS *vs*. those who died or underwent LT prior to day 21. A total of 37 (59.6%) subjects had the initial ^{13}C-MBT conducted on day 1 and an additional 21 subjects had the initial test on day 2 while the 4 remaining subjects had the first study after day 2. The reasons for a delay in testing included the need for other clinical procedures and the subject not having fasted for the required time period. Of the 58 who had ^{13}C-MBT data on day 1 or 2, 2 had missing outcome data which is how we came to 56 evaluable patients in Table 2.

Per **Table 2,** the mean PDR peak on Day 1 was significantly lower in 21 day non-survivors compared to transplant-free survivors (1.9 %/h (Standard deviation (SD): 0.6) vs 10.2 %/h (SD: 7.8) ; p<0.0001). Expanding the PDR peak values to include either day 1 or day 2 showed similar results (9.1 %/h (SD: 6.8) *vs.* 2.3 %/h (SD: 0.9); p<0.0001). When we evaluated the 21 patients with APAP overdose, the day 1 or 2 PDR peak was significantly higher in the 21-day TFS group compared to non-survivors (7.9 %/h vs 2.6%/h, p=0.001) and also in the 35 patients with non-APAP ALI/ ALF (10.1%/h vs 2.2 %/h, p =0.0003). When the cumulative PDR at 20 minutes (cPDR20) was compared between the two outcome groups, similar differences in the mean values were observed (Data not shown).

Assessment of serial PDR peak values revealed that the PDR peaks were consistently higher in transplant free survivors *vs.* non-survivors **(Figure 2).** This difference remained significant over time (p < 0.001). An example of the serial PDR peak values of an idiosyncratic drug induced liver injury (DILI) patient that survived and another DILI patient who required liver transplantation can be seen in **Figure 3**.

Transplant free survival in the combined cohort of ALI and ALF patients- In the 56 ALF and ALI patients with evaluable Day 1 or 2 PDR peak values, the area under the receiver operator characteristics curve (AUROC) was 0.88 (95%CI: 0.79, 0.97). The addition of favorable etiology, defined as hepatitis A, APAP overdose, ischemic injury not requiring extensive pressors or pregnancy- related ALF, to the ^{13}C-MBT results further improved the AUROC to 0.92 (95% CI: 0.85, 0.99). In contrast, the AUROC of the Kings

College Criteria (KCC) was 0.70 (95%CI: 0.58, 0.81) and the AUROC for the MELD score was 0.83 (95%CI: 0.72, 0.94) (**Figure 4**). Additional models that compared APAP to non-APAP etiologies and intubated to non-intubated patients or inclusion of admission lab values failed to provide any incremental predictive capabilities (Data not shown).

Transplant free survival models in ALF patients- In the 42 ALF only patients with evaluable Day 1 or 2 PDR peak values, the mean PDR peak was significantly higher in TFS vs non-TFS (8.5 %/h vs 2.4 %/h, p=0.0005) and the AUROC was 0.82 (95% CI: 0.68-0.96). The addition of a favorable etiology of ALF to the PDR peak value from the ^{13}C- MBT improved the AUROC to 0.89 (95% CI: 0.79, 0.98). In contrast, the AUROC of the ALFSG prognostic index was 0.90 (95% CI: 0.81, 1.0), the AUROC for KCC alone was 0.78 (95% CI: 0.66, 0.91); and, AUROC for MELD alone was 0.83 (95% CI: 0.69, 0.97) (**Figure 5**).

Safety assessments- Review of the serum APAP-cysteine adduct results obtained in the first 20 evaluable patients demonstrated no evidence of inadvertent APAP hepatotoxicity or accumulation of APAP-cysteine adducts in either non-APAP- or APAP-related patients (12). There were a total of 28 SAE's reported from 25 subjects, none of which were deemed related to the investigational product. In addition, 4 potentially related, non-serious adverse events with a reported severity of mild to moderate were noted including nausea, vomiting and two events of emesis which occurred in non-intubated patients during test administration.

DISCUSSION

The aim of our study was to determine the safety and accuracy of a bedside, non-invasive hepatic metabolic test to provide real time prognostic data for clinical decision-making in critically ill patients with ALF or severe non-APAP ALI. The initial ^{13}C-MBT results performed quite well in predicting 21-day TFS in our patient population (**Figure 2**). In addition, serial assessment of ^{13}C-MBT results demonstrated that subjects that died or underwent LT within 21-days had persistently lower values compared to patients with TFS, indicating that this test may be a useful dynamic measure of global hepatic function. Interestingly, the single subject with a persistently low ^{13}C-MBT PDR peak who did not die or undergo LT had Wilson Disease and recovered within 21-days. We presume that the low ^{13}C-MBT results in this patient may have been due to unsuspected advanced fibrosis or cirrhosis which is present in most subjects with Wilson disease who present with ALF (15).

Acetaminophen is minimally absorbed from the stomach but rapidly absorbed by the small intestine via passive diffusion (16, 17). Following absorption, ^{13}C-methacetin undergoes extensive first pass clearance in the liver via metabolism of hepatic CYP1A2 into APAP and $^{13}CO_2$. The BreathID device is based upon continuous measurement of $^{13}CO_2$ and $^{12}CO_2$ concentrations by molecular correlation spectroscopy that can detect variations less than 1:1000 in the $^{13}CO_2/^{12}CO_2$ ratio. Use of the ^{13}C-MBT in critically ill patients requires the study staff to bring the portable equipment into the patient room. In 31% of the 185 tests initiated, the ^{13}C- methacetin was given *via* nasoenteric tube. One potential concern of using an oral substrate-based breath test in intubated patients receiving sedation is the potential for gastroparesis which could delay absorption of the substrate and impact test results. In the current study, the ^{13}C-MBT PDR peak reliably identified those more likely to survive whether they were intubated (22 patients, 10.5 %/h versus 2.7 %/h, p =0.03) or not intubated at the time of test administration (34 patients, 8.6 %/h versus 1.9 %/h, $p < 0.001$). An unrelated study has previously demonstrated that intubated medical ICU patients receiving vasopressors and opiates frequently had evidence of impaired gastric emptying (18). Another study of intubated trauma patients with intracranial hemorrhage reported that the median gastric emptying time was 13.9 hours compared to 3.0 hours in healthy volunteers (19). Furthermore, small bowel motility was also severely impaired in these ICU patients (19). Lastly, a single dose of narcotics in healthy volunteers has been shown to significantly delay the emptying of acetaminophen from the stomach (16, 17). With these data, we were concerned that many of our ALI and ALF patients may have had functional gastroparesis leading to a reduction in ^{13}C- methacetin absorption. Although none of the enrolled patients had a history of known gastroparesis, there were 3 instances of gastrointestinal adverse events. Two of these events occurred during the ^{13}C-methacetin administration suggesting that some of these critically ill patients may have had functional gastroparesis. However, individual patients with low ^{13}C-MBT test results generally remained low during serial assessments over subsequent days. We also did not find an association between the use of mechanical ventilation (p=0.34) or vasopressor administration (p=0.09) at study admission with ^{13}C-MBT results nor with the route of substrate administration via oral vs naso-enteric/orogastric route (p=0.37). Therefore, we presume that the ^{13}C-MBT provides a robust index of hepatic function in critically ill ALF and non-APAP ALI patients being cared for in the ICU. Other groups have reported on the use of intravenous administration of ^{13}C-methacetin with periodic collection of exhaled $^{13}C0_2$ in patients with cirrhosis undergoing surgery and ALF patients (20, 21). However, most of these data are in small, retrospective cohorts and the kinetics of ^{13}C-methacetin hepatic metabolism are very different when given intravenously compared to orally. In addition, a larger dose of methacetin is required for intravenous

administration and needs to be dosed by patient body weight leading to additional complexity and safety concerns as well as challenges with manufacturing sterile ^{13}C methacetin for human use (22). Therefore, due to its rapid absorption in the fasted state from the small intestine, extensive hepatic extraction and first pass metabolism in the liver, and simplicity of fixed dosing, we believe that oral administration of ^{13}C-methacetin may lead to a more accurate and reproducible measure of global hepatic function (23).

The accurate determination of prognosis in ALF has been an elusive goal, with many investigators using regression analyses of clinical features to identify which patient will die without a liver transplant (3, 14). Such prognostic indices have generally suffered from poor accuracy. The ^{13}C-MBT, however, does not rely on static clinical features but rather actual metabolic capacity of the liver. Other groups have identified that serial arterial lactate levels as a modification to the KCC can also be of value in identifying APAP overdose patients with a poor prognosis (24-26). In addition, the ^{13}C-MBT not only reflects CYP450 activity and thus the severity of hepatocyte injury/necrosis, but also hepatocyte regeneration, as shown by the recovery of activity over days after admission. The clinical importance of serial ^{13}C-MBT measurements deserves emphasis, as an increase in PDR peak over time provides objective evidence of recovery, and suggest waiting another day for a final decision regarding liver transplantation (**Figure 3**). The present study, therefore, represents the first attempt to predict the outcome of ALF and non-APAP ALI using a truly dynamic liver function assessment tool.

Other prognostic models in ALF using widely available clinical and laboratory data include the King's College criteria and the MELD score. A recent meta-analysis of these indices showed that the King's College criteria performed better than the MELD score in patients with APAP hepatotoxicity (26, 27). In contrast, the MELD score had a superior prognostic value for non-survival in non-APAP patients. The ALFSG Prognostic Index was recently shown to have superior performance to both the King's College and MELD score in a large derivation and validation cohort of North American ALF patients (3). This index includes 5 variables including the etiology of ALF (favorable *vs.* unfavorable as defined above), use of vasopressors, encephalopathy grade, total bilirubin, and INR level. In the current study, the ^{13}C-MBT plus etiology had comparable AUROC to the ALFSG Prognostic Index whether one grouped etiology as APAP versus non-APAP or favorable versus unfavorable. Furthermore, the dynamic nature of the ^{13}C-MBT makes it more attractive for medical decision making. The combination of the ^{13}C- MBT PDR peak and the ALFSG prognostic index variables showed a minimal improvement in prediction of 21-day TFS performance characteristics in our limited cohort. This is primarily due to the high AUROC that each

measure had alone. In order to validate its utility, testing of the ^{13}C-MBT in additional large, cohorts of ALI and ALF patients will be needed.

There were several important lessons learned from this study including the challenges of enrolling patients into a clinical trial in patients with a rare, sudden onset disease. Per Figure 1, informed consent was not able to be obtained from 16% of the screened patients. Other studies of critically ill patient populations have demonstrated a consent failure rate of 1 in 3 to 1 in 4 particularly when a therapeutic intervention is not being offered as was the case here. In addition, several patients were initially excluded due to use of concomitant medications like acyclovir, famotidine, and statins which could have theoretically impacted the ^{13}C-MBT results via competitive inhibition of CYP1A2. However, after further review with the manufacturer and regulatory authorities, we were able to remove this exclusion criteria to allow more patients to be enrolled. In addition, we increased the upper age of enrollment to 80 and also allowed for enrollment of non-APAP ALI patients who have a limited 21 day TFS to further help reach recruitment goals. Other challenges with this study included device malfunction that prohibited the collection of ^{13}C- MBT data in 5 of the 76 enrolled participants. In order for the BreathID device to operate, the equipment must come to the ambient temperature of the room. In addition, it was at times challenging to complete the ^{13}C-MBT in critically ill patients who were receiving enteral feedings. However, reliable data using the T-shaped ventilator hose adaptor could be obtained from intubated patients. Therefore, use of the ^{13}C- MBT appears to be feasible in an ALI and ALF population. Finally, the testing for serum APAP-Cysteine adducts demonstrated that there was no evidence of new or worsening acetaminophen exposure with the small, 75 mg test dose of ^{13}C methacetin needed to conduct the study (13). Other studies have also demonstrated no adverse effects from the small dose of methacetin used (9,11). Finally, there were no device related SAE's reported although as mentioned four patients did experience transient nausea and emesis which may have been related to underlying gastroparesis.

Limitations of the current study include the modest sample size that did not meet our intended recruitment goal of 200 patients. This arose due to the presence of multiple exclusion criteria in many patients who were receiving various concomitant contraindicated medications. In addition, it can be difficult to establish a good rapport with family members of ALF and ALI patients due to the sudden nature of the illness and looming concerns regarding the need for urgent liver transplantation. During the study, we were able to eliminate some of the medication exclusion criteria but early on this factor proved to be problematic. Overall, there was a clear difference in breath test results between the TFS

and those needing urgent LT and dying. The observation that a given trajectory over time associates with their outcome suggests that additional studies of serial ^{13}C-MBT are needed.

In conclusion, we demonstrated that the ^{13}C-MBT is a feasible and promising means of risk stratifying critically ill ALF and non-APAP ALI patients for adverse outcomes. Further studies with broader inclusion criteria are indicated to further develop the use of this simple, non-invasive bedside technology which can provide instantaneous objective information to clinicians and hopefully reduce the need for unnecessary transplants in ALF and non-APAP ALI patients.

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Table 1. Baseline characteristics of the patient population

Parameter	Overall group N=62	ALF group N=47	ALI group N=15
Median age (yrs)	43 (18, 73)	40 (18, 73)	50 (25, 66)
Female (%)	61%	70%	33%
Race/ ethnicity (%)			
White	79%	79%	80%
Black	18%	19%	13%
Asian	3%	2%	7%
Hispanic	7%	6%	7%
Etiology (%)			
APAP	42%	55%	0%

Hepatitis A	**11%**	**2%**	**40%**
DILI	**11%**	**9%**	**20%**
Hepatitis B	**8%**	**6%**	**13%**
Autoimmune	**5%**	**4%**	**7%**
Shock *	**5%**	**4%**	**7%**
Indeterminate	**5%**	**4%**	**7%**
Pregnancy	**2%**	**2%**	**0%**
Wilsons	**2%**	**0%**	**7%**
Other virus	**2%**	**2%**	**0%**
Other	**8%**	**11%**	**0%**
Median Entry labs			
ALT (U/L)	**2738 (141, 15,817)**	**2860 (141, 15,817)**	**1702(578, 8631)**
Bili (mg/dl)	**7.1 (0.6, 36.0)**	**5.5 (0.6, 36.0)**	**12.4 (4.3, 21.5)**
INR	**2.7 (1.4, 10.7)**	**2.9 (1.4, 10.7)**	**2.4 (2.0, 5.0)**
Creatinine (mg/dl)	**0.9 (0.4, 7.7)**	**1.2 (0.4, 7.7)**	**0.7 (0.5, 5.9)**
MELD	**30.0 (14, 47)**	**31.0 (14, 47)**	**26.0 (20, 39)**
Complications at entry			
Renal replacement	**21%**	**28%**	**0%**
Intubated	**34%**	**45%**	**0%**
Vasopressors	**18%**	**21%**	**7%**
NAC (%)	**90%**	**96%**	**73%**
Grade ½ HE	**34%**	**45%**	**0%**
Grade ¾ HE	**42%**	**55%**	**0%**
Ever listed for LT	**32%**	**34%**	**27%**
First 21 day outcome			
Liver Transplant**	**21%**	**23%**	**13%**
Death	**15%**	**20%**	**0%**
Transplant-free survivor	**61%**	**55%**	**80%**
Unknown***	**3%**	**2%**	**7%**

* Shock was an allowable etiology if not severe at study entry; that is, on no pressors (n=2) or one pressor (n=1). Data presented as Median (range)

** One subject died post transplant; data reported as median (range) NAC= N-acetylcysteine; HE = hepatic encephalopathy ALT= alanine aminotransferase; DILI= drug induced liver injury

*** Two unknown 21-day outcomes due to consent withdrawal and lost to follow up

Table 2: Maximal Percent Dose Recovery (PDR peak) and cumulative PDR at 20 minutes (cPDR20) by 21 day Outcomes

	Outcome	
	21-day Transplant-Free survivor ^	**21-day non-Survivor ^**
Primary Outcome*	**N=23**	**N=12**
Day 1 Mean PDR peak %/h (SD	10.2 (7.8)	1.9 (0.6)
Difference in Means (90% CI)	8.3 (4.4, 12.1)	
P value	<0.0001	
Secondary Outcomes*	**N=35**	**N=21**
Day 1 or 2 Mean PDR peak %/h (SD)	9.1 (6.8)	2.3 (0.9)
Difference in Means (90% CI)	6.8 (4.8, 8.8)	
P value	<0.0001	
Day 1 or 2 mean cPDR20 %/h (SD)	1.4 (1.3)	0.2 (.2)
Difference in Means (90% CI)	1.2 (0.8, 1.6)	
P value	<0.0001	

*Two participants missing outcome due to consent withdrawal and lost to follow up.

^ Data reported as mean (Standard deviation)

**P value is based on a two-sided Student's t-test at an alpha level of 0.10.

Figure legends

Figure 1- Overview of eligible and enrolled patients. A total of 280 ALF and ALI patients were screened for the study and 76 were enrolled. The analysis population included 62 patients with available ^{13}C-MBT results and 21 day outcome of which 56 had a day 1 or 2 ^{13}C-MBT test result for efficacy analysis.

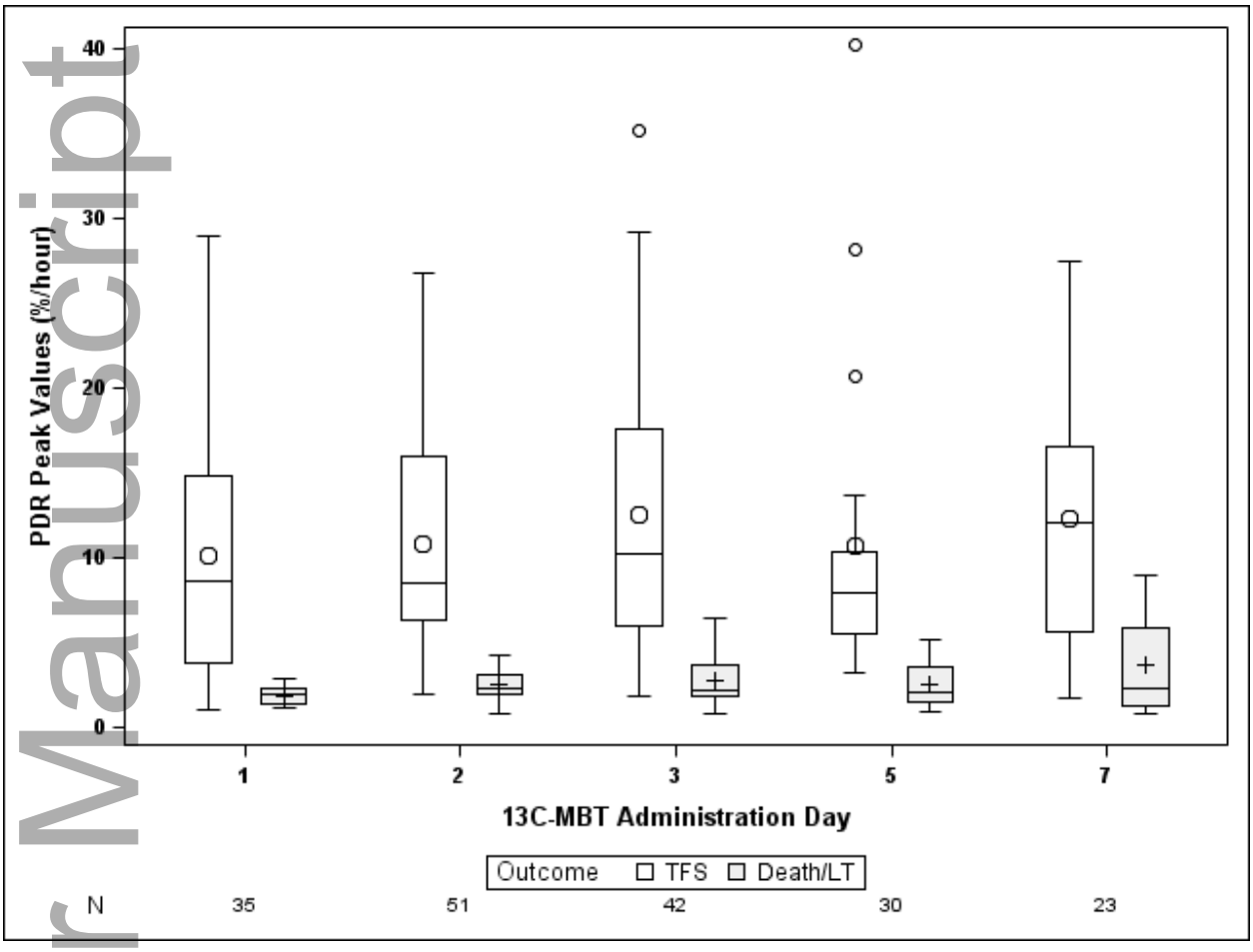
Figure 2- PDR peak by outcome and ^{13}C-MBT administration day. The PDR peak values were significantly lower in the non-survivors compared to the transplant free survivors ($p < 0.001$, repeated measures analysis)

Figure 3- Serial ^{13}C- MBT results in an ALF DILI patient who survived and an ALI DILI patient who required liver transplantation. A) A 52 year old female with presumed DILI due to tizanidine and ropinirole was enrolled with a MELD score of 26 and a total bilirubin of 9.3 mg/dl, INR 2.8, and grade 3 hepatic encephalopathy. Her ALFSG prognostic score was 7.7% likelihood of 21-day transplant free survival. She received intravenous N-acetylcysteine on day 1 and was on famotidine starting on day 2 but never on pressors nor intubated. Over time her clinical status improved and by day 7 her HE grade was 1 and she was discharged home at day 14. Her ^{13}C-MBT results improved from day 2 through day 7. B) A 53 year old female with DILI due to clindamycin was enrolled with ALI and no encephalopathy. During follow-up, she rapidly deteriorated and was listed for liver transplantation. Her initial MELD score was 30. She was never on pressors but was intubated on day 6. She eventually underwent LT on study day 7 and discharged home on day 20. Her ^{13}C-MBT results demonstrated a very low PDR peak that never improved.

Figure 4- 21-day transplant free survival in the combined ALF/ ALI cohort. Amongst 56 ALF and ALI patients with Day 1 or 2 measures, the AUROC for the ^{13}C-MBT PDR peak was 0.88 which improved to 0.92 with the inclusion of the etiology of ALI/ ALF. The AUROC for 21-day survival was 0.79 for the King's College criteria and 0.83 for the day 1 MELD score.

Figure 5- 21-day transplant free survival in the ALF patients alone. Amongst the 42 ALF patients with available data, the AUROC for the ^{13}C-MBT PDR peak was 0.82 which improved to 0.89 with inclusion of ALF etiology and to 0.93 when combined with the ALFSG prognostic index. The AUROC was 0.78 for King's college criteria and 0.83 for MELD scores.

Figure 2: PDR peak by Outcome and MBT Administration Day



Large circles indicate means, boxes interquartile ranges, horizontal line within the boxes medians, bars 1.5 times the interquartile rate and small circles outliers.

Figure 3. Serial ^{13}C- MBT results in an ALF DILI patient who survived and an ALI DILI patient who required liver transplantation.

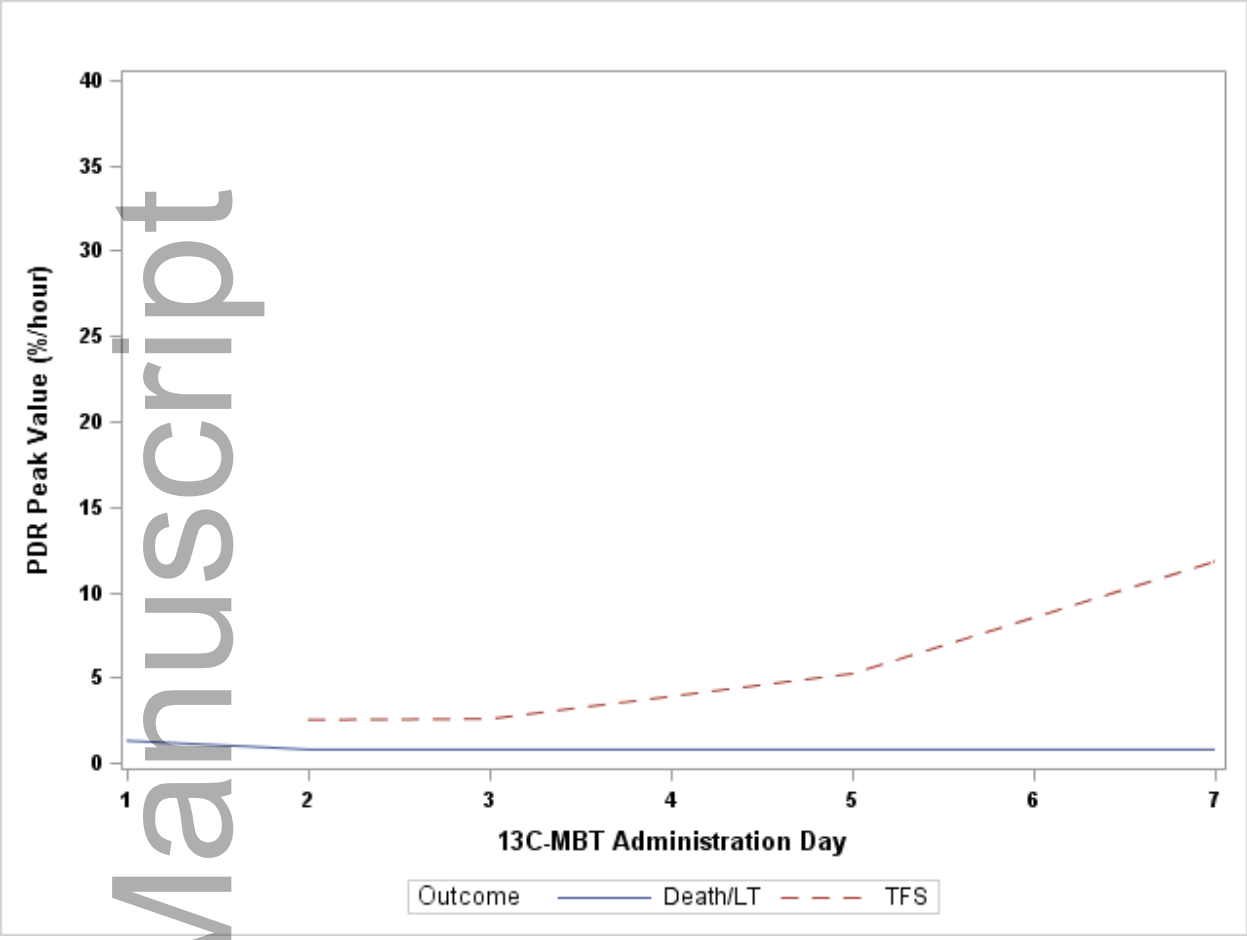


Figure 4: 21-day transplant free survival in the combined ALF/ ALI cohort

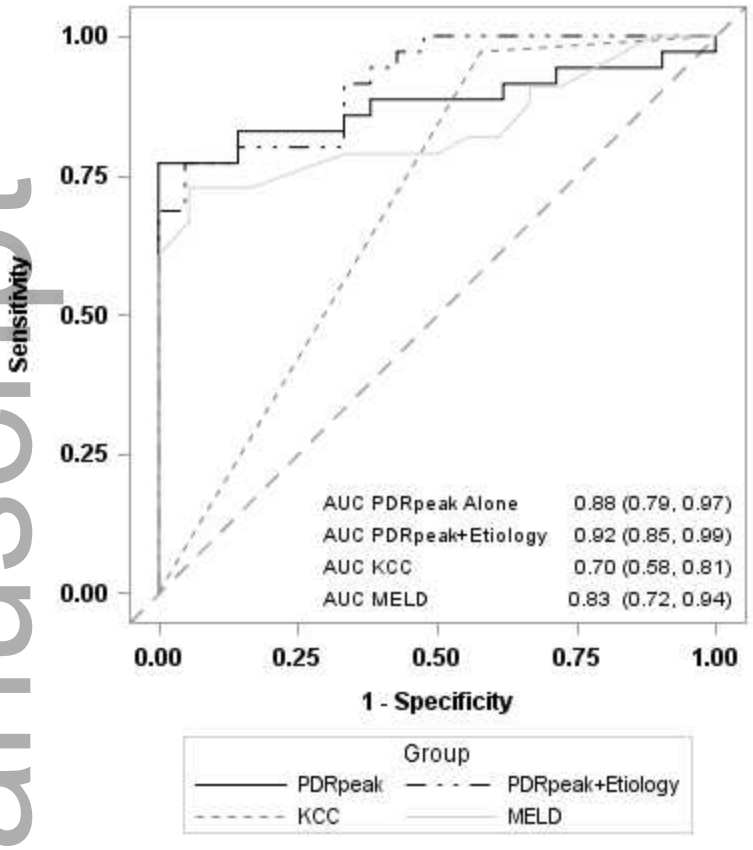


Figure 5: 21-day transplant-free survival in ALF only patients

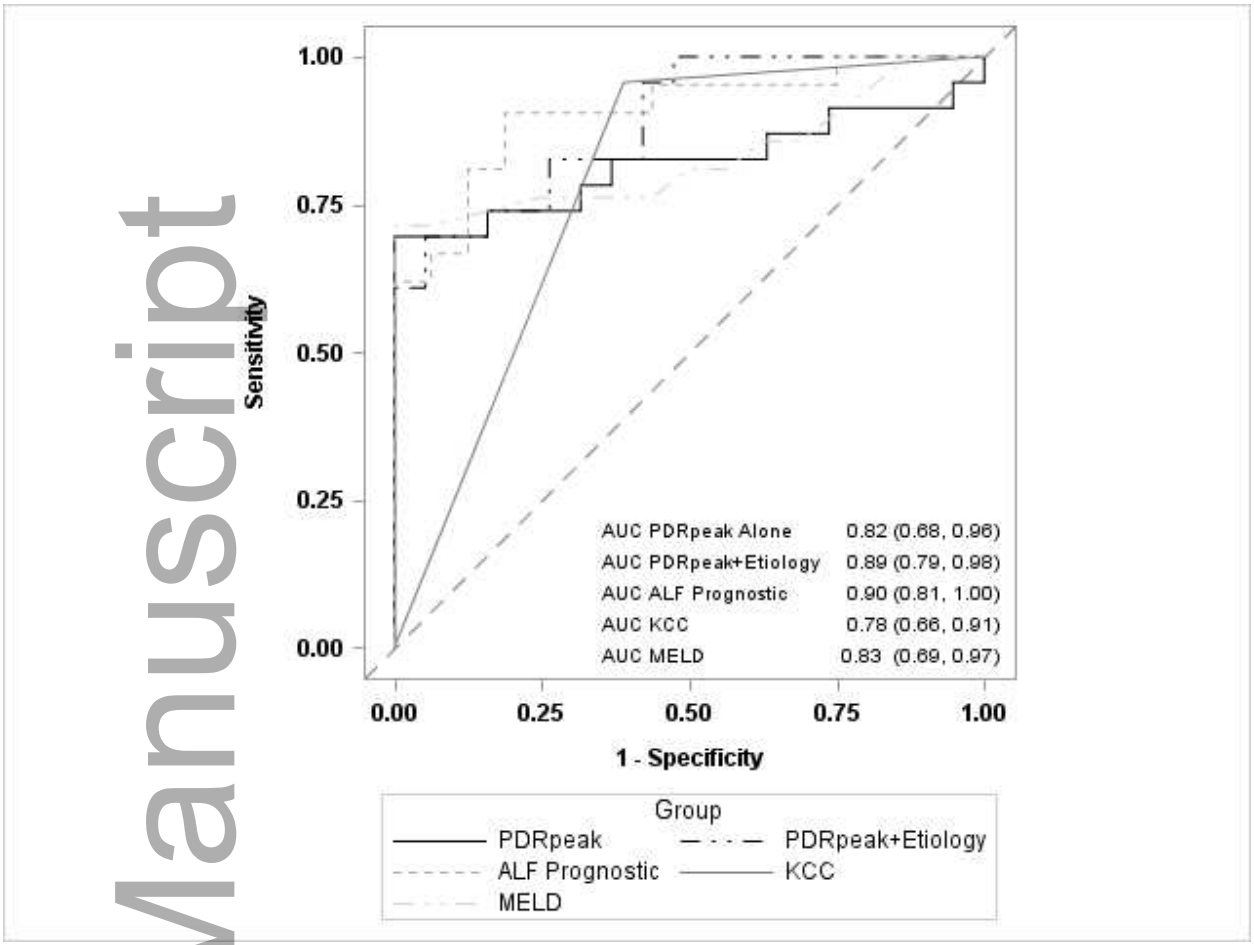


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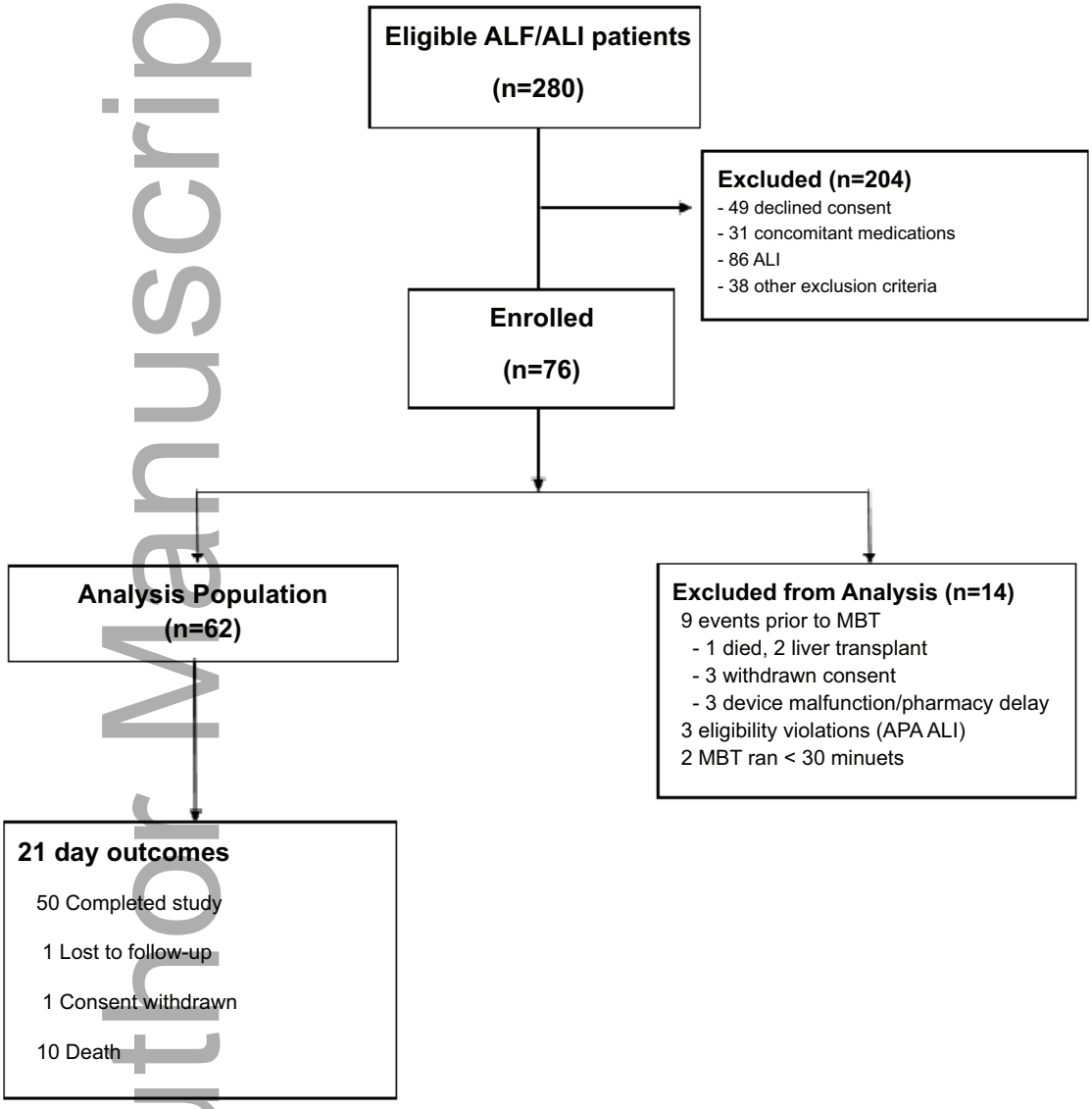
Figure 1



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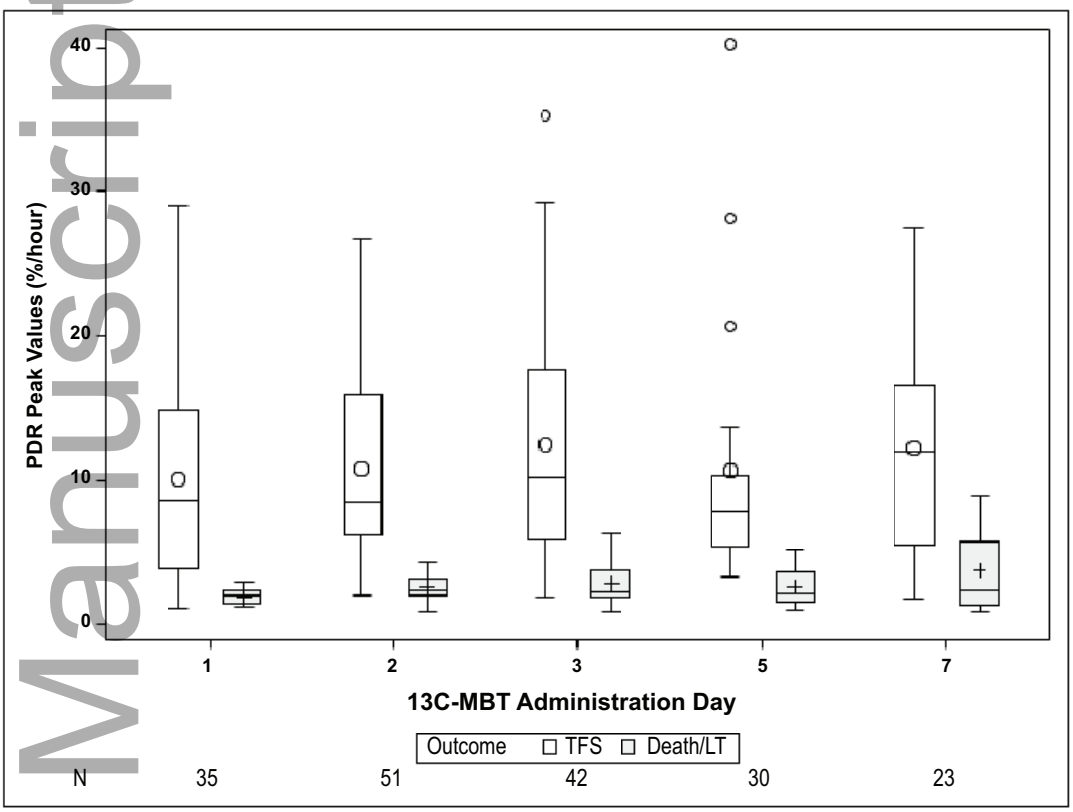

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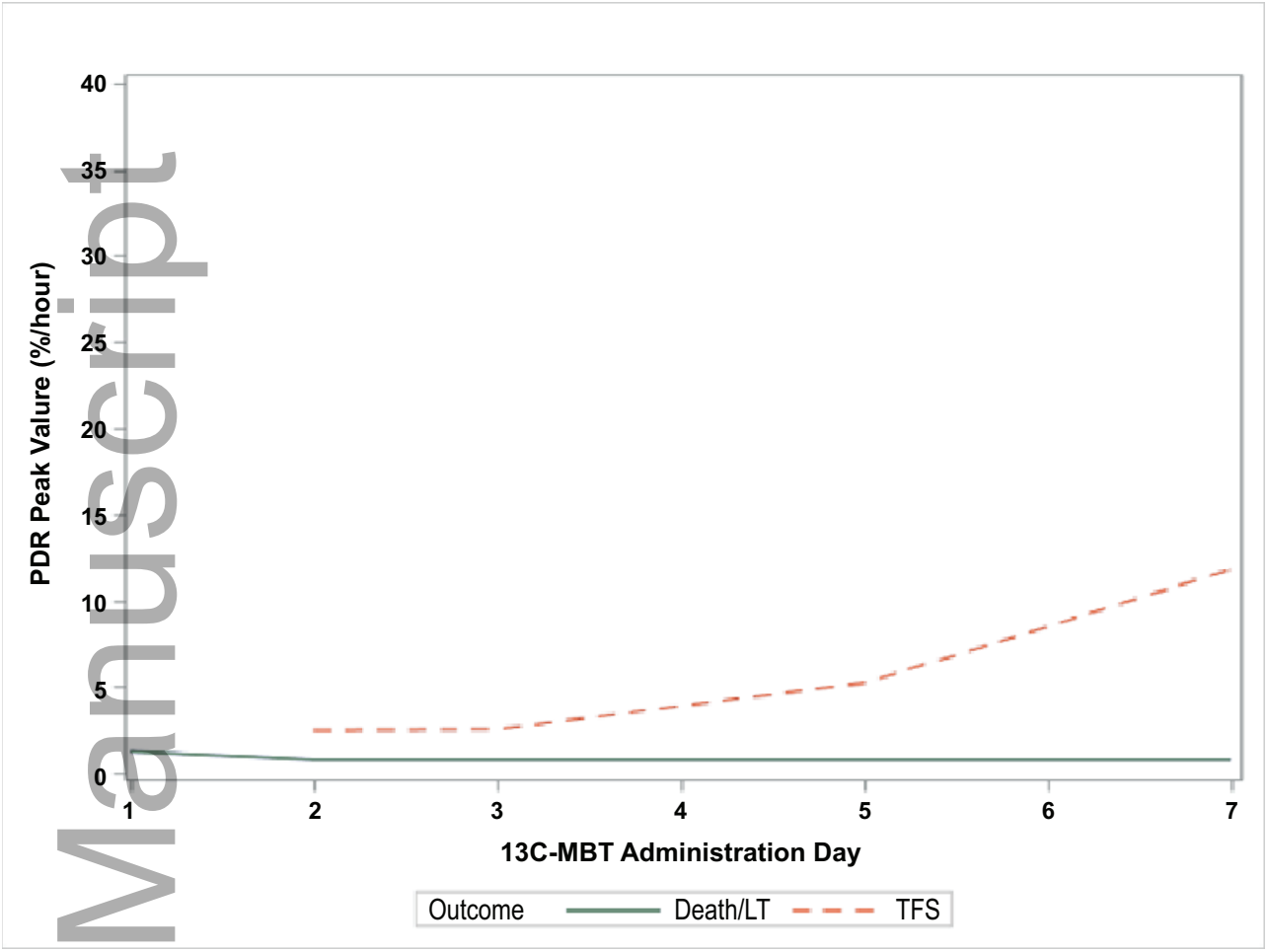
Figure 3



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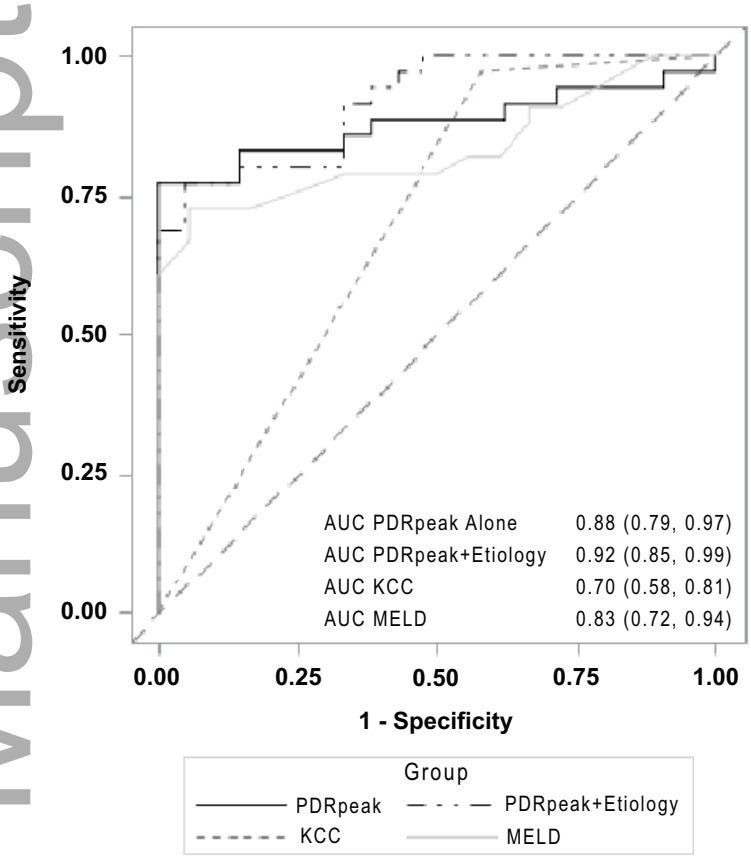

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