Protocol Title: Non-invasive assessment of liver function in patients undergoing heart and liver transplant evaluation

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Background and Goals:

Dilated Cardiomyopathy

Biventricular heart failure results in chronic passive hepatic congestion. This congestion through a non-inflammatory process will in time result in fibrosis and development of cirrhosis, also known as cardiac cirrhosis. The time for the liver to become impaired and fibrotic is variable, with initial stages of fine fibrosis potentially reversible with improvement of heart function. Unfortunately for some patients by the time they need a heart replacement by transplantation , the liver not only may be at an irreversible stage of cirrhosis but the impaired liver may not have reserve to sustain injury through hypoperfusion at time of transplant and therefore patients need to be evaluated to have a combined liver and heart. This liver evaluation is at present challenging in selecting patients appropriately to have either heart only or combined heart and liver transplantation. Traditional testing and predictors (i.e. MELD score) are often not helpful.

Fontan-Associated Liver Disease

Due to advances in medical care and surgical techniques, the population of adults with congenital heart disease (CHD) has been growing exponentially since the 1950s. Survivorship to adulthood is estimated to be greater than 90% of children born with congenital heart disease; with over 1 million adults living in the United States with CHD. One of the more severe forms of CHD is that of functional single ventricle, including hypoplastic left or right heart syndrome, tricuspid or pulmonary atresia. Without palliative surgery, the life expectancy of neonates born with single ventricle physiology is extremely poor with mortality reaching more than 90% within the first year of life. The Fontan procedure, first performed in 1968 as a palliative surgery for tricuspid atresia, has been widely implemented as a staged palliative surgery for single ventricle patients. Although there has been a significant decrease in mortality in the first 25 years post-Fontan, it has been associated with significant cardiac and non-cardiac complications resulting in delayed morbidity and mortality in young adults.

The Fontan procedure allows for passive pulmonary blood flow by creating a direct connection with the inferior and superior vena cava to the lungs. Over time, there is an increase in both pulmonary vascular resistance and ventricular end-diastolic pressure. The pathophysiology of hepatic fibrosis likely shares similarities with typical cardiac hepatopathy with downstream effects causing hepatic venous and sinusoidal congestion resulting in hepatic fibrosis and possible cirrhosis. Hepatic dysfunction has been well documented amongst adults with Fontan physiology with outcomes worsening as a patient is farther out from the surgical intervention (Figure 1).

Liver in the Fontan

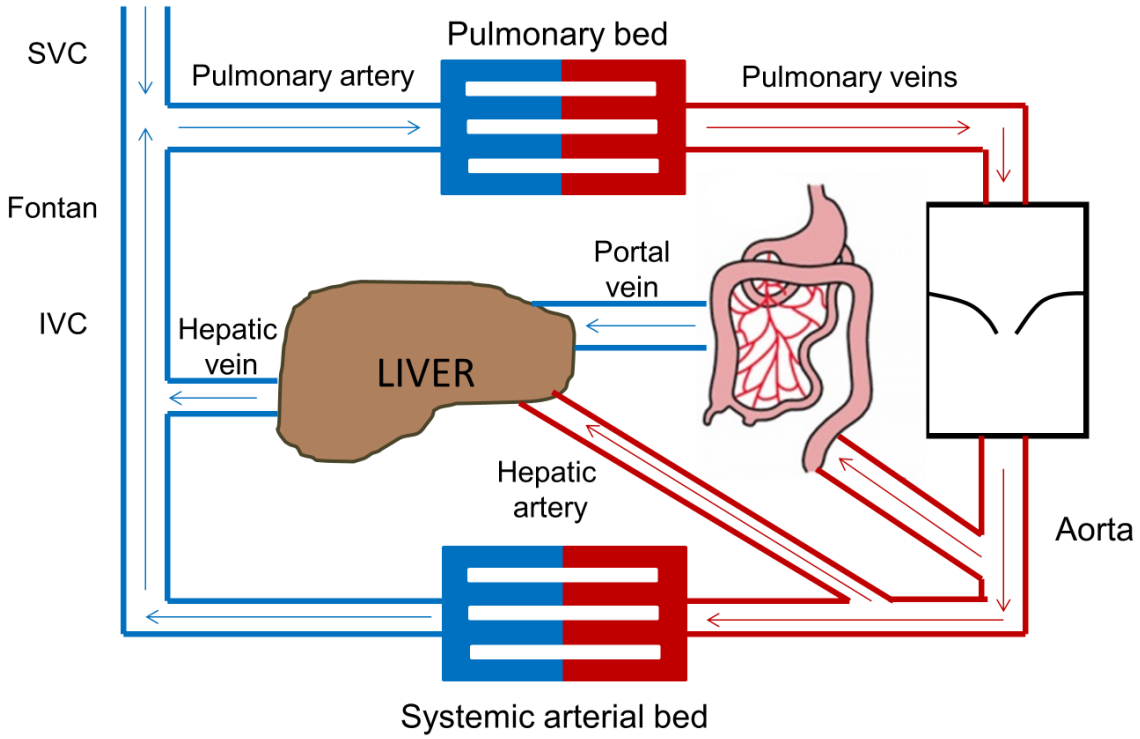

Figure 1. Hemodynamic relationship of the liver in relation to the Fontan circulation. The liver receives approximately 75% of its afferent blood flow from the portal vein which delivers efflux from the mesenteric circulation. The remaining 25% of blood flow to the liver is derived from the hepatic artery. The hepatic vein drains the liver into the IVC which connects into the Fontan circulation.

What is not known is the relative amount of portal vein flow and hepatic artery flow to the liver in the Fontan circulation. It is postulated that the combination of elevated central venous pressure and low cardiac output may compromise portal venous flow such that hepatic artery blood flow increases as a compensatory mechanism of autoregulation. Elevated transhepatic resistance can result in venous-venous collaterals between the portal and hepatic venous systems, decompressing portal hypertension. However, the transhepatic gradient has not been shown to be elevated in the Fontan which could be result of such collaterals or simply reflective of portal hypertension coupled with high downstream hepatic venous pressure.

Evidence over the past few decades has shown that liver injury is universal in the Fontan patient. Over time, the cumulative effects of high central venous pressure/hepatic congestion coupled with relatively lower cardiac output results in liver fibrosis, cirrhosis and even hepatocellular cancer. Hepatic fibrosis appears to be time-related but other risk factors have been identified including elevated systemic venous pressure, decreased cardiac output, decreased heart rate, and older age at initial Fontan operation.

Non-invasive imaging such as computed tomography (CT) and magnetic resonance imaging (MRI) as well as biopsy specimens commonly demonstrate advanced disease. Despite marked morphologic abnormalities, laboratory investigations such as liver function tests are usually minimally affected.]For example, total bilirubin and alkaline phosphatase can be mildly elevated as seen in hepatic congestion but AST and ALT abnormalities are minimal or absent while CT or MRI can show “cardiac cirrhosis.” Simultaneous, transient elastography and MR elastography demonstrate significant abnormalities in liver stiffness, another striking aspect of FALD. This uncoupling of liver function (measured by conventional laboratory means) from liver structure as assessed by imaging and biopsy in FALD continues to be a major area of research. To date, there is no biomarker or test to accurately measure liver function in the Fontan.

Fontan-associated liver disease (FALD) is a poorly understood comorbidity of the Fontan circulation, manifest radiographically and histologically as hepatic congestion, fibrosis, and cirrhosis.[1] Of great concern is a small but growing number of case reports describing hepatocellular cancer in Fontan survivors. Clinically, these marked morphologic findings of fibrosis and cirrhosis are out of proportion to the degree of laboratory markers of hepatic injury and synthetic dysfunction (i.e. conventional liver function tests, albumin, INR) [5]. There is no good predictor of functional liver reserve in patients who will undergo cardiac surgeries such as Fontan revisions or even cardiac transplantation, further underscoring the elusive nature of FALD. The decision to recommend cardiac transplant only or combined heart liver transplant is challenging and requires better tools than currently available.

Congestive Dilated cardiomyopathy (**DCM**) is a condition in which the heart becomes enlarged and cannot pump blood effectively.[3]Symptoms vary from none to feeling tired, leg swelling, and shortness of breath.[2] It may also result in chest pain or fainting.[2] Complications can include heart failure, heart valve disease, or an irregular heartbeat.[3][4]

The aim of this project is to assess a non-invasive functional liver test in patients with the Fontan circulation or with DCM that may be used for prognostic purposes. Specifically, we aim to determine whether there are alterations in a Methacetin breath test (MBT) in the Fontan or DCM patient and if so, whether it is related to conventional tests of liver and cardiac function. The hypothesis is that the MBT parameters in the Fontan patient is abnormal as a result of alterations in liver perfusion, liver cell metabolic capability and transhepatic resistance secondary to hemodynamics unique to the Fontan as well as end-organ liver damage. Due to lack of robust biomarkers or other risk stratification schemes, we aim to determine whether there is prognostic value in hepatic MBT parameter in the Fontan patient.

Aims - The aims of this study are three-fold:

1. To measure MBT parameter in a cohort of patients with Congestive (Dilated) Cardiomyopathy and a group of Fontan patients and compare results to published normal controls.
2. To explore any association between MBT parameter and clinical parameters already available (both baseline values and changes in values after 12 months), including Fontan hemodynamics as assessed by either of the following tests: cardiac catheterization, echocardiography, non-invasive imaging of the liver (CT or MRI), non-invasive assessment of liver stiffness (ARFI, MRE or Fibroscan), laboratory investigations, and clinical characteristics (i.e. age of patient, time since Fontan operation, type of Fontan etc., onset of DCM) . The clinical parameters should be within 3 months of the MBT.
3. To determine whether MBT is predictive of clinical outcomes: heart failure, clinically significant ascites, and time to transplant or death.

Research Design and Methods Study Design: This is a prospective pilot cohort study in 20 patients with one group of 10 subjects with Dilated Congestive Cardiomyopathy (DCM) and one group of 10 adult subjects with Fontan. All subjects will be recruited from an adult congenital heart disease clinic in a tertiary care center. Patients enrolled in the study will be prospectively followed for 3-5 years and date of liver transplantation as well as survival status will be recorded.

The first Breath Test will be performed after consent is obtained. This first test (visit 1) will be scheduled as close as possible to the day of consent. The MBT test will be repeated one year later (visit 2) provided no contraindications are present.

Subjects:
Inclusion Criteria
- Written informed consent obtained prior to any study-specific assessments
- Adults patients ≥ 18 years of age
- Liver disease secondary to congenital heart disease or cardiomyopathy

Exclusion Criteria
- Females of child-bearing potential that are pregnant or breastfeeding
- Participation in other interventional trials
- Pregnant or breastfeeding female
- Allergy to acetaminophen based medications

Study-Wide Number of Participants: We anticipate accruing 20 patients from our center.

Study-Wide Recruitment Methods: N/A

Data Collection and Analysis:
Predictor variables
Congestive cardiomyopathy:
- *Age*
- *Etiology: Viral, Alcohol, Amyloid, Unknown* NYHA functional class

Include all or some of the following tests collected as part of their standard medical care:
- Six minute walk test
- Cyanosis (saturations <90%)
- Ejection fraction or systemic ventricular function (normal, mild, moderate, severe)
- Degree of atrioventricular regurgitation (none, mild, moderate, severe)
- Cardiac index (L/min/m2), transhepatic gradient, end-diastolic pressure, PVR, SVR
- Cardiac medications (beta-blocker, calcium channel blockers, ACE/ARB, diuretics)
- History of hepatitis, alcoholism, large volume paracentesis, varices, GI bleed, hepatic encephalopathy
- History of heart failure (defined as admission for volume overload)
- Laboratory tests: comprehensive metabolic panel, CBC, PT/INR, NTproBNP, AFP, and troponin T
- Child-Pugh score
- MELD, MELD-Na, and MELD-XI scores
- Abdominal CT or MRI findings consistent with cirrhosis or portal hypertension
- Liver biopsy
- Fibroscan, ARFI or MR Elastography score

Adult Fontan Associated Liver Disease patients.

- Age
- Age at Fontan
- Time from Fontan
- Type of Fontan (atrio-pulmonary, lateral tunnel, extracardiac, other)
- History of Fontan revision
- Underlying congenital heart disease lesion
- Systemic right ventricle or left ventricle
- NYHA functional class
- Six minute walk test
- Cyanosis (saturations <90%)
- Ejection fraction or systemic ventricular function (normal, mild, moderate, severe)
- Degree of atrioventricular regurgitation (none, mild, moderate, severe)
- Fontan pressure (mmHg), cardiac index (L/min/m2), transhepatic gradient, end-diastolic pressure, PVR, SVR
- Cardiac medications (beta-blocker, calcium channel blockers, ACE/ARB, diuretics)
- History of hepatitis, alcoholism, large volume paracentesis, varices, GI bleed, hepatic encephalopathy
- History of heart failure (defined as admission for volume overload)
- Laboratory tests: comprehensive metabolic panel, CBC, PT/INR, NTproBNP, AFP, and troponin T
- Child-Pugh score
- MELD, MELD-Na, and MELD-XI scores
- Abdominal CT or MRI findings consistent with cirrhosis or portal hypertension
- Liver biopsy
- Fibroscan, ARFI or MR Elastography score

Other than the MBT, all testing will be obtained as part of the standard of care evaluation of heart and liver disease in patients with Fontan circulation or DCM.

Study Timelines: We anticipate that it will take approximately 2 years to recruit patients who are to be included in this study. Patient will followed 3-5 years.

Study Endpoints: The aim of this project is assess a non-invasive functional liver tests in patients with the Fontan circulation or DCM that may be used for prognostic purposes.

PROCEDURES INVOLVED:

Methods

Screening and consent

Eligible patients will be screened and recruited through the outpatient Hepatology Clinic, Heart Failure Clinic and the Adult congenital heart disease clinic. They will be approached the day of the scheduled outpatient appointment by the PI or a study coordinator in a private room.

Informed consent will be obtained by the principal investigator, a sub-investigator or a member of the study team who is authorized to give consent. This will allow the potential subject sufficient opportunity to consider whether or not to participate and this will minimize the risk of influence or coercion. The subject will be informed of what the research entails. Any foreseeable risks or discomforts will be discussed with the subject. The protection of confidentiality will be discussed with subjects as well as the subject will be given the name(s)/number(s) of persons to contact should they have any further questions before signing the informed or consent or once they have enrolled in the study. It is reiterated to the subject that participation is voluntary and not participating will not affect present or future medical care. The subject will ask and have

answered any and all questions before signing the informed consent. The subject is given a signed copy of the informed consents(s) and HIPAA authorization(s).

MBT testing

The ^{13}C-Methacetin Breath Test (MBT) is a non-invasive test for assessing liver metabolic function. The BreathID® MCS System consists of the BreathID® MCS device and a test kit containing a breath collection cannula and a non-radioactive isotope ^{13}C-Methacetin solution. It measures and computes the ratio between $^{13}CO_2$ and $^{12}CO_2$ in the patient's exhaled breath. The components of the MBT system include the

1. MBT BreathID® MCS unit;
2. ^{13}C-Methacetin solution.

A dedicated flash memory stick for automatic raw data download will be provided to all participating investigative sites.

All components of the MBT system will be provided by Exalenz Bioscience Ltd.

<u>The MBT BreathID® MCS Unit</u>

Exalenz Bioscience Ltd. has developed a molecular correlation spectrometer, based on specific optical-radiation emission and absorption by $^{13}CO_2$ and $^{12}CO_2$ gases. This device continuously senses exhaled breath and analyzes CO_2 in real-time through a breath collection cannula connected to the patient. Based on Molecular Correlation Spectrometry (MCS), the BreathID® MCS device continuously measures $^{13}CO_2$ and $^{12}CO_2$ concentrations from the patient's breath and establishes the $^{13}CO_2$ /$^{12}CO_2$ ratio. The cannula continuously transports the breath sample from the patient to the BreathID® MCS device. Results are displayed in real time on the device screen and are printed after the completion of the test. The BreathID® MCS device calculates the delta over baseline (DOB), which can be translated into the percent dose recovery (PDR) and the cumulative percent dose recovery (CPDR) for each time point derived from the ratio difference compared to baseline (normalized/adjusted to the patient's body weight and height as well as substrate dose) throughout the course of the test. The ^{13}C-Methacetin metabolism begins almost immediately since liquid passes through the stomach to the duodenum, where it is absorbed, without delay. The default breath collection test time is one hour after ingestion of ^{13}C-Methacetin. The light sources are $^{13}CO_2$ and $^{12}CO_2$ discharging lamps. By using $^{13}CO_2$ and $^{12}CO_2$ discharging lamps as light sources, light absorption will be solely due to the existence of $^{13}CO_2$ and $^{12}CO_2$ in the gas mixtures. Furthermore, by using $^{13}CO_2$ and $^{12}CO_2$ discharging lamps as light sources, background radiation will be substantially reduced, leading to highly sensitive absorption curves. These highly sensitive absorption curves enable detection of very small variations in $^{13}CO_2$ and $^{12}CO_2$ concentrations and the $^{13}CO_2$/ $^{12}CO_2$ ratio. The BreathID® MCS System can detect variations of less than 1/1000 in the $^{13}CO_2$ / $^{12}CO_2$ ratio measurement. The actual breath collection is automatically done by the device and is not operator dependent. If the patient is not connected properly (e.g. the breath does not reach the device), the BreathID® MCS device will prompt the operator to adjust the nasal cannula. The cannula transports the breath sample from the patient to the BreathID® MCS device. The cannula test kit is a single-use kit and is comprised of a plastic sampling line with an in-line hydrophobic filter (used to reduce the amount of moisture present from the patient's breath) and a Luer-lock connector for connection to the BreathID® MCS device. The device is designed to accommodate an extended range of respiratory rates.

<u>^{13}C-Methacetin</u>

Exalenz Bioscience Ltd. will supply a ready to use, non-radioactive isotope ^{13}C-Methacetin solution for single-use oral administration (75 mg in 150 ml purified water), dispensed in thermoplastic polyester (PTE) bottles sealed with a plastic child resistant stopper. The ^{13}CMethacetin solution can be administered via typical oral. ^{13}C-Methacetin meets all of the

qualifications for a liver function test substrate: it is a nontoxic small molecule; is administered orally; is rapidly absorbed; and is exclusively metabolized by the liver. Furthermore, ^{13}C can be easily synthesized into a key location within this molecule. No related adverse events have been reported when using this substance, including in vulnerable populations, and the compound remains stable over time. The ^{13}C-Methacetin substrate is a well-known diagnostic reagent that has been described in the literature and used for over 30 years by researchers around the world. ^{13}C-Methacetin is rapidly absorbed and metabolized by the hepatic mixed function oxidase, via O-demethylation. This process is carried out by hepatic cytochrome P450 enzymes that produce two products, acetaminophen and formaldehyde, which is transformed through two successive oxidative steps to $^{13}CO_2$. Toxicology testing results in animals and other clinical information support the safe use of Methacetin in humans. Based on the acute toxicity studies in mice and rats where relatively high LD50 values of 1190mg/kg were administered, the Methacetin dose administered in human breath tests in adults of 75 mg, or approximately 1mg/kg, has a safety ratio in excess of 1000- fold (10). There have been no reports of any complications with the use of this substance in over 2500 patients tested worldwide (see IDE filing). The main metabolite of Methacetin is acetaminophen which has wide routine clinical use at much higher doses than orally administered dose of ^{13}C-Methacetin of 75mg used in this study.

Preparation of ^{13}C-Methacetin

Exalenz will provide 75 mg ^{13}C-Methacetin in a 0.05% solution in 150mL purified water supplied in amber thermoplastic polyester (PET) bottles. No preparation is needed.

Investigational Product Handling

The Investigator and/or Research Pharmacist (if relevant) will be provided with Investigational Product Handling Guidelines that will provide details regarding the packaging and labeling requirements, receipt of investigational product, dispensing and accountability procedures, preparation instructions, storage and stability of Investigational product.

^{13}C-Methacetin Accountability

The Investigator is responsible for ensuring that all study substrate supplies received at the site are inventoried and accounted for throughout the study. The dispensing of study substrate to the subject must be documented in the respective accountability form. The study Investigational Product must be handled in strict accordance with the protocol and the container label and will be stored in a limited access area or in a locked cabinet under appropriate environmental conditions. Study substrate should be dispensed under the supervision of the investigator, or a qualified member of the investigational staff.
After completion of the study, the device must be returned to Exalenz Bioscience Ltd. and the study drug will be destroyed according to local regulations with written confirmation.

Requirements for Preparation before MBT:

- The subject should be fasting from drink and solid food for a minimum 8 hours Subject has not ingested acetaminophen-related medications (e.g. Tylenol) within the past 48 hours (subjects with acetaminophen intoxication may be included 24 hours after ingestion).
- Subject has not had any products containing caffeine for 24 hours.
- Subject has not taken any oral (by mouth) medications for at least 1 hour before the test.
- Subject has not to have any Tylenol or acetaminophen for 48 hours prior to the scheduled test.
- Subject has not received general anesthesia in the last 24 hours.
- Subject should not have taken within last 48 hours any of the following drugs: Acyclovir , allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein,

(herbal) disulfiram, echinacea, enoxacin, famotidine, fluvoxamine, methoxsalen, mexiletine, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlopidine, thiabendazole, verapamil, zileuton, as they are metabolized by CYP1A2 .

Breath is collected before and after the substrate is administered to the subject; no waiting period is required once the substrate has been administered. Breath is collected automatically via a nasal cannula for a total of 75 minutes (up to 15 minutes for baseline measurement and 60 minutes following ingestion of Methacetin). All test-specific equipment will be supplied by Exalenz

Study Feasibility: The Clinical Research Unit will provide essential resources for the conduct of the proposed research. Study coordinator already experienced form other studies with the Breath ID machine and protocol will be required for administration of the test. Subjects are expected to spend approximately 2 hours in the CRU for administration of MBT test.

Study Limitations: One major limitation to this study is the unknown ability to correlate MBT with various clinical data, specifically variables derived from cardiac catheterization, if not available. Cardiac output, which is measured by cardiac catheterization, is a determinant of hepatic blood flow and likewise, Fontan pressure can influence hepatic perfusion. Cardiac catheterizations are not routinely performed in healthy Fontan patients and any analysis using this variable represents a selection bias towards a symptomatic and potentially sicker subgroup. There is merit in adding a non-invasive test like MBT when looking at FALD/DCM patients, given the difficulty to predict liver outcome in these patients.

It is entirely possible that none of the secondary end-points will be achieved within the time period specified given the infrequency of such events and that the cohort may need to be followed for 3-5 years or longer to determine such outcomes.

Data Banking: Data will be stored on a password protected excel spreadsheet. Only the Investigators will have access to this password protected file. This file will be kept on the FSM Transplant Surgery server only and/or storage that is maintained on Northwestern Vault or other NU-own storage consistent with FSM policies. No subject identifiers will be included in this spreadsheet. All clinical data will be kept completely confidential and PHI will be de-identified at the earliest opportunity. All study documents will be retained on site (electronically or otherwise) for at least 2 years after the study has been formally terminated at the NU IRB. The investigator(s) will work with the department and FSM to ensure electronic data is erased and any paper documents are stored in Iron Mountain Record Retention Center.

Provisions to Monitor the Data to Ensure the Safety of Participants: The investigator will permit study-related monitoring, audits, and inspections by the NU IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents.

<u>Adverse Events</u>
In this trial, any medical conditions not present prior to the initial administration of the test substrate but that emerge after the initial test substrate is administered and for 24 hours post administration are considered Adverse events (AEs). AEs encountered will be recorded, as well as Serious Adverse Events will be recorded. All study personnel are responsible for entering any and all nonserious and serious adverse events, regardless of severity or relationship to the 13C-Methacetin solution and device, and including updates to adverse event information. A follow up phone call will be made to all subjects approximately 24 hours post administration of test substrate.

Requirements for Stopping Criteria for Administration of MBT Test

- Prior occurrence of any adverse events related to 13C-Methacetin solution and device
- Change in subject's health which makes continued participation unsafe. PI will see subject prior to administration of test.
- Subject has not fasted from solid food or drink for 8 hours
- Subject has consumed caffeine products within the past 24 hours
- Subject has not taken any oral (by mouth) medications for at least 1 hour before the test.
- Subject has not to have any Tylenol or acetaminophen for 48 hours prior to the scheduled test.
- Subject has undergone general anesthesia within the past 24 hours
- Subject has taken excluded medications within the past 48 hours. These medications include: Acyclovir, allopurinol, carbamazepine, cimetidine, ciprofloxacin, daidzein, (herbal) disulfiram, echinacea, enoxacin, famotidine, fluvoxamine, methoxsalen, mexiletine, montelukast, norfloxacin, phenylpropanolamine, phenytoin, propafenone, rifampin, terbinafine, ticlopidine, thiabendazole, verapamil, zileuton, as they are metabolized by CYP1A2.

Early Termination of the Breath Test

Key personnel will be trained how to terminate the breath test early if a subject is unable to complete the full 75 minutes of breath collection. In the following situations, the breath test collection will be terminated:

- The subject vomits.
- The subject has to be disconnected from the nasal cannula/ collection device for more than 1 minute due to an urgent procedure.
- The subject is inadvertently disconnected from the BreathID® MCS device.
- The BreathID® MCS device malfunctions.

Requirements for stopping the study

The study will be stopped if there is a pattern seen of adverse events related to the MBT test among the subjects. Since this is a small pilot study if adverse events related to the MBT test are seen in one subject the study will be stopped.

Withdrawal of Participants: Subjects who consent but wish to withdraw will be asked to write to the PI for reporting purposes.

Risks and Alternatives: Risk of MBT administration: To date, MBT (methacetin) that will be used in this study has not been associated with any complaints or side effects. However, they are still considered experimental and there may be unknown risks.

Women of childbearing age must demonstrate a negative urine pregnancy test in order to participate.

Loss of Confidentiality - Anytime information is collected, there is a potential risk for loss of confidentiality. Every effort will be made to keep all subject information confidential.

Potential Benefits to Participants: Subjects will not receive any potential benefits from this study.

Vulnerable Population: N/A

Community-Based Participatory Research: N/A

Sharing of Results with Participants: The data obtained will be accessible only to the study investigators. The results of the investigation will not be released to each individual patient.

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