**Structured Research Abstract**

Introduction: Sepsis is a common condition seen in emergency departments across Canada with a high rate of morbidity and mortality. Currently there are few accurate diagnostics to rapidly identify sepsis with a high sensitivity and specificity. Collecting biofluids from patients with sepsis before they receive any treatment or therapies can allow for identification of new uncorrupted biomarkers of this disease state. Using non-invasive urinary metabolomic analysis, small molecule biomarkers can be identified from septic and septic shock patients.

Rationale: Early identification of septic biomarkers can change initial treatments and therapies. Patient outcome is most favourable when treatment is initiated for sepsis at the earliest possible timepoint. Currently there are no definitive laboratory test to identify sepsis with high sensitivity and specificity.

Research Question: Can urinary biomarker metabolomic patterns be identified and used to diagnose and prognosticate patients with early sepsis and septic shock presenting to the emergency department.

Methods: Urine samples will be collected from all patients presenting to a tertiary emergency department with signs of sepsis or septic shock. Urine will be processed to standardize samples for NMR metabolomic analysis. Once NMR spectra is obtained from the samples individual urinary metabolites will be identified using pattern recognition software. Metabolites essential for diagnosing sepsis will be determined by multivariant statistical analysis.

Potential Impact: Urinary biomarkers and metabolite patterns that are identified can be used to develop future point of care diagnostic technology allowing for early identification and treatment of all patients with sepsis.

**Research in Depth**

**Introduction and Rationale:** Sepsis places a huge burden on hospitals across Canada (excluding Quebec) with over 30,000 hospitalizations due to sepsis occurring from 2008 to 2009 [1]. Identifying patients most at risk of worsening morbidity and mortality is essential to improving outcomes and decreasing hospital length of stay. Currently septic patients may be recognized by primary care physicians, emergency medical services, or upon arrival at an emergency department.

Acute care physicians have turned to simple clinical scores consisting of vital signs, laboratory results, and clinical judgment to help rapidly identify patients that may or may not be septic [2-4]. The addition of specific biomarkers of sepsis has the potential to aid in more accurate identification and effective treatment of patients with sepsis. In the acute care setting serum lactate levels are the most commonly used biomarker for diagnosing end organ dysfunction and sepsis prognostication [5]. A new emerging field of small molecule biomarker identification, metabolomics, has the potential to identify new targets for diagnostic testing and medical therapies to treat patients with septic infections [6]. Metabolomics uses common biochemical analytical techniques like mass spectroscopy and nuclear magnetic resonance.
spectroscopy to identify known compounds, and biochemical patterns in human biosamples such as cerebral spinal fluid, serum, and urine. As of now there have been several metabolomic studies on patients with sepsis, and those that have been reported have been primarily focused on septic patients in the ICU [7-9]. Unfortunately these studies are focused on patients long past the initial development of their septic infection, and therefore have more complex and confounding metabolomic biomarker profiles. Identification of sepsis biomarkers at the earliest possible time point will allow for identification of more precise markers with fewer false positives. Current efforts have been directed at serum-based biomarkers but very few have targeted urinary metabolites [7]. Urine based biomarkers offer a non-invasive method of sampling biofluid at regular time points over the course of an illness. 1H NMR analysis offers the opportunity to identify and quantify known biomarkers, as well as identify patterns related to yet unidentified biomarkers [10, 11].

**Research Question:** Can urinary biomarker metabolomic patterns be identified and used to diagnose and prognosticate patients with early sepsis and septic shock presenting to the emergency department.

**Primary Objective:**
- To determine the feasibility to assess urinary biomarkers in patients with early sepsis and septic shock presenting to the emergency department.

**Primary Outcome:**
(1) Feasibility will be determined to be successful if ≥75% of identified patients are able to provide urine samples points in the ED.

**Secondary Objectives:**
- To identify common urinary biomarker patterns for diagnosing patients with early sepsis and septic shock.
- To identify specific urinary biomarker patterns for diagnosing patients with specific sources of early sepsis and septic shock.

**Secondary Outcomes:**
(1) Identification and quantification of known metabolites in urine samples.
(2) Identification of NMR spectral imaging patterns and bins correlated to unknown metabolites specific for early sepsis and septic shock.

**Aims:**
1. To identify urinary biomarkers associated with early sepsis and septic shock in patients in the ED.
2. To use biomarker profiles to differentiate patients with early sepsis and septic shock,

**Methods:**
**Study Design:** This will be a prospective observational cohort study, conducted at the emergency departments (ED) in both the Hamilton General Hospital (HGH) and the Juravinski Hospital and Cancer Centre (JHCC) in Hamilton.

Patients in the ED will be identified and recruited by research and clinical staff recruiting for the SEPSIS ED Study under Dr. Alison Fox-Robichaud. The SEPSIS ED is a multi-centre prospective study to identify patients with early sepsis and septic shock, and obtain biomarker samples including cell free DNA and urine for NMR metabolomic analysis.
The Sepsis ED study has ethics approval from the Hamilton Health Sciences Research Ethics Board.

**Study Population – Inclusion Criteria:** Adult patients presenting to the ED at the HGH and JHCC with a potential infection and suspected sepsis or septic shock:

A. Sepsis (2 of 3) (as per qSOFA)

- Respiratory rate ≥22/min
- Altered mentation or level of consciousness
- Systolic blood pressure ≤100 mm Hg

OR

Patients with suspected infection and have:

- Hamilton Early Warning Score (HEWS) >5 [12]

B. Septic Shock

Patients must fulfill criteria for A (above) and despite adequate volume resuscitation:

- have persistent hypotension requiring vasopressors to maintain MAP ≥65 mm Hg and
- have a serum lactate level >2 mmol/L

**Study Population – Exclusion Criteria:**

- Age < 18 years
- Limitation of care, palliative measures only.

**Consent:** These research coordinators will be responsible for obtaining consent from patients or substitute decision makers (SDM). In the event that the patient or SDM is unable to give consent before specimens are obtained, the research coordinator will employ a deferred consent approach and see consent when the opportunity arises.

**Sample Collection – Urine Collection:** Patients admitted to the ED and identified to have early sepsis/septic shock will have urine samples collected at the earliest possible time point after triage.

**Sample Collection – Urine Storage:** Urine will be collected in sterile specimen cups by either midstream catch or sterile catheter, (100 mL), with screw top lid for a tight seal (e.g. Dynarex). The approved urine specimen cups (100mL) are coated with sodium azide (100 μl of 10% NaN3) and dried at room temperature overnight in a sterile fume hood [13]. Cups will be dry stored in a zip-lock plastic bag at normal refrigerator temperature. Under these conditions cups are considered usable for up to 6 months, after which containers not used in that time period will be disposed of. Once the sample has been collected the specimen cup will be immediately sealed and placed into a specially designated refrigerator (2-4°C) by clinical staff. It is imperative that all samples be cooled immediately and transferred to an -80°C freezer as directly and quickly as possible.
Sample Processing: Urine will be thawed with up to 10mL of raw urine being transferred to a 15mL sterile conical tube. A 1mL aliquot of urine will be placed in a sterile 2mL eppendorff tube, with the remaining raw urine being immediately frozen at -80°C until required for further study. The 1mL raw urine sample will then have its pH recorded using a pH meter and if needed, adjust the urine pH to ~7.0 ± 0.2 using NaOH or HCl (0.1 M stock solutions). The tube will then be centrifuged at 14,000 rpm (~10,000 g) in a refrigerated microfuge (4°C) for 5 minute. 900 μL of pHed urine is removed from the supernatant, and transferred to a sterile 2mL eppendorff tube containing 100 μL of Chenomx (Chenomx Inc. Ed. AB), standard solution. Care is taken not to disturb the bottom of the tube whether there is a visible pellet or not. The tube containing the remaining ~100 μL and pellet are disposed of. The sample is then directly refrigerated before for use in the magnet or frozen at -80°C if data is not to be acquired immediately.

Sample 1H-NMR Data Acquisition: The prepared urine samples will be thawed and placed in either a refrigerator or on ice immediately before 1H-NMR data acquisition. The samples will again have their pH measured and recorded before 750 μL of the prepared urine sample is transferred into clean glass NMR tube. The sample will be analysed in either a 14.1 Tesla (600 MHz) nuclear magnetic resonance (NMR) spectrometer (Varian/Agilent Inc.) utilizing a Varian768 automatic sampling handling system, or a 18.8 Tesla (800 MHz) spectrometer. A 2D-1H,1H-NOESY (metnoesy) pulse sequence will be collected in one-dimensional mode using either 32 (3.5 mins) or 192 scans (12 mins) per experiment depending on sample concentration.

1H-NMR Data Analysis: Chenomx NMR Suite Professional software package (Chenomx Inc. Edmonton, Alberta), will be used to process the raw NMR spectra. Metabolite profiles available in the Chenomx NMR suite software database will be used to identify known human metabolites. The metabolite concentration will be normalized to the creatinine resonance in the spectra to control for the patient’s hydration status. Metabolomic patterns will be identified by segmenting the spectra into consecutive non-overlapping regions (bins). This will be achieved by sectioning the data on regular 0.04 ppm width bins using the Chenomx NMR Suite software. Once the metabolite and binning data is collected an unsupervised analysis of the data, using Principal Component Analysis (PCA) will be performed. PCA is an unsupervised method of data analysis that reduces the complexity of the spectral data and represents the data as a plot along X and Y axes. Two clusters on the PCA plot indicate that there are significant metabolite differences between the cases (e.g. septic shock vs. sepsis) and controls (e.g. septic shock vs. stable pneumonia). Following PCA, a supervised analysis using partial least squares discriminant analysis (PLS-DA) will be performed. PLS-DA creates mathematical models using a known set of de-blinded samples. These models are then used to test samples in which the clinical condition remains unknown. Both PCA and PLS-DA will be done using SIMCA software (SIMCA-P 13, Sartorius Stedim Biotech, Sweden).

1H-NMR Data and Clinical Data Interpretation: Once statistical analysis has been completed samples will be unblinded and variations will be interpreted by clinically correlating biomarkers with clinical information such as past medical history, medication regimen, physiological data, and overall clinical outcome.

Follow-up: Patient charts will be screened for 28 and 90-day follow-up to monitor their outcomes. Patient or SDM will be contacted via phone to determine outcome for which hospital data is not available.

Sample Size: Currently there is no consensus in metabolomics in regard to the minimal number of samples per patient group for untargeted metabolomic analysis [14]. Current recruitment efforts at the HGH site has resulted in 93 urine samples being collected from patients with either sepsis or septic shock.
Timeline and Impact
Timeline: All samples will be collected over the calendar year January 1st 2017 to January 1st 2018. Analysis of the sample will be run within the next calendar year from January 1st 2018 to January 1st 2019, with manuscript preparation occurring over this time as well.

Relevance and Future Directions:
Given that sepsis and septic shock is a multi-factorial disease with high patient mortality and efforts to predict the disease using clinical criteria, cytokines, and inflammatory mediator biomarkers have not offered a definitive diagnostic; there is an urgent need to explore and develop new methods for diagnosing and prognosticating septic shock. Urinary metabolomics offers a new perspective on biomarker identification compared to standard testing methods with the benefits of being non-invasive, providing rapid test results, and having a low per sample cost. Importantly, urine is the ideal biological fluid for metabolomics studies making it especially appealing for pediatric research. Using urinary metabolomic profiles, our innovative and novel study will attempt to establish the feasibility and identification of early predictors of sepsis and septic shock in ED patients.

Project Budget

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<tr>
<th>Material/Service</th>
<th>Cost per unit (CDN)</th>
<th>Cost of items (CDN)</th>
<th>Cost (CDN)</th>
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<tr>
<td>Chromex Analysis</td>
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<td>Chromex NMR Standard (50mL)</td>
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<tr>
<td>Miscellaneous lab costs</td>
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<td>NMR Tubes + Caps</td>
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The cost for collection, processing, and analysis of 150 urine samples with NMR.