

Diagnostic Potential of Coagulation-Related Biomarkers for Sepsis in the Emergency Department: Protocol for a Pilot Observational Cohort Study

BACKGROUND: Between 75% and 80% of patients with sepsis arrive in the hospital through the emergency department. Early diagnosis is important to alter patient prognosis, but currently, there is no reliable biomarker. The innate immune response links inflammation and coagulation. Several coagulation-related biomarkers are associated with poor prognosis in the ICU. The role of coagulation biomarkers to aid in early sepsis diagnosis has not previously been investigated. The objective of our study is to determine the individual or combined accuracy of coagulation and inflammation biomarkers with standard biochemical tests to diagnose adult septic patients presenting to the emergency department.

METHODS: Sepsis in the Emergency Department is a prospective, observational cohort study with a target enrolment of 250 suspected septic patients from two Canadian emergency departments. The emergency physicians will enroll patients with suspected sepsis. Blood samples will be collected at two time points (initial presentation and 4 hr following). Patients will be adjudicated into septic, infected, or not infected status in accordance with the Sepsis-3 definitions. Patient demographics, cultures, diagnosis, and biomarkers will be reported using descriptive statistics. Optimal cut off values with sensitivity and specificity for each biomarker will be determined using C-statistics to distinguish between septic and nonseptic patients. Stepwise multiple logistic regression analysis with exclusion of nonsignificant covariates from the final model will be used to establish a panel of biomarkers.

CONCLUSIONS: Our protocol describes the processes and methods for a pragmatic observational biomarker study in the emergency department. This study will seek to determine the potential diagnostic importance of early coagulation abnormalities to identify additional tools for sepsis diagnosis.

KEY WORDS: a Disintegrin and Metalloprotease with Thrombospondin type 1 motif, member 13 protein; cell-free deoxyribonucleic acid; Early Warning Scores; neutrophil extracellular traps; protein C; von Willebrand factor

BACKGROUND AND RATIONALE

Sepsis is a life-threatening dysregulated systemic response to infection leading to tissue damage, organ failure, and death (1). Sepsis is a significant contributor to the global health burden with approximately 48.9 million new cases of sepsis reported in 2017 (2, 3). One of every 120 adults visiting an emergency department (ED) in the United States is diagnosed with sepsis every year, with an increasing

Jaskirat Arora, MBBS¹

Jennifer A. Klowak, MD, MSc, FRCPC^{1,2}

Sameer Parpia, MSc, PhD³

Marcelo Zapata-Canivilo, MD^{1,4}

Walaa Faidi, MSc¹

Christopher Skappak, MD, PhD⁵

Rachael Gregoris, BHS¹

Colin A. Kretz, BSc, PhD¹

Dhruva J. Dwivedi, BSc, MSc, PhD¹

Kerstin de Wit, BSc, MSc, MB ChB, MD, FRCPC, FRCM, MRCP^{1,5}

Michelle Welsford, BSc, MD, FACEP, CCPE, FRCPC⁵

Alison Fox-Robichaud, MSc, MD, FRCPC^{1,4}

on behalf of the Canadian Critical Care Translational Biology Group

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trend (4, 5). The average length of hospital stay for sepsis is twice as long as the average stay of any other potentially fatal condition, and the in-hospital mortality remains as high as 20% (2, 6). Early and aggressive treatment of sepsis is essential to reduce mortality (7).

The diagnosis of sepsis is often challenging. Procalcitonin and lactate, the most widely used ED identifiers of sepsis, lack specificity and sensitivity (8). One important mechanism in the complex pathophysiology of sepsis is the simultaneous activation of the inflammation and coagulation pathways, collectively termed immunothrombosis (9). Biomarkers involved in sepsis-associated immunothrombosis have been investigated as prognostic tools (10). Coagulation markers with inflammatory variables could help diagnose septic patients (11, 12). Neutrophil extracellular traps (NETs), cell-free DNA (cfDNA), and histones down-regulate the natural anticoagulants (such as activated protein C [PC]) and augment fibrinolysis (13, 14). Deoxyribonuclease is an important regulator of NETs (15). Concomitantly, the von Willebrand factor (VWF) cleaving protease, a Disintegrin and Metalloprotease with Thrombospondin type 1 motif, member 13 (ADAMTS13), is reduced in sepsis, and levels correlate with poor patient outcome (16, 17). Recent studies have shown coagulation biomarkers levels modulate the early pathophysiologic changes among septic patients in the ED (18). We hypothesize the biomarkers involved in sepsis-associated immunothrombosis would aid in early diagnosis of sepsis.

Many research studies have enrolled patients at a later stage of sepsis, often from the ICU and hospital wards due to the pressurized and often overburdened ED environment. Therefore, emergency research requires unique considerations, protocols, and guidelines for the successful implementation of a research study. Our protocol provides a rationale for exploring the inflammatory and coagulation-related biomarkers in combination with the standard biochemical analysis to diagnose sepsis in the ED.

OBJECTIVES

Primary Objective

To investigate the diagnostic potential of coagulation biomarkers to identify adult patients with sepsis in the ED.

Secondary Objective

To determine if a panel of biomarkers (including coagulation and inflammatory), in conjunction with standard laboratory tests, vital signs, and organ dysfunction, can improve sepsis diagnosis in the ED.

MATERIALS AND METHODS

Study Design

Sepsis in the ED (Sepsis-ED) will be a prospective, observational cohort study (**Fig. 1**).

Study Setting

Study Sites. Patients will be recruited from the EDs of two academic, tertiary care hospitals (Juravinski Hospital and Hamilton General Hospital) in Hamilton, ON, Canada.

Consent and Protocol Compliance. Waiver of informed consent for enrollment of ED patients with suspected sepsis has been approved by the Hamilton Integrated Research Ethics Board (HiREB) (study number 0660). Informed consent will be taken from healthy volunteers before blood sample collection (Research Ethics Board number: 12-712-T).

Participants. Emergency physicians will identify patients with suspected sepsis (**Table 1**). Suspected sepsis patients will be defined as having evidence of infection and end-organ dysfunction or presence of an elevated Hamilton Early Warning Score (21, 22).

Patient Enrollment and Sample Collection. Patients will be enrolled when the emergency physician signs a Sepsis-ED order set. The order set will include prompts for standard ED testing and treatment, along with instructions to draw two blood samples (one citrate and one heparin tube) for research analysis at presentation (preantibiotic) and 4 hours later. All ED clinical personnel will be educated on the order set in-person with follow-up e-mails. Posters with study eligibility will be posted on the walls of the ED. The samples will be labeled and transferred to the core clinical chemistry laboratory for centrifugation and freezing of the plasma as per the standard operating procedure. The collected patient samples will be deidentified in the research laboratory and relabeled with new study identifiers. A urine sample will be collected in sodium azide containers for future metabolomics analyses.

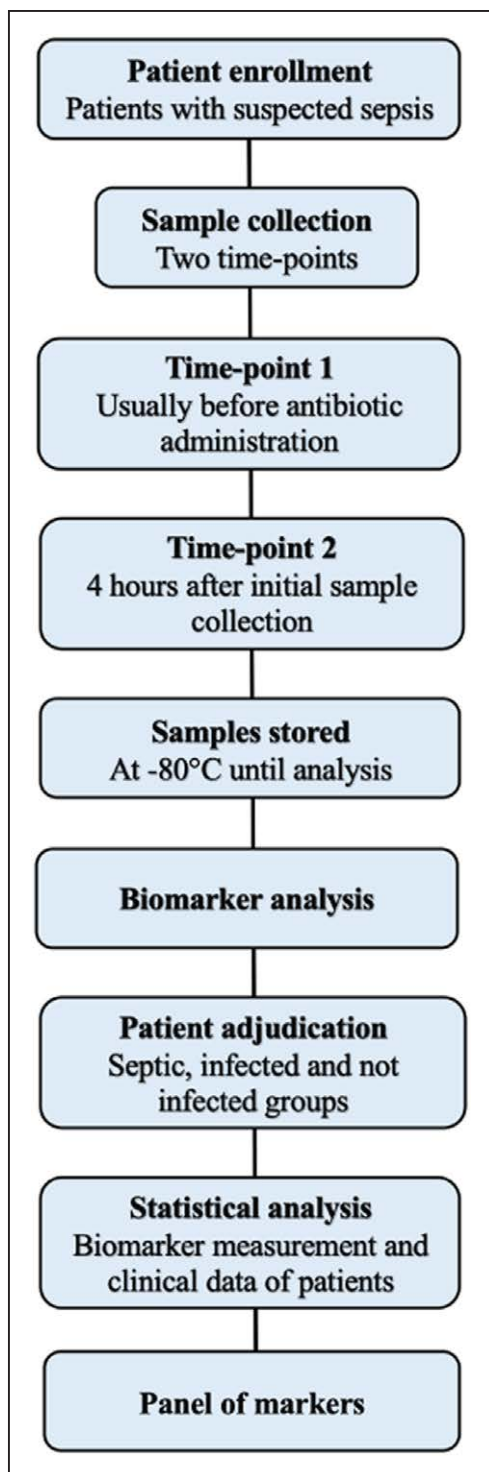


Figure 1. Sepsis in the Emergency Department (Sepsis-ED) study design. Blood samples will be collected from suspected sepsis patients at a two time point and stored at -80°C until biomarker analysis. The patients will be adjudicated into septic, infected, and not infected by two physicians independently. The gathered data will be analyzed to identify a panel of markers, including abnormal routine clinical variables of patients and biomarker measurements, to diagnose Sepsis-ED.

Sample Size. A total of 250 patients with both time point research blood samples will be enrolled in the inception cohort. The study size is based on a pragmatic, convenience sampling method (19, 20). We will retain the single time point patient samples to be used as a validation cohort.

Source and Method of Patient Data Collection. Clinical data will be collected from the patient's electronic medical records (EMRs) using case report forms, including patient demographics, symptoms, vital signs, laboratory results, microbiology results, treatment, and in-hospital mortality at 28 days by trained data abstractors.

Adjudication Process. Two physicians will independently (blind to the research blood test results) review the patients data collected within an 8-hour window of ED admission to classify patients as septic, infected, or not infected using Sepsis-3 criteria (1, 21) (Fig. 2). A third adjudicator will review any discrepancies. Agreement between data abstractors will be assessed with a kappa value.

Healthy Controls. We will collect plasma samples from a group of healthy volunteers to set normative values for some of our exploratory biomarkers. The healthy volunteers will be recruited via mass e-mail at the research institute. The collection and storage methods will be the same as described for study samples. The volunteers will not be taking prescribed medications, which could influence the concentrations of the studied biomarkers at the time of sample collection. Cases and controls will not be matched. These samples will act as healthy controls for biomarker analysis and interpretation.

Laboratory Protocols. We will conduct an exploratory analysis to understand the change in the coagulation biomarkers related to sepsis's inflammatory biology. The interim results of laboratory analysis would determine the inclusion or exclusion of more biomarkers relevant to confirm our findings. The number of biomarkers measured will depend on sample availability and available funding. We will include the following biomarkers: cfDNA, deoxyribonuclease I levels, PC, citrullinated Histone H3 (citH3), procalcitonin, VWF, and ADAMTS13 for analysis. CfDNA levels will be extracted from plasma using the QiAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) by the spin protocol as per the manufacturer's instructions. Enzyme-linked immunosorbent assays will be

TABLE 1.
Eligibility Criteria of Sepsis in the Emergency Department Study

Inclusion criteria	
1) Adult patient (18 yr or older) presenting to the emergency department with suspected sepsis. (Suspected sepsis patients will be defined as having evidence of infection and end-organ dysfunction or presence of an elevated Hamilton early warning score [19, 20].)	
2) The patient meets criteria for suspected sepsis A) or suspected septic shock B) as below:	
A) Suspected sepsis (need 2 of 3)	B) Suspected septic shock (need 2 of 2)
1) 2/3 quick Sequential Organ Failure Assessment requirements (1).	1) Persistent hypotension requiring vasopressors to maintain mean arterial pressure \geq 65 mm Hg and
2) Hamilton Early Warning Score \geq 5 (20).	2) Serum lactate level \geq 2 mmol/L.
3) Physician-suspected sepsis based on organ dysfunction.	
Exclusion criteria	
1) Admission for palliative care or	
2) Patient transferred from another hospital to the emergency department.	

used to quantify deoxyribonuclease I, procalcitonin, citH3, PC, and ADAMTS13 levels. Additional biomarkers may be added based on new evidence.

Analysis. Patient demographics, clinical and laboratory data, and biomarker levels will be the study variables. The expected outcomes are summarized in **Table 2**. Quantitative data will be described using mean and SD or median and interquartile range as appropriate. Categorical data will be reported as absolute numbers and percentages. Wilcoxon signed-rank test will compare the distribution of the biomarker values in the same patients at two time points. Mann-Whitney *U* test will be used to compare the difference in the distributions of demographic and biomarker values between septic and nonseptic samples, and the Kruskal-Wallis H test to determine the mean difference between septic, nonseptic, and healthy controls. The impact of different combinations of biomarkers to diagnose sepsis will be evaluated using a multivariable logistic regression model with adjusting of patient demographic data (such as age and sex). The performance of individual and combined biomarker panel in identifying sepsis will be assessed as an area under a receiver operating characteristic (ROC) curve (23). The larger area under the ROC curves (AUCs) will indicate better discrimination between sepsis and nonsepsis. For each

biomarker, optimal cut off values will be defined at the point of the curve, to maximize the Youden index (defined as: sensitivity + specificity–1) (24). AUCs will be expressed with their 95% CIs.

Potential Confounders and Effect Modifiers. Some effect modifiers may modulate the levels of the included biomarkers and will be considered in the analysis. For instance, due to the procalcitonin's thyroid origin, the levels of procalcitonin may vary based on the predisposing thyroid condition of the patient. Similarly, other predisposing factors, such as underlying conditions, may impact the analysis and will be considered on an individual patient basis.

Enrollment is dependent on the identification of eligible patients by clinical personnel; therefore, spectrum bias is possible. For example, the clinical team may enroll less critically ill patients due to acuity or conversely less mildly sick patients due to a lack of recognition of sepsis. All patients will be reviewed for the Sepsis-3 criteria and severity of illness. We will minimize measurement bias through blinding data abstractors and personnel performing laboratory analysis. Even though we aim to evaluate patients early in the disease process by using the ED, the exact onset of sepsis cannot be accurately determined due to the heterogeneity of the disease process.

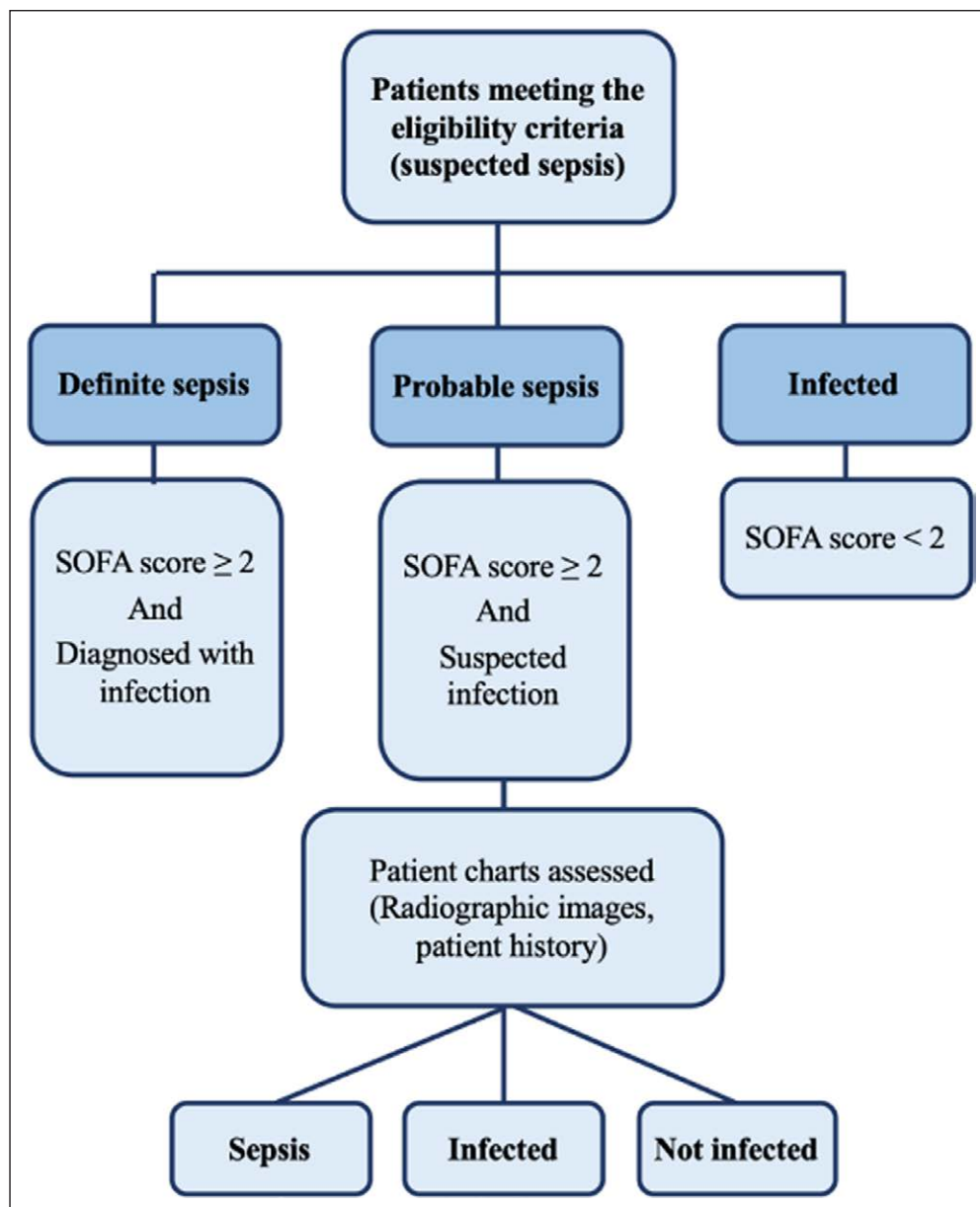


Figure 2. Adjudication plan of Sepsis in the Emergency Department study. Two physicians will independently assess patient charts. Patients with a Sequential Organ Failure Assessment (SOFA) score of two or more with a definite infection will be adjudicated as “septic.” A patient with a SOFA score of less than 2 will be described “infected.” If the SOFA score is equal to or more than 2 and without definite infection, the patient charts would be assessed for other signs of infection such as radiographic findings. In the absence of clear signs of infection, the patient will be adjudicated as “not infected.”

DISCUSSION AND CONCLUSIONS

Early identification of sepsis is essential to initiate timely intervention and reduce the economic and patient important outcome burden. A scan of the current literature only found 11 studies exploring potential diagnostic biomarkers among septic patients admitted to the ED; none examined coagulation markers (25–35). This protocol article describes a pragmatic recruitment approach

to recruit patients as we investigate the diagnostic potential panel of biomarkers from septic patients in the ED. We will analyze different biomarkers known to be important in sepsis pathogenesis and shown to be important in prognosis (such as cfDNA, deoxyribonuclease I levels, PC, citH3, procalcitonin, VWF, and ADAMTS13), to identify a panel that can be used to diagnose sepsis in the ED. This study will include an inception cohort that would use patient samples at two time points. Results of the inception cohort patients will be confirmed in the validation cohort by using single time point samples. A large multicenter study will be planned to verify the results.

However, this study will have several limitations. Our results will require a larger scale multicenter study to determine the clinical utility of the biomarkers. This study is limited by moderate study size and the presence of only two study sites, which may result in biases in the study patient population. There may also be a selection bias of the ED staff recruiting patients.

Our adjudication of patients will be limited by the data available through the EMRs. Unmeasured confounders reported in the EMRs can over- or underrepresent the health of patients. We will use two time points to diagnose sepsis, which is not always practically possible in an emergency setting. Verification of our results using the additional pool of single time point samples will be important. Although the patient samples will be collected early in the disease process, the precise onset of sepsis remains unknown.

TABLE 2.
The Objectives, Outcome, and Method of Analysis of Sepsis in the Emergency Department Study

Objectives	Outcomes	Method of Analysis
1) Primary objective Determine the potential utility of novel blood biomarkers for diagnosing sepsis in the ED.	1) The levels of biomarkers in adult septic patients presenting to the ED. 2) The difference in the levels of biomarkers between septic and nonseptic patients.	Laboratory analysis. Mann-Whitney <i>U</i> test.
2) Secondary objective Determine a panel of biomarkers (including coagulation and inflammatory), with standard biochemical tests and vital signs and organ dysfunction.	Diagnostic potential of a panel of biomarkers to diagnose sepsis in the ED.	Receiver operating characteristic curve and multivariable logistic regression.

ED = emergency department.

ETHICS AND DISSEMINATION

Patient Confidentiality

This study has been approved for a waiver of informed consent by the HiREB. All patient information will be stored electronically within the research institution's network with firewalls and additional security measures. Only investigators of the study will have access to the patient data for adjudication purposes. The physical case report forms will be stored in a locked cabinet in a secure office area.

Dissemination

The results of the study will be shared with the Canadian Critical Care Translational Biology Group. We will seek peer-reviewed publications and presentations at scientific national and international conferences and meetings.

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2 Department of Pediatrics, McMaster Children's Hospital, Hamilton, ON, Canada.

3 Department of Health Research Methods Evidence and Impact, McMaster University, Hamilton, ON, Canada.

4 Department of Medicine, Division of Critical Care, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada.

5 Department of Medicine, Division Emergency Medicine, Faculty of Health Sciences, McMaster University, Hamilton, ON, Canada.

This work was performed at 1) Hamilton General Hospital, Hamilton, Ontario, Canada; 2) Juravinski Hospital, Hamilton, Ontario, Canada; and 3) Thrombosis and Atherosclerosis Research Institute, Hamilton, Ontario, Canada.

Dr. Arora conducts the emergency department (ED) staff education and orientation, sample transfer, entry, and labeling; she drafted the article, which was subsequently reviewed and edited and approved by all authors for submission. Drs. Arora, Klowak, and Zapata-Canivilo prepared the study case report forms and adjudication process. Dr. Skappak enrolls eligible patients to the study. Drs. de Wit and Welsford contributed to the study design and patient enrollment in the ED. Drs. Arora, Kretz and Dwivedi, and Ms. Faidi and Gregoris are involved in the laboratory biomarker analysis plan. Dr. Parpia contributed to the statistical analysis plan. Dr. Fox-Robichaud created the study concept and design and supervises the conduct of the study.

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For information regarding this article, E-mail: afoxrob@mcmaster.ca

1 Thrombosis & Atherosclerosis Research Institute (TaARI), Department of Medicine, McMaster University, Hamilton, ON, Canada.

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