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Impact of Venous CONgestion on Organ Function and Outcomes in Sepsis (ICON-Sepsis): a prospective observational cohort study protocol

Robert R. Ehrman ,[1] Robert L. Sherwin,[1] Christian A. Reynolds,[1] Steven J. Korzeniewski,[1] Robert D. Welch ,[1] Jeffrey A. Kline ,[1] Hao Ying,[2] Phillip D. Levy[1]

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[1]Department of Emergency Medicine, Wayne State University School of Medicine, Detroit, Michigan, USA
[2]Electrical and Computer Engineering, Wayne State University, Detroit, Michigan, USA

Correspondence to
Dr Robert R. Ehrman;
rehrman@med.wayne.edu

ABSTRACT

Introduction Sepsis is a common condition with significant morbidity, mortality and annual costs of care in the billions of dollars. Despite innumerable studies on the causes of, and therapies for, sepsis, the mortality rate has not changed substantially in the last 20 years. Treatments remain generic, with current guidelines recommending the same approach for all patients, regardless of the litany of differences that exist at baseline. Moreover, the blanket administration of 30 cc/kg of intravenous fluid (IVF) to all patients is recognised as being directly harmful to some. Patient-level heterogeneity in prior sepsis trials is recognised as a substantial contributor to all these problems, yet no prior investigation has attempted to identify volume-informed septic phenotypes, a necessary first step towards precision care.

Methods and analysis Predicated on prior studies demonstrating detectability of organ-level congestion, we hypothesise that central venous hypertension (1) is deleterious to the function of the lungs, liver, kidneys and vascular endothelium; (2) is worsened by cardiac dysfunction and IVF administration; and (3) contributes to adverse organ-specific and overall outcomes. Beginning in the emergency department, cardiac function will be assessed with echocardiography while congestion in the lungs and kidneys will be assessed using previously validated sonographic markers of congestion. Biomarkers for each organ will be collected concurrently, thereby increasing the fidelity of our phenotypic profiles by pairing indicators of macroscopic and microscopic stress and dysfunction. Data will also be collected at 24 hours and 7 days (or discharge, whichever comes first) after presentation. Classical and machine learning approaches will be used to analyse our large data stream and develop a rule-based system to identify distinct subpopulations of patients with sepsis who have greater risk/likelihood of both organ-specific and overall adverse outcomes.

Ethics and dissemination This project has been approved by the Wayne State University Institutional Review Board, with patient enrolment beginning in April 2024. Findings will be reported and disseminated via conference presentations and open-access publications.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ Prospective collection of concurrent sonographic and biomarker indicators of congestion across multiple organs.
⇒ Initial data captured at the time of emergency department presentation minimises confounding by treatments/interventions.
⇒ Longitudinal data collection allows description of trajectories of congestion with associated clinical outcomes.
⇒ Confounding by patient-level and disease-level factors will likely remain, given overall small sample size.
⇒ Single-centre study in an urban, largely African American population may limit generalisability and transportability.

INTRODUCTION

Our long-term goal is the provision of transformative sepsis care by phenotype subclassification to optimise intravenous fluid (IVF) volume. As a starting point, we propose a line of investigation for sepsis care that leverages information from biophysical and biochemical markers of pulmonary, renal, cardiac and endothelial function to identify the subset of patients who have heightened risk for organ congestion from IVF-induced central venous hypertension (CVH). Our robust exposure profiling strategy will not only enable us to identify clinical factors as potential contraindications to IVF, but we will also carefully probe positive and negative social determinants of health (SDoH)[1] as potential modifiers or mediators alongside traditional patient-level demographics (eg, sex as a biological factor), anthropometrics (eg, body mass index), behavioural factors (eg, smoking) and medical histories.

The proposed data-driven approach to IVF loading will maximise benefit while

minimising harm by addressing the clinical heterogeneity that has plagued the quest for novel interventions to reliably improve sepsis outcomes.[2 3] Our central hypothesis is that early CVH from resuscitation is (1) deleterious to the function of the lungs, kidneys and vascular endothelium; (2) worsened by cardiac dysfunction and IVF administration; (3) adds to adverse organ-specific and overall outcomes and (4) social/environmental stressors moderate these relationships. This project will identify sepsis phenotypes that have increased risk for adverse outcomes, including those precipitated or worsened by IVF administration (eg, death, acute respiratory distress syndrome (ARDS), need for renal replacement therapy (RRT)).

Sepsis is a common, costly, but incompletely characterised syndrome

In 2001, sepsis-related mortality was as high as 46%[4]; while this has dropped substantially, it has remained static at 23% for over 10 years.[5]African Americans are more often affected and have worse outcomes than whites.[6 7] Basic interventions introduced 20 years ago are still the primary treatments: IVF, antibiotics and vasopressors. Importantly, the most fundamental therapy—IVF—is administered generically (30 cc/kg[8]), without regard for potential benefit or risk of fluid overload, which carries strong associations with worse outcomes, including death.[9–12] This approach is based on consensus guidelines[8] due to the profound absence of reliable, informative data to support an individualised approach.

IVF in sepsis and its sequelae

Despite known harms (increased ventilator days, hospital stay and mortality[9 11 13 14]), fluid overload is common.[14 15] Yet, these harms are difficult to avoid as putative risk factors (left ventricular dysfunction, end-stage renal disease) are not reliable predictors.[16–19] Indeed, the goal of volume expansion is amelioration of the effects of sepsis-induced hypoperfusion, reflected by serum lactate. Greater elevation is associated with increased mortality,[20–22] and while existing care bundles improve lactate clearance and reduce mortality for some,[23 24] achieving it can result in fluid overload. To improve sepsis-related outcomes, a shift to an individualised, rather than a 'one-size-fits-all' approach, is needed. However, lack of knowledge about patient-level differences in physiological response to volume loading—heterogeneity of treatment effect—and multiple confounding influences in patients with sepsis[25 26] have thus far precluded individualised resuscitation. Moreover, scant data exist on the effect of environmental factors on disease progression, particularly in urban areas, thereby limiting health equity research capacity.[27–29]

Limitations of Sequential Organ Failure Assessment

The Sequential Organ Failure Assessment[30] (SOFA) score demonstrates the additive impact of individual organ dysfunction, but ignores complex causal interdependencies among component factors. While biochemical crosstalk is well-known, only individual—not interactive—congestive dysfunction has been explored in sepsis,[31 32] and never in a longitudinal fashion. The central venous compartment and vascular endothelium link the heart, lungs and kidneys such that congestion of one organ may be transmitted to, and affect the function of, another. This protocol is informed by prior studies that found deleterious effects of shared congestive dysfunction in unselected patients in the intensive care unit (ICU).[33 34] We narrow the focus to septic patients and expand on the idea by positing that sonographic markers of organ dysfunction have concurrent biomolecular signatures that are associated with clinical outcomes. Biomarker profiling is recognised as an avenue by which sepsis care will be improved in the future via identification of unique subgroups that have different treatment needs and different outcomes.[35–38]

CVH and indicators of pulmonary, renal, cardiac and endothelial dysfunction

Central venous pressure alters perfusion gradients across organs, and CVH can cause organ congestion, the effects of which are recognised in sepsis and other conditions.[39–42] Despite extensive study of varied approaches (done mostly in ICUs, after initial resuscitation[43–46]), neither a definition of optimal resuscitative volume, nor clearly delineated sepsis phenotypes to guide individualised therapy have been established. The clinical progression of sepsis is typically characterised by multiple indicators of organ dysfunction that are both common and associated with worse outcomes:

- *Septic cardiomyopathy* (SC) detected by echocardiography occurs in up to 44% of patients[47 48] with increased mortality when present[49–52] ; biochemical derangements (troponin elevation) are ubiquitous and also associated with greater mortality.[53]
- *Sepsis-associated acute kidney injury* (sAKI)[54] occurs in up to 60% of patients. It portends a worse prognosis,[55] with mortality up to 46%,[56] and 60% when RRT is needed.[57] Many biomarkers of sAKI have been investigated, but no single reliable candidate has been identified.[58]
- *Sepsis-associated lung injury* occurs frequently (>190 000 annual cases in the USA), with substantial mortality.[59 60] Numerous lung injury phenotypes exist but remain mechanistically unexplained, leading to a call for increased endotyping studies in this area.[61]
- *Sepsis-associated endothelial dysfunction* (sED) contributes to organ failure and adverse outcomes.[62 63] IVF administration is postulated to exacerbate sED,[64 65] but incomplete understanding of the varied mechanisms (glycocalyx degradation, mechanosensory disruption from altered microcirculatory flow[65–68]) precludes identification of those at the greatest risk of additional harm.

Table 1 Variables collected and outcomes assessed

System assessed	Variables measured	Traditional lab values	Novel biomarkers	Outcomes
Organism	► Demographics, vital signs, medical history		► Tumour necrosis factor-α, interferon-γ	► SOFA score, fluid balance, mortality, LOS (hospital/ICU)
Heart	► LV: EF, E, e', LS, E/e', 3D volume ► RV: free-wall LS, 3D volume, RVSP	► Troponin, B-type natriuretic peptide	► Bioactive lipids from eicosanoid lipidome (150 species)	
Lung	► B-lines/EVLW (quantified pulmonary oedema)		► Bioactive lipids from eicosanoid lipidome (150 species)	► Ventilator days, ARDS, P/F ratio, oxygen needs
Kidney	► Lobar renal vein Doppler	► Creatinine, GFR	► Urinary NGAL	► RRT days, progression to CKD and ESRD
Endothelium and glycocalyx			► PECAM, ICAM, VCAM, E-selectin, hyaluronan, heparan sulfate, syndecan-1	

ARDS, acute respiratory distress syndrome; CKD, Chronic kidney disease; e', mitral annular velocity; E, trans-mitral flow velocity; EF, ejection fraction; ESRD, end-stage renal disease; EVLW, extravascular lung water; GFR, glomerular filtration rate; ICU, intensive care unit; I/V/PECAM, intercellular/vascular/platelet-endothelial cell adhesion molecule; LOS, length of stay; LS, longitudinal strain; LV/RV, left/right ventricle; NGAL, Neutrophil Gelatinase-associated Lipocalin; P/F ratio, PaO_2/FiO_2; RRT, renal replacement therapy; RVSP, RV systolic pressure; SOFA, Sequential Organ Failure Assessment.

Novelty of our approach

The novelty of our approach is (1) capturing patients in the emergency department (ED) in order to minimise the potential confounding effects of initial resuscitation; (2) assessing patients throughout hospitalisation; (3) pairing direct measures of organ congestion and CVH with a diverse panel of concomitantly obtained organ-specific biomarker data, thus informing the molecular consequences and drivers of congestion; and (4) incorporating a robust suite of SDoH data into multilevel models that are developed to discriminate high-risk from low-risk patients. Detroit is home to a large population who identify as racial and ethnic minorities and those with extreme social vulnerabilities and adverse clinical outcomes, resulting in a distinctive confluence of biological and social determinants.[1 69 70] We are therefore uniquely positioned to explore interactions of these factors and how they relate to heterogeneity in the outcomes of sepsis and its sub-phenotypes.

METHODS AND ANALYSIS

Protocol summary

The initial goal of the project is the creation of a repository of clinical and social data that will allow characterisation of outcome-based (table 1) septic phenotypes at the individual organ, and organismal, level. Macroscopic congestion will be measured with ultrasound: pulmonary oedema, by quantification of extravascular lung water (EVLW); capacitance and pressure of the central venous compartment, measured by size and collapsibility of the inferior vena cava (IVC); renal congestion, measured by pulsed-wave Doppler (PWD) of intralobar renal veins; and cardiac function, measured by transthoracic echocardiography. Novel and traditional biomarkers (table 1) of organ and endothelial function will be measured on concurrently obtained serum/urine samples in order to link macroscopic and microscopic (dys)function. To account for disease progression and the effects of treatments, assessments will be performed at presentation to the ED and repeated at 24 hours and 7 days (or at discharge if <7 days). All US examinations will be performed by a qualified research sonographer, per organ-specific society guidelines.

Target organ considerations and research questions

Endothelium and glycocalyx

Endothelial cell (EC) activation in sepsis includes expression of chemokines and adhesion molecules.[62 71] Deactivation occurs via reduced expression or shedding into the circulation, which lessens leucocyte attraction/adhesion and other cell–cell interactions.[72 73] Excess EC activation[63] and IVF-induced glycocalyx disruption occur,[65 73 74] but causal links are poorly understood. A potential mechanistic explanation lies in the fact that ECs are flow-adapted: regular exposure to laminar shear stress promotes vascular health,[75 76] and reduced or turbulent flow activates ECs.[77] While initially beneficial (eg, ischaemia-induced neovascularisation[78]), prolonged activation is detrimental: deranged morphology, permeability and function–sepsis-associated endothelial dysfunction.[79 80] Our project will investigate a mechanosensory link between CVH, IVF and sED by measuring markers of cellular adhesion/permeability and glycocalyx disruption. We will thus focus on the following key question:

► Is there a dose–response relationship between CVH, IVF and markers of EC/glycocalyx dysfunction?

Cardiac

While described nearly 40 years ago,[81 82] the pathophysiology and prognostic implications of cardiac dysfunction in sepsis remain unclear. Longitudinal assessment, begun at presentation, will capture disease progression and the effects of IVF and other treatments. While the causative agents of SC have not been identified,[83 84] bioactive lipids, which freely cross cell membranes, are promising contributors[85–88]; time-varying expression patterns have been reported in sepsis survivors versus non-survivors.[89 90] To explore the link between lipids and development of SC, we will perform longitudinal, untargeted analysis of the lipidome (fatty acyls, sterols, prenols, sphigolipids, saccharolipids and glycerolipids). Differential expression patterns could be useful for diagnostic panels or as the first step in development of targeted interventions.

Functional assessment will include standard 2D measures, plus 3D chamber volumes, which can better detect subtle changes in response to resuscitative interventions.[91] Where available, gender-specific normal function parameters[92] will be used for analysis. Capacitance/congestion of the central venous compartment will be assessed by size and collapsibility of the IVC.[33 93] A full list of variables to be collected is shown in table 1.

This portion of the programme will address knowledge gaps through focus on the following *key questions*:

- Does cardiac function at presentation impact disease progression and in-hospital outcomes?
- How does cardiac function change in response to treatment and does this affect outcomes?
- Do subtypes of cardiac dysfunction have unique bioactive lipid profiles?

Lung

Pulmonary dysfunction in sepsis is common, multifactorial and associated with worse outcomes.[60 94] Infection leads to capillary leak, interstitial oedema, impaired compliance and, in severe cases, to ARDS. IVFs worsen pulmonary oedema, particularly in those with left heart dysfunction,[45] yet a quantified relationship is elusive. Minimising iatrogenic injury is critical as the only treatment is supportive (lung protective ventilation), with ARDS mortality up to 46%.[60 95 96] As with SC, differential patterns of lipid expression identify varying severity of lung disease, including ARDS.[97–99] Respiratory–renal interactions occur but remain unexplored: direct haemodynamic effects (CVH from fluid retention or right-heart dysfunction), changes in acid–base status and neurohormonal effects.[100]

Pulmonary dysfunction will be measured with US by quantifying EVLW as B-lines,[101–103] a lung-specific marker of fluid overload, but in a pattern distinct from infection.[104 105]

This portion of the programme will address knowledge gaps through focus on the following *key questions*:

- Does a dose–response relationship exist between IVF administration, cardiac function and development of pulmonary oedema, regardless of age/gender/comorbidities/source of infection?
- Does CVH-induced renal congestion affect the development or worsening of pulmonary oedema?
- Do pulmonary biomarker profiles change prior to the onset of pulmonary oedema/clinical deterioration?

Renal

Sepsis is the most common cause of AKI (increased creatinine, decreased glomerular filtration rate/urine output[54]) in critical illness.[106] sAKI is associated with increased costs, length of stay and mortality.[107–109] Treatment is supportive, and some patients develop end-stage renal disease, further increasing morbidity and cost.[110 111] sAKI is initiated by microvascular dysfunction (thrombus, disrupted cell membranes[112]) rather than reduced blood flow,[113 114] but hypoperfusion worsens kidney function due to reduced cardiac output and CVH.[42 115 116] IVF administration can elevate central venous pressure and further diminish perfusion, while positive cumulative fluid balance is associated with worsened AKI and mortality.[9 12] Markers of renal stress and injury (eg, neutrophil gelatinase-associated lipocalin)[117] have been identified, but precise mechanisms are not known. Congestion will be measured by evaluating flow in the interlobar renal veins with PWD.[118]

This portion of the programme will address knowledge gaps through focus on the following *key questions*:

- How does renal congestion impact in-hospital kidney-related and overall outcomes?
- Is there a link between renal congestion, fluid administration/fluid balance and cardiac function?
- Does congestion move proximal to distal (heart→lungs→kidneys)?
- Does renal congestion correlate with traditional or novel markers of kidney function?

Social determinants of health

We will leverage PHOENIX—Wayne State's Population Health OutcomEs aNd Information eXchange programme[119]—to ascertain information about patient exposures to SDoH. PHOENIX is specifically designed to remove bottlenecks in the data-to-action pipeline. The programme maintains an SDoH data mart in its virtual data warehouse to de-identify, geocode, aggregate and integrate disparate data sources for the purposes of multilevel data analysis. We will use PHOENIX to geocode home addresses to identify residential census tracts which will be used to merge social vulnerability (eg, poverty and inadequate healthcare access or housing) data and related factors (eg, systemic racism, characteristics of the natural and built environments).

Inclusion criteria

- Patients≥18 years of age who have the ability of patient/legally authorised representativeto provide written informed consent.

- Are being treated for suspected infection *plus* systolic blood pressure≤100 mm Hg or lactic acid>2 mmol/L from suspected infection.

Exclusion criteria

- Incarceration (owing to vulnerable population status).
- History of cardiac transplant or mechanical circulatory device.
- Plans for transfer to another institution.
- Pregnancy (owing to altered physiology and fluid balance compared with non-pregnant state).

Ultrasound protocol

Bedside ultrasound (BU1) will occur as soon as possible after patient presentation to the ED. While every attempt will be made to perform BU2 at 24 hours from BU1, and BU3 at the same time of day as BU1, some patients may be getting other diagnostic tests, having a procedure or otherwise be unavailable for study exams at the correct chronologic time. As such, follow-up exams (BU2 and BU3) can be completed in a ±4-hour window.

All echocardiograms will begin by entering the subject's study identification number into the ultrasound system. Clip length will be set to 5 s in order to capture a minimum of three cardiac cycles. A parasternal window will be used to capture images of the left ventricle in the long axis; clips from the basal, mid-chamber and apical levels of the heart in the short axis will also be recorded. The transducer will then be moved to the apical position with patient repositioning as necessary (left lateral decubitus, arm overhead) to obtain an optimal apical four-chamber (A4C) image; a 5 s clip will be recorded. From the A4C view, PWD, with the sample volume positioned at the tips of the mitral valve leaflets, will be used to capture and record trans-mitral flow velocity. Finally, tissue Doppler imaging will be used to record septal and lateral mitral annular velocities during diastole. We will also store 5 s clips from the apical three-chamber and two-chamber view for global longitudinal strain analysis. From the apical windows, clips of sufficient length to generate 3D images will also be collected. The IVC will be imaged from the subcostal window, with an M-mode clip stored to capture respirophasic variation. This imaging sequence will be repeated for each study BU.

- After completion of the cardiac portion of the exam, lung ultrasound will be performed to quantify EVLW. A total of four zones in each hemi-thorax will be examined (anterior–superior, anterior–inferior, posterior–superior and posterior–inferior) for a total of eight zones per exam. To quantify the number of B-lines visualised, the intercostal space with the greatest number of B-lines within each zone will be used for scoring. Each zone will be given a B-line score of 0–20 based on the maximum number of B-lines counted during one respiratory cycle. Discrete or narrow B-lines will be counted individually. For B-lines that were wide or fused together, the score will be determined by multiplying the percentage of the intercostal space filled with confluent B-lines by 20.
- Finally, renal ultrasound will be performed. Renal vasculature will be visualised with the probe in the posterior axillary line, with the Doppler gate placed to detect the flow of the interlobar or arcuate renal veins in the renal cortex, outside the hilum of the kidney. A normal renal Doppler pattern shows arterial pulsations generating regular retrograde peaks, and renal veins generating a continuous, smooth anterograde flow. As venous congestion increases, venous pulsations become visible, creating anterograde pulsations observable during systole and diastole, and eventually, only diastole. A smooth venous baseline is considered normal. Biphasic anterograde pulsations reflecting systole and diastole are considered mildly abnormal, and monophasic pulsation, corresponding only with diastole, is considered severely abnormal.

At each time point, CVH is defined as *absent* (IVC<2 cm), *mild* (IVC≥2 cm), *moderate* (IVC≥2 cm plus mild PWD profile) and *severe* (IVC≥2 cm plus severe PWD profile). Blinded grading by two reviewers will occur offline, with a third adjudicating any disagreements.

While treating clinicians will be blinded to study ultrasound findings, it is possible that clinician knowledge of study performance could affect their treatment decisions, including IVF administration. To assess for such an effect, we will compare median volume administration from study participants to historical institutional data for patients with sepsis.

Biospecimen handling and analysis

Samples for biomarker analysis will be drawn at the time of each US examination. Blood will be collected in lithium heparin tubes (three tubes of 5 mL each, totaling 15 mL per draw) and remain upright for 1 hour to clot. Once clotted, they will be centrifuged at 4000 RPMs for 7 min to separate serum, which is then aliquoted into cryovials in 0.5 mL increments and stored at −80°C. All the proposed biomarker assays can be done using even the most restrictive IRB limits on research blood draws (50 mL per 7 days). When possible, urine samples will be collected along with blood samples. Urine will be collected as a 'clean catch', a bedpan, a urinal or from a Foley catheter bag (when the patient has one). We will attempt to collect urine for as many patients as possible, but provision of urine at each time point is not a requirement for study participation. Biomarker analyses will be conducted in our CLIA (Clinical Laboratory Improvement Amendments)-certified research laboratory.

Lipidomic analysis will be completed using tandem liquid chromatography-coupled mass spectrometry analysis of the fatty acyl lipidome, performed using a QTrap6500 mass spectrometer (Sciex, Singapore) at the Lipidomic Core Facility at Wayne State University. We will perform multireaction monitoring (MRM) to detect unique molecular ion–daughter ion combinations. The data are collected using Analyst software

(Sciex), and the MRM transitions and chromatograms are quantitated using MultiQuant software (Sciex). The internal standard signals in each chromatogram are used for normalisation of overall recovery as well as relative quantitation of each fatty acyl lipid. The quantified lipids are positively identified by comparing HPLC (high performance liquid chromatography) retention times with authentic standards (Cayman Chemicals, Ann Arbor, Michigan, USA) and specific parent–daughter ion combinations as well as MS/MS mass spectra obtained from information-dependent acquisitions.

Sample size calculation

For the primary outcome of 28-day mortality modelled with logistic regression, 268 patients detect an OR increase of 1.6 per one category increase in 24-hour CVH profile (primary predictor) at 80% power and α=0.05. Covariates are 24-hour SOFA score, age and 24-hour IVF ≥ vs <4.2 L. Power calculation is informed by institutional data, literature review and our preliminary data: 28-day mortality 20%, median 24-hour SOFA score 3.8 (IQR 4), mean age 61 years (SD 16). Dichotomisation of IVF addresses non-linearity between volume and mortality, and 4.2 L is the ideal cut-point for alive/dead in our data, consistent with prior literature.[120] For 28-day mortality, estimated adjusted OR per one-point SOFA increase is 1.2,[121] 1.1 per 5-year age increase and 1.5 for 24-hour IVF>4.2 L. To prevent undersampling, we estimated the correlation between covariates and CVH class at 0.4. We anticipate the recruitment of five patients/month to achieve the target sample size over approximately 5 years.

Analytic plan for secondary outcomes

There is strong evidence of harm from fluid overload,[9 11 13] but no data to identify those at greatest risk or to guide fluid management decisions at the bedside. Our three-part analytic approach will address these, and other, knowledge gaps by leveraging our longitudinal, multimodal data stream from the patient and organ level.

1. At each time point, we will describe profiles of each biomarker by assessing central tendency, normality and conducting bivariable analyses (ie, pairwise scatter plots, tests for differences in proportions/distributions). Quantile regression models will be used to estimate the expected 2.5th, 5th, 10th, 25th, 50th, 75th, 90th and 97.5th biomarker percentiles. Potential covariates for the models include standard clinical data plus CVH profile and interventions (IVF, vasopressors).
2. For each outcome in table 1, we will use appropriate regression models to estimate the magnitudes of association with biomarker levels at each time point (binomial, logistic, Cox PH, regression). Multivariable model selection will be performed according to time-oriented analysis of risks.[122–124] Specifically, candidate co-variables will be grouped according to known or presumed time order and examined in a succession of time-sequenced clusters (epochs). Factors significantly associated in each epoch will be retained in all subsequent models, regardless of the changes in statistical association for the retained variable(s). This strategy ensures that variables whose influences operate early are not supplanted by those measured later as our goal is the earliest possible prediction of adverse outcomes. Final adjusted models will be used to determine if any individual biomarker contributes additional information about outcome risk beyond standard clinical factors. We will also explore mediation and moderation of observed associations between sepsis and the targeted biomolecules to determine whether the relationship between biomarkers and outcomes is affected by exposure to social vulnerability.[125–130]
3. Single biomarkers often provide insufficient discrimination between cases and non-cases to be clinically useful, and thus multiple biomarkers, combined in an algorithm, are likely advantageous.[131–133] The greatest improvement in predictive performance comes from combining parsimonious sets of markers with negative correlation.[131] For sepsis, it may be that multiple biomolecules are associated with different facets or phases of the disease. We will perform an evaluation of the potential utility of multiple time-varying biomarkers for predicting patient and organ-specific outcomes (table 1). We will use standard Fisher's linear discriminant functions for linear combination of biomarkers to maximise sensitivity.[134] This allows evaluation of the 'value' of adding additional molecules by determining the ΔpartialAUC for a multi-marker versus single-marker algorithm. We will also use automated machine learning (ML) approaches to patient classification. As explicability and clinical utility are important, we will use rule-based models and decision trees to identify distinct subpopulations of patients with sepsis with greater risk/likelihood of outcomes. In addition, fuzzy logic may be used to represent and process ambiguous patient data. We anticipate missing data for both sonographic and biomarker measures. To address this, missingness patterns will be analysed and addressed with multiple imputation, where appropriate. Finally, the additive and/or comparative value of standard-of-care clinical laboratory results will also be considered in additional models.

Based on institutional data, with 268 patients, we estimate at least 50 patients per outcome. While the 804 serum samples, plus additional clinical measures, provide adequate data to train our ML algorithm, external validation will be necessary. Within the scope of this project, 268 patients support the discrete (28-day mortality) and experimental goals (1–3, above) of the project.

Patient and public involvement

There was no involvement of patient or public entities in the design of this study protocol.

ETHICS AND DISSEMINATION

This project has been approved by the Wayne State University Institutional Review Board (M1 panel, protocol # IRB-23-03-5647). Enrollment began in April 2024. Findings will be reported and disseminated via conference presentations and open-access publications. All publications will follow the Strengthening the Reporting of Observational Studies in Epidemiology guidelines.[135]

Contributors RE: conceptualisation, writing original draft, editing and revising, analysis plan development and guarantor. RLS: sepsis content expert; conceptualisation, writing original draft, editing and revising. RW: analytic plan development; writing and revising. SJK: analytic plan development and revision with focus on integration of SDoH data, editing and revising protocol drafts. JK: project advising, editing and revising original draft. CR: lipidomic content expert; writing and revising lipid-related portions of protocol. HY: computer scientist and ML expert; advising, design, writing and revising of ML components of original draft. PL: project conceptualisation and advising, writing and revising original draft.

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ORCID iDs
Robert R. Ehrman http://orcid.org/0000-0001-9730-8471
Robert D. Welch http://orcid.org/0000-0003-0358-2811
Jeffrey A. Kline http://orcid.org/0000-0001-7190-3109

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