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A Prospective Cohort Protocol for the Remnant Investigation in Sepsis Study

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BACKGROUND: Sepsis is a common and deadly syndrome, accounting for more than 11 million deaths annually. To mature a deeper understanding of the host and pathogen mechanisms contributing to poor outcomes in sepsis, and thereby possibly inform new therapeutic targets, sophisticated, and expensive biorepositories are typically required. We propose that remnant biospecimens are an alternative for mechanistic sepsis research, although the viability and scientific value of such remnants are unknown.

METHODS AND RESULTS: The Remnant Biospecimen Investigation in Sepsis study is a prospective cohort study of 225 adults (age ≥ 18 yr) presenting to the emergency department with community sepsis, defined as sepsis-3 criteria within 6 hours of arrival. The primary objective was to determine the scientific value of a remnant biospecimen repository in sepsis linked to clinical phenotyping in the electronic health record. We will study candidate multiomic readouts of sepsis biology, governed by a conceptual model, and determine the precision, accuracy, integrity, and comparability of proteins, small molecules, lipids, and pathogen sequencing in remnant biospecimens compared with paired biospecimens obtained according to research protocols. Paired biospecimens will include plasma from sodium–heparin, EDTA, sodium fluoride, and citrate tubes.

CONCLUSIONS: The study has received approval from the University of Pittsburgh Human Research Protection Office (Study 21120013). Recruitment began on October 25, 2022, with planned release of primary results anticipated in 2024. Results will be made available to the public, the funders, critical care societies, laboratory medicine scientists, and other researchers.

KEY WORDS: biospecimen; precision medicine; remnant; sepsis

In 2017, there were 48 million cases of sepsis worldwide (1). The case fatality rate in sepsis is 10–40% (2) and accounts for 230,000 U.S. deaths annually. Among survivors, many suffer disability, and one in three are readmitted in 90 days (3).

The biology of sepsis is complex and not characterized by a single deterministic pathway (4). Thus, a new approach to investigate complex sepsis mechanisms is needed. Mechanistic work in sepsis tends to focus on a single pathway, single "omic" dataset, or explore broad molecular readouts. For example, recent work by the Molecular Diagnosis and Risk Stratification of Sepsis (multiple affinity removal system [MARS]) consortium in The Netherlands revealed distinct sepsis subtypes using whole blood transcriptome analyses (5). These unsupervised data are not linked to detailed clinical phenotypes, treatment responses in electronic health record (EHR), or mapped to other "-omic" analyses. They are a single, unsupervised snapshot in time. Many other examples follow this approach (6, 7). A contrasting approach that targets a single cytokine or receptor pathway has led to many failed clinical trials (e.g., activated protein C) (8, 9), as it ignores the redundancy and complexity

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KEY POINTS

Knowledge Gap: In patients with community sepsis, what is the accuracy, integrity, and comparability of proteins, small molecules, lipids, and pathogen sequencing measured in remnant biospecimens compared to paired biospecimens obtained according to research protocols?

Proposed Study Design: In a prospective cohort study of 225 adults (≥ 18 yr old) presenting to the emergency department with community sepsis-3 within 6 hours of arrival, plasma biospecimens will be obtained in sodium–heparin, EDTA, sodium fluoride, and citrate tubes from two sources in each patient, 1) remnant samples from the clinical laboratory, and 2) coordinator collected samples at the bedside. Analyses will include comparative protein, metabolomic, and lipidomic biomarkers for both sample types.

Study Implications: If comparable in accuracy and integrity, remnant biospecimens may offer a scalable and less costly approach for sepsis mechanistic investigation.

of the immune response (10). A new foundation for biologic inquiry is required to develop precision medicine treatments for sepsis.

Current Knowledge

An ideal repository for mechanistic research in sepsis would 1) be large in size, 2) generalizable in case mix and race/ethnicity, 3) be linked to clinical data in the EHR, 4) sample biospecimens from multiple organs over time, 5) be prepared for integrated, multiomic analyses, and 6) be affordable. There are many challenges on the path to this ideal (**Table 1**). We propose, therefore, to change the paradigm from traditional biospecimen sampling to light-touch, remnant collection for studies of sepsis biology (16).

The first step is to develop a translational laboratory to determine the scientific value of remnant biospecimens for mechanistic research in sepsis, compared to traditional research sampling. Traditional research collects biospecimens prospectively using informed consent, in modest sample sizes, at substantial cost, and at prespecified time points. We have the tools to move beyond this approach and leverage clinical specimen remnants from treatment-relevant time points in

TABLE 1.
Challenges for Ideal Biospecimen Repository in Sepsis

Challenge	Explanation
Pace	Sepsis is a life-threatening emergency (11) and changes occur in hours not days
Permission	The prospective investigation of biospecimens for research requires informed consent and Health Insurance Portability and Accountability Act of 1996 authorization. These steps, required by many regulatory review boards, may reduce generalizability, and many patients may decline to participate
Price	The infrastructure, collection, and storage costs can exceed millions of United States dollars for just one repository before any assay costs
Uncertainty	The classification of patients as septic or not, or one subtype or another is not well-defined (12). Nor does it remain the same over time. Biorepositories must be linked to clinical data robust enough to characterize diagnostic uncertainty and temporal changes (13)
Academic bias	Traditionally, prospective sepsis research is conducted at large, tertiary-care academic centers. The recruited patients and their pathogens may not be generalizable to community-level healthcare facilities or austere environments
Recruitment	Research staff often collect samples during daylight, and may limit collection by a funding-based enrollment cap (14). This introduces bias, noting the diurnal variations in host response (15)
Aging samples	The new investigation of sepsis mechanisms cannot rely on re-analysis of existing samples alone. These repositories may be 5–10 yr old, with uncertain stability and integrity, and changing temporal practices

generalizable cohorts. Potential applications include multiple priorities of the National Advisory General Medical Sciences Working Group on Sepsis (17), such as 1) unpacking the underlying mechanism(s) of clinical sepsis phenotypes, 2) linking sepsis subtypes to EHR-captured treatment response, 3) exploring and integrating multiomic readouts for mechanistic insight, and 4) mapping the trajectories of both pathogen adaptation and host response in sepsis.

Why use remnant samples? Thousands of biospecimens are acquired in hospital clinical laboratories each day. For example, in a 22-hospital integrated health system, hundreds of thousands of biospecimens are processed and assayed per month (16). And then the remaining sample is wasted. After a mandatory hold of 40–48 hours for follow-up clinical questions, this valuable resource is discarded. These specimens have notable features, 1) sampled concurrent with the trajectory of clinical disease, 2) obtained for clinical care and may be studied with fewer burdensome regulatory requirements, 3) a broad case mix of septic patients, at academic and community hospitals, at all times of day or night, and with broad racial/ethnic and gender diversity, and 4) less concern about sample aging and freeze/thaw as with secondary analyses of existing biorepositories.

To date, little work has focused on remnant biospecimens in sepsis or critical illness. Small cohorts use remnant blood for traditional biomarker assays or commercially available pathogen detection kits. They do not assess accuracy or stability compared to research-collected samples. We conducted a pilot study to test the feasibility and cost of a remnant specimen biorepository at a single hospital at UPMC. Steps included: 1) obtain preliminary waiver of informed consent, 2) develop local laboratory protocol to acquire remnants, 3) securely label, aliquot, and freeze biospecimens, 4) use EHR-embedded screen for Sepsis-3 continuously in the emergency department (ED) with staff alerting, and 5) assess remnant sample volume and quality. We enrolled greater than 1,000 patients in this pilot in 21 months and found adequate feasibility in this proof-of-concept and substantial cost reduction compared to our traditional sampling (16). We demonstrated that remnant biospecimens in sepsis could be located, processed, and meet basic quality control requirements. Yet, the integrity and scientific value of these samples compared to traditional biospecimens are unknown.

Research Study Aims

We will conduct a prospective cohort study to demonstrate the feasibility of remnant sample measurement in a large, integrated health system. Next, we will determine the scientific value of a remnant biospecimen repository in sepsis compared to paired specimens obtained using research protocols. Supported by the National Institute of General Medical Sciences-funded laboratories at the University of Pittsburgh and Vanderbilt University, we will measure candidate readouts of sepsis biology, governed by a conceptual model, and determine the precision, accuracy, integrity, and comparability of proteomics, small molecules, lipids, and pathogen sequencing in remnant biospecimens compared to biospecimens obtained according to research protocols (**Fig. 1**). These data will inform best practice for scalable, mechanistic studies in remnant biorepositories.

MATERIALS AND METHODS

The study has received approval from the University of Pittsburgh Human Research Protection Office (Study 21120013, REMISE study: REMnant biospecimen Investigation in Sepsis, July 18, 2022). Procedures will be followed in accordance with the ethical standards of the responsible committee on human experimentation (institutional or regional) and with the Helsinki Declaration of 1975.

This is a single-site, prospective, observational cohort study at UPMC that began enrollment on October 25, 2022. Study patients enter the cohort after they are registered in the ED and are assessed for inclusion/exclusion criteria (**Table 2**). Patients will be flagged initially using an embedded screening tool based upon 2 or more Sequential Organ Failure Assessment score points present within 6 hours of arrival to the ED (18). Automated alerting to research personnel will occur by secure email, after which clinical adjudication for Sepsis-3 criteria will take place by the research team. Sepsis-3 adjudication will follow international criteria and include a review of all relevant clinical notes, laboratory data, orders, radiology, and assessments by independent critical care clinicians. Discordant adjudication will be resolved by in-person discussion among 2–3 reviewers.

When a patient is deemed eligible and satisfies clinical adjudication, trained research personnel will

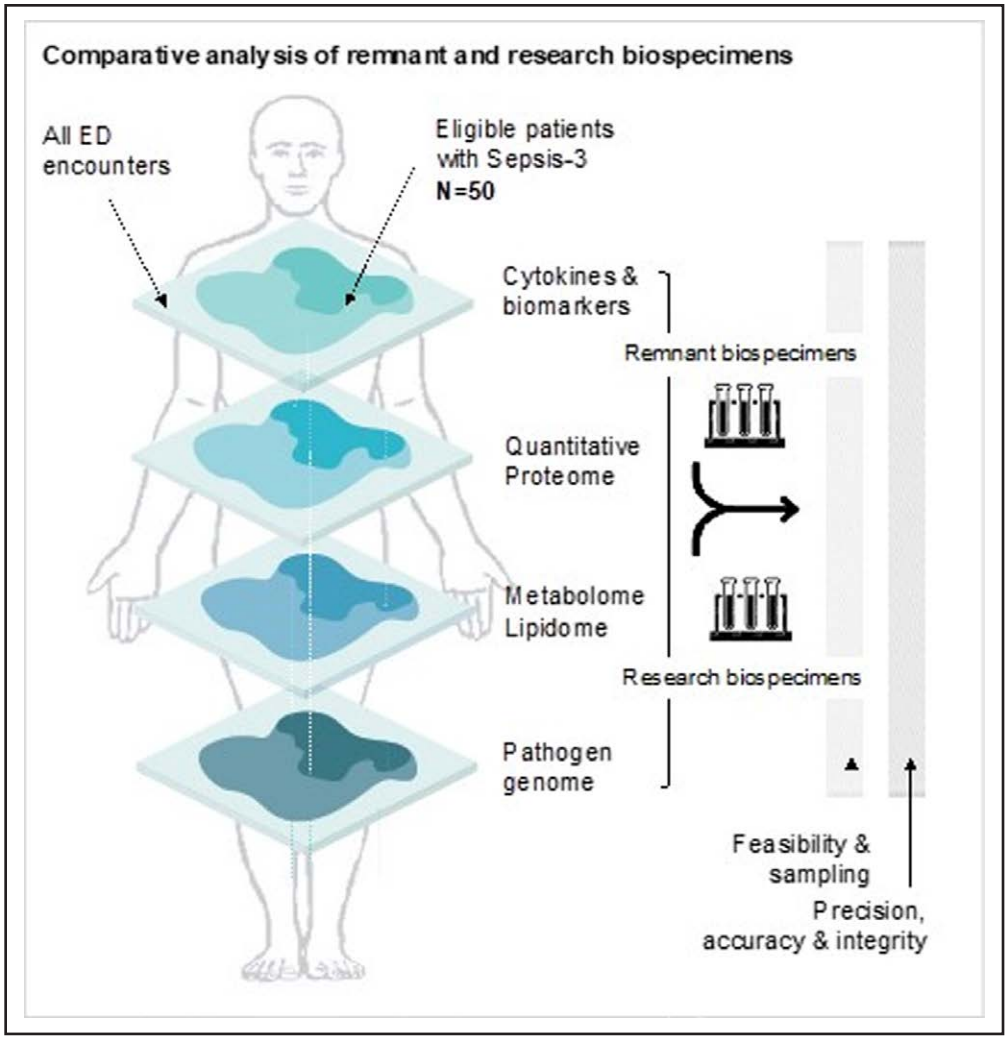

Figure 1. Conceptual overview of Remnant Biospecimen Investigation in Sepsis protocol, including paired biospecimens for measurement of cytokines, biomarkers, proteomics, lipidomic, metabolomic, and pathogen sequencing. ED = emergency department.

obtain informed consent from the patient or their legal next of kin within 72 hours of arrival. At the time of enrollment, baseline data will be collected including demographics, severity of illness, and patient location. Additional data will be linked retrospectively to enrolled patients in batches at study conclusion, including admission diagnoses, comorbidities, vital signs, laboratory values, and clinical outcomes. Outcomes will include hospital discharge disposition, in-hospital mortality, and hospital length of stay (**Fig. 2**).

Biospecimen Collection

We collect two types of biospecimens for analysis, those that are remnants from the UPMC Clinical Laboratory ("Remnant") and those prospectively sampled from the same subject by research coordinators ("Research"). Among eligible patients, remnant biospecimens are obtained directly from the Clinical Laboratory by research staff after the hospital-specific window for mandatory hold (40–48 hr). During the mandatory hold in the laboratory, biospecimens are stored at 4°C before release to the study team. Remnant samples include plasma from sodium–heparin, EDTA, sodium fluoride, and citrate tubes. When samples are reviewed, they are assessed for tube type, sample volume, and other measures of quality control, after which they are centrifuged, aliquoted, and barcode labeled for storage at the Clinical Research, Investigation, and Systems Modeling of Acute Illness (CRISMA), Clinical Research Biospecimen Core (CRBC) Laboratory. Research samples are sampled prospectively among eligible patients by research staff who engage with the bedside care team. Research kits are prepared to sample the same tube types as remnants within a specified time window. Samples are transported to the CRISMA laboratory and once received, are centrifuged at 2,000*g* for 10 minutes to remove cellular debris. All samples are checked visually for hemolysis, lipemia, and icteria. Samples that appear grossly hemolyzed, lipemic, or icteric are annotated and excluded from further study. Samples are aliquoted into 500 µL tubes and stored at −80°C until analysis.

Selection of Biomarkers for REMISE

To inform selection of biomarkers for REMISE, we collaborated with the National Institutes of Health Big

TABLE 2.
Remnant Biospecimen Investigation in Sepsis Study Inclusion and Exclusion Criteria

Eligibility Criteria for Remnant Biospecimen Investigation in Sepsis
Inclusion
• Adult patient, greater than 18 yr old
• Evaluated at participating emergency department (ED)
• Sepsis-3 criteria met within 6 hr of arrival
• Suitable biospecimens obtained for clinical measurements by clinical team
• Less than 72 hr since arrival at the ED
Exclusion
• Traumatic injury
• Cardiac arrest
• Stroke or intracranial haemorrhage
• Unable to obtain intravenous access
• Unable to consent or contact legal representative
• Subject declines to participate
• Inadequate biospecimen remnant sample
• Comfort measures only
• Known or suspected pregnancy
• Prisoner

Data to Knowledge (BD2K)-funded Center for Causal Discovery at the University of Pittsburgh (19) to adapt a framework of infection and disease tolerance in a unifying conceptual model for sepsis (20). This model relates the host, resistance, pathogen, tolerance, direct and indirect damage, and unknown pathways. It incorporates multiple treatments for different conceptual targets and forms a scaffold by which markers were selected. We chose to measure the concentration of eight inflammatory and endothelial-related biomarkers in duplicate. We will use customized assays to measure interleukin-6, pentraxin-related protein, soluble fms-like tyrosine kinase-1, E-selectin, P-selectin, soluble programmed cell death ligand 1, and vascular endothelial-cadherin (Luminex LX200 [Luminex Corp, Austin, TX] and Luminex Human Discovery Assay [R&D Systems, Minneapolis, MN]). Heme-oxygenase-1 (HO-1) will be quantified using enzyme-linked immunosorbent assays (HO-1 DYC3776, R&D Systems).

Pathogen Sequencing

Our approach to identify and genotype pathogen(s) within samples will depend on the amount of microbial DNA that can be recovered from the sample, relative to host DNA. Low-biomass samples will be analyzed by amplification and sequencing of the full-length gene encoding 16S ribosomal RNA and/or the fungal ribosomal internal transcribed spacer gene on the Oxford Nanopore MinION platform. Strain-level genotyping and diversity profiling of polymicrobial infections will be conducted using adenosine deaminase 2 deficiency. Higher biomass (> 30 ng/μL) samples will be analyzed by shotgun DNA sequencing on an Illumina NextSeq 550 (Illumina, San Diego, CA). We will extract DNA using the Qiagen BiOstic kit (Qiagen, Germanton, MD), sequencing libraries will be prepared using diluted Nextera Flex reactions (Illumina), and samples will be pooled and sequenced to minimum of 1 gigabase pair coverage. Reads mapping to contaminating microbes from the skin or the environment will be excluded.

Proteomic Analyses

We will use a semiautomated plasma proteomics protocol to measure ~2,500 proteins in a discovery mass spectrometry (MS)-based assay and mine the data to search for previously identified proteins in community-acquired sepsis patients and those with primary intra-abdominal (21) or urinary tract infections. We will subject plasma samples (40 μL) to immunodepletion with a MARS Top 14 column. The immunodepleted protein fraction will be digested using trypsin/Lys-C endopeptidase mix (Promega, Madison, WI) and peptides will be chemically labeled with commercial Tandem Mass Tags (TMTpro reagents). Sample preparation steps will be handled using an automated Biomek i7 liquid handling device (Beckman Coulter, Indianapolis, IN). Each TMT multiplex batch consists of up to 16 samples of paired remnant and traditional samples that are randomized and blinded across the channels and two quality control samples. Labeled peptides will be separated further into 24 concatenated fractions using high pH reversed-phase liquid chromatography (RPLC) and each fraction will be subjected to low pH RPLC on a nanoflow LC system (Thermo Ultimate 3000+, ThermoFisher Scientific, Waltham, MA). coupled to an Orbitrap Fusion Lumos or

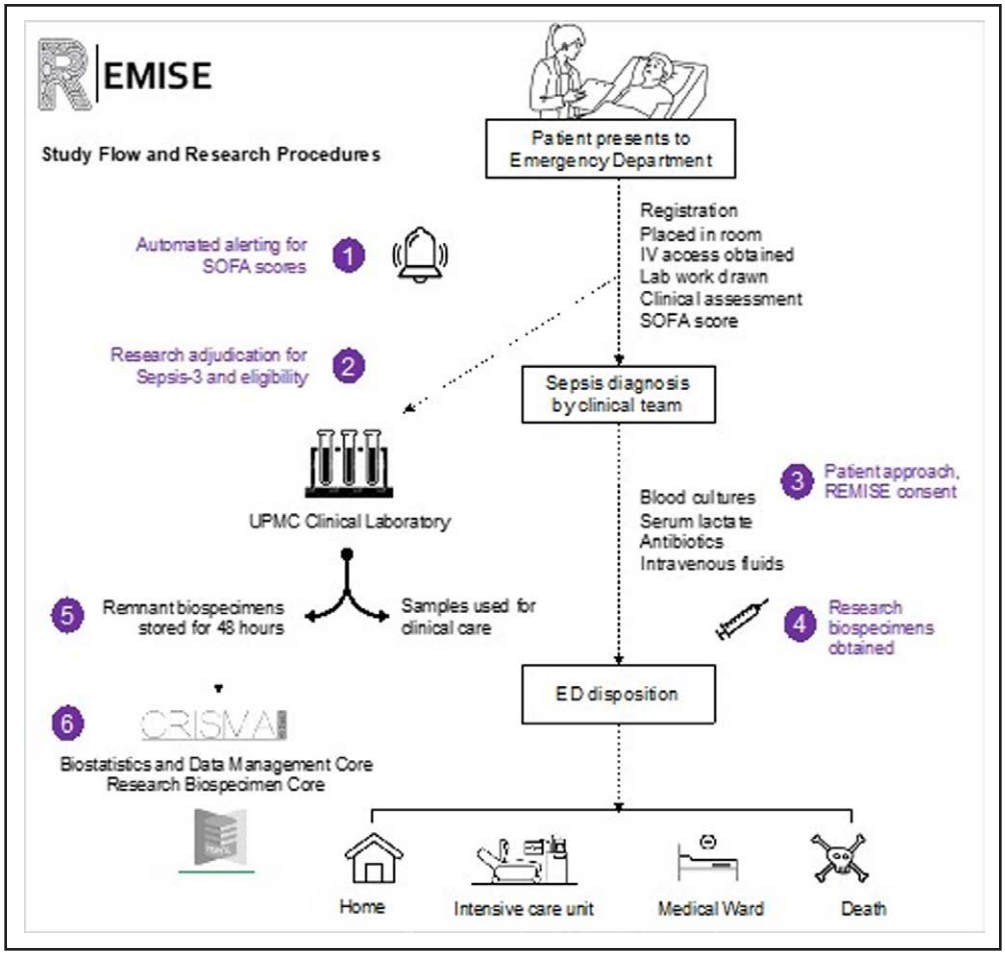

Figure 2. Procedures for Remnant Biospecimen Investigation in Sepsis (REMISE) recruitment, showing research team steps (*purple*), step 1 is automated screening using embedded Sequential Organ Failure Assessment (SOFA) score alerting, step 2 is clinical adjudication for sepsis-3 and eligibility, step 3 is patient approach and informed consent, step 4 is research specimen acquisition according to laboratory protocols, step 5 is remnant sample retrieval from the clinical laboratory, step 6 is secure, deidentified, linked storage of biospecimens and electronic health record at the Clinical Research, Investigation, and Systems Modeling of Acute Illness Research Cores.

Orbitrap QExactive mass spectrometer (ThermoFisher Scientific, Waltham, Massachusetts, USA). Peptides will be subject to MS and MS/MS analysis and duplicate injections. Database searching (Uniprot Human database) will be done with Proteome Discover v2.5 software and normalized reporter ion intensities will be stratified based on remnant or plasma patient samples or other clinical parameters of interest.

Metabolomic Analysis

Organic acids and amino acids will be separated and analyzed using a Vanquish ultrahigh-performance liquid chromatography (UHPLC) coupled to a high-resolution Exploris 240 hybrid quadrupole-Orbitrap mass spectrometer (ThermoFisher Scientific) (22–24). Untargeted methodology will be employed to collect discovery datasets. Quantitative targeted profiling will be used such that organic acids and amino acids of known identifications (matched to in-house library identifications) will be normalized to stable isotope-labeled internal standards and quantified against calibration curves. Metabolic quenching and polar metabolite pool extraction will be performed by adding ice-cold MeOH:EtOH (1:1) at a ratio of 1:4 plasma:solvent. The solvent contains deuterated internal standards (10 µM) including creatinine-d_3, alanine-d_3, taurine-d_4, and lactate-d_3 (Sigman Aldrich, Burlington, MA). After 3 minutes of vortexing, the supernatant is cleared of protein by centrifugation at 16,000*g*. Cleared supernatant (2 µL) will be subjected to online LC-high-resolution mass spectrometry (HRMS) analysis. Briefly, samples will be injected via a Thermo Vanquish UHPLC and separated over an RP Thermo HyperCarb porous graphite column (ThermoFisher Scientific) (2.1 × 100 mm, 3 µm particle size) maintained at 55°C. For the 20-minute LC gradient, the mobile phase will consist of the following: solvent A (H_2O/0.1% formic acid) and solvent B (acetonitrile [ACN]/0.1% formic acid). The gradient is as follows: 0–1 minutes 1% B, increasing to 15% B over 5 minutes, and then increasing to 98% B over 5 minutes, holding at 98% B for 5 minutes, and equilibrating at initial conditions for 5 minutes. The Exploris 240 hybrid mass spectrometer (ThermoFisher Scientific) will be operated in both positive and ion mode, scanning in

ddMS^2 mode (2 µscans) from 70 to 800 *m/z* at 120,000 resolution with an AGC target of 2e5 for full scan, 2e4 for MS^2 scans using higher-energy collisional dissociation fragmentation at stepped 15,35,50 collision energies. Source ionization settings include spray voltages at 3.0 and 2.4 kV, respectively, for positive and negative modes. Source gas parameters are 35 sheath gas, 12 auxiliary gas at 320°C, and 8 sweep gas. Calibration will be performed before analysis using the Pierce FlexMix Ion Calibration Solutions (ThermoFisher Scientific). Integrated peak areas will then be extracted manually using Quan Browser (ThermoFisher Xcalibur, ver. 2.7) and reported in units of Molarity based on the calibration curve for each respective analyte.

Lipidomic Analysis

Phospholipids will be separated and analyzed using a Vanquish UHPLC coupled to a high-resolution ID-X tribrid mass spectrometer (ThermoFisher Scientific) (25, 26). Analyses were performed by untargeted LC-HRMS to collect discovery datasets; however, phospholipids will be quantified using stable isotope dilution methodology. To 50 µL of thawed serum, a stable isotope internal standard mix containing phosphatidylinositol, phosphatidylcholines, glycerophospholipid, phosphatidylethanolamine, and phosphoglyceride UltimateSplash deuterated phospholipid internal standards at biologically relevant plasma concentrations (Avanti Polar Lipids, Alabaster, AL), will be added before using a modified Folch extraction ($CHCl_3$:MeOH:H_2O, 2:1:1). Samples will rest on ice for 10 minutes before phase separation via centrifugation at 2,500*g* for 15 minutes. The organic phase (700 µL) is dried under nitrogen gas and resuspended in 1:1 ACN:IPA. Sample (3 µL) will be injected via a Thermo Vanquish UHPLC and separated over an RP Thermo Accucore C18 column (2.1 × 100 mm, 5 µm particle size) maintained at 55°C. For the 30-minute LC gradient, the mobile phase will consist of the following: solvent A (50:50 H_2O:ACN with 10 mM ammonium acetate/0.1% acetic acid) and solvent B (90:10 IPA:ACN with 10 mM ammonium acetate/0.1% acetic acid). The initial gradient conditions will start at 30% B and increase over the first 2 minutes to 43% B followed by an increase to 55% B in 0.1 minutes. The organic will increase to 65% B over 10 minutes and continue to 85% B over 6 minutes, finally increasing to 100% B during the wash phase over 2 minutes, which is held for 5 minutes before equilibration at initial loading conditions. The Thermo ID-X tribrid mass spectrometer (ThermoFisher Scientific) is operated in both positive and negative electrospray ionization mode. A data-dependent MS^2 method scanning in Full MS mode from 200 to 1500 *m/z* at 120,000 resolution with an automatic gain control (AGC) target of 5e4 for triggering MS^2 fragmentation using stepped HCD collision energies at 20,40, and 60 in the Orbitrap at 15,000 resolution will be used. Source ionization settings will be 3.5 kV and 2.4 kV spray voltage, respectively, for positive and negative modes. Source gas parameters will be 35 sheath gas, 5 auxiliary gas at 300°C, and 1 sweep gas. Calibration is performed before analysis using the Pierce FlexMix Ion Calibration Solutions (ThermoFisher Scientific). Phospholipid species and stable isotope-labeled stable isotope-labeled dilution peak areas will be extracted manually using Quan Browser (ThermoFisher Xcalibur, ver. 2.7) and phospholipid species will be quantified using the appropriate corresponding calibration curve. Untargeted differential comparisons will be performed using LipidSearch 4.2 (ThermoFisher) to generate a ranked list of significant lipid compounds at the class- and species-specific levels.

Statistical Analysis

We will determine the feasibility of the remnant biospecimen repository by measuring sample integrity and quality. The integrity of samples will be a continuous variable for sample volume (mL), and categorical variables for hemolysis, lipemia, or icteria. Then, we will measure "scientific value" of remnant biospecimens and will study accuracy, precision, and integrity compared to the gold standard, research-collected specimens. We define the following, 1) accuracy is defined as the closeness of the value to the true value. Accuracy will be assessed by comparing the results of the remnant samples with the research sample as the gold standard, 2) precision is defined as how well methods provide similar results when a single remnant or research sample is tested repeatedly, and 3) integrity of remnant and research specimens will be quantified using the sample volume (mL), presence of hemolysis,

lipemia, or icteria, continuous and categorical parameters, as appropriate.

We will analyze five biologic "layers" for scientific value: traditional protein biomarkers (n = 8 markers), quantitative proteomics (n = 2,500), quantitative metabolome (n = 40), quantitative lipidome (n = 60), and pathogen genomics. To measure accuracy, we will assess differential expression between the remnant biospecimen value to the gold standard specimen values. We will use appropriate tests for differential expression based on the model distributions of the markers. The overall accuracy of the markers will be determined by false-positive rate after adjusting for multiple tests based on the R package limma (27). Accuracy will indirectly measure "stability," as research specimens are not subject to mandatory hold in the clinical laboratory. To measure precision, we will estimate consistency among repeated measurements using intraclass correlation coefficients (ICC), which is the proportion of the variation between measurements that is due to consistent differences between the individuals measured. We will use generalized linear models with link functions specified based on the distributions of the markers. We will then test ICC using F test. The categorical variables will be compared using McNemar's tests and the continuous variables will be compared using paired t tests or nonparametric rank-sum (or signed rank) tests, as appropriate.

Sample Size

We propose to enroll 225 patients. We estimate the following effect sizes for 80% power for paired readouts and comparison tests mentioned above: 0.4 SD of the mean difference for comparing means; McNemar's odds ratio ranging from 2.4 to 5.8 with 20 to 90% discordant proportions for comparing proportions; and the width of the 95% CI from 0.11 to 0.54 with intraclass correlation ranging from 0.1 to 0.9.

Methodological Issues

There are potential limitations to this protocol. First, we considered but rejected transcriptomic analyses. Remnant samples are, 1) not collected in tubes with additives that stabilize intracellular RNA, leading to possible degradation during postcollection time, thus not reflecting in vivo processes, and 2) not handled in RNAse-free environments, leading to possible degradation. Second, we anticipate the scientific value of remnant biospecimens will be adequate for traditional biomarker assays. However, it is possible that assays such as quantitative proteomics or metabolomic profiling, which require more rigorous pre-analytic processing, may return values that are missing between patient channels. In this case, we will expand our assays to a broader selection to determine if this finding is specific to the markers we chose or a major barrier. Third, the timing of sampling for remnants and research biospecimens will be minimized to allow comparability but may not be immediate. We will quality-check this difference during the study to maintain a window of less than 12 hours. Fourth, clinical adjudication for sepsis is known to be difficult. However, our team is experienced in the derivation and identification of Sepsis-3 in the EHR, assuring internal validity.

Data Management and Oversight

Study investigators will take responsibility for the conduct of REMISE. They will supervise the day-to-day operation of the project and assure compliance with regulatory and laboratory protocols. Members of the research team will monitor weekly reports of enrollment, exclusions, and informed consent procedures. Monitoring will ensure protocol compliance, study management, and biospecimen handling best practices.

Protocol and Registration

The REMISE protocol is approved as minimal risk by the University of Pittsburgh Human Research Protection Office (21120013) and registered on ClinicalTrials.gov (NCT05684133).

Data Storage and Security

Clinical data from the research team will be entered electronically via a password-protected web-based data entry system. The system will be created using ASP.NET programming, with the data stored using MS SQL Server. The data entry process will begin during the online enrollment after automated screening alerts, and we will scrape approximately 90% of REMISE clinical data directly from the Cerner Millennium database at UPMC. This process uses already developed Oracle SQL, MySQL, and R scripts

to Export, Transform, and Load data for reporting purposes in a Microsoft Azure environment, whereas leveraging standard vocabulary mapping tables developed within CRISMA will facilitate interoperable exchange of study data.

Data will be stored at the CRISMA Biostatistics Data Management Core on password-protected electronic servers. Identifiers will be maintained separately from research data and samples and a link connecting deidentified data to identifiers will be separately stored. Biospecimens will be stored indefinitely in a dedicated −80°C freezer in the CRBC Laboratory for batched subsequent analyses. Specimens shared outside of this laboratory will only be shared with proper agreements. The CRBC uses a customized inventory tracking system, Biospecimen Inventory and Operations System for tracking and reporting functions and linking biospecimens to clinical data.

Dr. Seymour takes full responsibility for the integrity of the data and the accuracy of the data analysis. Drs. Seymour, Urbanek, and Palmer were involved in the drafting of the article. Drs. Seymour and Angus were involved in the study supervision. All authors participated in the study concept, design, and critical revision of the article for important intellectual content.

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