**Title:** The MicroRESUS Study: An Observational Study to Examine the Effects of Circulatory Shock and Resuscitation on Microcirculatory Function and Mitochondrial Respiration After Cardiovascular Surgery.

NCT05330676
Unique Protocol ID: 843614

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**Abstract**

**Background**

Despite current resuscitation strategies, circulatory shock and organ injury after cardiac surgery occur in 25-40% of patients. Goal-directed resuscitation after cardiac surgery has generated significant interest, but clinical practice to normalize hemodynamic variables including mean arterial pressure, cardiac filling pressures, and cardiac output may not reverse microcirculation abnormalities and do not address cellular dysoxia. Recent advances in technology have made it possible to measure critical components of oxygen delivery and oxygen utilization systems in live human tissues and blood cells. The MicroRESUS study will be the first study to measure microcirculatory and mitochondrial function in patients with circulatory shock and link these findings with clinical outcomes.

**Methods and analysis**

This will be a prospective, observational study that includes patients undergoing elective cardiovascular surgery with cardiopulmonary bypass (CPB). Microcirculation will be quantified with sublingual incident dark field videomicroscopy. Mitochondrial respiration will be measured by performing a substrate–uncoupler–inhibitor titration protocol with high resolution respirometry on peripheral blood mononuclear cells at baseline and serial timepoints during resuscitation and at recovery as a possible liquid biomarker. Plasma samples will be preserved for future analysis to examine endothelial injury and other mechanisms of microcirculatory dysfunction. Thirty-day ventilator and vasopressor-free days (VVFDs) will be measured as a primary outcome, along with sequential organ failure assessment scores, and other clinical parameters to determine if changes in microcirculation and mitochondrial respiration are more strongly associated with clinical outcomes compared to traditional resuscitation targets.

This will be the first prospective study to examine both microcirculatory and mitochondrial function in human patients with circulatory shock undergoing cardiac bypass and address a key mechanistic knowledge gap in the cardiovascular literature. The results of this study will direct future research efforts and therapeutic development for patients with shock.

## Introduction

Despite current resuscitation practices, circulatory shock and perioperative organ injury after cardiac surgery occur in 25-40% of patients [1,2]. Traditionally, causes of shock after cardiovascular surgery with cardiopulmonary bypass are classified by macrocirculatory derangements (e.g. low cardiac output, vasoplegia, hypovolemia) [3]. Interventions to normalize hemodynamic variables, including mean arterial pressure, cardiac filling pressures, and cardiac output restore the large vessel (macrocirculatory) pressure and flow targets but may not reverse underrecognized disruptions in microcirculatory blood flow and oxygen utilization. Current methods to estimate the balance of oxygen delivery ($DO_2$) relative to demand ($VO_2$) include blood gas-derived calculations and the measurement of downstream biomarkers of anaerobic metabolism such as blood lactate. Using these methods, previous literature has concluded that lactic acidosis after cardiac surgery is unlikely related to inadequate oxygen delivery [4]. Unfortunately these inferences fail to consider the presence of regional blood flow derangements caused by pathologic microcirculatory heterogeneity, which are also associated with severity of postoperative lactic acidosis and organ injury [5,6]. To resolve this important clinical discrepancy, a deeper understanding of the determinants of oxygen transport pathways and oxygen utilization during health, shock, and resuscitation are needed.

The microcirculation is composed of a network of vessels including arterioles, capillaries, and venules <100 μm in diameter where red blood cells (RBCs), leukocytes, and plasma components interface with the vascular endothelium to allow metabolic substrate exchange. Changes in microcirculatory blood flow can be caused by inflammatory-mediated vascular endothelial injury, microthrombosis, or an inadequate balance between vasoconstrictive and vasodilating agents leading to a global or heterogeneous reduction in capillary blood flow [7,8]. Incident dark field (IDF) videomicroscopy is a novel, handheld method that can directly image the human microcirculation in real time. Current generation IDF videomicroscopy has improved imaging resolution compared to previous generation devices, and can detect up to 30% more capillaries compared to side stream imaging [9,10]. As a result, important determinants of tissue oxygenation, such as microcirculatory diffusive and convective properties, can now be more accurately quantified.

Blood gas derived calculations of $VO_2/DO_2$ balance may provide false clinical reassurance as they cannot identify microvascular injury, microvascular shunting, and other mechanisms of dysoxia which may contribute to cell injury and organ dysfunction in patients with shock [11]. Advancements in high resolution respirometry now make it possible to quantify mitochondrial respiration rapidly and reliably in live tissues [12]. Nucleated blood cells (platelets and peripheral blood mononuclear cells) are readily accessible and can be used as surrogates to study cellular respiration in acute care illnesses such as acute heart failure, hemorrhagic shock, sepsis, and patients with ischemic reperfusion injury [13–15]. It is unclear if deficiencies in oxygen delivery and utilization occur independently or concomitantly in patients with shock. Studies that simultaneously examine mitochondrial respiration and microcirculatory function, which are also tied to clinical outcomes, are vitally important to guide future research efforts in therapeutic development and perform effective interventional trials.

## Materials and methods

### Study objective

The primary objective of the MicroRESUS study is to evaluate the microcirculatory and mitochondrial function in patients following elective cardiovascular surgery with cardiopulmonary bypass, to determine if these parameters outperform traditional biomarkers and global hemodynamic measurements to predict clinical outcomes.

### Study design and setting

This is a prospective, observational, single center study with repeated measures from baseline through 30-days after surgery at the University of Pennsylvania in Philadelphia, PA, USA.

### Patient screening

Patients will be screened 24-48 hours prior to surgery using the published operative schedule. First-case elective coronary artery bypass graft (CABG) or valvular surgeries will be considered for study enrollment, pending availability of research personnel to complete the study protocol. Efforts will be made to diversify subject enrollment to accurately reflect the general cardiovascular patient population.

**Informed consent**
Patients will be approached for consent by telephone one day prior to surgery or in person while in the preoperative area on the day of surgery. Consent will be obtained by the Principal Investigator or trained research personnel. Patient signatures will be obtained digitally via the REDcap data management system [16]. A digital copy of the consent form will be sent to the patient in addition to being retained by the study team.

**Sample size and power**
Using data from our previous foundational work as well as unpublished pilot data, we anticipate a 1:1 allocation of patients to the high (PVD > 22mm/mm$^2$) and low group (PVD $\leq$ 22 mm/mm$^2$). We will need a sample size of at least 134 subjects to detect at least a 2-day difference in ventilator and vasopressor-free days (VVFDs) with a $\beta$=0.8, using a one-sided t-test $\alpha$=0.05 [5,6]. We will enroll a total of 140 subjects to allow for a 5% loss to follow-up (surgical delay, ICU delay, cancelled surgery, etc.) and exclusion due inadequate microcirculation video quality (S1 Appendix).

**Inclusion criteria**
Adult patients ($\geq$18 years old) receiving elective CABG or valvular surgery requiring cardiopulmonary bypass are eligible for enrollment. Post-operative patients with circulatory shock will be identified by having:

1. Either vasopressor-dependent hypotension or low cardiac output requiring inotropic support
2. Signs of end-organ injury or impaired tissue perfusion defined by one of the following criteria:
    a. Normothermic patients with a capillary refill time > 3 seconds
    b. Serum lactate > 2 mmol/dL
    c. Mixed venous oxygen saturation (SvO2) < 60%

**Exclusion criteria**
Patients will be excluded if they are unable to tolerate sublingual microcirculatory flow imaging (e.g., non-intubated patients dependent upon oxygen by facemask, poor mouth opening), receiving an emergent procedure, have an active malignancy, or mitochondrial disorder.

**Timeline and techniques for data collection**
Data for each subject will be collected at time points outlined in **Error! Reference source not found.**. Prior to surgery, baseline biologic samples, microcirculation imaging, and clinical data will be obtained. Repeated measurements will be obtained in the ICU and surgical floor. Long-term clinical outcomes will be recorded at 30-days or upon discharge.
**Fig 1. Timeline of data collection.**

**Demographic and resuscitation data**
Demographic values including age, gender, and ethnicity will be recorded. Preoperative risk scores including the STS (Society of Thoracic Surgeons) mortality score and euroSCORE II (European System for Cardiac Operative Risk Evaluation) will be calculated [51,52]. Intraoperative data including cardiopulmonary bypass time, cross clamp time, blood product use, intravenous fluid administration, and vasoactive administration will be recorded. ICU clinical data (hemodynamics, laboratory testing, etc.), resuscitation data (intravenous fluids, vasoactive administration, blood transfusion, etc.), and clinical outcomes (VVFDs, ICU length of stay, hospital LOS, etc.) will be recorded as indicated in **Error! Reference source not found.**. Sequential organ failure assessment (SOFA) scores will be calculated prior to surgery (baseline), as well as 24 and 72 hours after surgery.

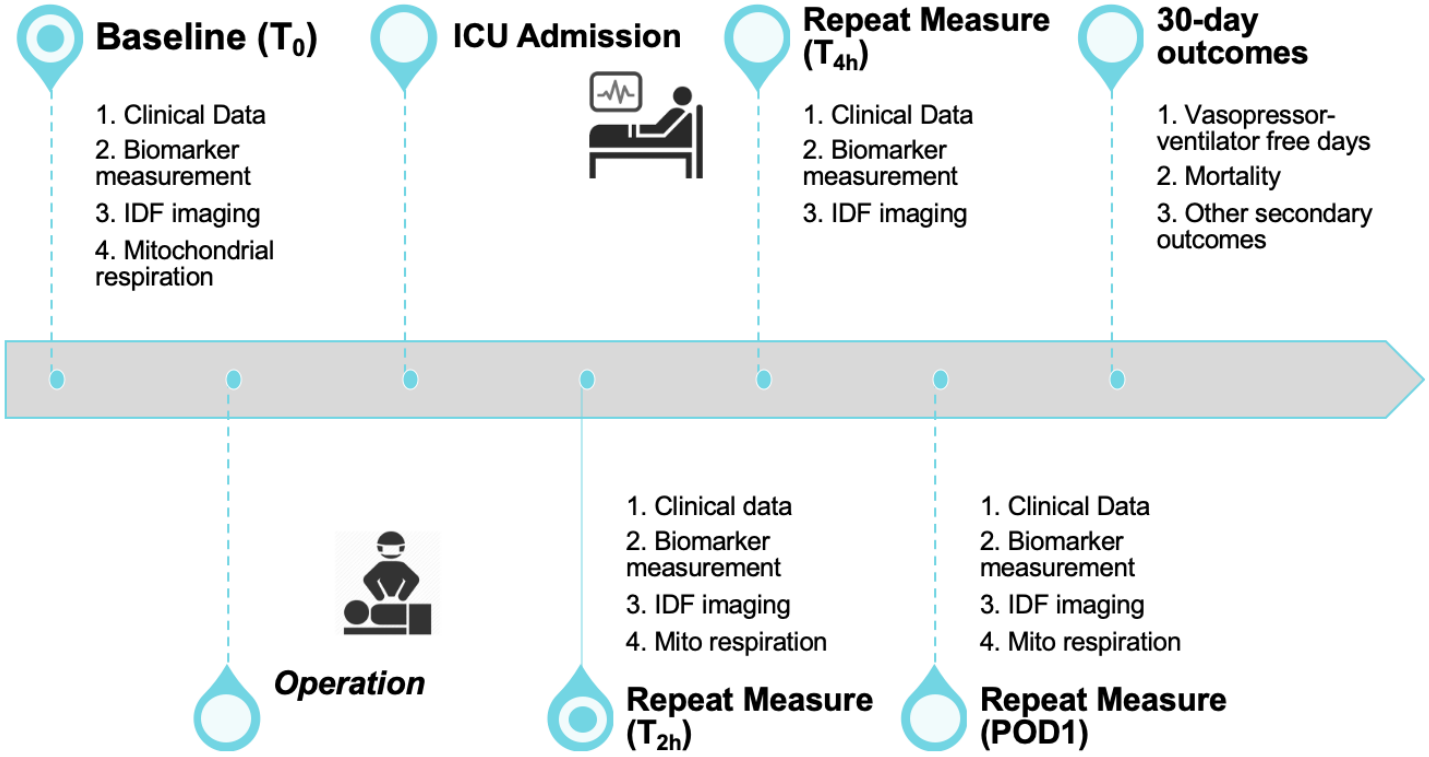


**Figure 1**

**Physiologic and pharmacologic data**
Systemic hemodynamic data, perfusion data, and blood gas measurements will be collected upon ICU admission, then hourly during the first 6 hours of postoperative care. Cardiac output (CO), cardiac index (CI), central venous pressure (CVP), pulmonary artery pressure (PAP), and mixed venous oxygen saturation ($SvO_2$) will be monitored continuously using a pulmonary artery catheter (Edwards Lifesciences LLC, Irvine, CA, USA). Arterial blood pressure will be measured using a standard invasive arterial line. Intraoperative and post-operative administration of blood products, intravenous fluids, vasoactive agents (vasopressor and inotropes), and sedatives will be recorded at each time point.

**Incident Dark Field Microscopy**
Sublingual microcirculation imaging will be performed using handheld incident dark field (IDF) videomicroscopy (CytoCam, Braedius Medical BV, the Netherlands) at four time points during the enrollment period. Imaging will be performed by the Principal Investigator or trained research personnel. Video sequences are obtained by placing the CytoCam device should sublingual space and maneuvered so that pressure and motion do not result in image artifact (**Error! Reference source not found.**). A series of successive video clips (3-5 clips of at least 120 frames or 6 seconds in length) should be captured in distinct areas of the sublingual space to account for vessel heterogeneity. Focus and lighting during video capture may be adjusted to optimize image acquisition. Baseline ($T_{0h}$) imaging will be obtained in the preoperative area prior to surgery on the day of the scheduled operation. Repeated measurements will be obtained upon arrival to the ICU (0-2 hours after surgery, $T_{2h}$), during ongoing resuscitation (2-4 hours post-op, $T_{4h}$), and on post-operative day one after recovery ($T_{24h}$).

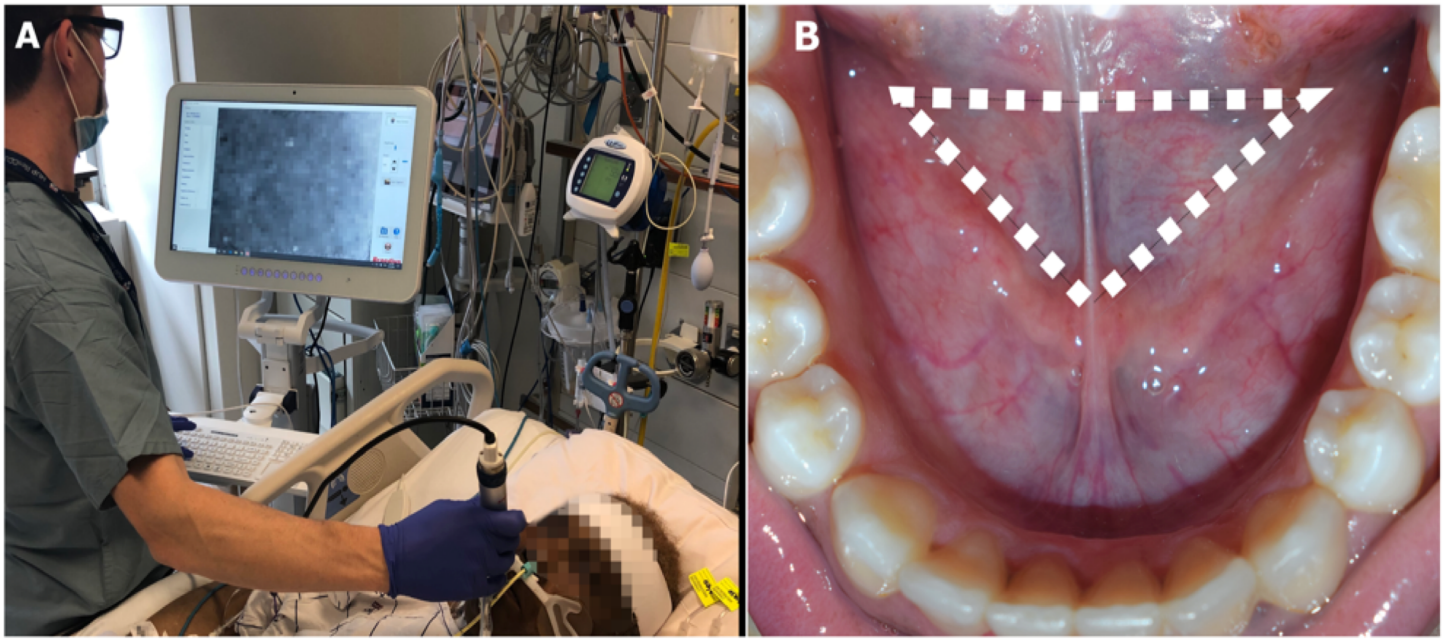


**Fig 2. A. Experimental setup with patient in supine position during IDF measurement. B. Anatomical sublingual triangle where measurements are obtained.**

**Analysis of IDF videomicroscopy**

Prior to analysis, video quality will be assessed using the 6-factor Massey quality score, which uses a semiquantitative assessment of each video for appropriate illumination, duration, focus, content, stability, and pressure. Only videos with Massey scores of <10 will be included for further analysis [17]. Three videos with the best quality score will be selected for further processing. All IDF images will be coded then analyzed using an offline, dedicated software (Automated Vascular Analysis v3.02, Microvision Medical, The Netherlands). Microvascular flow index (MFI), microcirculatory heterogeneity index (MHI), total vessel density (TVD), proportion of perfused vessels (PPV), and perfused vessel density (PVD). Individual microvessel flow will be scored as 0=no flow, 1=intermittent flow, 2=sluggish flow, or 3=continuous flow. Vessels will be considered perfused if they are scored as either sluggish or continuous flow. To ensure only vessels contributing to tissue gas exchange and metabolism are included, only vessels < 20 μm in diameter will be analyzed. This process follows the current standard for microcirculation measurement and analysis [18]. Interobserver variation will be tested periodically in at least 10% of the subject videos to ensure minimal scoring heterogeneity between investigators.

**Biological samples**

Blood gas samples will be drawn into a commercial, pre-heparinized 1 mL blood sampler then immediately analyzed by an ABL90 FLEX automatic blood gas analyzer (Radiometer America Inc., Brea, California, USA). Arterial blood gas samples will be obtained by clinical staff every 1-2 hours for the first 6 hours after ICU admission based on current clinical practice. Central venous blood gas measurements will also be obtained by the study team at the time of microcirculation measurement. Hemoglobin, hematocrit, pH, $PaO_2$, $PaCO_2$, and glucose are also reported on each blood gas analysis. After 6 hours, blood gas analysis will be performed as needed by the clinical team. For biomarker and mitochondrial function analysis, enrolled patients will undergo phlebotomy with volumes of 15 mL drawn into $K_2$EDTA tubes. Blood samples will then be centrifuged at room temperature using Ficoll-Paque™ PLUS (GE) and Leucosep tubes (Greiner Bio-one). Plasma specimens will be stored at -80ºC for later evaluation of the mechanisms of microcirculatory dysfunction. Peripheral blood mononuclear cells (PBMCs) will undergo further analysis and processing as detailed below.

**Mitochondrial respiration**

A population of PBMCs will be obtained and analyzed from the plasma buffy coat within 1 hour of blood draw. A cell count and viability will be calculated using the Cell Countess II (Invitrogen) with trypan blue exclusion. Between 4-5 x $10^6$ PBMCs will be used for respiration analysis and residual PBMCs will be processed and stored

at-80º C for future quantitative PCR. Unless otherwise specified, all reagents will be obtained from Sigma-Aldrich and Invitrogen.

Mitochondrial respiration will be analyzed using an Oroboros O2k-FluoRespirometer (Oroboros Instruments, Innsbruck, Austria) with a substrate–uncoupler–inhibitor titration (SUIT) protocol and MiR05 buffer [19]. The SUIT protocol measures oxidative phosphorylation capacity with electron flow through complex I (CI) and complex II (CI + CII) using malate, pyruvate, glutamate, and flavin adenine dinucleotide-linked substrate succinate in the presence of adenosine diphosphate. The addition of digitonin allows for the measurement of specific complex-linked activity. Oligomycin, an inhibitor of the ATP synthase, uncouples respiration from ATP-synthase activity to measure respiration where the $O_2$ consumption is dependent on the leakiness the mitochondrial membrane and back-flux of protons into the mitochondrial matrix independent of ATP synthase ($LEAK_{CI+CII}$). Maximal convergent non-phosphorylating respiration of $ETS_{CI+CII}$ is evaluated by titrating the protonophore, carbonyl cyanide p-(trifluoromethoxy) phenylhydrazone. $ETS_{CI+CII}$ is considered a stress test for mitochondria, a marker of mitochondrial respiratory reserve. Non-phosphorylating respiration specifically through CII ($ETS_{CII}$) is achieved through the addition of rotenone, an inhibitor of CI. The complex III (CIII) inhibitor antimycin-A is added to measure the residual non-mitochondrial oxygen consumption, and this value is subtracted from each of the measured respiratory states to provide only mitochondrial respiration. Complex IV (CIV)-linked respiration will be measured by the addition of ascorbate with N,N,N,N-tetramethyl-phenylenediamine. The CIV inhibitor sodium azide will be added to reveal the chemical background that is subtracted from the N,N,N,N-tetramethyl-phenylenediamine-induced oxygen consumption rate. Mitochondrial reactive oxygen species production will be measured using the Amplex UltraRed method [20]. All data will be acquired using DatLab 7 (Oroboros Instruments, Innsbruck, Austria) and respiration value will be normalized to cell count.

**Outcome measures**

Our hypothesis is that patients with poor post-operative sublingual functional capillary density (defined as a PVD < 22 mm/mm$^2$ and MHI > 0.4) will have a higher degree of postoperative cardiovascular and pulmonary organ injury compared to patients with normal postoperative microcirculation. The primary outcome for this study will be VVFDs during the first 30 days after surgery. This outcome was chosen because our elective cardiac surgery patients are managed using early recovery after surgery (ERAS) protocols, with a goal to be extubated and weaned off vasopressors within 24 hours after surgery [21,22]. VVFDs will be calculated as a reverse count of consecutive days without requiring ICU-level respiratory or vasopressor support (**Error! Reference source not found.**). Our research group has used a similar primary outcome in a previous clinical trial [23]. The day of operation (day zero) will not be included as all patients undergoing cardiovascular surgery with CPB require mechanical ventilation and vasoactive medications on the day of surgery. Ventilator days will include mechanical ventilation via endotracheal tube or tracheostomy, high-flow nasal cannula $\geq$ 40 liters/minute with an FiO2 > 40%, or non-invasive positive pressure ventilation not prescribed at home. Non-invasive positive pressure ventilation and HFNC will be included as these both require the patient to remain in our ICU. Vasoactives include epinephrine, norepinephrine, vasopressin, phenylephrine, dobutamine, or milrinone at any dose. Subjects in need of respiratory or vasopressor support on day 30, or die before day 30 will be assigned zero VVFDs. If the patient is discharged prior to hospital day 30 a “last status carried forward” approach will be used. Secondary outcomes will include sequential organ failure assessment (SOFA) score on day 1, day 3, diagnosis of acute kidney injury (defined as an increse in serum creatinine $\geq$ 0.3 mg/dL within 2 days), and hospital length of stay.

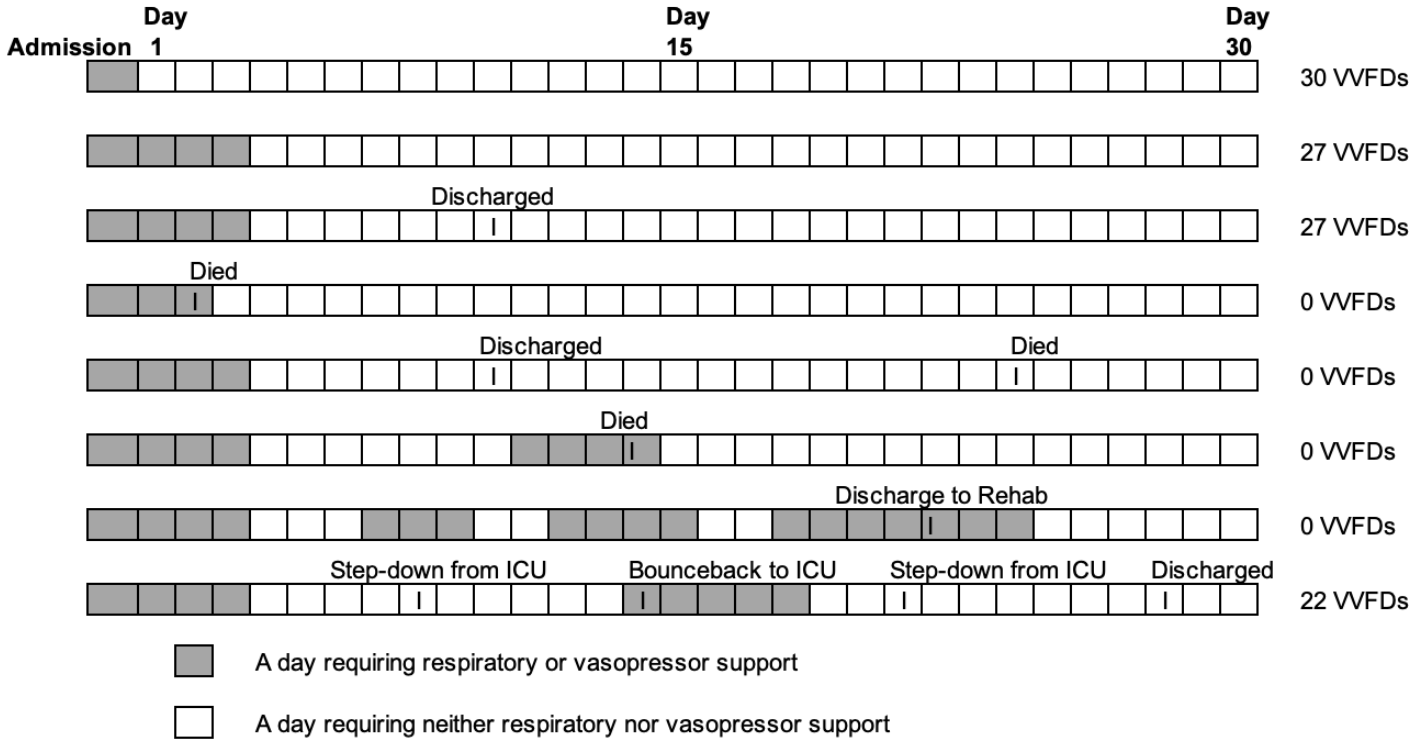


**Fig 3. Examples of VVFD scenarios to determine clinical outcomes.**

Exploratory outcomes will examine the relationship of microcirculatory function with common clinical biomarkers such as lactate, $SvO_2$, venous-to-arterial $PCO_2$ gap, capillary refill time, and lactate to pyruvate ratio. Markers of endothelial injury and inflammation such as soluble vascular cell adhesion molecule 1 (sVCAM-1), soluble intercellular adhesion molecule 1 (sICAM-1), e-selectin, IL-8, IL-10, and other inflammatory cytokines will be measured. We will also examine mitochondrial complex function in human blood cells, comparing individual complex (I, II, III, and IV) function and reactive oxygen species generation between patients with and without post-operative shock.

**Statistical and analysis plans**

Continuous variables characterizing demographical data, microcirculation data, and mitochondrial respiration measurements, and outcomes data will be reported as means with standard deviations if normally distributed or medians with interquartile ranges if not normally distributed. Categorical variables will be represented as frequencies and proportions. To examine the predictive performance of selected variables for the primary outcome, we will construct receiver operator characteristic curves for threshold values of PVD, MHI, lactate, $SvO_2$, mean arterial pressure, and cardiac index. A Youden index will be calculated to determine the best cutoff value for determining prolonged VVFDs.

Linear regression modeling will be used to examine the relationship between L/P ratio and postoperative microcirculation variables. We will perform univariate analyses on candidate predictor variables of L/P ratio including PVD, MHI, LFTs, creatinine, CPB time, cross clamp time, and catecholamine administration. Multiple linear regression analysis will be used to model the effect of significant predictors. Repeated measure ANOVA will be used to compare changes in microcirculatory variables, mitochondrial respiration, and mitochondrial reactive oxygen species production over time. To adjust for multiple comparisons, post-hoc pairwise Tukey Kramer t-tests will be performed. All analyses will use statistical software (SAS version 15.1, Cary, NC; Prism v 9.0, Graph-Pad Software, San Diego, CA).

**Ethics and dissemination**
This study is approved by the University of Pennsylvania Institutional Review Board (IRB # 829765) and informed consent will be obtained prior to enrollment. The dataset supporting the results of this study will be available in the Zenodo research data repository. This study is registered with ClinicalTrials.gov at NCT05330676.

**Status and timeline of the study**
Initial pilot testing, staff education, and laboratory calibration testing began recruitment of the participants began on September 1, 2020. The study is actively enrolling subjects at the time of this publication. Preliminary analysis of microcirculation images, mitochondrial respiration, and VVFDs will be conducted in 2022.

## SAFETY MANAGEMENT

**Clinical Adverse Events**
Clinical adverse events (AEs) will be monitored throughout the study.

**Adverse Event Reporting**
Since the study procedures are not greater than minimal risk, SAEs are not expected. If any unanticipated problems related to the research involving risks to subjects or others happen during the course of this study (including SAEs) these will be reported to the IRB AEs that are not serious but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the IRB at the time of continuing review.

**Safety considerations**
The Principal Investigator will be primarily responsible for the study conduction throughout protocol completion. Research personnel will be required to report any relevant protocol deviations or research related adverse events to the Principal Investigator. Risks associated with sublingual IDF imaging are low and no identifiable adverse events have been previously linked to sublingual IDF imaging.

## STUDY ADMINISTRATION

### Data Collection and Management

Data abstracted from the medical record will be managed and stored using the research-focused electronic data capture system REDCap (Research Electronic Data Capture) under an agreement with the software's development consortium, led by Vanderbilt University. We will use the REDCap server located at HUP.

Data will be managed and stored using the research-focused electronic data capture system REDCap, under an agreement with the software's development consortium, led by Vanderbilt University. REDCap supports two secure, web-based applications designed exclusively to support data capture for research studies.

REDCap as implemented at HUP includes daily destructive database backup files that are stored on the database server and are deleted only after successful backup of the entire database to file. Data and backups are stored in the HUP Research Information Systems Storage Area Network (SAN). Access to the SAN directories where data are stored will be limited to Research Information Systems personnel, with authentication performed using HUP's enterprise Active Directory service.

Each study patient will be given a study identification number (study ID). Only the study ID will be recorded on the CRF. A master list linking the study ID to identifying information will be kept separate from the CRF and stored as a locked Excel file on the hospital's secure servers. Dates (e.g., date of birth, date of hospitalization, date of study enrollment) will be recorded in REDCap in order to determine age, length of stay, etc. However, **only coded data will be exported from REDCap to be used for analysis of study results.** We will use the automated feature within REDCap to shift all dates by a value between 0 and 364 days. Date shifting is important for de-identification because dates are identifiers that can be used for identifying an individual and thus possibly exposing confidential personal information. Date shifting will prevent any dates from being used as identifiers while preserving the interval between dates so that age can be calculated. Date shifting will be used to de-identify data prior to sharing with colleagues at Penn. Access to PHI (i.e the master list and dates in REDCap) will be limited to the PHI and key study personnel at HUP. Those not involved in data entry will not have access to view PHI (i.e., dates) within REDCap (using REDCap's security features to limit data access) and will be limited to viewing coded data and shifted dates only. If paper versions of the CRF are needed (e.g., temporary loss of access to REDCap), these will be kept in a locked cabinet in the PI's office and will be coded using the study ID. Only study personnel authorized by the IRB will have access to study-related data and files.

The password-protected master list linking patient identifiers to the study ID will be retained indefinitely.

### Data sources

This study will in no way alter or interfere with the clinical care of any shock or control subject as determined by the medical care team. All clinical data will be abstracted from the paper or electronic (EPIC and Sunrise) medical record. To assist in this effort, we will perform manual review of the medical record for each study patient. At HUP, each data collector and the Data Coordinator have completed extensive initial training and monitor data quality quarterly, including inter-rater reliability.

### Confidentiality

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy and that the Investigator and other site personnel will not use such data and records for any purpose other than conducting the study. Safeguards to maintain subject confidentiality are described under Data Collection and Management.

Confidentiality of the data will be ensured by keeping a master list containing patient identifiers and study ID separate from data forms (paper and electronic). The master list will be kept in a locked file on the password-protected computer of the PI, with a paper copy stored in a locked file cabinet in the PI's office. All study-

related files will be password-protected. Patient identifiers, including the master list, will be destroyed within three years after publication.

For data security, a copy of all password-protected files will be maintained on the Investigator's office computer (which itself is password-protected), with the original in one of the Hospital's secure servers.

No identifiable data will be used for future study without first obtaining IRB approval. The investigator will obtain a data use agreement between provider (the PI) and any recipient researchers (including others at HUP) before sharing a limited dataset (dates and zip codes). Samples processed in the Metabolomics lab and the will be coded with a study ID only and PHI will not be shared.

**Regulatory and Ethical Considerations**

**Data and Safety Monitoring Plan**

The systems for storing and backing up data were described above. The principal investigator will be responsible for monitoring data collection and the safety of all study measurements. Given the minimal risks and observational nature of the study, we do not require an independent medical monitor of safety.

**Risk Assessment**

The risks for patients participating in this study are not greater than minimal risk and include those related to 1) blood collection for study measurements and 2) breach of confidentiality.

Since we will limit the volume of blood collection to less than 3 mL/kg or 50 mL and no more than two blood collections in one week, this falls under the provision of "not greater than minimal risk." For any patient whose clinical condition might be adversely affected by removal of the stated blood volume for this study (e.g., patients with significant anemia or compromised cardiac output), we will discuss further limiting the volume of blood withdrawn for research purposes with the patient's attending physician. Any patient concurrently enrolled in another study for who enrollment in this study would mean the combined blood volume collection would exceed IRB regulations will be excluded from participation in this study. Finally, since patients with shock typically undergo multiple blood draws per day and often have central and/or arterial vascular access devices, whenever possible blood sampling for study purposes will be timed with other clinically indicated blood draws or catheter access in order to limit pain from venopuncture, arterial puncture, and access to indwelling vascular catheters. Only caregivers with expertise and training in venopuncture, arterial puncture, or access of indwelling catheters will be permitted to collect blood specimens for this study.

In the rare cases where phlebotomy is performed for study purposes only, attempts will be made to mitigate patient discomfort by using topical anesthestics, such as EMLA. Also, risks will be minimized by limiting the number of attempts to obtain blood to 2 per patient per day and only individuals skilled at performing phlebotomy will be permitted to attempt.

To minimize a potential breach of patient confidentiality, CRFs will be coded with a study ID, all data will be password-protected, and the safeguards to ensure confidentiality as described.

**Potential Benefits of Study Participation**

There are no direct benefits to patients as a result of participation in this study.

The indirect benefits of this study are clear. The knowledge gained through participation may lead to improved diagnosis, prognosis, and therapy for adults with life-threatening shock and will advance the state of knowledge about progressive organ dysfunction in postoperative shock. The data gathered from this study will be used to inform subsequent studies in shock.

**Risk-Benefit Assessment**

Given the minimal risk profile associated with this study, the overall benefits to science, medicine, future generations of postoperative individuals, and society as a whole outweigh the risks.

**Informed Consent/Assent and HIPAA Authorization**
For patients who meet criteria, the patient or next of kin/power of attorney will be approached for study enrollment. The PI, co-investigators, or trained staff members will engage them in a discussion regarding reasons for the study, the study procedures, and the risks and benefits and answer all questions. Due to the often critical nature of the patients' conditions in the inpatient floor, preoperative clinic, or intensive care unit, this discussion may take place at the patient's bedside or in an alternative location (e.g., family conference room) at the next of kin or power of attorney's option and the study investigator's discretion. Also, since it is common for the next of kin or power of attorney to be away from the bedside, we anticipate the need to seek consent over the phone for many patients. Regardless of the where this discussion takes place (i.e., in person or via telephone), all reasonable safeguards to ensure patient privacy will be taken. Next of kin/power of attorney will be given sufficient (i.e., up to several hours) to make a decision to participate in this study.

Following the above conversation seeking informed consent from each patient or their surrogate decision maker and ensuring that this party understand the study objectives and procedures, we will obtain written permission on the combined consent-HIPAA authorization form for all patients for whom the parent/guardian is physically present. If the next of kin/power of attorney is not physically present, we seek a waiver of documentation of consent and HIPAA Authorization to obtain verbal consent, which will be documented on the standard consent form that will be read to subjects with a verbal consent documentation page. At any point during the study time period, patients will be able to withdrawal themselves from the study, as critically ill patients may regain capacity to make decisions regarding the study.

We will provide a copy of the combined consent-HIPAA authorization document to the party and patient and will write a note in the patient's medical record documenting the informed consent discussion.
If the next of kin/power of attorney consents to participate in the study, the next of kin/power of attorney and patient will be notified that any remaining blood samples may be stored in a coded fashion for possible use in future research. Next of kin/power of attorney and patients will have the opportunity to decline use of remaining blood samples for future research. All stored blood samples will be labeled only with a coded study identification number that will be linked to patient identifiers via a master list kept separate from these samples in the PI's locked electronic and/or paper files.

**Informed consent process for non-English speakers**
For non-English speaking next of kin/power of attorney and patients who speak fluent Spanish, Italian, Portuguese, Korean, Haitian Creole, Vietnamese, Chinese, Russian, and Arabic, an interpreter will assist in the informed consent discussion. The interpreter will be fluent in both English and the native language of the patient (next of kin/power of attorney) and may serve as the witness. Informed consent will be documented using the HUP standard short-form consent document in the appropriate native language and on a Study Summary Document written in English.

Speakers of languages other than English, Spanish, Italian, Portuguese, Korean, Haitian Creole, Vietnamese, Chinese, Russian, and Arabic will not be approached for informed consent until an appropriately translated consent form can be obtained (which may be submitted as a separate amendment).

**Payment to Subjects/Families**
No monetary or physical reimbursement will be provided to patients or their families for participation in this study. The cost of all study measurements will be covered by the investigational team.

**PUBLICATION**
The method and results of this study are expected to be presented at local and/or national/international scientific meetings and to be submitted for publication in a peer-reviewed journal. Only de-identified aggregate data will be published or presented.

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**LIST OF ABBREVIATIONS (alphabetical order)**

CABG: Coronary artery bypass graft
C1: Complex 1
CII: Complex II
CIII: Complex III
CIV: Complex IV
CI: Cardiac index
CO: Cardiac output
CPB: Cardiopulmonary bypass
CRF: Case report form
CVP: Central venous pressure
$DO_2$: Rate of oxygen delivery
ERAS: Early recovery after surgery
euroSCORE II: European System for Cardiac Operative Risk Evaluation
IDF: Incident dark field
LFTs: Liver function tests
MAP: Mean arterial pressure
MFI: Microcirculatory flow index
MHI: Microcirculatory heterogeneity index
PAP: Pulmonary artery pressure
PBMCs: Peripheral blood mononuclear cells
PPV: Proportion of perfused vessels
PVD: Perfused vessel density
RBCs: Red blood cells
SOFA: Sequential organ failure assessment
STS: Society of Thoracic Surgeons
SUIT: Substrate-uncoupler-inhibitor titration
$SvO_2$: Central venous oxygen saturation
TVD: Total vessel density
$VO_2$: Rate of oxygen consumption
VVFDs: Ventilator and vasopressor-free days