PROTOCOL

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This study protocol is organized in accordance with the recently published SPIRIT Guidelines, (1,2) with items corresponding to the SPIRIT 2013 Checklist. (1)

Administrative Information

1. Title: SQUEEZE Trial: a trial to determine whether septic shock reversal is quicker in pediatric patients randomized to an early goal directed fluid-sparing strategy vs. usual care (SQUEEZE)

2a. Trial Registry:
The trial will be registered at ClinicalTrials.gov: identifying number: NCT 03080038

2b. Items from the World Health Organization Trial Registration Data Set

Item	Description
1. Primary registry and trial-identifying number	Primary Registry: ClinicalTrials.gov Identifying Number: NCT 03080038
2. Date of registration in primary registry	Feb 28, 2017
3. Secondary identifying numbers	Clinical Trials Ontario Project #: 0833
4. Sources of monetary or material support	Monetary Support i) AFP AHSC Innovation Fund (HAHSO): $192,206 ii) Canadian Institute of Health Research Project Scheme Grant: $1,941,831 iii) Canadian Blood Services/Canadian Institutes of Health Research New Investigator Salary Award 2014-2019 (Award – Programatic Support including SQUEEZE: $300,000) iv) Canadian Child Health Clinician Scientist Program Award (Award – Programatic Support including SQUEEZE: $25,000)
5. Primary Sponsor	McMaster University
6. Contact for Public Queries	PI: Dr. Melissa Parker Associate Professor of Pediatrics, McMaster University Staff Physician, McMaster Children's Hospital 1280 Main St W, Room 3E-20 Hamilton, Ontario L8S4K1 Email: parkermj@mcmaster.ca Tel: (905) 521-2100 Ext 76651
7. Contact for Scientific Queries	PI: Dr. Melissa Parker Associate Professor of Pediatrics, McMaster University Staff Physician, McMaster Children's Hospital

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8. Public title	SQUEEZE Trial
9. Scientific title	SQUEEZE Trial: a trial to determine whether septic shock reversal is quicker in pediatric patients randomized to an early goal directed fluid-sparing strategy vs. usual care
10. Countries of recruitment	Canada
11. Health condition(s) or problem(s) studied	Pediatric Septic Shock
12. Intervention(s)	At all points, the caring physician is directed to target ACCM hemodynamic goals using the particular strategy to which the patient is allocated. 1. Usual Care Arm Tier 1: Following randomization, further fluid boluses may be liberally administered to treat persistent signs of shock. The need for and/or timing of initiation of vasoactive medication(s) is at the discretion of the treating physician, but vasoactive support should not be initiated until a minimum of 60 mL/kg (or 3 litres for participants ≥ 50 kg) of isotonic fluid bolus therapy [crystalloid (0.9% Normal Saline or Ringers Lactate) and/or colloid (5% Albumin)] has been administered (Includes fluid boluses received in the 6 hours prior to randomization). Tier 2: If vasoactive medication(s) are initiated, the decision to administer further isotonic fluid bolus therapy versus escalating vasoactive medication support to target achievement of recommended ACCM hemodynamic goals is at the discretion of the caring physician. No restrictions regarding volume or number of fluid boluses administered. Intervention end: When the patient is free from infusion of vasoactive medication support and shock is reversed. 2. Fluid Sparing Arm Tier 1: Vasoactive medication support should be initiated immediately following randomization for children with persistent signs of shock despite receiving a minimum of 40 mL/kg (or 2 litres for participants ≥ 50 kg) of isotonic fluid bolus therapy

	[crystalloid (0.9% Normal Saline or Ringers Lactate) or colloid (5% Albumin)] in the 6 hours prior to randomization. Tier 2: Once vasoactive medication(s) have been initiated, these should be preferentially titrated/escalated to target achievement of recommended ACCM hemodynamic goals. Further fluid bolus therapy should be provided only where intravascular hypovolemia is judged to be present in order to maintain adequate (but not excess) intravascular volume. Where further fluid bolus therapy is judged to be indicated, aliquots of 5-10 mL/kg (or 250-500 mL for participants ≥ 50 kg) of isotonic crystalloid or colloid can be given with the lowest acceptable volume preferred and the indication for administration documented. Intervention end: When the patient is free from vasoactive medication support and shock is reversed.
14. Key inclusion and exclusion criteria	Inclusion Criteria: 1.Age 29 days to <18 years of age * 2a. Persistent signs of shock defined as one or more of the following: Must select 'YES' in i), ii) or iii) for the patient to be eligible. i) Vasoactive Medication Dependence (need for vasoactive drug for hemodynamic support) ii) Hypotension (systolic and/or mean blood pressure < 5th percentile for age) iii) Abnormal Perfusion, defined as the presence of 2 or more of the following: abnormal capillary refill (CR < 1 second (flash) or CR ≥ 3 seconds (delayed), tachycardia (HR > 95th percentile for age), decreased level of consciousness, or decreased urine output). *2b. Suspected or confirmed septic shock *2c) Fluid Resuscitation Threshold Met. Patient has received within the previous 6 hours a minimum of: i) 40 mL/kg of isotonic crystalloid (0.9% Normal Saline or Ringer's Lactate), and/or colloid (5% albumin) as IV fluid bolus therapy for participants <50 kg. OR ii) 2 litres of isotonic crystalloid (0.9% Normal Saline or Ringer's Lactate), and/or colloid (5%

	albumin) as IV fluid bolus therapy for participants ≥50 kg. *Adapted from the International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. [1] Exclusion Criteria: i) Patient admitted to the Neonatal Intensive Care Unit (NICU) ii) Full active resuscitative treatment is not within the goals of care iii) Shock secondary to causes other than sepsis (i.e. obvious signs of cardiogenic shock, anaphylactic shock, hemorrhagic shock, spinal shock). iv) Patients requiring resuscitation in the Operating room or Post Anesthetic Care Unit. v) Previous enrolment in this trial, where known by the research team
15. Study type	Allocation: Randomized Blinding: Investigators, Research Staff, and Healthcare Providers are not blinded to participant assignment Assignment: Parallel group, 2 study arms Purpose: To determine which of the two resuscitation strategies results in the best outcome for infants and children treated for suspected septic shock. Phase: Phase III Trial Method of Sequence Generation: Computer Generated Allocation sequence Method of Allocation Concealment: Use of a Third party randomization technique
16. Date of First Enrolment	March 6, 2017
17. Target Sample Size	400 participants
18. Recruitment Status	Status of recruitment into definitive phase of trial: Enrolling. *Enrolling into pilot trial as of January 6, 2014. Pilot trial participants will be rolled in to final sample.
19 Primary Outcome(s)	SQUEEZE: Difference (in hours) in time to shock reversal between the two study groups. SQUEEZE-D: Predictive value of cfDNA to predict time to shock-reversal
20. Key Secondary Outcomes(s) Clinical Outcomes	SQUEEZE: 1. Outcomes related to clinical course, procedures and resource utilization e.g. PICU length of stay

	Organ dysfunction e.g. change in PELOD2 Score, Ventilator Free Days, Mortality 2. Adverse events related to fluid overload and vasoactive medications e.g. pulmonary edema, pleural effusion requiring drainage, abdominal compartment syndrome, signs of digital ischemia, revision amputation. SQUEEZE-D: Predictive value of cfDNA for 28-day mortality Predictive value of cfDNA for hospital mortality Correlation of cfDNA with change in PELOD2 score Determination of whether cfDNA predictive values are improved when combined with Protein C levels, platelet count and PELOD-2 scores

3. Protocol version and date: Version 6, 26-Jun-23

4. Funding

i) AFP AHSC Innovation Fund (HAHSO) 2016-2018: $192,206
ii) Canadian Institutes of Health Research Project Scheme Grant 2016-2020: $1,941,831
iii) Canadian Blood Services/Canadian Institutes of Health Research New Investigator Salary Award 2014-2019 (Award – Programatic Support including SQUEEZE: $300,000)
iv) Canadian Child Health Clinician Scientist Program CEP Award 2014-2019 (Award – Programatic Support including SQUEEZE: $25,000)

5a. Names, affiliations and roles of protocol contributors

Melissa Parker (PI) Dr. Parker is an Associate Professor of Pediatrics and Clinical Epidemiology and Biostatistics at McMaster University and a staff physician at McMaster Children's Hospital. Her primary area of affiliation and practice is the Pediatric Intensive Care Unit. She is also certified in Pediatric Emergency Medicine and practices in the Pediatric Emergency Department as part time staff. This protocol was developed by Dr. Parker with input and guidance from her Scientific Mentors.

Karen Choong (Co-I/Scientific Mentor) Dr. Choong is an Associate Professor of Pediatrics and Clinical Epidemiology and Biostatistics at McMaster University, and a staff physician at McMaster Children's Hospital. Her primary area of affiliation and practice is the Pediatric Intensive Care Unit. Dr. Choong contributed to development of this protocol and serves as a Scientific Mentor to Dr. Parker.

Lehana Thabane (Co-I/Scientific Mentor) is currently a Professor and Associate Chair in the Department of Clinical Epidemiology and Biostatistics, Director of the Biostatistics Unit at St. Joseph's Healthcare Hamilton, Director of the Biostatistics Services at Systems-Link Research Unit, Senior Scientist of the Population Health Research Institute (PHRI), Hamilton Health Sciences, McMaster University, Associate Member of the Departments of Pediatrics and Anesthesia. Dr. Thabane contributed to the development of this protocol and serves as a Scientific Mentor to Dr. Parker.

Alison Fox Robichaud (Co-I) is Associate Professor of Medicine and clinician

scientist/Intensivist with more than a decade of experience in sepsis basic research. She is the Lead Investigator for SQUEEZE-D.
Patricia Liaw (Co-I) is Associate Professor of Medicine and a member of the Thrombosis and Atherosclerosis Research Institute. Her research currently focuses on the role of cfDNA in thrombosis and inflammation with particular interest in critical illness and oncology.
Academy of Critical Care: Development, Evaluation and Methodology (ACCADEMY) This McMaster based critical care research group provided methodological input into the final protocol.
Canadian Critical Care Trials Group (CCCTG) This national critical care research group provided methodological input into the final protocol.
Pediatric Emergency Research Canada (PERC) This national pediatric emergency medicine research group provided methodological input into the final protocol.

5b. Trial Sponsor:

McMaster University
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5c. Role of Study Sponsor and Funders:

The study sponsor and funders have not been involved in the study design, and will not be involved in the collection, management, analysis, and interpretation of data. The study sponsor and funders will not be involved in the writing of the manuscript reporting study findings nor will they have any input into the decision to submit this for publication. The authority for overseeing trial conduct, analysis, interpretation and dissemination of findings will rest with the study investigators.

5d. Additional Roles and Responsibilities: Coordinating Centre: McMaster University.

Steering Committee (SC): The steering committee will consist of Drs. Parker (Principal Investigator and SC Chair) and the SQUEEZE Trial Co-Investigators [2]

End Point adjudication: We will adjudicate the time of resolution of septic shock, according to our study definitions.

Data management team: The members of the data management team include the investigators, the Research Staff, and the REDCap Super Administrator. Further details regarding data management and security and elaboration on the role of the REDCap Super Administrator are outlined under Item 19.

Research staff associated with the study:

SQUEEZE

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SQUEEZE-D
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Introduction

6a. Background, Rationale, and Research Question

Rationale:

Septic shock remains one of the most significant and potentially preventable causes of death in children world wide, with pediatric mortality rates ranging from 15-70%. [3,4] Current pediatric surviving sepsis guidelines [5] from the American College of Critical Care Medicine (ACCM) (Appendix 1) emphasize an early and goal-directed approach to resuscitation. [6-8] These guidelines suggest that fluid resuscitation should be aggressive with repeated intravenous (IV) fluid boluses of 20 mL/kg, such that some children may require as much as 200 mL/kg of fluid to achieve therapeutic endpoints. [4] The guidelines also recommend the initiation of vasoactive agents at the stage of "fluid refractory shock", i.e. when there is persistent hypoperfusion despite at least 60 ml/kg IV fluid. [5] Current evidence suggests that adhering to the resuscitation goals and guidelines of the ACCM may improve mortality and functional morbidity. [9,10] The fluid resuscitation guidelines from ACCM were derived primarily from observational studies and expert opinion. [5] Aggressive and ongoing fluid resuscitation in septic shock has recently been called into question. [11] Accumulating adult [12-15] and pediatric data [16-18] suggest that excessive fluid resuscitation in patients with septic shock is associated with increased morbidity and mortality. This has sparked a furious debate in both the adult and pediatric literature on how "aggressive" fluid resuscitation should be, given that the main morbidity and mortality in septic shock is due not to refractory hypotension, but end-organ failure due in part from massive fluid overload. The overall objective of our research programme is therefore to evaluate whether use of a more conservative fluid sparing strategy that involves the earlier initiation and escalation of vasoactive medications to achieve ACCM goal directed targets, results in improved clinical outcomes for children experiencing septic shock. The results of our research will enable us to support the ACCM guidelines with prospective trial evidence, and better define when the initiation of hemodynamic support should occur in order to optimize patient outcomes and survival in this devastating condition.

Relevant Literature:

The fundamental basis of resuscitation in septic shock is IV fluid with the rationale that volume expansion increases preload and thereby stroke volume, according to Frank-Startling principles. [19] Mortality in pediatric septic shock significantly improved since the introduction of rapid fluid resuscitation in the first "golden" hour of resuscitation. [7-10,20] Subsequent improvements in pediatric septic shock survival have been attributed to adherence to the first iteration of the ACCM septic shock guidelines, and the use of goal directed targets. [21,22] However, the largest and most

publicized pediatric trial of fluid resuscitation in children with suspected septic shock (FEAST Trial), published in NEJM in 2011, demonstrated an increased mortality among children treated with aggressive fluid resuscitation in comparison to the conservative fluid resuscitation arm. [16] These results sparked a flurry of commentaries and attempts to explain these unexpected findings. [23-25] The FEAST trial was conducted in sub-Saharan Africa, and enrolled a significant proportion of children with Malaria. As a result, the pediatric critical care community clearly acknowledges that these results, while important, are not necessarily generalizable to developed countries such as Canada. These results did, however, fuel further discussion and debate regarding the optimum fluid resuscitation in the course of goal directed therapy in septic shock. Emerging publications in the ICU literature suggest that excessive compared to conservative fluid administration in adults with septic shock worsens outcomes such as duration of mechanical ventilation, [18,26] complications related to the third-spacing of fluids, [27,28] length of ICU stay, [18,26] and mortality. [12-15] A systematic review published in August 2012 [15] reveals a paucity of randomized controlled trial (RCT) evidence other than the FEAST trial examining the impact of fluid resuscitation on mortality in children with septic shock. This raises the important question of whether children in developed countries would also benefit from a fluid sparing resuscitation strategy to achieve the ACCM goal-directed targets. Use of such a fluid sparing strategy would, by default, require earlier initiation and preferential escalation of vasoactive medications to meet ACCM hemodynamic goals. [5] There are potential adverse effects attributable to the earlier initiation of vasoactive medications that may outweigh those resulting from aggressive fluid administration strategy which is yet another justification for this study. [29-31] The optimal degree of fluid resuscitation and the timing of initiation of vasoactive support in order to achieve therapeutic targets in children with septic shock remains unanswered. No prospective study to date has examined this important question for children in developed countries including Canada.

Why is a trial needed now?

1) Early goal directed resuscitation in septic shock improves mortality and morbidity; 2) excessive fluid worsens morbidity and mortality in adults and children with septic shock; 3) the optimal extent of fluid resuscitation and the timing of initiation of vasoactive support to achieve goal-directed endpoints in septic shock in children is unclear. There is therefore significant rationale for a pediatric specific trial to determine if a fluid sparing strategy involving the earlier initiation and escalation of vasoactive support improves outcomes in infants and children with septic shock.

Overall Research Question:

In pediatric patients with septic shock, does a fluid sparing strategy to achieve ACCM therapeutic goals, result in improved clinical outcomes without an increased risk of adverse events, compared to the usual care of aggressive fluid resuscitation, as currently recommended by the ACCM guidelines.

Background for Nested Study: SQUEEZE-D

In adults, cell-free DNA (cfDNA) was predictive of mortality in a pilot study of patients with severe sepsis. [32] Drs. Fox-Robichaud and Liaw have completed an 800 patient observational validation study called DYNAMICS to determine the value of cfDNA levels in critical illness prognosis. SQUEEZE-D is the pediatric extension of this work. In addition to finding cfDNA levels increased and predictive of ICU mortality in adult patients with severe sepsis, levels of Protein C were found to be reduced.[32] The serial data from the study suggested that the

combination of Protein C, cfDNA and Multiorgan Dysfunction Syndrome (MODS) score may yield a stronger prognostic predictive power. We will therefore determine Protein C levels in SQUEEZE-D to determine if Protein C level enhances the predictive value of cfDNA in children for clinical outcomes of interest. The pediatric corollary of the MODS score is the PELOD score, and this is being collected in SQUEEZE trial participants.

Research Question for SQUEEZE-D:
Is plasma cell free DNA (cfDNA) in pediatric septic shock predictive of clinical outcome?

6b. Explanation for choice of comparators:

While the FEAST Trial demonstrated improved survival odds among children with hypoperfusion and suspected septic shock randomized to a) No Bolus compared to those randomized to b) Fluid Bolus, we do not believe that using a comparator of 'No Bolus' would be acceptable to practicing physicians in Canada (or likely in any other developed country setting). We do not think that 'No Bolus' would be acceptable to our physician colleagues who manage children with suspected septic shock (or to us for that matter) due to clear differences in the FEAST study population vs. children in Canada, combined with existing (although retrospective and low grade) evidence indicating that aggressive fluid resuscitation of children with septic shock is associated with improved survival odds. For this reason, the only sensible way to investigate the findings of the FEAST study in the Canadian context, while generating high quality RCT data, is to evaluate comparators of a 'fluid sparing strategy' vs. 'usual care – which is fluid liberal'. It is important to evaluate the signal from FEAST in Canadian children with septic shock because FEAST was a large, high quality, randomized controlled trial with good internal validity.

7. Objectives

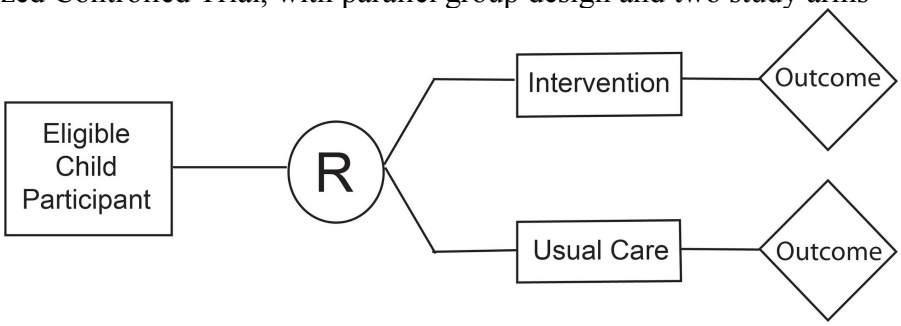
Primary Objective SQUEEZE Trial:
To determine whether time to shock-reversal is quicker in pediatric patients with septic shock treated with a Fluid Sparing resuscitation strategy vs. Usual Care.

Primary Objectives SQUEEZE-D:
To determine if plasma cell free DNA (cfDNA) in pediatric septic shock is predictive of clinical outcomes?

Secondary Objectives SQUEEZE Trial:
To determine whether a Fluid Sparing strategy impacts:
1. Clinical outcomes
2. Adverse events potentially attributable to fluid overload or inotrope/vasopressor use
3. Clinical course, resource use, and procedures
4. Health services outcomes

Secondary Objectives SQUEEZE-D:
1. To describe cfDNA levels in pediatric patients with septic shock in relation to participant baseline characteristics
2. To determine whether Protein C levels, platelet count, and organ dysfunction scores (PELOD-2) enhance the predictive value of cfDNA for clinical outcomes of interest.

8. Trial Design:
Pilot Randomized Controlled Trial, with parallel group design and two study arms



Rationale Study Design:
A randomized controlled trial design will provide the most robust data to determine which resuscitation approach leads to the best outcome. An initial pilot RCT has been conducted to determine the feasibility of and inform the appropriate methodological design of the larger multi-centre RCT to fully answer our research question. [33,34] The pilot trial protocol has been well received and few changes have been required. We therefore plan to roll in the pilot trial participants into the definitive trial sample.

Methods

9. Study Setting:
Pediatric Tertiary Care Hospitals. The Canadian sites listed below are planned to participate (pending REB approvals) in the multicenter phase of the trial. We will consider adding additional sites to expedite participant accrual to our target sample size should additional resources become available.

McMaster Children's Hospital (Lead Site; Hamilton, Ontario)
The Hospital for Sick Children (Toronto, Ontario)
Children's Hospital of Western Ontario (London, Ontario)
Winnipeg Children's Hospital (Winnipeg, Manitoba)
Alberta Children's Hospital (Calgary, Alberta)
Stollery Children's Health Centre (Edmonton, Alberta)
CHU Sainte-Justine (Montreal, Quebec)
Centre hospitalier de l'Université Laval (Quebec City, Quebec)

10. Eligibility Criteria:
Patients presenting to the Emergency Department, or admitted to an in-patient ward (including the Critical or Intensive Care Unit) with the following criteria:

Inclusion Criteria:
1.Age 29 days to <18 years of age

* 2a. Persistent signs of shock defined as one or more of the following:
Must select 'YES' in i) or ii) or iii) for the patient to be eligible.

i) Vasoactive Medication Dependence (need for vasoactive drug for hemodynamic support)
ii) Hypotension (systolic and/or mean blood pressure < 5th percentile for age)
iii) Abnormal Perfusion, defined as the presence of 2 or more of the following: abnormal capillary refill [CR < 1 second (flash) or CR ≥ 3 seconds (delayed)], tachycardia (HR > 95th percentile for age), decreased level of consciousness, or decreased urine output).

*2b. Suspected or confirmed septic shock

*2c) Fluid Resuscitation Threshold Met. Patient has received within the previous 6 hours a minimum of:
i) δ 40 mL/kg of isotonic crystalloid (0.9% Normal Saline or Ringer's Lactate), and/or colloid (5% albumin) as IV fluid bolus therapy for participants <50 kg.
OR
ii) 2 litres of isotonic crystalloid (0.9% Normal Saline or Ringer's Lactate), and/or colloid (5% albumin) as IV fluid bolus therapy for participants ≥50 kg.
*Adapted from the International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. [3]
δ Based on the adult surviving sepsis guideline initial targets for fluid resuscitation (35)

Exclusion Criteria:

i) Patient admitted to the Neonatal Intensive Care Unit (NICU)
ii) Full active resuscitative treatment is not within the goals of care
iii) Shock secondary to causes other than sepsis (i.e. obvious signs of cardiogenic shock, anaphylactic shock, hemorrhagic shock, spinal shock).
iv) Patients requiring resuscitation in the Operating room or Post Anesthetic Care Unit.
v) Previous enrolment in this trial, where known by the research team

11a. Interventions for each group with sufficient detail to allow replication, including how and when they will be administered:

Patients will be randomized to one of two study arms: 1) Usual Care arm, or 2) Fluid Sparing arm. Appendix 2 provides an illustration of the two study arms in ACCM guideline format. For all participants, care providers will be provided with a copy of the hemodynamic goals as specified in the ACCM Surviving Sepsis Guidelines and instructed that they should escalate treatment according to the intervention assigned in order to achieve these.

The Goal Directed Targets from the ACCM Guidelines are as follows:

Initial Goals (Often, but not exclusively in the Emergency Department setting)
Initial therapies should be directed toward restoring:

- Normal Mental Status
- Threshold Heart Rates
- Peripheral perfusion (capillary refill < 3secs)
- Palpable distal pulses
- Normal blood pressure for age.

Age Range	Threshold Heart Rates	*Threshold Perfusion Pressure MAP-CVP (mm Hg)
Term Newborn	120-180	55
Up to 1 year	120-180	60
Up to 2 years	120-180	65
Up to 7 years	100-140	65
Up to 15 years	90-140	65

*Perfusion Pressure = Mean Arterial Pressure (MAP) – Central Venous Pressure
*From a practical perspective, perfusion pressure data is frequently unavailable for children during the initial phase of septic shock resuscitation due to lack of presence of an arterial line and/or central venous catheter. Thus, initial goals of resuscitation may be limited to the other parameters outlined until invasive monitoring is available.

Subsequent Goals (Intensive Care Unit) Phase
Shock should be further evaluated and resuscitation treatment guided by hemodynamic variables:
Monitor CVP in PICU (ensure adequate preload)
Maintain Adequate Perfusion Pressure: Normal MAP-CVP
Maintain Oxygen Delivery: Mixed Venous Oxygen Saturation [SCVO2] > 70%
Maintain Oxygen Carrying Capacity: Hemoglobin Concentration > 10 g/dL

***Note: While clinicians are advised to follow the ACCM guidelines and to strive to achieve recommended goal directed targets, as a pragmatic trial strict guideline adherence is not required. The adequacy of resuscitation and end organ perfusion ultimately relies on clinician judgement.

Intervention Tier	Usual Care Arm	Fluid Sparing Arm
Tier 1	Usual Care	Early Initiation of Vasoactive Medications to Spare Fluid
[c]Bolus [a]Fluid Therapy	• Following randomization, further isotonic fluid bolus therapy [crystalloid (0.9% Normal Saline or Ringers Lactate) or colloid (5% Albumin)] may be administered in any volume and as requested by the caring physician	• Following randomization, further isotonic fluid bolus therapy [crystalloid (0.9% Normal Saline or Ringers Lactate) or colloid (5% Albumin)] should be avoided and provided only if required due to 1. Delay in the ability to immediately initiate vasoactive medication(s) and/or 2. To treat intravascular hypovolemia. The Reason/indication for administration of further fluid bolus therapy prior to initiation of vasoactive medications must be documented.
[b]Vasoactive Medication	• The decision to initiate vasoactive medication(s) is at the discretion of the treating	• Vasoactive medication(s) should be initiated immediately following randomization.

	physician. Vasoactive support should not be started until the participant has received a minimum of 60 mL/kg (3 litres for participants ≥ 50 kg) of isotonic fluid as boluses (Includes fluid boluses received in the 6 hours prior to randomization). • The choice of initial vasoactive medication and the initial dose is to be at the discretion of the caring physician	• The choice of initial vasoactive medication and the initial dose is to be at the discretion of the caring physician
Tier 2 [c]Bolus [a]Fluid Therapy	Usual Care • Further isotonic fluid bolus therapy may be administered at the discretion of the caring physician • The type and dose of any further isotonic fluid bolus therapy is at the discretion of the caring physician	Preferential Escalation of Vasoactive medications • Further isotonic fluid bolus therapy may be administered by the caring physician to treat documented inadequate intravascular filling/preload • If further isotonic fluid bolus therapy is provided, the DOSE provided should be in 5-10 mL/kg aliquots (250-500 mL for participants ≥ 50 kg) with the lowest acceptable volume preferred and the indication for administration documented. • Aliquots of isotonic fluid bolus therapy may be administered "back-to-back" if required to address inadequate intravascular volume status • The TYPE of Isotonic fluid bolus therapy provided is at the discretion of the caring physician
[b]Vasoactive Medication	• If initiated, vasoactive medication(s) may be titrated (increased, decreased, or discontinued) at the discretion of the caring physician • Additional vasoactive medication(s) may be initiated at the discretion of the caring physician	• Escalation of Vasoactive medications should be the first line to achieve hemodynamic goals (provided intravascular volume status is judged to be adequate) • The initiated vasoactive medication(s) may be titrated (increased, decreased, or discontinued) at the discretion of

		the caring physician • Additional vasoactive medication(s) may be initiated at the discretion of the caring physician
Intervention End	•When the patient is free from vasoactive medication support and shock is reversed OR the patient is placed on mechanical circulatory support e.g. ECMO OR Death occurs.	•When the patient is free from vasoactive medication support and shock is reversed OR the patient is placed on mechanical circulatory support e.g. ECMO OR Death occurs.

a. Fluid Therapy: Isotonic Crystalloid or Colloid solutions which include 0.9% Normal Saline, Ringers Lactate, and 5% Albumin.
b. Vasoactive Medications are administered by intravascular (IV or IO) infusion and include: Dobutamine, Dopamine, Epinephrine, Norepinephrine, Vasopressin, Phenylephrine, Milrinone
c. Bolus: A (fluid) bolus is a discrete volume of fluid prescribed to be administered intravascularly (IV or IO) over a defined period of time (ranging from STAT i.e. as fast as possible to *typically* no greater than 60 minutes). A fluid bolus *typically* ranges in size from usually not less than 5 mL/kg (250 mL for participants ≥ 50 kg) to 20 mL/kg (1 litre for participants ≥ 50 kg, although some clinicians may use per kilogram dosing in larger patients). A documented medical order is required for a fluid bolus. Routine fluid replacement is not considered to be bolus(es).

Note: We will track but not direct how other therapies that may be provided as part of the management of patients with septic shock are administered e.g. blood products other than 5% albumin, corticosteroids, dialysis.

11b. Criteria for Discontinuing or Modifying Allocated Interventions for a Given Trial Participant:

We will allow for exit criteria from the study protocol as follows:
i) Participant or their Substitute Decision Maker (SDM) withdraws consent for ongoing study participation in discussion with a member of the research team. Any requests for study withdrawal should be referred to the site Principal Investigator or their delegate (other site investigator, Research Coordinator) for discussion. Reasons for study withdrawal provided by the participant or their SDM should be documented if this occurs.
ii) Change in the medical goals of care for a study participant e.g. decision to limit escalation of resuscitative therapies and/or withdrawal of life sustaining supportive measures. Where new limitations on the goals of care do not impact upon protocol adherence e.g. decision for a one-way extubation, study participation may continue if the participant or SDM wishes.
iii) Confirmatory evidence that the participant is suffering from another form of shock other than septic shock e.g. occult hemorrhage. Diagnosis of hemorrhagic shock would indicate the need to switch to a different resuscitative management strategy.
iv) The Most Responsible Physician (MRP) believes that ongoing patient management according to the assigned intervention will lead to patient harm. In this instance, the MRP should contact a member of the Research Team to discuss their specific concerns. Where such a discussion results

in agreement to withdraw the participant from the study, clear and objective medical reason(s) for this should be recorded.

11c. Strategies to Improve Adherence to Intervention Protocols, and any Procedures for Monitoring Adherence:

For study participants, we will post an alert on the front of the medical chart advising their enrolment in the study as well as the assigned intervention. We will also post a sign indicating the assigned intervention in the participant's room e.g. at the head of the bed. The participant's chart will be reviewed on a daily basis to collect data regarding the size of any prescribed fluid boluses, and to collect data regarding trigger(s) for fluid bolus administration. Data regarding the use and titration of any vasoactive medication infusions, including triggers for initiation or escalation will also be collected. This data will be reviewed in light of the protocol for the assigned intervention and any protocol deviations along with the associated reasoning will be documented. The importance of protocol adherence will be routinely reinforced by members of the study team.

11d. Relevant Concomitant Care and Interventions that are Permitted or Prohibited during the Trial:

There will be no restrictions with respect to concomitant care and interventions. Any such interventions should be provided at the discretion of the responsible medical team in accordance with the current ACCM guidelines.

12. Study Outcomes

Outcome	Analysis
SQUEEZE Primary Time to Shock Reversal (in hours)	t-test
SQUEEZE-D Primary Predictive value of cfDNA for time to shock-reversal (in hours)	ROC curve
SQUEEZE Secondary **1. Clinical Outcomes.**	
Ventilator Free Days	Chi-square test
Peak PELOD-2 Score	t-test
Change in PELOD-2 Score	t-test
Acute Kidney Injury	Chi-squared test
Length of PICU Stay	t-test
Length of Hospital Stay	t-test
Mortality (28-day)	Chi-squared test
Mortality (90-day)	Chi-squared test
Mortality (Hospital Mortality)	Chi-squared test
2. Adverse Events *Complications possibly attributable to fluid overload or third spacing of fluid during the intervention period except where otherwise specified*	
Pleural effusion requiring drainage	Chi-squared test
Intra-abdominal hypertension	Chi-squared test

Highest Bladder Pressure	t-test
Abdominal Compartment Syndrome	Chi-squared test
Soft Tissue Edema	Median Rank
Pulmonary Edema	Chi-square test
Total Lasix Exposure (mg/kg) – from enrolment until 7 days after shock-reversal	t-test
Maximum Daily Lasix Exposure (mg/kg) - from enrolment until 7 days after shock-reversal	t-test
Other Diuretics Used - from enrolment until 7 days after shock-reversal	Chi-square test
Complications possibly attributable to inotrope/vasopressor use	
Clinical signs of digital soft tissue ischemia	Chi-square test
Digital ischemia requiring revision amputation – censored at the earliest of hospital discharge or 90 days following enrolment.	Chi-square test
Clinical signs of compromised bowel perfusion as determined by the pediatric surgical consultation service	Chi-square test
3. Descriptive Information Regarding Clinical Course and Procedures	
Invasive Mechanical Ventilation	Chi-square test
Dialysis	Chi-square test
Arterial Line placement	Chi-square test
Central Line placement	Chi-square test
Chest tube	Chi-square test
Peritoneal Drain	Chi-square test
Any form of mechanical circulatory support e.g. ECMO during the intervention period to treat refractory shock?	Chi-square test Descriptive
Positive cultures	Descriptive
Cause of Death	Descriptive
4. Health Services Outcomes	
PICU admission rate	Chi-square test
<u>SQUEEZE-D Secondary</u>	
Predictive value of cfDNA to predict 28-day mortality	ROC Curve
Predictive value of cfDNA to predict hospital mortality	ROC Curve
Correlation of cfDNA with PELOD-2 score	Correlation
Predictive values of cfDNA when combined with Protein C level, platelet count and organ dysfunction scores (PELOD-2) for clinical outcomes of interest: time to shock reversal, 28-day mortality, hospital mortality.	ROC Curves

Ascertainment of Time to Shock Reversal – measured in hours.

Where a participant is placed on ECMO or death occurs during the intervention phase, shock will be treated as never reversed.

Time zero: Allocation time.
The intervention should be begun as soon as possible and within 1 hour of allocation. Allocation time will be obtained in a consistent manner e.g. from the methods centre, and we will therefore use this as time zero.

Shock Reversal:
When a patient has achieved all of the following in the absence of mechanical circulatory support:
1) Free from all vasoactive medication support
2) Normalization of HR (less than the 95th percentile for age)
3) Normalization of Blood Pressure (SBP and MBP greater than the 5th percentile for age)
4) Normalization of Capillary refill (<3 seconds).
These outcomes will be assessed and documented in the flowsheet of the medical record by the assigned bedside nursing staff at least every 4 hours, in keeping with routine assessment of patient vital signs. Where there is clear and reliable documentation in the medical record that a participant's baseline vital sign(s) deviate from normal age-expected values then return to baseline value(s) will be the endpoint for determining when shock is reversed.

Measurement of cfDNA and Protein C (SQUEEZE-D)
Our plan is to collect samples using CPT tubes (2mL volume) as the source of plasma for cfDNA and Protein C determination. The cfDNA and Protein C levels will be measured in the laboratory of Drs. Fox-Robichaud and Liaw at the Thrombosis and Atherosclerosis Research Institute. Please see Item 33 (Biological Specimens) for further details on specimen handling, storage, and diagnostic testing.

Baseline, demographic, and other data to be collected for descriptive purposes

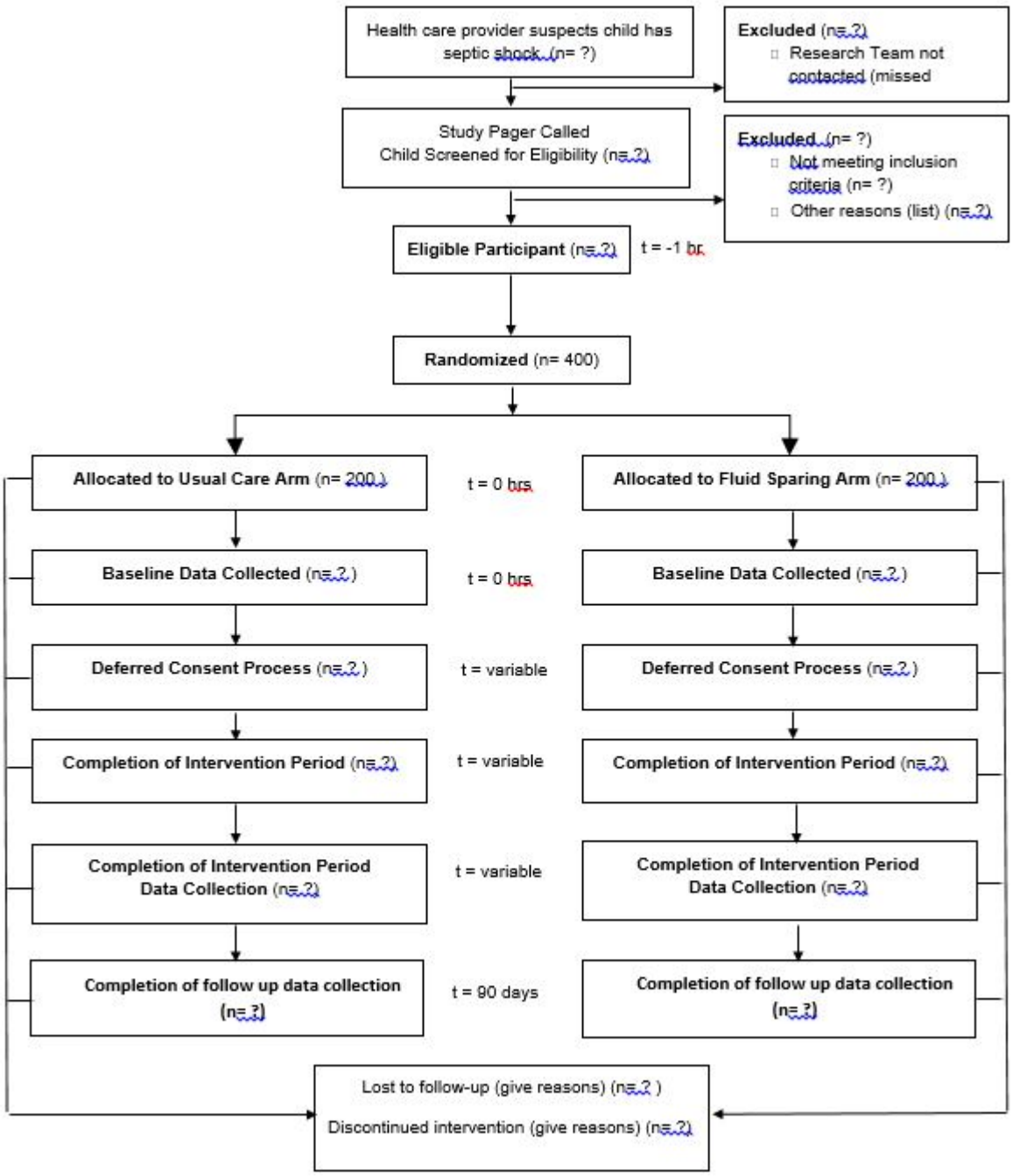
Demographic data	**Aggregation**
Age in Months	Mean (sd)
Gender (male or female)	Simple proportion
Weight (in kilograms)	Mean (sd)
Admission diagnosis to hospital	Descriptive
Location Patient met Eligibility Criteria (ER, Hospital Ward, PCCU)	Simple proportion
Patient arrived at your site within the past 48 hours (Y/N)	
If yes, select: a) Patient presented from home OR b) Patient	Simple proportion
transferred in from another medical facility	Simple proportion
Admission PRISM IV Score	Mean (sd)
Month of Presentation	Descriptive
Year of Presentation	Descriptive
Hospital ID Number (delinked from other data collected)	Not Applicable
Previously Diagnosed Medical Co-morbidities	Simple proportion
Baseline data	
Heart Rate	Mean (sd)
Systolic Blood Pressure	Mean (sd)
Diastolic Blood Pressure	Mean (sd)
Mean Blood Pressure	Mean (sd)
Capillary refill Time	Mean (sd)
Mental Status at Baseline	Descriptive

Respiratory Rate	Mean (sd)
SpO2	Mean (sd)
Body Temperature	Mean (sd)
pH	Mean (sd)
Lactate	Mean (sd)
HCO3	Mean (sd)
Glucose	Mean (sd)
Potassium	Mean (sd)
Documented positive test for Malaria	Descriptive
Cardiac Dysfunction	Descriptive
Acute Kidney Injury	Descriptive
Sodium	Mean (sd)
Chloride	Mean (sd)
Data to characterize fluid intake, output, and fluid balance	
Information Related to Fluid Administration and Fluid Balance for Participants for the 24 hour Period Immediately Prior to Randomization and every 12 hours During the Intervention Period	
Total fluid intake as IV maintenance fluids	Mean (sd)
Total fluid intake as IV Total Parenteral Nutrition	Mean (sd)
Total fluid intake as fluid bolus therapy	Mean (sd)
Total fluid intake as enteral fluid and nutrition	Mean (sd)
Total fluid intake as blood products	Mean (sd)
Total fluid intake as IV Medication administration	Mean (sd)
Total fluid intake as scheduled IV fluid replacement	Mean (sd)
Actual total fluid intake	Mean (sd)
Total fluids in operating room	Mean (sd)
Fluid Bolus Therapy data for each event	
Fluid Bolus Volume (mL/kg)	Mean (sd)
Fluid Bolus Type (NS, RL, or 5% Albumin)	Descriptive
Justification for Fluid Bolus (for fluid sparing)	Descriptive
Fluid Losses or Removal	
Urine output (ml/kg/hr)	Mean (sd)
Output from Drains (ml/kg/hr)	Mean (sd)
Dialysis (net fluid removal)	Mean (sd)
Losses as Vomit/Stool	Mean (sd)
Central Venous Pressure and Related Data	
Highest CVP	Mean (sd)
Lowest CVP	Mean (sd)
Central Venous Catheter Location	Descriptive
Highest Mean Airway Pressure	Mean (sd)
Data to characterize inotrope/vasopressor use	
First Inotrope/Vasopressor Used	Simple proportion
Documentation of Vasoactive Medications and doses every 12 hrs until discontinued and shock reversed	Descriptive
Vasoactive Medication(s) used and highest dose	Descriptive
Highest Vasoactive Medication Score	Mean (sd)

Other treatment and clinical variables	
Antimicrobials received	Descriptive
IV Steroid Administration	Simple proportion
Echocardiography performed during the intervention period?	Simple proportion
Site of Infection	Descriptive
Laboratory Data	
Collected daily during the intervention period	
Neutrophils	Mean (sd)
Lymphocytes	Mean (sd)
Platelet Count	Mean (sd)
Band Count	Mean (sd)
Lowest pH value	Mean (sd)
HC03 value corresponding to lowest pH value	Mean (sd)
Lowest Hemoglobin	Mean (sd)
Lowest Fibrinogen	Mean (sd)
Highest Sodium during intervention period*	Mean (sd)
Highest Chloride during intervention period*	Mean (sd)

*Collected only once during intervention period

13. Participant Timeline



14. Sample Size:
400 subjects (200 per arm) are required for a definitive multicenter trial to detect a 30% difference in the time to shock reversal based on two-sided t test, type I error (α) at 0.05 and power at 80%. Based on pilot trial estimates, a 30% difference for the time in shock corresponds to 15 hrs, a clinically important time difference as patients in shock require intensive care monitoring that is costly and resource intensive. We estimate trial recruitment can be completed within 30 months based on a conservative estimate of 8 sites enrolling 1.5subject/site/month and roll-in of pilot trial participants.

15. Screening and Recruitment:
Patients will be screened for eligibility from the following patient care areas: Emergency Department, Medical and Surgical Wards, and the Pediatric Critical Care Unit (PCCU). Posters and information sessions will be used to promote the study to physician and nursing staff, and pediatric postgraduate trainees. The research coordinator and/or Site Investigator (PI) will be paged for any potentially eligible patient, e.g. suspected sepsis, receiving a fluid bolus. If/when a patient is screened and determined to meet study eligibility criteria, the randomization procedures will be executed immediately and the patient enrolled. This trial will use a deferred consent model, due to the inability to obtain informed consent in the resuscitative setting and by virtue of the time sensitive nature of initiation of the intervention (see Ethical Considerations).

16a. Allocation Sequence Generation:
The allocation sequence will be computer generated (REDCap).

16b. Allocation Concealment Mechanism:
The allocation sequence will be implemented through a third party computer-based process accessible on a 24 hour basis.

16c. Responsibility for Allocation Sequence Generation, Participant Enrolment, Assignment of Interventions:
The allocation sequence will be computer generated (REDCap) according to parameters input by the Biostatistics Unit and this information will be kept secret from the investigators. A Research Assistant or one of the site investigators will enroll participants into the study and they will also be responsible for communicating the assignment of interventions.

17a. Blinding:
The investigators, research staff, and treating health care providers will all be blinded to the allocation sequence. It will not be possible to blind the investigators, treating physicians, or bedside nursing staff from participant treatment assignment as these individuals will need to be aware of and operationalize the intervention. The data analysts will be blinded to treatment assignment through use of a numeric code in the database.

17b. Procedures for Unblinding:
This is not applicable as the investigators, bedside clinical staff, and participants will not be blinded to the assigned intervention.

18a. Data Collection Methods:
Participant demographic data and SQUEEZE outcome data will be collected from the hospital chart by a Research Assistant or one of the investigators, all of whom will be trained in use of the data collection forms. Dr. Fox-Robichaud will be responsible for oversight of SQUEEZE-D outcome data collection.

18b. Activities to Promote Participant Retention and Follow-up:
We do not anticipate difficulties with participant follow-up given that those enrolled will be receiving close monitoring and management for suspected septic shock. It is expected that all of these patients will be admitted to hospital, with many admitted to the Pediatric Intensive Care Unit. Data collected prior to approaching participants/SDMs for written consent to continue study participation will be retained for all participants enrolled in the trial, including for those where consent for ongoing participation is declined. Where a participant experiences a deviation from the assigned protocol, this information including the reason for this will be noted and data collection will otherwise continue according to protocol. All cause hospital mortality for all enrolled subjects will be collected and this information will be available from hospital electronic medical records.

19. REDCap data management program, data location, and data security considerations:
(Research Electronic Data Capture) is a secure web application for building and managing online surveys and databases (www.project-redcap.org). (36) As in several Canadian pediatric academic centers, REDCap has been setup in the Department of Pediatrics at McMaster University to support Faculty members' research. Data is stored on a secure, firewall protected server with regular backup in the Faculty of Health Sciences Computer Services Unit with only the https port available to the internet. Data can be entered by designated users or survey respondents from any computer with an internet connection. The main role within the REDCap system that controls the set-up of projects is the Super Administrator; only one person in the Department of Pediatrics at McMaster University is designated as a 'Super Admin'. Together with the PI, this person also establishes user accounts and user rights for the research team, which are customized for each study. User accounts include electronic signatures comprised of a username and password and an audit trail is generated for all activity within each REDCap project. The Computer Services Unit has access to the database, however this is only for the purposes of IT support including regular server maintenance and software updates.

SQUEEZE-D outcome data will be stored in the Team sepsis database, which is located at the Thrombosis and Atherosclerosis Research Institute (TaARI).

Data entry and quality considerations:
Data entry will be performed by the trained research staff. Remote monitoring of data fields will be performed and range checks will be used for data values to aid in the detection of any errors.

20a. Statistical methods for analyzing primary and secondary outcomes:

The process of subject selection and flow throughout the study will be summarized using a flow diagram. Baseline characteristics will be reported as mean (standard deviation) or median (interquartile range) for continuous variables, and count (percent) for categorical variables. Continuous and dichotomous outcomes will be analyzed using two group t-test or logistic regression respectively. The statistical significance will be set at $\alpha \leq 0.05$. The intention-to-treat principle will be used to guide the analysis. For all analyses statistical significance will be set at alpha = 0.05. We will adopt the CONSORT guidelines for reporting of RCT results and an intention-to-treat principle to analyze all outcomes. [37, 38]

For SQUEEZE-D, Baseline characteristics and cfDNA and Protein C will be summarized by descriptive statistics. ROC curves will be generated to determine the predictive value of cfDNA to predict length of time in shock and mortality outcomes. ROC curves will also be generated to determine the utility of selected variables (Protein C levels, platelet count, organ dysfunction scored (PELOD-2 score)) to improve the predictive value of cfDNA for clinical outcomes of interest (length of time in shock, mortality outcomes).

20b. Methods for any Additional Analyses:

1. We will conduct exploratory analysis to assess for association between location of participant eligibility and study outcomes.
    a. Emergency Department location vs other hospital location

2. We will conduct exploratory analyses to assess for association between volume of isotonic fluid bolus therapy [crystalloid (0.9% Normal Saline or Ringers Lactate) and/or colloid (5% Albumin)] received in the 24 hours prior to randomization and study outcomes.
Subgroups of interest include:
a. Participants who received ≤60 mL/kg as fluid bolus therapy prior to randomization
b. Participants who received ≤80 mL/kg as fluid bolus therapy prior to randomization

20c. Analysis Population and Plans for Handling of Missing Data:

We will adopt an intention-to-treat principle to analyze all outcomes. Imputation of missing data will be utilized in the analysis.

21a. Data Safety and Monitoring Board:

A Data Safety and Monitoring Board (DSMB) will be struck as is required for studies involving vulnerable populations including children. The steering committee will report serious adverse events to the DSMB, and the DSMB will perform blinded interim analyses for safety at pre-specified recruitment milestones during the trial.

21b. Description of any Interim Analyses and Stopping Rules:

The DSMB will perform two blinded interim analyses for safety at the following recruitment milestones: n=200, ~50% accrual; and n=320, ~80% accrual. Safety will be assessed based on the differences in mortality and revision amputation between the two groups.

22. Harms:

The number and type of serious adverse events (SAE) that occur during the intervention period

will be monitored by the trial Steering Committee. The published complications that can normally occur as a result of septic shock and/or its treatment include: refractory shock, organ dysfunction, multiorgan failure, respiratory failure precipitating a need for mechanical ventilation, renal failure precipitating a need for dialysis, blood derangement(s) precipitating a need for administration of blood products, disseminated intravascular coagulation with resulting complications related to bleeding and/or thrombosis, digital/limb ischemia with resultant tissue necrosis that may require revision amputation, cardiac arrhythmias, cardiac arrest, and death. Patients may also experience morbidity related to fluid overload and/or medications, including but not limited to vasoactive medication infusion(s) that are frequently required as part of routine septic shock management. The vast majority of children in North America now survive septic shock: they may fully recover OR they may be left with residual/permanent disability, which may be severe. The decision as to whether an adverse event (AE) experienced by a trial participant is serious will be at the discretion of the PI. The PI, in consultation with other members of the Steering Committee, will judge whether any identified SAE is attributable to the patient's underlying condition(s) or whether this should be attributed to trial interventions/procedures. All SAE's along with the Steering Committee's interpretation of attribution, will be reported to the Research Ethics Board and the DSMB.

23. Auditing:

There are no planned audits for this trial. Our study may be subjected to audit by the Research Ethics Board(s) of participating sites. As a clinical trial, our study may also be subjected to audit by Health Canada.

Ethics and Dissemination

24. Plans for Seeking Research Ethics Board Approval:

Approval has been obtained from the Hamilton Integrated Research Ethics Board to conduct this trial. Research Ethics Board approval must be obtained for trial participation at a given site prior to enrolment of any participants at that site.

25. Plans for Communicating Protocol Modifications:

Any modifications to trial procedures must be communicated to, reviewed by, and approved by the Hamilton Integrated Research Ethics Board, as well as the Research Ethics Boards providing oversight for of any other participating sites through a formal amendment request. The ClinicalTrials.gov website, where this trial has been registered, will be updated to reflect any such modifications to the trial protocol. The Principal Investigator will be responsible for communicating approved changes in the trial protocol to the Research Coordinator and other site investigators. Where relevant/applicable, such changes will be communicated to physicians, nurses, postgraduate trainees and other research staff at participating sites who are involved in the identification of potentially eligible subjects and implementation of the allocated treatment assignment.

26a. Consent and Assent Procedures:

Given that pediatric septic shock is a recognized medical emergency and that these patients require prompt and active resuscitation, and given that our study will evaluate a time-sensitive resuscitation protocol we plan to use a process of deferred consent in order to achieve timely

enrolment, randomization, and initiation of study procedures. Use of a deferred consent process for research evaluating treatment of emergency conditions has precedent and is supported in the TCPS2 (Chapter 3, Section 8). [40] A deferred consent process has been previously approved and employed in other Canadian [41,42] and international resuscitation trials, [43] including those evaluating fluid therapy in patients experiencing septic shock. [41,43] A deferred consent approach has been feasible to implement and well received in the SQUEEZE Pilot Trial. Use of deferred consent to conduct this study is ethical because some research cannot be conducted without use of a deferred consent model. [40]

At the earliest appropriate opportunity, we will inform the participant or the SDM for the participant (where the participant is incapable of consent) that they have been enrolled into a trial through providing a one-page 'Information Sheet for Parents and/or Legal Guardians'. This document briefly outlines the nature of the study, the name of the Principal Investigator, the Research Ethics Board study status, and states that a time will be arranged to discuss the study in detail with study staff. The timing of discussions regarding the nature of the study is important to allow for the effective provision of information and optimizing the likelihood of true comprehension of the potential risks and benefits associated with ongoing study participation, which is critical for consent to be truly fully informed.

At the earliest appropriate opportunity, as determined based on ongoing communication between the research team and the Most Responsible Physician (or their delegate) and the nurse responsible for the patient, the Research Coordinator or one of the Study Investigators will approach the participant or SDM to provide information about the study and seek consent for ongoing participation. All efforts will be made to have the informed consent process conducted by someone not concurrently responsible for the medical care of the child (Site investigators could potentially find themselves in this position). It will be made clear that ongoing study participation is voluntary and any decision regarding further participation will not influence the medical care provided. Participants who are incapable of providing consent will be approached for assent to ongoing study participation if/when they are able to communicate verbally or non-verbally with the Research Coordinator or one of the study Investigators. Similarly, we will also monitor participants on an ongoing basis for signs of dissent. Dissent will be somewhat difficult to gauge given the nature of this study, because the study intervention consists primarily of treatment that would be otherwise administered as part of pediatric septic shock management e.g. withdrawal from the study would not preclude the need for administration of intravenous fluid and/or vasoactive medications, but may impact how these are administered/escalated. SQUEEZE-D is embedded within SQUEEZE and does not require any additional testing or bloodwork apart from that which occurs naturally as part of clinical care based on established guidelines. Please see item 33 for further details concerning this translational research.

Where a participant or SDM indicates that they are considering withdrawing from trial participation, an approach consistent with that outlined in Appendix 3 is recommended to allow the site investigator an opportunity to answer any remaining questions. This process also provides follow-up and support for members of the healthcare team who facilitated participant enrolment and protocol implementation.

In the event that a participant dies before full informed consent discussions can occur, the parent/legal guardian will be notified of their child's enrolment by the Most Responsible Physician (where the Information Sheet for Parents and/or Legal Guardians has not already been provided) and/or permission will be sought for the Site Principal Investigator to contact the parent/legal guardian at a time of their preference to discuss the study in detail. Following on any such discussions, the Site Principal Investigator will document on the study consent form the

parent/legal guardian's wishes with respect to use of their child's data, any collected biological specimens, and any further research-related contact. Where the parent/legal guardian does not wish to be contacted by the Site Principal Investigator to discuss the study, further data collection will cease and any collected biological specimens will be destroyed, however data collected up until this point will be retained.

26b. Additional Consent Provisions: Not applicable.

27. Plans for Collection and Use of Personal Health Information:
Collection of participant identifiers will be limited to those determined to be necessary for trial purposes. Participants will be assigned a unique identifying code number, with participant identifiers kept separate from other trial data collected.

28. Declaration of Financial and Other Competing Interests:
Melissa Parker – none
Karen Choong – none
Lehana Thabane – none
Alison Fox-Robichaud – none
Patricia Liaw – none

29. Access to Data:
It is the intent of the trial investigators to eventually make the final trial data set publicly available, once doing so will not interfere with their scientific interests.

30. Ancillary and post-trial medical care:
Ancillary and post-trial medical care will be dictated by the medical status of any given participant and determined by the most responsible physician (MRP) in charge of their clinical care and arranging suitable medical follow-up. It is unlikely that participants in this trial will suffer harm attributable to trial participation. However, in the trial information and consent form, we advise participants and/or their SDMs that should they have questions or concerns regarding the participant's rights in relation to the study, they may wish to contact an independent health and disability advocate.

31a. Communication of Trial Results:
Plans for communication of trial results include presentation at one or more national or international scientific meetings, publication in conference abstract form, and publication of a full manuscript outlining study findings in a peer reviewed journal. We will seek to publish our study findings in an open access journal, which will serve to make these accessible to the public. There are no publication restrictions or data sharing arrangements for this study.

31b. Authorship Eligibility:
Criteria for authorship on any manuscript disseminating study results will be determined in accordance with the statement from the International Congress Medical Journal Editors. [44] Medical/professional writers will not be involved in manuscript preparation.

31c. Plans for Public Access to the Full Protocol, Participant-level Dataset, and Statistical Code:

We plan to publish our study protocol in the peer reviewed literature to make this accessible to interested parties. We will consider publishing our full and anonymized study data set, including anonymized participant-level data, after our study results have been published and at a point at which this will not jeopardize the scientific interests and plans of the investigators. We recognize that investigators are increasingly encouraged to publish their datasets to allow for third party data analysis and a number of options currently exist for on-line data archiving e.g. Dryad (http://datadryad.org). [45] We will include our plans for eventual publication of anonymized participant level data in the study information and consent form.

Appendices

32. Informed consent materials (attached)

i) Information Sheet and Consent Form for Substitute Decision Makers
ii) Information Sheet and Consent Form for Capable Participants
iii) Information Sheet and Assent Form for Participants Incapable of Consent

33. Biological Specimens:

A. SQUEEZE-D

Is a translational sub-study nested within the SQUEEZE trial. Pilot work for SQUEEZE-D conducted as part of the SQUEEZE pilot trial was funded by the Team Sepsis Bench to Bedside HHS Strategic Initiative to Dr. Alison Fox-Robichaud. The Team Sepsis Strategic Initiative is a knowledge translation (KT-1) grant whose primary goal is to move the basic science research discoveries of HHS and McMaster scientists into clinical practice. The objective of SQUEEZE-D is to describe the levels of plasma cell free DNA (cfDNA) in pediatric septic shock. Our recently funded CIHR Project Scheme Grant includes funding for SQUEEZE-D activities during the definitive multicenter phase of SQUEEZE.

Two samples will be collected during routine blood work. Sample A will be drawn within 6 hours of randomization and Sample B will be collected 24-48 hours post randomization. Pediatric CPT tubes (2 mL volume) will be used. The cfDNA and Protein C levels are routine assays in the laboratory of Drs. Fox-Robichaud and Liaw at the Thrombosis and Atherosclerosis Research Institute and measured as previously described.[32] Briefly, total plasma DNA is isolated using a commercially available kits from citrated plasma stored at -80C and quantified by UV spectroscopy or Nanodrop technology. Protein C is measured from citrated plasma (can be stored at -80C indefinitely) by enzyme immunoassay. Samples will be stored for 15 years. Future research may be conducted on those samples if approved by the local research ethics board.

B. PERSEVERE

Is a translational research study being led by Principal Investigator, Dr. Hector Wong, Professor of Pediatrics, and Director of the Division of Pediatric Critical Care at Cincinnati Children's Hospital. Please see Appendix 4 (attached) for a detailed outline of this ancillary study. The objective of PERSEVERE is to use a novel validated pediatric sepsis biomarker risk model

(PERSEVERE; PEdiatRic SEpsis biomarkEr Risk modEl) to predict 28-day mortality in children with septic shock. With the approval of the Hamilton Integrated Research Ethics Board, enrollment into the PERSEVERE ancillary study will occur in conjunction with enrollment into the parent SQUEEZE Trial, and will consequently leverage the SQUEEZE Trial consent and enrollment procedures. Enrollment into the PERSEVERE ancillary study does not incur any additional risk to the study subjects because the two blood specimens will be drawn during routine blood work on Day 1 and 3 respectively. The samples shipped to the PERSEVERE PI's laboratory will be stored for up to 15 years. Future research may be conducted on those samples if approved by the local research ethics board. The PERSEVERE PI will be responsible for all costs related to this ancillary study.

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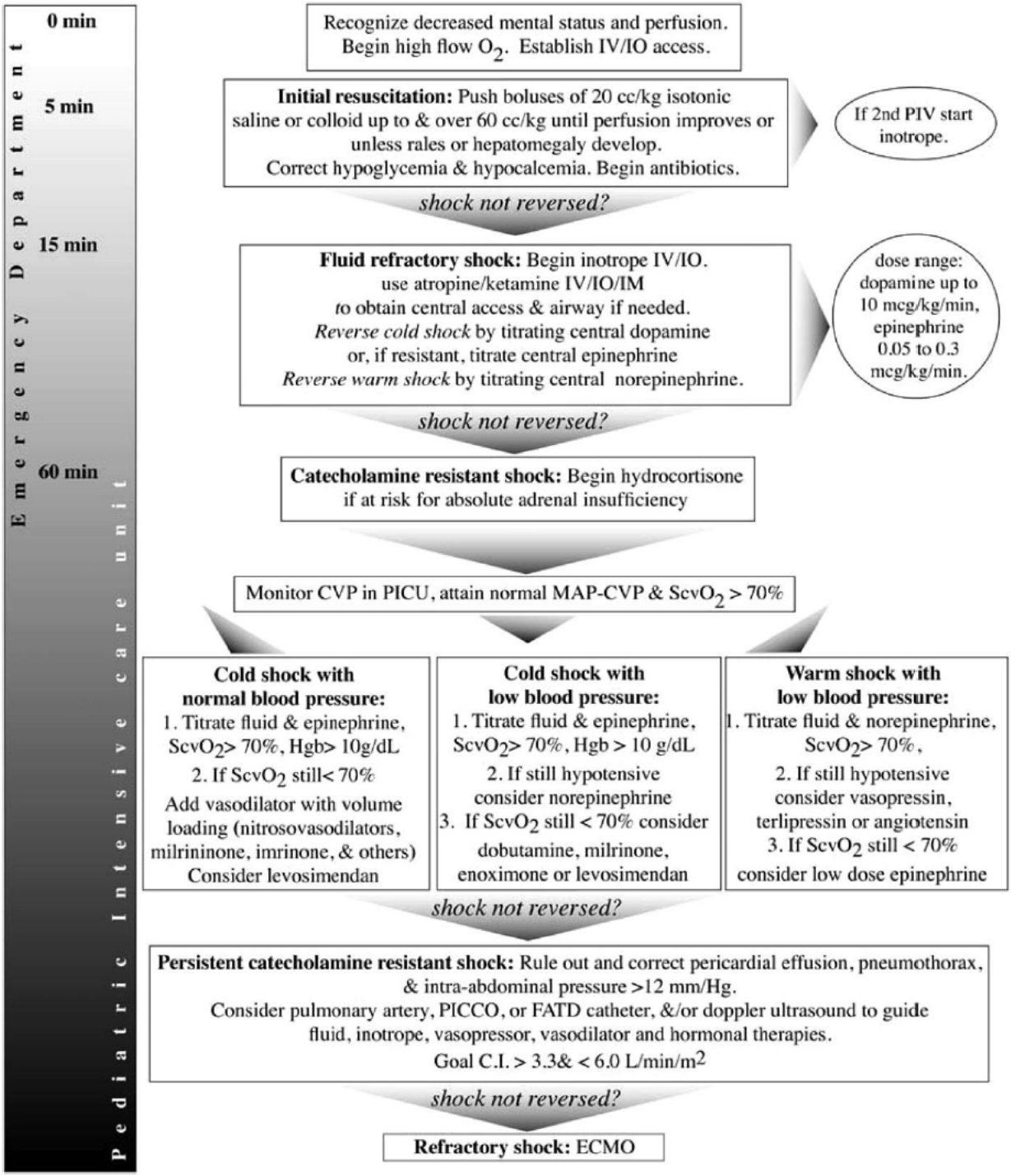
Fig 1. Schedule of enrolment, interventions, and assessments

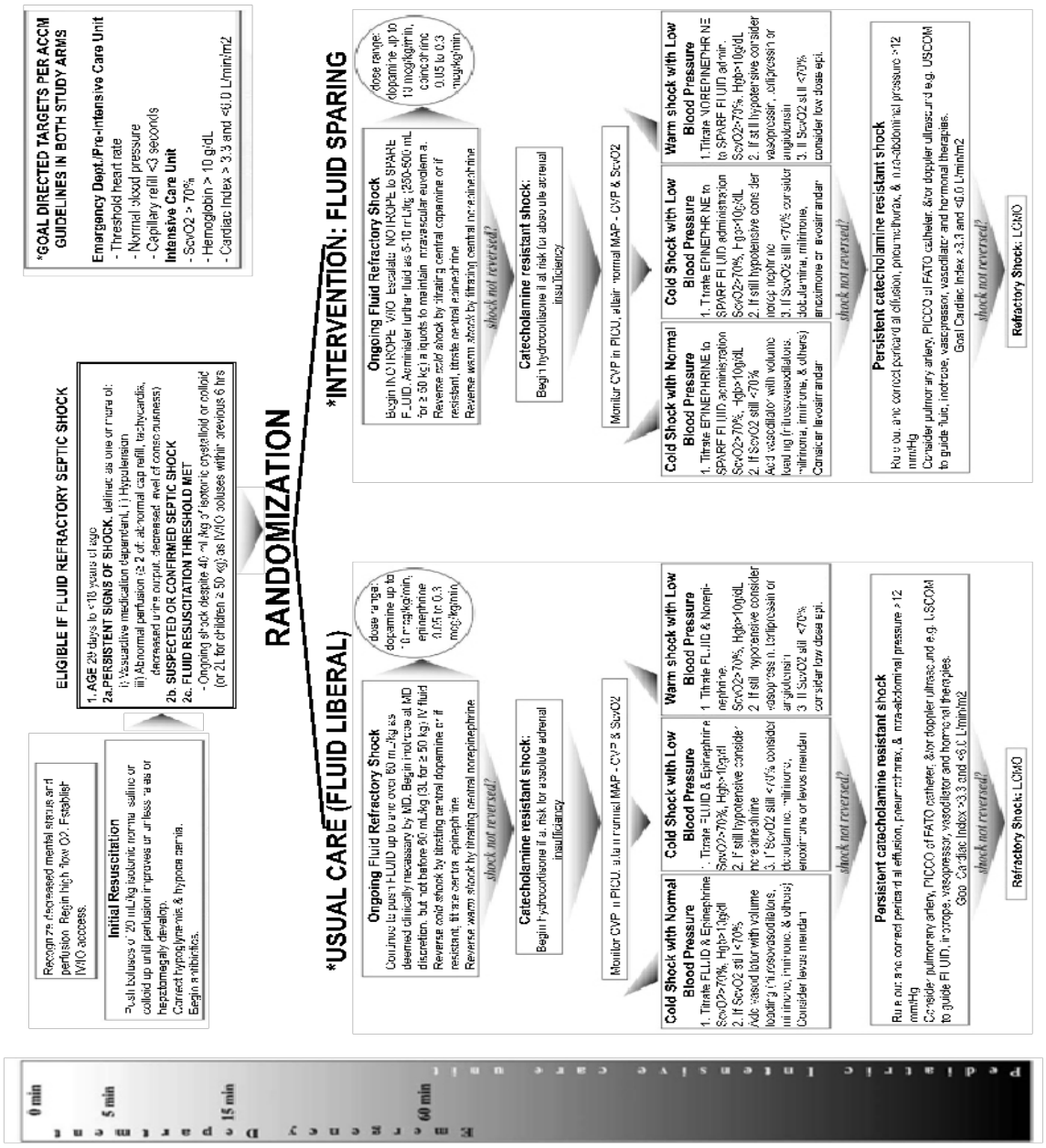
		STUDY PERIOD							
	Enrolment	Allo-cation	Post Allocation						Close -out
Timepoint	-t1	0	t1	t2	t3	t4	t5	t…	tx	
Enrolment:										
Eligibility Screen	X									
Informed Consent			→	→	→	→	→	→	→	
Allocation		X								
Interventions:										
Usual Care		→	→	→	→	→	→	→	→	
Fluid Sparing		→	→	→	→	→	→	→	→	
Assessments:										
Baseline variables	X	X								
Initiation of study procedures			X							
Shock Reversal Outcome Data			→	→	→	→	→	→	→	
Hemodynamic Outcome Data			→	→	→	→	→	→	→	
*Fluid adminis-tration, losses, and fluid balance outcome data			→	→	→	→	→	→	→	→
Vasoactive medication outcome data			→	→	→	→	→	→	→	→
Adverse Events Related to Fluid Overload					X		X	X	X	X
Adverse Events Related to Vasoactive Medications					X		X	X	X	X
*Positive cultures Antimicrobials					X		X	X	X	X
Laboratory Results					X		X	X	X	X
Clinical Course and Procedures Data									X	X

-t1: Enrolment
0: Time zero (Allocation)
t1: 1 hr post allocation
t2: 12 hrs post allocation
t3: 24 hrs post allocation
t4: 36 hrs post allocation
t5: 48 hrs post allocation
t… schedule repeats according to t2-t:5 until shock reversal is achieved.
Tx: 24 hrs post determination participant has achieved shock reversal
* Data gathered will include data from the 24 hours immediately prior to enrolment, if available.

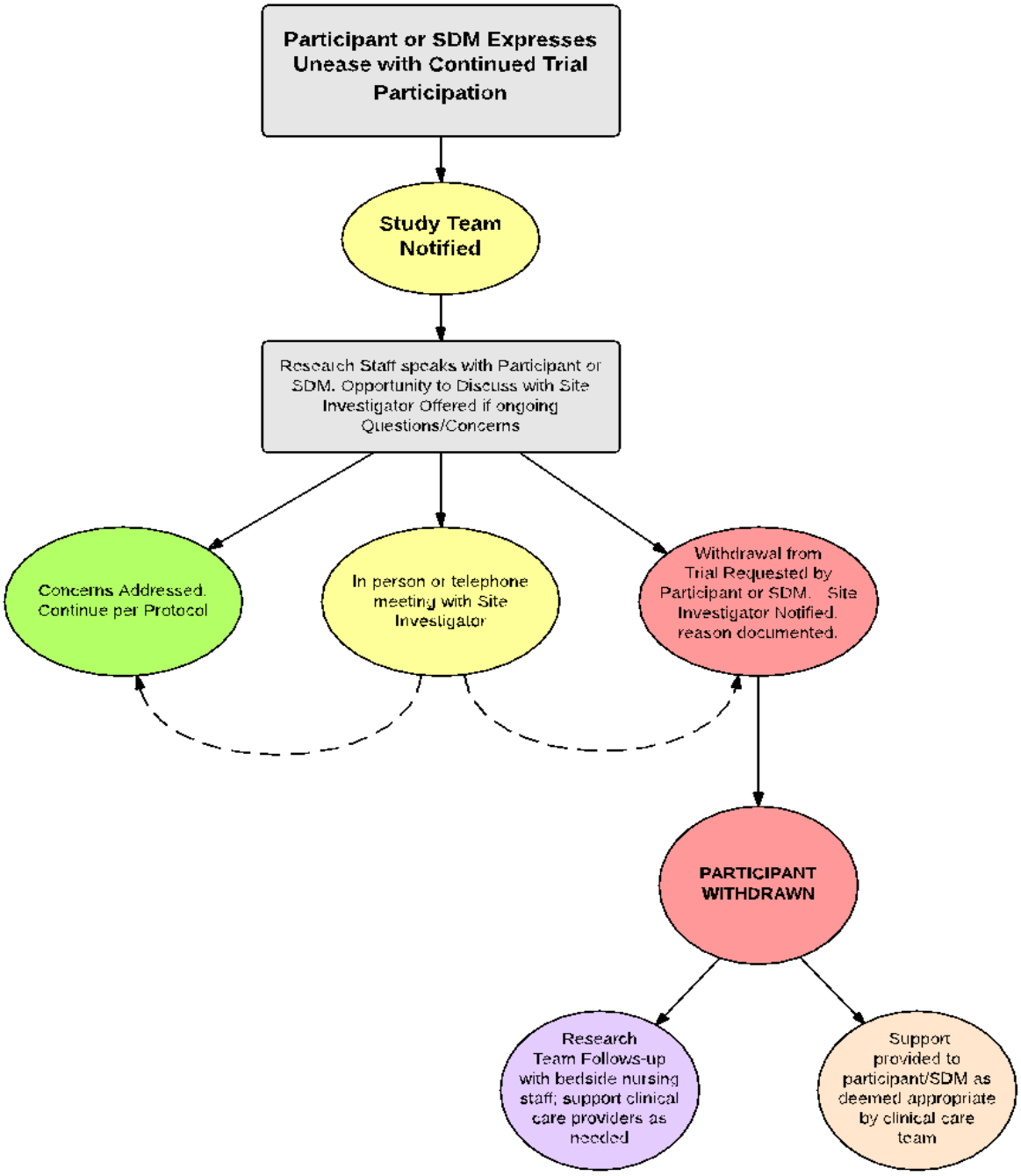
Appendix 1. ACCM Guideline for the Management of Infants and Children with Septic Shock (5)



Appendix 2: Study Algorithm illustrated in ACCM Guideline Format



Appendix 3: Recommended Approach in the setting of Participant/SDM Contemplation of or Request for Withdrawal from Continued Participation in SQUEEZE



Appendix 4: PERSEVERE Protocol: Ancillary Study to the SQUEEZE Trial

Background
Heterogeneity is a major feature of pediatric septic shock, including widely variable mortality risk. In the absence of tools to accurately assess mortality risk, investigators have little objective information to adjust for risk in analyses of clinical data and risk stratify patients for interventional clinical trials. To meet this need, we have derived and validated the pediatric sepsis biomarker risk model (PERSEVERE; PEdiatRic SEpsis biomarkEr Risk modEl) (1, 2). PERSEVERE was derived using a Classification and Regression Tree (CART) approach to predict 28-day mortality. The derivation selected five biomarkers and age, from among twelve biomarkers (serum proteins) and clinical variables potentially associated with outcome. Importantly, PERSEVERE was derived and validated using data measured during the first 24 hours of presentation to the pediatric intensive care unit (PICU) with septic shock, which is an optimal time for risk stratification. In addition, participants were drawn from multiple centers in the United States (3-10). Recently, PERSEVERE was used to conduct a risk stratified analysis of the association between positive fluid balance and pediatric septic shock outcomes (11).

We have also derived and validated a temporal version of PERSEVERE (tPERSEVERE) (12). tPERSEVERE takes into account biomarker data at the first and third day following presentation with septic shock to estimate the probability of poor outcomes. We anticipate that tPERSEVERE can serve as an adjunct monitor for therapeutic effectiveness and/or as a surrogate outcome variable for Phase 1/2 interventional clinical trials.

Hypothesis
PERSEVERE-based mortality risk stratification will inform *post hoc*, secondary analyses of SQUEEZE Trial data.

General Study Procedures
Enrollment into the PERSEVERE ancillary study will occur in conjunction with enrollment into the parent SQUEEZE Trial, and will consequently leverage the SQUEEZE Trial consent and enrollment procedures. Enrollment into the PERSEVERE ancillary study does not incur any additional risk to the study subjects because samples will be obtained during routine blood work. There will not be an extra "poke" to obtain the samples.

Specimen Handling Procedures
For the PERSEVERE ancillary study, two blood specimens (2mL collected in gold top tubes) will be required for all patients enrolled and consented into the SQUEEZE Trial. The two blood specimens will be obtained during routine blood work in the PICU or ED.. The first sample ("Day 1") will represent a time point as close as possible to the time of enrollment in the SQUEEZE Trial. The second sample ("Day 3") will represent approximately 48 hours after the timing of the first sample.

After centrifugation per standard laboratory procedures, fifty (50) microliters of serum/plasma will be removed from the gold top tubes and placed in de-identified Eppendorf tubes supplied by the PERSEVERE PI. The de-identified Eppendorf tubes will then be stored at -80° C. The de-identified label will contain the study ID and the respective study day (Day 1 or Day 3).

There is no expectation that samples will be obtained and frozen immediately. Our experience indicates that the PERSEVERE biomarkers can be assayed reliably using samples stored at 4° C in the clinical laboratory, for up to 72 hours, before being aliquoted and frozen at -80° C.

PERSEVERE Workflow Process
De-identified serum samples will be shipped to the PERSEVERE PI's laboratory at the Cincinnati Children's Hospital Research Foundation, Cincinnati, Ohio. Samples will be shipped on dry ice in batches of approximately 50 samples (the optimal batch size will be determined by the SQUEEZE Trial investigators).

The PERSEVERE biomarkers include C-C chemokine ligand 3 (CCL3), interleukin 8 (IL8), heat shock protein 70 kDa 1B (HSPA1B), granzyme B (GZMB), and matrix metallopeptidase 8 (MMP8) (1, 2, 12). Serum concentrations of these biomarkers will be measured using a multi-plex magnetic bead platform (MILLIPLEX™ MAP) designed for this project by the EMD Millipore Corporation (Billerica, MA). Biomarker concentrations will be measured in a Luminex® 100/200 System (Luminex Corporation, Austin, TX), according the manufacturers' specifications. Assay performance data were previously published (1).

The PERSEVERE decision tree includes age as a lower level decision rule. Accordingly, the study subjects' ages will be required in order to generate a PERSEVERE-derived mortality probability for each subject. A research subject identification or code will also be required in order to link the mortality probability to the specific subject. No other patient information will be required to generate the mortality probability.

The Day 1 biomarker data and age will be used to classify the study subjects according to the PERSEVERE decision tree. The decision tree consists of eight terminal nodes that provide a range of mortality probabilities (1, 2). After classification, the PERSEVERE PI will provide the SQUEEZE Trial investigators with the following data:
- The overall mortality probability for the study cohort, with 95% confidence intervals.
- Mortality probabilities for individual subjects.

A similar procedure will incorporate the Day 1 and Day 3 biomarker data into tPERSEVERE.

When the SQUEEZE Trial is completed, the SQUEEZE Trial investigators may wish to know how well the model performed at the individual patient level. In order to determine model performance, the PERSEVERE PI will require 28-day outcome data (mortality/survival). With this information, the PERSEVERE PI can provide the SQUEEZE Trial investigators with a breakdown of false positive/negative, and true positive/negative subjects. This information could potentially be included in *post hoc* analyses and may inform the design of a future trial.

Initial Use of the PERSEVERE Data
The use of the PERSEVERE data will be at the discretion of the SQUEEZE Trial investigators. Ideally, the PERSEVERE data could be used to test the hypothesis stated above. If the PERSEVERE data is used in this manner, the publication process will also be at the discretion of the SQUEEZE Trial investigators. Suggested strategies for publication include a secondary analysis within the main SQUEEZE Trial manuscript, or a separate manuscript describing the

secondary analysis. For either strategy, the PERSEVERE PI has no expectation of primary or senior authorship.

Future Use of the PERSEVERE Data
An important feature of the PERSEVERE decision tree is that it is amenable to periodic calibration as more subjects are classified. Accordingly, the data generated through this ancillary study could inform future calibrations of the model. Such use, including publication and presentation at scientific meetings, will require permission from the SQUEEZE Trial investigators. Any publication resulting from this use will include the appropriate SQUEEZE Trial investigators as co-authors.

Future Use of Clinical Samples
The samples shipped to the PERSEVERE PI's laboratory will be stored for up to 15 years. Future research may be conducted on those samples if approved by the local research ethics board.

Study Related Costs
The PERSEVERE PI will be responsible for all costs related to this ancillary study.

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