Imperial College London

FLIP-SepTiC

FLuid strategies and Inflammatory Phenotypes in the SepTiC trial

Study Management Group

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Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Research Governance and Integrity.

This protocol describes the FLIP-SepTiC study and provides information about procedures for entering participants. Every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study. Problems relating to this study should be referred, in the first instance, to the Principal Investigator.

This study will adhere to the principles outlined in the Data Protection Act 2018 and General Data Protection Regulations (Europe) and other regulatory requirements as appropriate.

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1. INTRODUCTION

1.1 Summary

What do we want to test in this study?
Serious infection, known as sepsis, causes 52,000 deaths a year in the UK with 80,000 people suffering life-changing after-effects. Sepsis involves inflammation, where the immune system overreacts to infection, but the amount varies between people. People with sepsis need fluid, usually salt water, given through their veins to ensure adequate blood supply to organs, like the kidneys. Too much and not enough fluid might both be harmful, but there has not been enough research to know what the right amount is. We are looking at two ways to give fluid to see which is better within the SepTIC trial:

1) The amount of fluid which is normally given to people (which may be too much)
2) Or using less fluid and giving drugs to take extra fluid away from people's bodies

Research shows that people with severe lung damage, many of whom have sepsis, respond differently to these two fluid approaches. Patients with more inflammation had better survival if they had more fluid. But people with less inflammation, had better survival if they had less fluid. In this study we will look to see if patients with sepsis in the SepTIC trial will respond differently to the two fluid approaches depending on how much inflammation they have.

How will we do this research?
We will use blood samples collected from 3000 patients in the SepTIC trial. From these samples we will measure chemicals called cytokines that cause inflammation. We will put these tests together with information that is normally collected in hospital, for example, blood test results and measurements like blood pressure. Then a mathematical approach called 'latent class analysis' will be used to separate patients into groups using all of this information without using information about the SepTIC treatment group they are in or if the person later dies. We will look to see how people in each group responded to being given standard amounts or less fluid in terms of survival at 90-days. Understanding if different groups of people respond differently to these treatments is important so that doctors know how to save the most lives.

1.2 Background

Sepsis causes 11 million deaths yearly (1). Despite years of research, no new therapies have improved outcomes, partly because sepsis is a heterogeneous condition (2), meaning a treatment that benefits one patient may not benefit all. Identifying patients who benefit from treatments is a research priority (3–5). Sepsis has a significant patient burden (6–8) and improving outcomes is a WHO priority (9). In the UK 245,000 people per year are affected by sepsis leading to 48,000 deaths (more than bowel, breast and prostate cancer combined) and 80,000 people with life changing morbidity. Sepsis costs £15.6 billion annually. It is recognised that some treatments may benefit some patients but harm others (2).

Fluid is a cornerstone of initial sepsis management and The Surviving Sepsis Campaign (10) suggests at least 30ml/kg of fluid is given to patients with sepsis induced hypotension. This is controversial as it is based on low quality evidence and fluid accumulation has been associated with mortality (11–13). It is unlikely that the same approach to fluid management is appropriate in all patients (14,15). For example, the CLASSIC trial (16) found no difference in survival between restrictive and standard

fluid strategies overall but patients with or without respiratory support responded differently to the fluid strategies, supporting heterogeneity of treatment effect. However, it is unknown how to identify the best strategy for different patient groups. Identifying which patients benefit from therapies is a research priority as identified by the Intensive Care Foundation's James Lind Alliance Research prioritisation exercise (3), which took into account views of clinicians, patients and the public, and the Surviving Sepsis Campaign (4,5).

Observational studies (11–13) have suggested an association between fluid accumulation and increased mortality in critical illness. There are several strategies to reduce fluid accumulation in critical illness from restrictive early fluid resuscitation to active removal of fluid using diuretics (de-resuscitation). However, prospective randomised trials have not always been able to demonstrate a survival benefit from more restrictive fluid approaches. Findings from other studies suggest that there may be heterogeneity of effect with regard to the benefit of conservative fluid strategies within populations of patients with sepsis and other critical illness.
In a 400-patient observational study in the UK and Canada (13), 87% of patients in ICU had a positive fluid balance by day 3. Although mortality was low in those patients undergoing de-resuscitation and achieving a negative fluid balance (18%, p=0.01) the highest mortality was in those who underwent de-resuscitation but remained in a positive balance (52%, p<0.01). The RADAR-2 randomised feasibility study (15), comparing a conservative fluid strategy combined with de-resuscitation to standard care, found no difference in mortality at 28-days (21% vs 16%) between the two strategies in critically ill patients. However, in those with sepsis, mortality was higher (35%) in the conservative arm than usual care (12.5%), p = 0.03, although small patient numbers and baseline imbalances could have influenced this finding. The CLASSIC trial compared a conservative fluid approach to standard care in 1545 septic patients. Although there was no difference in 90-day mortality between the two arms (42.3% conservative vs 42.1% standard, p=0.96), patients requiring respiratory support showed reduced mortality with the conservative approach (46% vs 52%), whilst patients without respiratory support had higher mortality with a conservative approach (36% vs 33%), p-value for heterogeneity 0.03 (16). Despite these promising findings of heterogeneity of the benefit of a conservative fluid approach, it is unknown how best to identify sub-populations who will gain maximal benefit from this approach and those who may come to harm.

Critical illness phenotypes
Latent class analysis (LCA) is a statistical procedure used to identify subgroups within populations. It has been applied successfully in acute respiratory distress syndrome (ARDS)(14,17–20), a common precipitant of which is sepsis and infection. Across all studies, LCA of a combination of clinical variables and inflammatory mediators has consistently identified two subphenotypes, one of which is characterised by higher levels of IL-6, IL-8 and sTNFr1, lower protein C and bicarbonate and more frequent use of vasopressor drugs than the other. They are known as hyper- and hypoinflammatory subphenotypes. The hyperinflammatory subphenotype is associated with lower survival than the hypoinflammatory subphenotype. Similar subphenotypes are seen in patients without ARDS (21–23) and in two sepsis trials (VANISH (24) and LeoPARDS (25)) LCA identified a similar hyperinflammatory subphenotype with high levels of circulating inflammatory mediators and worse clinical outcomes (26). Similarities in the pattern of elevation of IL-6, IL-8 and sTNFr1 between the subphenotypes identified in these sepsis trials and those found in ARDS suggest that similar sub-phenotypes exist in both syndromes.

Differential Response to Fluid Strategies
Separating patients from randomised trials into inflammatory subphenotypes has shown that they may respond differently to treatments (17,19). The Fluid and Catheter Treatment Trial (27)

compared a liberal (standard) fluid approach to a conservative approach, which included de-resuscitation with frusemide, in patients with ARDS, many of whom (70%) also had sepsis or pneumonia. When patients were divided based on inflammatory subphenotypes, there was a significant interaction between subphenotype and treatment on 90-day mortality (p = 0.004). Hypoinflammatory patients had a mortality of 26% with the liberal strategy, versus 18% with the conservative strategy. In contrast, hyperinflammatory patients had a 40% mortality with the liberal fluid strategy, versus 50% in the conservative fluid group (14). Although a large proportion of these patients had sepsis or infection, it is unknown if such heterogeneity of treatment effect is seen in populations made up of only patients with sepsis, not all of whom will have ARDS. However, preliminary data from a small observational cohort in Uganda suggest that an LCA approach in sepsis has promise to identify subgroups who have a differential response to fluid strategy (28).

SepTiC Trial

The SepTIC trial (ISRCTN 80791572, REC: 23/LO/0339) is investigating three interventions in sepsis (a conservative fluid strategy, a rapid microbial diagnostic and GM-CSF, see trial protocol attached as an appendix). We will use serum samples and data collected during this trial to answer the question of differential response to a conservative fluid strategy. We will measure biomarkers in samples collected as part of the trial and, once combined with clinical variables from the trial database, we use established methods (14,17–19) to identify subphenotypes that have previously shown promise to respond differently to standard and conservative approaches to fluid (14). We will investigate differences in treatment effect between subphenotypes and fluid strategy in the fluid domain of SepTIC on the primary outcome of 90-day mortality.

1.3 Study Rationale

Hypothesis: Patients with sepsis can be divided into two or more subphenotypes based on measurement of circulating inflammatory mediators and routine clinical variables that will respond differently to a conservative fluid strategy compared to standard care regarding 90-day mortality.

Aim: To determine the differential response to a conservative fluid strategy with active de-resuscitation of latent class analysis defined inflammatory subphenotypes in the patients enrolled into the SepTiC randomised clinical trial.

2. STUDY OBJECTIVES

1. Measure circulating biomarkers, including inflammatory mediators such as cytokines, in blood samples collected at enrolment into the SepTiC trial.

2. Identify subphenotypes of septic patients present in the SepTiC trial population using latent class analysis of clinical and biomarker measurements.

3. Explore differential treatment responses to standard and conservative fluid strategies on 90-day mortality (primary outcome) between subphenotypes.

4. Explore differential treatment responses between subphenotypes and the secondary study outcomes.

5. Exploratory objectives will include comparing subphenotypes identified by latent class analysis with other relevant phenotyping models described in the literature and exploring novel ways to

describe the phenotypes, for examples as a continuous variable describing likelihood of class membership.

3. STUDY DESIGN

This is an observational study utilising samples and data collected during the SepTiC trial. Serum biomarkers including, but not limited to, inflammatory proteins such as IL-6, IL-10, sTNFR1 and protein C, will be measured in serum samples collected during the SepTiC trial. Other biomarkers will be measured based on preliminary results from this research and published literature as required. The research biomarker data will be combined with clinical variables such as clinical laboratory test results and physiological parameters (for example heart rate and blood pressure) recorded during the SepTiC trial to determine underlying sepsis sub-groups. We will then explore if these sub-groups respond differently to the SepTiC fluid intervention.

Duration: 48 months
Subjects: 3000 subjects enrolled into the fluid domain of the SepTiC trial

Inclusion Criteria:
• Randomised into the fluid domain of the SepTiC trial as per the SepTiC inclusion and exclusion criteria
• Serum sample collected during the SepTiC trial

Exclusion Criteria:
• No serum sample collected

3.1. Study Outcome Measures

The study outcomes in FLIP-SepTiC will reflect those captured in the SepTiC trial:

Primary: 90-day mortality

Secondary:

- 90-day mortality combined with clinical state (in-hospital with organ support, in-hospital without organ support, discharged from hospital) over time
- Duration of mechanical ventilation, shock, renal replacement therapy during index hospital admission up to 90 days
- Length of stay in ICU and hospital up to 90 days
- Adverse events and adverse drug reactions during index hospital admission up to 28 days
- Health-related Quality of Life (EQ-5D-5L) and cognitive function (MoCA-Blind) at 6 months
- 1-year mortality

- Exploratory comparison of latent class analysis defined phenotypes with other phenotyping methods described in the literature.

3.2. Statistical Analysis

A statistical analysis plan will be written prior to data analysis. A summary of proposed analysis is provided below.

Sample size

We anticipate 80% of patients enrolled into SepTiC will also have a research blood sample collected, providing 3000 patients for FLIP-SepTiC. SepTiC is projected to recruit 120 patients per month over 60 sites.

In studies of inflammatory sub-phenotypes the hyper-inflamed subphenotype represents 27-37% of patients with ARDS, however, in the same studies when only patients with sepsis as a precipitant of ARDS are considered this proportion increases to 48-56% (14,17–20) with similar proportions having been seen in our own analysis in sepsis (26). As such we anticipate two subphenotypes within our population containing similar numbers of patients.

The mortality rate expected in the control arm of the SepTiC trial has been estimated at 30% based on published data (6) and the ICNARC case mix programme and accounting for the trial inclusion criteria. Previous studies have shown that the hyperinflammatory subphenotypes has a 90-day mortality rate of 1.8 to 2.8 times that in the hypoinflammatory group (14,17–20). Limited data are available in the sub-populations with sepsis, so we have used a conservative estimate of an increased mortality in the hyperinflammatory group of 1.4 times that in the hypoinflammatory group for the purpose of our power calculations to give a 90-day mortality rates of 25% and 35% in the hypo and hyperinflammatory subphenotypes respectively in the control arm of the study.

The primary sample size calculation was done considering a balanced distribution of patients in each treatment group and subtype [assuming a prevalence of 50% across treatment arms and two subtypes]. If the response reflects that seen in ARDS with the hyper-inflamed group having a 10% increase in mortality with a conservative strategy and the hypo-inflammatory group having an 8% decrease which equals an odds ratio for interaction of 2.4, 3000 patients would provide more than 99% power to detect a differential response to the conservative fluid strategy with an alpha of 5% and assuming an OR of subtype of 1.7 to 2.8 and an OR of treatment of 0.6 to 0.7 based on the published data by Famous et al. (14). At the same time, this sample size will provide 80% to 99% power to detect more conservative effects ranging from odds ratio for interaction of 1.6 to 2.0 with the same assumptions.

We repeated the calculations considering a prevalence of 50% for treatment and 30% for the hyper-inflammatory subtype [30% hyper and 70% hypo] as reflected in Famous study (14). We will still have 0.85 to 0.99 power to detect odds ratio for interaction of 1.7 to 2.4.

Latent Class Analysis

Inflammatory mediator results will be combined with routinely collected clinical variables recorded at enrolment into the SepTiC trial. These will include but are not limited to laboratory values such as bicarbonate, creatinine, bilirubin, lactate and platelet count and clinical parameters such as PaO2:FiO2 ratio, mean arterial blood pressure, respiratory rate and temperature. Clinical variables have been chosen for inclusion in this analysis based on their previous association with inflammatory phenotypes (14,17–20,26).

Latent class analysis (LCA) is a well-validated statistical technique based on mixture modelling that is used to find the best fitting model for a dataset, with the hypothesis that it contains a number of

unobserved groups. Traditional regression analysis is used to understand the relationship of pre-specified independent variables to a known outcome. In contrast, LCA models seek to determine if there are subgroups of patients defined by a combination of variables, without consideration of the outcome.
We will closely follow the methods described previously (14,17–20,26). Variables with a high degree of missingness, and one of a pair of correlated variables will be excluded from the modelling. Retained clinical data and biomarker levels will be considered as class-defining variables in the LCA model. Importantly, the LCA will be conducted without consideration of clinical outcomes or treatment allocation. Since the scales of the retained clinical and biomarker variables will vary widely, all continuous measures will be rescaled to a common z-scale where the mean is set to 0 and the standard deviation to 1. Optimal model selection will be based on the measures such as the Bayesian Information Criteria, the Vuong-Lo-Mendell-Rubin likelihood ratio test, entropy, and the size of the smallest class. To determine how distinct the classes are from each other, we will calculate the average probabilities for belonging to each class (the likelihood of belonging to each class for a given subject). Each patient will be assigned to the class to which they have the highest probability of class membership.

Baseline analysis
Baseline demographic, clinical and inflammatory variables will be summarised for each arm and LCA defined phenotypes to describe the study population distribution. Continuous variables that follow an approximately normal distribution will be summarised using means and standard deviations. Skewed continuous variables will be summarised using medians and inter-quartile ranges. Categorical/binary variables will be summarised using frequencies and percentages.

Interaction Analysis
After extracting subtypes using LCA, logistic regression will be used to assess the interaction between subphenotype (hyper-inflammatory versus hypo-inflammatory) and fluid strategy (conservative versus standard) on the primary outcome (90-day mortality). Odds ratios for main effects and interaction will be reported with 95% confidence intervals. As a supplementary analysis, we will also assess the interaction between subphenotype and fluid strategy on time to death using the Cox regression model and reporting hazard ratio with 95% CI.

Exploratory analysis of secondary outcomes will be performed using relevant statistical methods. For example, a longitudinal Proportional Odds model can be used to analyse day-90 outcome taking into account transition between clinical states (discharged from hospital, remaining in hospital without organ support and remaining in hospital requiring organ support).
Outcomes expressed as incidences or incidence rates can be examined using a suitable mixed effects regression model such as logistic, Poisson or negative binomial and outcomes where it is important to account for competing events such as death, can be analysed as time-to-event or time-to-recovery data as appropriate, using sub-distribution hazard models (29) to account for the dependent competing risk of censoring when subjects transition to a less favourable clinical state (e.g. death). Quality of Life (EQ-5D-5L) and cognitive function (MoCA-Blind) can be analysed using linear mixed models and logistic regression can be used to assess the interaction effect between inflammatory subphenotypes and fluid strategy on binary outcomes. In all analysis an interaction term will be included to investigate the interaction of inflammatory subphenotype and fluid strategy and related measure of effect with 95% CI will be reported.

Primary analysis will focus on assessing differential treatment effect based on the latent class analysis defined sub-groups. However, class membership is likely to be stronger for some patients

than others. To investigate this we will also conduct exploratory analysis of heterogeneity of treatment effect where class membership is expressed as a continuous scale, for example by using the probability of class membership.

Further statistical analysis will be performed as required and will be detailed in a statistical analysis plan written prior to data analysis. This will take into account the most up to data literature and evidence in this area.

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

4. SECONDARY DATA

Data and samples will be used as collected in the SepTiC trial for which we have permission of the Chief investigator of the SepTiC trial (Prof Anthony Gordon) who is a co-investigator on this project. Data needed for the analysis in FLIP-SepTiC will be obtained from the Imperial College Clinical Trials Unit. All data and samples will be pseudoanonymised and will be stored under a unique study identifier from the SepTiC trial on Imperial College OneDrive which will be accessible via the cloud.

5. REGULATORY ISSUES

5.1 Ethics approval

The Principal Investigator has obtained approval from the Head of Department and approval from the Research Governance and Integrity Team (RGIT).

5.2 Consent

Patients enrolled into the SepTiC will be asked to consent for their data and samples to be used in future research studies, the SepTiC consent forms have been included as an appendix to this protocol.

5.4 Funding

The National Institute of Health and Care Research Efficacy and Mechanism Evaluation Programme (NIHR153209) are funding this study. There are no per participant fees.

5.5 Audits

The study may be subject to inspection and audit by Imperial College London under their remit as sponsor and other regulatory bodies.

6. STUDY MANAGEMENT

The day-to-day management of the study will be co-ordinated through Dr Antcliffe at Imperial College London.

7. PUBLICATION POLICY

- Results of this study will be disseminated as conference presentations to international organisations such as the Intensive Care Society, the European Society of Intensive Care Medicine and the American Thoracic Society.
- The results will be published as an independent manuscript in a major peer-reviewed open access journal.
- To maximise impact and ensure clinical adoption, we will disseminate results to a wide audience. We will produce a lay summary and an infographic style report to ensure that the results are accessible. Results from FLIP-SepTIC will be provided via the SepTIC website using an accessible style and animated clips to provide patients and relatives with information about the findings.
- We will ensure a wide awareness of the findings from FLIP-SepTIC by ensuring that the results are presented at meetings that attract both academic and clinical audiences.

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