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LIPid Intensive Drug therapy for Sepsis Pilot (LIPIDS-P): Phase I/II clinical trial protocol of lipid emulsion therapy for stabilising cholesterol levels in sepsis and septic shock

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ABSTRACT

Introduction Sepsis is a life-threatening, dysregulated response to infection. Both high-density lipoprotein and low-density lipoprotein cholesterol should protect against sepsis by several mechanisms; however, for partially unknown reasons, cholesterol levels become critically low in patients with early sepsis who experience poor outcomes. An anti-inflammatory lipid injectable emulsion containing fish oil is approved by the Food and Drug Administration as parenteral nutrition for critically ill patients and may prevent this decrease in serum cholesterol levels by providing substrate for cholesterol synthesis and may favourably modulate inflammation. This LIPid Intensive Drug therapy for Sepsis Pilot clinical trial is the first study to attempt to stabilise early cholesterol levels using lipid emulsion as a treatment modality for sepsis.

Methods and analysis This is a two-centre, phase I/II clinical trial. Phase I is a non-randomised dose-escalation study using a Bayesian optimal interval design in which up to 16 patients will be enrolled to evaluate the safest and most efficacious dose for stabilising cholesterol levels. Based on phase I results, the two best doses will be used to randomise 48 patients to either lipid injectable emulsion or active control (no treatment). Twenty-four patients will be randomised to one of two doses of the study drug, while 24 control group patients will receive no drug and will be followed during their hospitalisation. The control group will receive all standard treatments mandated by the institutional sepsis alert protocol. The phase II study will employ a permuted blocked randomisation technique, and the primary endpoint will be change in serum total cholesterol level (48 hours − enrolment). Secondary endpoints include change in cholesterol level from enrolment to 7 days, change in Sequential Organ Failure Assessment score over the first 48 hours and 7 days, in-hospital and 28-day mortality, lipid oxidation status, inflammatory biomarkers, and high-density lipoprotein function.

Ethics and dissemination Investigators are trained and follow good clinical practices, and each phase of the study

Strengths and limitations of this study

► This novel study will test the hypothesis that the proposed lipid injectable emulsion will be capable of stabilising cholesterol levels in patients with early sepsis.
► The study will use Bayesian optimal interval design in the phase I trial so that the two doses of study drug used in the phase II randomised controlled trial are informed by the results of the phase I sequential, dose-escalation study.
► The study will not be blinded, and as such could be subject to bias.
► Blinding of data abstractors to study allocation and use of a numerical primary endpoint of the phase II trial will reduce the potential for bias.
► The primary endpoint of the phase II trial is change in total serum cholesterol level, not a clinical endpoint.

was reviewed and approved by the institutional review boards of each institution. Results of each phase will be disseminated through presentations at national meetings and publication in peer-reviewed journals. If promising, data from the pilot study will be used for a larger, multicentre, phase II clinical trial.

Trial registration number NCT03405870.

INTRODUCTION

Recent US estimates report up to 850 000 emergency department sepsis visits per year,[1] costing nearly $17 billion.[1–3] Sepsis is a lethal condition, resulting in death in approximately one of every four cases and nearly 215 000 deaths per year in the USA. Sepsis also leads to significant long-term morbidity, late mortality and chronic critical illness (CCI), characterised by intensive care unit (ICU) stays ≥14 days, progressive cachexia,

manageable organ dysfunction and frequent indolent death.[4 5] The rate of late mortality and CCI is approximately 50% based on previous studies.[4–6] Both low-density lipoprotein (LDL) and high-density lipoprotein (HDL) may be protective against sepsis. Specifically low-density lipoprotein cholesterol (LDL-C) facilitates bacterial toxin clearance in sepsis via hepatic LDL receptors (LDLRs) or via HDL (reverse cholesterol transport).[7–10] High-density lipoprotein cholesterol (HDL-C) may protect against sepsis by several mechanisms, including bacterial toxin binding,[8 11] prevention of inflammatory cytokine release,[12 13] inhibition of vascular and intercellular adhesion molecule expression,[14 15] and stimulation of endogenous corticosteroid release.[16 17]

Numerous observational studies have shown that low levels of both HDL-C and LDL-C are predictive of poor outcomes, including worsening organ dysfunction.[8 18–22] There are several potential mechanisms to explain these decreased cholesterol levels. Downregulation of key enzymes needed for cholesterol maturation (lecithin cholesterol acyltransferase and cholesteryl ester transfer protein) and upregulation of hepatic and endothelial lipases lead to reduced levels of HDL–C and LDL-C.[23] Levels of very low-density lipoproteins and triglycerides increase due to adipose tissue lipolysis, which is stimulated by catecholamines, cortisol and growth hormone, possibly to meet energy needs or for bacterial toxin binding.[8 24 25] Although some have attempted to demonstrate that these changes in cholesterol levels are not causal,[26] there may be a direct association between cholesterol levels and poor outcomes, as both cholesterol levels and functional status are predictive of poor outcomes.[22 27–29] The physiological rationale for the protective effects of cholesterol is strong, and thus we believe a pilot investigational study is warranted.

We propose the design for the LIPid Intensive Drug therapy for Sepsis Pilot trial (LIPIDS-P), a novel phase I/II pilot study of a lipid injectable emulsion (LIE) for stabilising cholesterol levels in patients with sepsis and septic shock. The proposed anti-inflammatory LIE was chosen as it contains a combination of soybean oil, medium-chain triglycerides, olive oil and fish oil that have the potential to support de novo cholesterol synthesis while having the additional anti-inflammatory benefits of long-chain ω−3 fatty acids derived from fish oil that can potentiate inflammation resolution.[30–34] We hypothesise that the proposed LIE will be capable of stabilising cholesterol levels during the critical early period of sepsis when cholesterol levels reach low levels (first 48–72 hours) by supplementing LIE during the first 2 days of hospital admission. The primary objective of the phase I trial is to evaluate increasing doses of LIE and their effects on 48-hour cholesterol levels postenrolment while evaluating for potential dose-limiting toxicities (DLTs). The results of the phase I study will inform the phase II randomised controlled trial, which will test the two most efficacious doses of LIE at stabilising 48-hour cholesterol levels in comparison with controls.

METHODS AND ANALYSIS
Design
This is a pilot clinical trial of early infusion of LIE in patients with early sepsis with moderate organ dysfunction, defined as Sequential Organ Failure Assessment (SOFA) score ≥4. The LIPIDS-P clinical trial will assess the following: (1) safety and tolerability of LIE and adverse effects, (2) the drug's ability to stabilise cholesterol levels, and (3) preliminary measures of biological activity and clinical outcomes.

The study has a phase I/II design, where up to 16 patients will be enrolled in the phase I study to evaluate for optimal dose and DLTs. For the phase I dose-escalation study there will be no control group, with doses starting at 1.0 g/kg and increasing incrementally by 0.2 g/kg to a maximum dose of 1.8 g/kg based on body weight, with two patients per group. All proposed doses are within the Food and Drug Administration (FDA)-approved dose range for nutritional purposes and are not expected to result in clinically relevant toxicity. Dose escalation or de-escalation will occur based on whether DLTs are observed at a specific dose with the threshold for toxicity set at 10% using the Bayesian optimal interval design, which has been shown to be safer than the standard 3+3 design for phase I trials.[35] Once two patients have successfully completed both doses of the study drug, the next sequence in the dosing scheme occurs. We chose this design and a conservative toxicity threshold to mitigate risk in this critically ill patient population. Phase I dosing will follow the schedule set in table 1, and prespecified DLTs are outlined in table 2. Dosing will be based on actual body weight in kilograms, except in cases of morbid obesity, defined as actual body weight (ABW) >200% ideal body weight (IBW). For morbidly obese patients, adjusted body weight (AdjBW) will be used based on the following formula: AdjBW=IBW + 0.4 (ABW – IBW).[36]

Table 1 Phase I dose escalation schedule

Number of patients treated	2	4	6	8	10	12	14	16
Escalate if number of DLT ≤	0	0	0	0	0	0	1	1
De-escalate if number of DLT ≥	1	1	1	1	2	2	2	2
Eliminate if number of DLT ≥	NA	2	2	3	3	3	4	4

Dose escalation or de-escalation will occur based on whether dose-limiting toxicities (DLTs) are observed at a specific dose and will follow the above schedule according to the Bayesian design. Dose escalation will occur after two patients at each specified dose level if there are no observed DLTs. If at any point in the first eight patients one patient experiences a DLT, de-escalation will occur and two more patients will be enrolled at the previous dose. For patients 10 through 16, two patients would have to experience a DLT for de-escalation to occur. Thresholds for elimination of doses and study stoppage are conservatively set at a DLT rate of 10% and follow the bottom row of the table.
NA, not applicable.

Table 2 Dose-limiting toxicities, expected adverse events and sepsis-related events

Toxicity	Monitor	Time point	Action
Drug-related, dose-limiting toxicities or SAEs			
Respiratory distress (occurring soon after drug start).	Bedside (nurse, doctor, RC).	First 60 hours.	Contact safety monitor immediately and PI.
Hypoxia (occurring soon after drug start).	Bedside (nurse, doctor, RC).	First 60 hours.	Contact safety monitor immediately and PI.
Fat overload.	Pharmacist, nutritionist, doctor.	First 60 hours.	Contact safety monitor immediately and PI.
Hypertriglyceridaemia (>1000 mg/dL).	Pharmacist, nutritionist, doctor.	First 60 hours.	Contact safety monitor immediately and PI.
Hepatitis (elevation of LFTs or total bilirubin).	Pharmacist, nutritionist, doctor.	First 60 hours.	Contact safety monitor immediately and PI.
Expected adverse events			
Serum triglycerides, fluid and electrolyte status, blood glucose, liver and kidney function, blood count including platelets, and coagulation parameters.	Pharmacist, nutritionist, doctor.	First 60 hours.	Document findings.
Tachypnoea, dyspnoea, increased blood triglycerides, anaemia, device-related infection, rash, urticaria, erythema, flushing.	Bedside (nurse, doctor, RC).	7 days or discharge.	Document findings.
Sepsis-related events exempted from regulatory reporting			
Dyspnoea, chest pain, fever, hypoxaemia, rapid pulse, rapid respiratory rate, dizziness, syncope, altered mental status, seizure, confusion, anxiety, generalised weakness, anorexia, nausea, abdominal pain, back pain, constipation, vomiting, pneumonia, skin infection, cancer, surgery not related to treatment of sepsis, electrocardiography abnormalities (atrial arrhythmias, right bundle branch block, and ST and T wave changes), elevated troponin level, elevated BNP or NT ProBNP level, high white cell count, pulmonary infiltrate, pleural effusion, cardiomegaly, need for oxygen therapy, need for vasopressor, need for blood product transfusion, need for inotropic therapy, need for mechanical ventilation, and need for physical or occupational therapy.			

Table of drug-related dose-limiting toxicities, including monitoring, time point for reporting and action to be taken if observed. List of potentially related expected adverse events and sepsis-related events exempted from regulatory reported.

BNP, brain natriuretic peptide; LFTs, Liver Function Tests; NT ProBNP, N-terminal **pro** b-type natriuretic peptide; PI, principal investigator; RC, research coordinator; SAE, Serious Adverse Event.

After evaluating for DLTs in the phase I study, the two most efficacious and safest doses will be used to randomise 48 patients to either LIE or no active treatment. Thus, the phase II arm will include 24 patients randomised to one of the two doses of the study drug, while the control group will consist of 24 patients who receive no drug but are followed while in-hospital (active controls) (figure 1). The control group will receive standard treatments guided by the institutional sepsis alert protocol.[37] For purposes of statistical outcomes analysis, the control group will be the comparison group. The phase II trial primary endpoint is delta (48 hours – enrolment) serum total cholesterol between groups.

Population

Patients who meet the following inclusion and exclusion criteria will be approached for enrolment.

Inclusion criteria include (1) age >18, (2) primary diagnosis of sepsis and within 24 hours of sepsis recognition, (3) SOFA score ≥4 and (4) screening total cholesterol ≤100 mg/dL or HDL-C + LDL C ≤70 mg/dL. A screening lipid panel will be drawn and paid for by the study prior to enrolment to ensure that patients meet the requirement of total cholesterol ≤100 mg/dL or HDL-C + LDL C ≤70 mg/dL. This cut-off is based on preliminary studies that suggest that patients at increased risk of adverse outcomes from sepsis who may respond to cholesterol stabilisation have low enrolment cholesterol levels.[22 28 29] Our data also show that nearly 50% of patients with sepsis with SOFA score ≥4 meet the proposed screening cholesterol criteria. Exclusion criteria include (1) total bilirubin >2 mg/dL, (2) serum albumin <1.5 mg/dL, (3) hypersensitivity to fish, egg, soybean or peanut protein, or to any of the active ingredients or excipients, (4) severe hyperlipidaemia or severe disorders of lipid metabolism with serum triglycerides >400 mg/dL, (5) alternative/confounding diagnosis causing shock or critical illness (eg, myocardial infarction or pulmonary embolus, massive haemorrhage, trauma), (6) significant traumatic brain injury (evidence of neurological injury on CT scan and a Glasgow Coma Scale (GCS) <8), (7) refractory shock
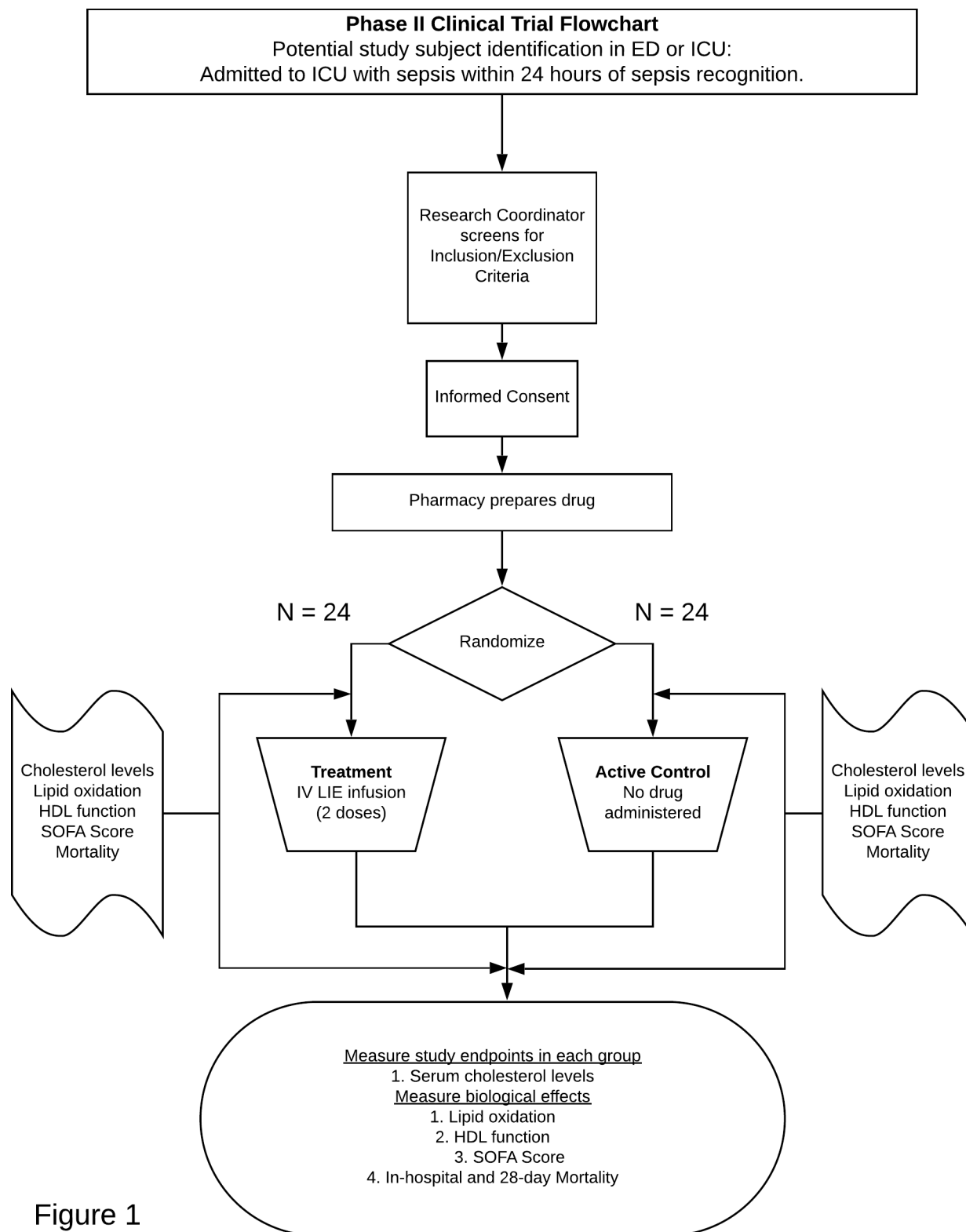
Phase II Clinical Trial Flowchart
Potential study subject identification in ED or ICU:
Admitted to ICU with sepsis within 24 hours of sepsis recognition.

Research Coordinator
screens for
Inclusion/Exclusion
Criteria

Informed Consent

Pharmacy prepares drug

N = 24   N = 24

Randomize

Cholesterol levels
Lipid oxidation
HDL function
SOFA Score
Mortality

Treatment
IV LIE infusion
(2 doses)

Active Control
No drug
administered

Cholesterol levels
Lipid oxidation
HDL function
SOFA Score
Mortality

Measure study endpoints in each group
1. Serum cholesterol levels
Measure biological effects
1. Lipid oxidation
2. HDL function
3. SOFA Score
4. In-hospital and 28-day Mortality

Figure 1

Figure 1 Flow chart of phase II pilot clinical trial enrolment, including screening, randomisation and outcomes. ED, emergency department; HDL, high-density lipoprotein; ICU, intensive care unit; IV, intravenous; LIE, lipid injectable emulsion; SOFA, Sequential Organ Failure Assessment.

(likely death within 12 hours), (8) advanced directives restricting aggressive care or treating physician deems aggressive care unsuitable, (9) anticipated requirement for surgery that would interfere with drug infusion, (10)

severe primary blood coagulation disorder, (11) acute pancreatitis accompanied by hyperlipidaemia, (12) acute thromboembolic disease, (13) uncontrollable source of sepsis (eg, irreversible disease state such as unresectable

dead bowel), (14) severe immunocompromised state, (15) pregnancy or lactation, (16) concurrently receiving intravenous lipid formulations (eg, parenteral nutrition, propofol), (17) Child-Pugh class B/C liver disease, and (18) actively on extracorporeal membrane oxygenation (ECMO) or anticipated need for ECMO within 48 hours of enrolment.

Study settings

This is a two-site study and patients will be enrolled from either University of Florida (UF) Health Jacksonville or UF Health (Gainesville, Florida) hospitals. UF Health Jacksonville is a not-for-profit, academic medical centre serving an urban, inner-city population and is a 695-bed level 1 trauma centre and regional referral centre with 142 intensive care beds. UF Health Jacksonville patients will be primarily enrolled from the adult emergency department, and medical and surgical ICUs. UF Health (Gainesville) is a level 1 trauma centre and academic, quaternary care medical centre that serves as the primary teaching and research hospital for UF College of Medicine. UF Health (Gainesville) patients will be enrolled from the Department of Surgery's surgical tertiary-care trauma and surgery ICUs. The UF Sepsis and Critical Illness Research Center will provide administrative resources for patient enrolment as well as a central laboratory for sample storage and analyses.

Eligible non-enrolled/non-randomised patients

We will report any of the following reasons for failure to enrol or randomise eligible patients in this research study, including (1) patient or legal representative refusal of consent, (2) lack of available consent, (3) clinician refusal to allow the patient's entry into the study, or (4) any other reason.

Informed consent

The research team will review eligibility criteria and will seek consent when criteria are met. Written informed consent and written authorisation will be obtained from all patients or their legal representatives in compliance with the Declaration of Helsinki and the Health Insurance Portability and Accountability Act (HIPAA) privacy rule prior to performing any study procedures. Eligible patients must be enrolled within 24 hours of sepsis recognition.

Randomisation and allocation concealment

The phase II study employs a permuted blocked randomisation technique with patients stratified by centre. Permuted blocked randomisation has several advantages in that it supports group balance at the end of the trial, and it supports continuous balance during trial progression by assuring that sequential patients are distributed equally between groups. The study statistician will generate the randomisation sequence.

Experimental procedures

LIE therapy

The LIE is Smoflipid (20% lipid emulsion for injection; Fresenius Kabi), which contains soybean oil, Medium Chain Triglycerides (MCTs), olive oil and fish oil in a 30:30:25:15 ratio. Smoflipid also contains 0.163–0.225 mg/mL of all-rac-α-tocopherol. The initial infusion rate of LIE will occur at a rate of 0.5 mL/min for the first 30 min to minimise exposure to the drug should the patient experience any side effects. If tolerated, the infusion will proceed at the maximum FDA-approved rate of 0.11 g/kg/hour, and not to exceed 0.5 mL/kg/hour. The specified dose (dose-escalation level for phase I or randomised dose for phase II) of the study drug will be administered twice over the first 48 hours after enrolment at 24-hour intervals, so that each patient will receive two doses of the study drug at each dose level. The proposed doses of LIE are based on current manufacturer-recommended dosing for nutritional purposes for this specific drug. The FDA-approved dose range is 1–2 g/kg/day, and not to exceed 2.5 g/kg/day. All infusion bags and hospital equipment that will be used for administration of LIE are standard use in the hospital and approved by the FDA, and non-PVC (polyvinyl chloride) tubing and a 1.2-micron inline filter will be used. All study treatments will be administered through a dedicated intravenous line, either via an existing central venous catheter or via a single lumen peripheral intravenous catheter. This approved method of administration is safe.

Study monitoring

Only patients with sepsis or septic shock admitted to an ICU will be enrolled in the study. For the first hour after initiation of drug administration, patients will be closely monitored for any adverse effects temporally related to drug administration. After this, patients will continue to have ICU-level monitoring during the period of drug administration (ranges from 10 to 16 hours according to drug dose). At 24 hours after enrolment, a study critical care pharmacist or nutritionist who has familiarity with the drug will review laboratory parameters including serum electrolytes, triglycerides, liver and kidney function, glucose monitoring, platelets, and coagulation parameters for any clinically significant laboratory abnormalities prior to approving administration of the second dose. At 48 hours, 72 hours and 7 days, all adverse events will be summarised and reviewed by the study monitor. Serious and likely related or related adverse events will be reported to the safety monitors and institutional review board within 5 business days. A decision to terminate the trial will be made by the two safety monitors (emergency medicine-toxicologist and cardiologist/clinical trialist) and study statistician as specified in the Data Safety Monitoring Plan, in conjunction with the principal investigator and in consultation with the institutional review board. Reviews by the Data Safety Monitoring Board (DSMB) of enrolled patients will occur after enrolment of the fourth and eighth patients and at completion of the phase I trial

and again after the 30th and 45th patients and at phase II completion.

Monitoring for adverse reactions

Patients will be closely monitored for the following adverse reactions to LIE: nausea, vomiting, hypergly-caemia, flatulence, fever, abdominal pain, increased blood triglycerides, hypertension, dyspepsia, anaemia, device-related infection, headache, sweating, dizziness, flushing, rash, urticaria, erythema, fish-like taste in mouth and coagulation defects. In addition, any reactions temporally related to drug administration or not pre-existing prior to the time of drug administration will be reported.

Investigational measurements

Blood will be drawn for cholesterol levels, lipid oxidation, HDL function, inflammatory biomarkers and SOFA score. Inflammatory biomarkers will include plasma monocyte chemotaxis (MCP-1), Growth-related oncogene (GRO), interleukin (IL)-6, IL-8 IL-10, tumour necrosis factor-α and IL-12. These have been chosen due to previous associations between proinflammatory HDL with MCP-1 and previous studies showing relationships between dysfunctional HDL and these specific biomarkers. Blood will be drawn for serial lipid panels on enrolment, at 24, 48 and 72 hours, and on day 7. For patients discharged from the hospital prior to day 7, a phlebotomy service will be sent to the patient's place of residence (home, nursing facility and so on) to draw their blood. Tests of lipid oxidation including dysfunctional HDL using the cell free assay, a measure of the antioxidant capacity of HDL, will be performed as in previous studies[38] and will be performed on blood drawn at enrolment, at 48 hours, as well as 7 days later to assess change over time. To prevent oxidative degradation of the samples, an antioxidant buffer will be added to samples prior to storage.[39] The cholesterol efflux assay is a quantitative assessment of HDL function and measures the ability of HDL to move lower density lipids from peripheral macrophages to the liver for clearance. HDL-mediated cellular cholesterol efflux assays will be performed to assess potential improvements in HDL function with LIE versus control as in previous studies.[27] SOFA score assessment will occur prior to enrolment and 48 hours later as well as at 7 days to determine change in SOFA score over time as well as serum lactate.

Blinding

Because this is a pilot study, and because the lipid emulsion appears white and will be visible to the treatment team, blinding to LIE is difficult and costly. Data abstractors, however, will be blinded to treatment allocation. As the primary outcome is numerical cholesterol levels, the likelihood of bias is low. If the pilot study leads to a larger multicentre trial, blinding procedures will be developed and applied.[40]

Outcomes

Consent rate

We expect 50% of patients or their legal representative to consent to participate in the study. Standardised consent forms will be used as well as standardised measures for determining patient capacity for consent in this critically ill population. Patients whose legal representatives provide consent for them will be reconsented as soon as feasible after regaining decisional capacity. Reasons for refusal to participate will be recorded and will be addressed on an as-needed basis.

Recruitment

We expect to recruit two to three patients per month at each clinical site. The 'Possible Sepsis' notification system as well as the 'Sepsis Alert' systems will be used to identify patients for prospective enrolment into sepsis research studies. The 'Possible Sepsis' notifications send hourly pages and generate a daily list of potentially septic patients based on vital signs measurements throughout the hospital and is currently used by the Hospital Sepsis Taskforce. The 'Sepsis Alert' system is activated by a provider when a patient is highly suspected of sepsis and is being managed with the hospital sepsis care bundle.

Protocol adherence

Protocol adherence will be defined by enrolment into the study with completion of both doses of the study drug at enrolment and at 24 hours with a lipid panel drawn at 48 hours for assessment of the primary endpoint. For enrolled patients who do not meet these requirements for the phase I study, an additional patient will be enrolled at the same dose to assess for efficacy and DLT. Data for the phase II study will be analysed using the intent-to-treat principle, regardless of completion of the initial 48-hour period.

Clinical outcomes

The phase II trial primary endpoint is delta (48 hours – enrolment) serum total cholesterol between groups. Phase II trial success will be defined by a statistically significant difference in delta serum total cholesterol between groups. Secondary endpoints include change in cholesterol level from enrolment to 7 days, change in SOFA score over the first 48 hours and 7 days, in-hospital and 28-day mortality, lipid oxidation status (HDL inflammatory index), inflammatory biomarkers, and HDL function (cholesterol efflux capacity).

Data collection

Data collected will include demographics, comorbid conditions (Charlson Comorbidity Score, thyroid disease), medication use (statin use), vitals, lab values, antibiotics and vasopressors through chart reviews and interviews with the patient or legal representative. We will also record the type and formulation of enteral feeds, rate of administration, supplemental protein and lipid kilocalories per day for the first 7 days of the study. At the time of enrolment, data will be collected and will

continue through day 7. On day 28, a mortality check on the patient or their legal representative will be completed by phone. Information is documented on standard case report forms and then entered into Research Electronic Data Capture (REDCap), a secure web-based database at the UF.

Sample size and data analysis

Our preliminary data suggest that with the proposed enrolment criteria, patients experienced an average decrease of 17 mg/dL (SD=23) in cholesterol at 48 hours. If the treatment group can stop the decline or increase the total cholesterol by 2 mg/dL, which corresponds to a between-group difference with Cohen's effect size of 0.74 or 0.83, then at a significance level of 0.05, 24 patients in each arm (n=48) will be able to detect the difference at 71% or 80% power, respectively.

The primary method of analysis will be using an unpaired t-test of change in mean lipid levels between the groups (48 hours – enrolment) and Wilcoxon rank-sum test between groups for change in SOFA score (48 hours – enrolment). For other secondary endpoints, normally distributed numerical data will be compared using unpaired t-test, while non-normally distributed data will be compared using Wilcoxon rank-sum. Finally categorical data will be compared using Pearson's χ^2 test. For all statistical tests $p < 0.05$ will be considered significant.

DISCUSSION

A large body of evidence supports the relationship between low cholesterol levels and poor outcomes after sepsis, including worsening organ failure and death. However, the causality of this relationship and whether early decreases in cholesterol levels can be prevented or treated are unknown. With this pilot study, we propose to test the hypothesis that a lipid emulsion will be able to stabilise cholesterol levels in patients with early sepsis and low enrolment total cholesterol levels in comparison with patients not receiving lipid emulsion therapy.

There are numerous signals of evidence that support our hypothesis. Phillip Dellinger and colleagues[41] performed a multicentre randomised controlled trial of a different drug (a phospholipid emulsion) for Gram-negative severe sepsis with the idea that the drug would be able to facilitate endotoxin clearance. Although the study initially demonstrated no effect on mortality,[41] a secondary post-hoc analysis was performed that was limited to patients with albumin ≥1.5 g/dL and either total cholesterol ≥40 mg/dL or HDL-C ≥20 mg/dL and showed potential reduced mortality of 6.6% (p<0.025) and 10.8% (p<0.005), respectively.[42] That study suggests that adequate liver function and a minimum quantity of circulating cholesterol are needed for effective lipid-mediated defence against sepsis, and guided the development of our inclusion/exclusion criteria. Our study proposes to carefully select for potential responders and metabolisers of LIE by only enrolling patients with low baseline total cholesterol (<100 mg/dL) and by excluding patients with severe liver dysfunction (total bilirubin >2 mg/dL or albumin <1.5 g/dL). Importantly, these screening criteria will still allow enrolment of nearly half of patients with sepsis based on our previous observational studies.[28]

We have chosen to study the proposed fish oil-containing LIE because there is a robust amount of preclinical and clinical data supporting its therapeutic efficacy. There are also few other lipid-based therapies that are readily available for testing in human sepsis trials that are likely to work. Recent attention has been given in the sepsis literature to proprotein convertase subtilisin/kexin type 9 (PCSK9) and its effect on LDL-C clearance in sepsis. PCSK9 is a zymogen in the proprotein convertase family involved in the regulation of hepatic LDLRs and therefore affects LDL and LDL-C clearance. A single gene produces PCSK9, which autocleaves in the endoplasmic reticulum to a mature form that, on release from cells, can bind LDLRs for endocytosis and lysosomal degradation. PCSK9 inhibitors are a recently developed class of potent lipid-lowering drugs that effectively and acutely reduce LDL-C levels, and Walley and colleagues[10 43] have shown that these may have a role in facilitating endotoxin clearance via the endotoxin binding to LDL-C and subsequent clearance through the LDL-R. Although PCSK9 inhibitors are a strong candidate for future testing, we have safety concerns as PCSK9 inhibitors will result in further LDL-C lowering in critically ill patients with sepsis with already low LDL levels, and this has been associated with poor outcomes in our studies and preliminary data.[22] We propose that stabilising cholesterol levels in carefully selected patients with sepsis who have the ability to metabolise the drug and clear bacterial toxins is a safer first step, and that future studies may attempt to combine PCSK9 inhibitors with lipid emulsion therapy after initial cholesterol stabilisation. In support of the concept that lowering cholesterol may increase sepsis risk, a study of torcetrapib (a cholesterol ester transfer protein inhibitor that lowers cholesterol levels) in patients with high cardiovascular disease risk showed increased all-cause mortality in the treatment group, who demonstrated significantly lower LDL-C and higher HDL-C levels.[44] When evaluating mortality cases, nearly half of them were due to 'infection' in the torcetrapib group.

Fish oil lipid emulsions have been shown to reduce acute kidney and acute lung injury, suppress inflammation, and favourably modulate immune function in septic mice.[45–47] These emulsions consist of only fish oil, as opposed to Smoflipid, which contains 15% fish oil. There are numerous clinical studies that have shown potential benefit for fish oil lipid emulsions. A randomised controlled pilot trial of 60 ICU patients with sepsis demonstrated clinical efficacy with regard to improved organ failure and mortality (prespecified subset of patients) after administration of a fish oil-only lipid emulsion.[48] In another single-centre, randomised controlled trial, a fish oil-only lipid emulsion was compared with a medium-chain and long-chain triglyceride emulsion in

patients with sepsis and showed significant reductions in inflammatory cytokines in the fish oil group.[49] In a large study of 661 critically ill patients, of whom 292 had sepsis, fish oil doses of 0.1–0.2 g/kg/day showed significant favourable effects on survival, infection rates and length of stay.[50]

It should be noted that we are proposing to use LIE in this study as a drug, with dosing targeted towards stabilising cholesterol levels, rather than as a nutritional agent for which it is intended. This has not been attempted in previous clinical trials, and for this reason we believe the negative results of nutritional studies in critically ill patients such as the OMEGA trial and MetaPlus trials may not be entirely relevant. The OMEGA trial was performed in patients with acute lung injury requiring mechanical ventilation, and provided twice-daily enteral supplementation of ω−3 fatty acids, γ-linolenic acid and antioxidants in comparison with an isocaloric control supplement.[51] There are several differences between LIPIDS-P and the OMEGA trial. First, all patients in OMEGA had a nutritional requirement, which is not true for the LIPIDS-P trial. Our proposed patient population of patients with early sepsis would not necessarily receive enteral or parenteral nutrition otherwise. Second, only 23% of patients in the experimental arm and 21% of patients in the control arm of the OMEGA trial were septic and our hypothesis is specific to the sepsis population. Third, the focus of serially evaluating and stabilising cholesterol levels has not been looked at in any prior sepsis clinical trial. Finally, we proposed a parenteral rather than enteral route of providing lipids to stabilise cholesterol levels as we believe these lipids would be more immediately available for conversion to cholesterol in patients with adequate hepatic function. We also believe that the use of parenteral fish oil containing lipid emulsions is supported by the study by Hall and colleagues[48] noted above. The MetaPlus trial treated mechanically ventilated critically ill patients with either high-protein enteral nutrition enriched with glutamine, ω−3 fatty acids, selenium and antioxidants compared with standard high-protein enteral nutrition and found an increased adjusted 6-month mortality in the experimental arm.[52] Again, differences include the use of an enteral formulation, the presumed need for enteral nutrition within 48 hours of ICU admission and a low proportion of patients with sepsis as their primary diagnosis (21% experimental arm, 23% control arm). These differences between nutrition studies and our proposed trial therefore do not preclude us from testing our hypothesis of cholesterol stabilisation and the anti-inflammatory effects of ω−3 fatty acids in this carefully selected sepsis population.

Summary, future directions and conclusions

In summary, with this pilot clinical trial we propose to demonstrate that cholesterol stabilisation using a fish oil-containing LIE in early sepsis is possible in what is to our knowledge the first study of its kind. Studies also suggest that lipid function, in addition to the quantitative decline in lipid levels, may play a role in adverse outcomes after sepsis. It remains unknown whether cholesterol stabilisation with an exogenous lipid emulsion can mitigate these adverse outcomes. Although this preliminary pilot study seeks to alter cholesterol levels while also evaluating organ function and inflammation, future work will be aimed at modulating organ failure, an important patient-oriented outcome in patients with sepsis and septic shock.

ETHICS AND DISSEMINATION

Patients were not involved in the design of this pilot study. All ethical procedures will be upheld in the conduct of this study, and results will be disseminated through presentations at national meetings and publication in peer-reviewed journals. If promising, data from this pilot study will be used as a basis for a larger, multicentre, phase II clinical trial.

PATIENT AND PUBLIC INVOLVEMENT

Our previous studies have informed the design of this study, with particular regard to quality of life after sepsis. Early organ failure, one of the main clinical outcomes after sepsis, is the main clinical outcome of the study and greatly affects patients' lives, as patients with persistent organ failure may need dialysis and frequent use of medical facilities for additional care, which may reduce quality of life. Patients were not involved in the design of the study. We plan to inform our enrolled participants by sending them a letter or an email after completion of the study with a summary of the study results and thanking them for their participation. The burden of the intervention was not assessed by patients.

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are contributing to the conduct of the trial. All others agree to be accountable for the integrity of the work performed in this trial.

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