Defining ARDS and sepsis sub-phenotypes: a re-analysis of two trials to inform stratified medicine approach with future trials

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Abstract

Background

Acute Respiratory Distress Syndrome (ARDS) and sepsis are the two most common illnesses in the critically ill, with increasing incidence and high mortality. The ARDS and sepsis literature is replete with randomised controlled trials (RCTs) that show no difference between the intervention and standard care groups in the overall study population, despite promising pre-clinical data that a therapeutic effect from the intervention may exist.

Recently, we completed two RCTs funded by the NIHR EME and RfPB programmes: simvastatin therapy in ARDS (HARP-2) and vasopressin and steroids in septic shock (VANISH). The HARP-2 trial hypothesized that the immunomodulatory effects of statins would reduce pulmonary dysfunction, resulting in more ventilator free days. The VANISH trial hypothesised that vasopressin therapy and the immunomodulatory effects of corticosteroids would reduce shock duration, resulting in greater kidney-failure free days.

Indeterminate RCTs could result from the clinical and biological heterogeneity producing variation in treatment response. Identifying homogenous groups (sub-phenotypes) such as patients with certain clinical or biological features or treatment response characteristics is a research priority. This will inform future trial design by enriching patient populations likely to benefit the most from interventions.

Hypotheses

Within ARDS and sepsis cohorts, there are between and within patient differences in pathobiology, treatment effects of interventions and outcomes, referred to as 'heterogeneity'. This heterogeneity is considered an important reason for indeterminate (statistically negative) randomized clinical trials (RCTs).

Thus, in the context of indeterminate RCTs, we plan to address two distinct hypotheses.

Hypothesis 1:

First, the differences in treatment effect of intervention over varying baseline risk of outcome is referred as heterogeneity in the treatment effect, which could explain the indeterminate results of HARP-2 and VANISH trials. Specifically, the absence of statistically significant findings overall in the HARP-2 and VANISH trial may mask effects in baseline subgroups that are identifiable by investigating heterogeneity of treatment effect.

Hypothesis 2:

Second, the differences in pathobiology when related to clinical and outcome characteristics will provide biologically separate sub-groups of ARDS and sepsis patients. These subgroups may differ in their response to the tested treatments, which could also explain the indeterminate results of HARP-2 and VANISH trials. These biologically distinct patient subgroups are referred to as sub-phenotypes. Thus, a statistical interaction to tested treatment within these biomarkers' derived subgroups would potentially identify treatment

responsive sub-phenotypes for statins in ARDS within the HARP-2 trial ARDS population and steroids in septic shock within the VANISH trial septic shock population.
Specifically, latent treatment-responsive sub-phenotypes exist to be derived and validated in both ARDS and sepsis populations.

Aims

- To identify ARDS and septic shock sub-phenotypes using baseline characteristics, clinical data, cytokine, neutrophil and endothelial injury, organ specific marker profiles.
- To identify treatment responsive sub-phenotypes in ARDS and septic shock.
- To delineate mechanisms of action of statins in ARDS and hydrocortisone in septic shock using cytokine, neutrophil and endothelial injury, organ specific marker profiles.

Methods

First, we will assess how the treatment response varies by baseline risk deciles within HARP-2 and VANISH cohort by generating baseline risk of death model using pre-randomisation clinical and biological data. Second, to delineate mechanisms of actions we will measure selected patterns of key cytokines driving neutrophil activation, lung endothelial and organ injury markers using stored serum samples from the two RCTs. Ethical approval is in place to use the stored samples for proposed analysis. Then we will replicate the ARDS sub-phenotypes reported previously using latent class analysis (LCA) from the baseline samples collected in HARP-2 trial and generate septic shock sub-phenotypes using the same LCA approach in the VANISH trial. We will then extend these analyses to identify treatment responsive sub-phenotypes to simvastatin, and to hydrocortisone in the respective cohorts. We will then investigate the potential mechanism of action of the therapies within treatment responsive sub-phenotypes, followed by validation in three recently completed trials.

Background

What is sepsis and ARDS?

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection. Septic shock is a subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to increase mortality[1, 2].

ARDS refers to onset of hypoxaemia with bilateral infiltrates on chest radiology that is of non-cardiac origin, within a week on onset of clinical insult, resulting in respiratory failure [3].

Definitions of syndromes seen in critically ill patients such as Acute Respiratory Distress Syndrome (ARDS) and sepsis use a trade-off between feasibility and reliability[2, 4]. Although both definitions identify clinical phenotypes with predictive validity[3], due to the underlying biological differences within this overall broad clinical phenotype, pharmacological interventions are frequently not effective in randomised controlled clinical trials (RCT) [5, 6]. Thus identifying sub-phenotypes, informed by biological and clinical characteristics, which are more likely to benefit from targeted interventions is a research priority.

ARDS and Sepsis are common conditions

The two conditions that are being studied (ARDS and Sepsis) are common in critical care settings 10.4% and 25% respectively, with a high mortality[7, 8]. The estimated crude population incidence of ARDS was 78.9 per 100,000 person-years[9] and increasing[7]. The estimated global population incidence of sepsis is 148 (98 – 226) per 100,000 person-years[10], with extrapolated adult population incidence of sepsis in England at 83/100,000 population[8] and increasing. These incidence data, when taken together, makes these conditions more common than heart disease and individual cancers.

Overlaps in sepsis and ARDS biology

The most common aetiology of ARDS is sepsis[7]. The initiating insult in both these conditions is the host innate immune response to danger signals in the form of conserved motifs on pathogens' surface (pathogen associated molecular patterns) and from tissue damage (damage associated molecular patterns). The endothelial involvement in both sepsis and ARDS is characterised by increased vascular permeability, endothelial damage, and

endothelial activation. Changes similar to the endothelium are also seen in epithelial tissues. Multi organ failure that characterises both these syndromes is a combination of end organ cellular damage, impaired tissue oxygen utilisation resulting in oxygen debt and potentially tissue shut down as a protective mechanism. These large overlaps in biology lend the two syndromes to be evaluated concurrently for similar patient sub-phenotypes [11-15].

Acute and long-term care of ARDS and sepsis patients is resource intensive

The hospital length of stay for ARDS and sepsis patients are similar with estimated median (IQR) 17 (9 - 32) days[7] and 16 (8 – 33) days[8] respectively. One in five ARDS[16] and sepsis[17] patients who survive hospitalisation die in the first year following discharge[16]. Both ARDS and sepsis patients have impaired quality of life, multiple readmissions, functional, cognitive and physical disabilities[17-22], that persists beyond the acute illness. Thus interventions that have the potential to improve short and long-term outcomes are important.

ARDS and Sepsis are heterogeneous conditions with no specific treatments

As syndromes, ARDS[3] and sepsis[1, 2] case definitions are a trade-off between feasibility and reliability[2, 4], which contributes to clinical and biological heterogeneity[5, 23]. Clinical heterogeneity refers to differences in aetiology, pathogens, age, gender, genetic makeup, comorbidities, and concurrent medications. The biological heterogeneity refers to the differences in host responses both within and between organ systems. Therefore identifying patients groups at higher risk of adverse outcomes or those with greater treatment response or those with a well characterised biological pathway/ target is important to identify effective treatments from indeterminate trials.

Indeterminate trials are common in the ARDS and sepsis

Indeterminate trials refer to randomised controlled trials (RCTs) where no statistically significant difference in average treatment effect was observed in the whole study population. It is being increasing recognised that within a heterogeneous study population there may subgroups of patients who may actually benefit from the intervention while at the same time

there are others subgroups without effect or who may even potentially be harmed[6]. In an RCT, the overall result depends on the sample size, the observed treatment effect and the variations in treatment effects (sub-phenotypes) amongst the patients enrolled in a trial [24].

Hypothesis

Within ARDS and sepsis cohorts, there are between and within patient differences in pathobiology, treatment effects of interventions and outcomes, referred to as 'heterogeneity'. This heterogeneity is considered an important reason for indeterminate (statistically negative) randomized clinical trials (RCTs).

Thus, in the context of indeterminate RCTs, we plan to address two distinct hypotheses.

Hypothesis 1:

First, the differences in treatment effect of intervention over varying baseline risk of outcome is referred as heterogeneity in the treatment effect, which could explain the indeterminate results of HARP-2[25] and VANISH[26] trials. Specifically, the absence of statistically significant findings overall in the HARP-2 and VANISH trial may mask effects in baseline subgroups that are identifiable by investigating heterogeneity of treatment effect.

Hypothesis 2:

Second, the differences in pathobiology when related to clinical and outcome characteristics will provide biologically separate sub-groups of ARDS and sepsis patients. These subgroups may differ in their response to the tested treatments, which could also explain the indeterminate results of HARP-2 and VANISH trials. These biologically distinct patient subgroups are referred to as sub-phenotypes. Thus, a statistical interaction to tested treatment within these biomarkers' derived subgroups would potentially identify treatment responsive sub-phenotypes for statins in ARDS within the HARP-2 trial ARDS population and steroids in septic shock within the VANISH trial septic shock population.

Specifically, latent treatment-responsive sub-phenotypes exist to be derived and validated in both ARDS and sepsis populations.

Aims

We plan to analyse sepsis and ARDS trial populations separately.

Our aims are

1) How does the response to the treatments tested in the two trials (statin in HARP-2 trial and steroids in VANISH trial) vary with differences in severity of illness, referred as heterogeneity in treatment effect (HTE)? To test for the first time using actual trial data, whether heterogeneity in treatment effect (HTE) reported previously using only simulations, explain indeterminate RCTs in critically ill patients.
2) To identify ARDS and septic shock sub-phenotypes using baseline characteristics, clinical data, selected cytokines driving neutrophil activation, epithelial and endothelial injury marker profiles by re-analysing two recently completed NIHR funded indeterminate RCTS [25, 26]
3) To identify treatment responsive sub-phenotypes in ARDS and septic shock.
4) To delineate mechanisms of action of statins in ARDS and hydrocortisone in septic shock by using patterns of cytokines, neutrophil activation and endothelial injury markers in treatment responsive sub-phenotypes.
5) Validate this analyses in two further trials – LeoPARDS[27] and VACS trials[28]

Objectives

Our objectives are

a) To assess presence of HTE by baseline risk deciles in the two RCTs[25, 26] by developing baseline risk of death model using pre-randomisation data.
b) Derive ARDS sub-phenotypes from HARP-2 study[25] using latent class analysis (LCA) to test whether these groups match previous reports [5, 29]. Since the outline stage, we are well on our way to completing this objective. For replication and validation of ARDS sub-phenotypes reported previously[5, 29] and to understand treatment response mechanisms for statins and steroids, the inflammatory state will be assessed using interleukins (1L-1B, IL-6, 1L-8, IL-10, 1L-17, IL-18), and soluble tumour necrosis factor receptor. As surrogates for neutrophil activation and endothelial injury with consequent

end organ dysfunction, we will use myeloperoxidase, soluble intracellular adhesion molecule, angiotensin-2, troponin and b-natriuretic peptide.

c) Using LCA, derive septic shock sub-phenotypes using VANISH study[26] and compare to ARDS subphenotypes

d) Describe patterns in cytokine profile, endothelial and epithelial injury and organ dysfunction markers in ARDS and septic shock to define correlates to ARDS resolution and septic shock resolution.

e) Identify the sub-phenotypes directly related to simvastatin effect as ARDS resolution and to hydrocortisone effect as septic shock resolution, by keeping the randomization intact. With high probabilities of class membership, participants will be assigned to their most likely phenotype, and an additional approach may be required accounting for uncertainty in class membership. Regression methods with likelihood ratio tests will be used to assess the association of classes with clinical outcomes with randomization kept intact, and extended to compare response amongst randomised treatments.

Design

To address our hypothesis and aims, we propose a re-analysis of existing data from two trials: simvastatin in ARDS (HARP-2)[25] and steroids and vasopression in septic shock (VANISH)[26].

First we will use baseline clinical data to test for heterogeneity in treatment effect (HTE) to ascertain whether the average treatment effect varies by the cohorts' baseline risk.

Secondly we plan to measure selected cytokine, epithelial and endothelial injury markers that will identify more homogeneous sub-phenotypes using latent class analysis (LCA).

We will then test for treatment effect within the sub-phenotypes. Furthermore, we will measure these biomarkers over time in serial samples to help inform biological mechanisms associated with treatment responses to simvastatin and hydrocortisone.

We will then validate this using two further trials.

Study population

Critically ill adults with ARDS and septic shock

ARDS cohort is from the HARP-2 trial[25]

- The HARP-2 trial that tested the hypothesis that treatment with simvastatin would improve clinical outcomes in patients with ARDS.
- Methods: In this multicentre, allocation concealed, double-blind clinical trial, patients with an onset of ARDS within the previous 48 hours were randomised in a 1:1 ratio to receive enteral simvastatin 80mg or placebo once daily for a maximum of 28 days.
- Outcomes: The primary outcome was the number of ventilator-free days to day 28. Secondary outcomes included number of non-pulmonary organ failure free days to day 28, mortality and safety.
- Study population: Patients were eligible if they were intubated and mechanically ventilated and had a partial pressure of arterial oxygen to fractional inspired oxygen concentration (PaO_2/FIO_2) ratio of 300 mmHg or less, if bilateral pulmonary infiltrates consistent with pulmonary oedema were present on chest radiograph, and if there was no evidence of left atrial hypertension. The study was amended to permit enrolment of patients receiving macrolides and for the level of alanine aminotransferase and/or aspartate aminotransferase for eligibility to be increased from more than five times to eight times the upper limit of the normal range.
- Intervention: Patients received once daily enteral route simvastatin 80mg or identical placebo tablets for up to 28 days, discharge from critical care, death or discontinuation of active medical treatment, development of a clinical condition requiring immediate treatment with a statin or withdrawal of the patient from the study. The study drug was stopped on safety grounds if the attending clinician determined that this was required, if the levels of creatine kinase were more than ten times the upper limit of the normal range or if the levels of alanine aminotransferase and/or aspartate aminotransferase were more than eight times the upper limit of the normal range.
- Results: The study recruited 540 patients with 259 patients allocated to simvastatin and 281 patients to placebo. The groups were well matched with respect to demographic and baseline physiological variables. There was no significant

difference between study groups in mean (± SD) ventilator-free days (12.6±9.9 with simvastatin and 11.5±10.4 with placebo, P = 0.21), non-pulmonary organ failure free days (19.4±11.1 with simvastatin and 17.8±11.7 with placebo, P = 0.11) or in 28-day mortality (22.0% with simvastatin and 26.8% with placebo, P = 0.23). There was no significant different in ICU, hospital and or 1 year mortality. There was no difference in the incidence of severe adverse events between the groups.

The septic shock cohort is from the VANISH trial[26]

- The VANISH trial compared the effect of early vasopressin versus norepinephrine and/or hydrocortisone versus placebo on kidney failure in patients with septic shock.
- Methods: VANISH was a 2x2 factorial, double-blind, randomized clinical trial conducted in 18 general adult intensive care units in the United Kingdom between February 2013 and May 2015, enrolling adult patients who had septic shock requiring vasopressors despite fluid resuscitation within a maximum of 6 hours after the onset of shock.
- Outcomes: The primary outcome was kidney failure–free days during the 28-day period after randomization, measured as (1) the proportion of patients who never developed kidney failure and (2) median number of days alive and free of kidney failure for patients who did not survive, who experienced kidney failure, or both. Rates of renal replacement therapy, mortality, and serious adverse events were secondary outcomes.
- Study population: Adult patients (≥16 years) who had sepsis (2 of 4 systemic inflammatory response criteria due to known or suspected infection12) and who required vasopressors despite adequate intravenous fluid resuscitation, as assessed by clinical examination, central venous pressure, oxygen saturation, or other physiological parameters using repeated fluid challenges were eligible for the trial.
- Interventions: Patients were randomly allocated to vasopressin (titrated up to 0.06U/min) and hydrocortisone (n = 101), vasopressin and placebo (n = 104), norepinephrine and hydrocortisone (n = 101), or norepinephrine and placebo (n = 103).

- Results: 409 patients were included in the study, with a median time to study drug administration of 3.5 hours after diagnosis of shock. The number of survivors who never developed kidney failure was 94 of 165 patients (57.0%) in the vasopressin group and 93 of 157 patients (59.2%) in the norepinephrine group (difference, −2.3% [95% CI, −13.0% to 8.5%]). There was less use of renal replacement therapy in the vasopressin group than in the norepinephrine group (25.4% for vasopressin vs 35.3% for norepinephrine; difference, −9.9% [95% CI, −19.3% to −0.6%]).

Ethical arrangements

29th November 2017:

After discussion with Sponsor post award of the grant, we were asked to a HRA submission. The reason being, the project was considered as a fresh hypothesis testing on previously collected samples with appropriate consents in place.

The HARP-2 trial was approved by a national research ethics committee and by the research governance department at each study site in the United Kingdom and by the institutional research ethics committee at each study site in Ireland. All the patients or their representatives provided written informed consent. The VANISH trial was approved by the Oxford A research ethics committee. In view of the emergency nature of the trial, a waiver of initial consent was granted and retrospective written consent was sought once the patient regained decision-making capacity. This consent also included collection of serial plasma samples from patients enrolled in the two trials, which will be used for bioassays described next in this project [25, 26].

The plasma samples will be stored in secure laboratories, with remote temperature monitoring. Standard operating procedures will be used to store, transport and analyse samples. The assays will be carried out in the research laboratories at Imperial College London for VANISH trial samples and at Queens University Belfast for HARP-2 samples. HARP-2 samples were stored with ethical permission for future use and permission to undertake the planned assays under the previous ethical approval (ORECNI REC B 10/NIR02/36) is in place until 30th June 2017. We have confirmed with the study sponsor that to use the study samples for any additional analyses only local QUB ethics is required and we

will submit this if needed. The sponsor has confirmed no other governance approval is required.

The REC number for VANISH is 12/SC/0014 and this included approval for “analysis of blood, plasma and urinary biomarkers of renal function and inflammation (including genetic polymorphisms)”. Similarly, the trials planned for validation also have ethics approvals. VACS trial (10/H0604/35)and LeoPARDS trials (ISRCTN12776039).

NIHR acknowledgement and disclaimer:

Acknowledgement

This project is funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership.

Disclaimer

The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NHS, NIHR or the Department of Health.

Measurement of bioassays and rationale

Calfee et al[5, 29] reanalyzed three RCTs in ARDS completed over ten years ago[30-32]; using clinical data and measuring similar biological biomarkers to those we propose, and reported two consistent sub-phenotypes in all three RCTs[5, 29]. The more inflammatory sub-phenotype responded differently to mechanical ventilation and fluid therapy[5, 29]. This observation led us to hypothesize that anti-inflammatory drugs (simvastatin and hydrocortisone[33]) may have a different treatment effect within inflammatory sub-phenotypes in ARDS (HARP-2)[25] and in septic shock (VANISH)[26]. Due to overlaps in biology [34, 35] and etiology of ARDS and sepsis, we hypothesised the presence of similar sub-phenotypes in both these conditions.

To explore this, we have chosen two drugs (simvastatin and hydrocortisone) with overlapping pleotropic effects on cytokines driving neutrophil activation, epithelial and endothelial injury. We used a hypothesis driven approach to select the most appropriate biomarkers to ensure comparability to preliminary reports addressing similar research questions[5, 29]. For example, both these drugs decrease the typical pro-inflammatory interleukin-6 and increase

the typical anti-inflammatory interleukin-10. It is therefore biologically plausible that we are likely to see ARDS and septic shock patients with differences in effect of these two interventions. Furthermore, our study will be the first unbiased assessment of the magnitude of effect of these two drugs on biomarker profiles that determine treatment effects in ARDS and sepsis.

Table-1: Biomarker choice and key rationale

Biomarkers	Biological rationale in sepsis and ARDS
Organ dysfunction - PaO2/FiO2 ratio - Creatinine - Platelets - Inotropes - Bilirubin	All variables required for SOFA score Marker of ARDS severity Marker of acute kidney injury Marker of haematological abnormality Cardiovascular or shock Hepatic dysfunction
Inflammation markers - IL-1B - IL-6 - IL-8 - IL-10 - IL-17 - IL-18	 Pro-inflammatory cytokine for host-defence to infection and injury Pro-inflammatory cytokine as surrogate for illness severity Chemokine, strongly associated with neutrophil chemiotaxis Anti-inflammatory cytokine with Th1 responses Potent regulator of neutrophils via CXC chemokine induction Influences production of interferon-γ from T-cells and natural killer cells
Leukocytes - Myeloperoxidase - sICAM	 Increased in early ARDS due to neutrophil activation; Enhances protein leakage at alveolar capillary membrane through by altering claudins resulting in increased tight junction permeability soluble Intracellular adhesion molecule (sICAM) that is involved in leukocyte migration
Endothelial injury - Angiotensin-2	 Affects microvascular permeability and vascular tone
Cardiovascular - Troponin - B-natriuretic peptide	 Marker of myocardial injury with additional prognostic value Marker of cardiac dysfunction

Table-2: Biomarkers in the three trials already measured (represented as 'Y" and to be measured (represented as 'to do') as part of this study. We also list 'Other markers*' that have been already measured as part of ongoing research. These markers will further enhance the granularity of the sub-phenotypes.

Biomarker	HARP-2 trial	VANISH trial	LeoPARDS and VACS trials trial
Number of patients	540	409	516 in LeoPARDS and 62 patients in VACS trials
Organ dysfunction - PaO2/FiO2 ratio - Creatinine - Platelets - Inotropes - Bilirubin	 Y Y Y Y Y	 Y Y Y Y Y	 Y Y Y Y Y
Inflammation markers - IL-1B - IL-6	 To do Y	 Y Y	 To do Y

- IL-8 - IL-10 - IL-17 - IL-18	To do To do To do To do	Y Y Y Y	Y Y To do To do
Leukocytes - Myeloperoxidase - sICAM	 To do To do	 To do To do	 To do To do
Endothelial injury - Angiotensin-2	 To do	 To do	 To do
Cardiovascular - Troponin - B-natriuretic peptide	 To do To do	 To do To do	 Y Y
Other markers* - sTNFr-1 - Lactate - CCL2	 Y Not planned Not planned	 To do Y Not planned	 Y Y Y

Laboratory methods

We plan to measure three groups of markers to help delineate specific biological effects and illness characteristics. First, a limited cytokine profile will be done to assess the balance between pro and anti-inflammatory state using interleukins (1L-1B, IL-6, 1L-8, IL-10, 1L-17, IL-18), and soluble tumour necrosis factor receptor. The state of neutrophil and endothelial injury will be assessed using myeloperoxidase, soluble intracellular adhesion molecule, and angiotensin-2. For organ dysfunction, in addition to the Sequential organ dysfunction score (SOFA) variables[36] we have collected as part of trial data, we will measure troponin and b-natriuretic peptide for cardiac dysfunction. Sequential measurements will be undertaken at baseline and on days 3.

These measurements will use ELISA methods and we have lab specific standard operating procedures for these measurements.

Statistical methods

Statistical methods use two methods to explore how indeterminate RCTs could result from the clinical and biological heterogeneity producing variation in treatment response namely heterogeneity in treatment effect and latent class analysis.

Trials enroll patients with different illness characteristics that is not completely controlled by the inclusion criteria. This results in heterogeneity. This heterogeneity could influence treatment response to the intervention tested and the outcome studied in the trial independent of the intervention.

The LCA tests the hypothesis that there exist unobserved (latent) sub-phenotypes that explain the relationship between lack of overall treatment effect in indeterminate RCTs – i.e. there exists sub-groups of patients with positive and negative treatment response to the intervention tested, which gives an overall indeterminate trial result. Finally, we have identified further data sources for validation. First, dataset is the recently completed trial of levosimenden for the prevention of Acute Organ dysfunction in sepsis (LeoPARDS Trial)[27]. This double-blind, 1:1 randomized clinical trial recruited 516 patients; with 259 patients assigned to levosimenden and 259 to standard care arm. The addition of levosimendan to standard treatment in adults with sepsis was not associated with less severe organ dysfunction or lower mortality. Plasma samples from 500 patients enrolled in the LeoPARDS trial (all with sepsis and 131, 26% had ARDS at baseline) are available that can be used to validate the presence of the same hyperinflammatory phenotype in an independent dataset. These have been collected in a similar manner to the VANISH samples. This trial samples will be used to validate the findings for sepsis population. We plan to supplement our primary analyses with multiple sensitivity analyses. The primary analyses will derive phenotypes using the whole VANISH trial population. The rationale being there was no evidence of an interaction between vasopressin and corticosteroids in the main trial. We will do a sensitivity analyses using the steroid only population and assess the consistency with results of primary analyses. We will also provide an interpretative descriptive validation using the LeoPARDS trial cohort to provide further evidence for the derived sub-phenotypes. We plan to supplement our primary analyses with multiple sensitivity analyses. The primary analyses will derive phenotypes using the whole VANISH trial population. The rationale being there was no evidence of an interaction between vasopressin and corticosteroids in the main trial. As highlighted by the EMEM board, we will do a sensitivity analyses using the steroid only population and assess the consistency with results of primary analyses. We will also provide an interpretative descriptive validation using the LeoPARDS trial cohort to provide further evidence for the derived sub-phenotypes.

- **Heterogeneity in treatment effect**

How does the response to the treatments tested in the two trials (statin in HARP-2 trial and steroids in VANISH trial) vary with differences in severity of illness, referred as heterogeneity

in treatment effect (HTE)? Ioannidis and Lau in a landmark study proposed a standardized way of evaluating baseline risk in the population enrolled in clinical trials. They proposed a four-stage approach: First, all characteristics potentially influencing the 'risk of outcome' are identified. Second, using a predictive model, the predicted risk of each patient is generated. Third, a histogram of risk distribution in the intervention and control arm is generated. Fourth, generate an appropriate measure of risk or odds to compare the outcomes from treatment[37].

We test for the first time using actual trial data in critically ill patients, whether heterogeneity in treatment effect (HTE) [6], explains indeterminate RCTs in critically ill patients. HTE will be assessed from the interaction between arm and the deciles derived from the model on trial outcome. Sub-phenotype effects will be assessed in the same way, and treatment effects estimated within sub-phenotypes with 95% confidence intervals. Dependence effects between decile generation and outcome modelling will be assessed with 2-fold cross-validation.[18] In view of the outcome data type, and for comparability, Cox proportional hazards regression of 28-day mortality will be used to assess both sub-phenotypes and to asses heterogeneity in treatment effect. As the mortality rates are 24% in HARP-2 and 29% in VANISH, there will be approximately 100 events in each trial cohort. Covariates will initially be univariately screened ($p<0.20$) to be considered for entry into a backwards model selection procedure, where the event numbers comfortably support models of ten covariates. The area under the nonparametric ROC curve c-index of the predicted hazards of death, derived from the model coefficients, will be taken to indicate the performance of the model. This approach is extendable to other trial outcomes set out within the objectives.

- **Latent class analysis**

Latent class analysis (LCA) of the baseline variables will be used to identify classes and replicate the previous work on ARDS trials published[5, 29]. We will derive ARDS and septic shock sub-phenotypes by applying latent class analysis to data from the HARP-2 and VANISH trials. These data variables will come from subjects in all trial arms, without the influence of arm, and consist of baseline characteristics, clinical data, cytokine, epithelial and endothelial injury marker profiles. For ARDS, the resulting sub-phenotypes will be compared

with those two derived independently in three trials previously[5, 29] and, therefore, as close to the same methodological approach will be maintained for sub-phenotype derivation and assessment for HARP-2, and for septic shock from VANISH.We will derive ARDS and septic shock sub-phenotypes by applying LCA to data from the HARP-2 and VANISH trials. These data variables will come from subjects in all trial arms, without the influence of arm, and consist of baseline characteristics, clinical data, cytokine, epithelial and endothelial injury marker profiles. For ARDS, the resulting sub-phenotypes will be compared with those two derived independently in three trials previously [5, 29] and, therefore, as close to the same methodological approach will be maintained for sub-phenotype derivation and assessment for HARP-2, and for septic shock from VANISH.

The inclusion of variables, and any adaptation to their form, will depend on their robustness for their multivariate purpose, which will be assessed by screening the univariate and bivariate data distributions for influential outliers, marked skewness and multi-collinearity, for categorical variables with extreme prevalence, and for variables contributing to the accumulation of missing data. We will closely follow the approach reported in the published supplement of Calfee et al [5, 29]. This will lead to establishing the principal dataset for the latent class analysis of each trial, where the variables are further standardised to the z-scale to have mean zero and unit variance, accounting for their differing units of measurement. A small number of data decisions might be less clear-cut, such as the exclusion of a variable with modest to moderate missing data, or categorical with extreme prevalence. We expect this because, in the paper by Calfee et al [5, 29], 4 of the 27 variables exceeded a 10% missing data rate. This will be added in an amendment to the statistical analysis plan as a sensitivity analysis to be undertaken after the principal modelling in order to check that sub-phenotypes are robustly derived. Omitted and included patients will be characterized and compared.

The latent class modelling stage will involve the estimation of linear combinations of the standardised variables to identify a number of underlying classes. The number of classes will be determined formally by using the Bayesian Information Criterion and other model selection criteria, and by assessing the clinical interpretability of the classes as sub-phenotypes. We will summarise the concordance of HARP-2 sub-phenotypes with the sub-phenotypes in

these two trials by calculating the rank correlation and by assessing the most influential variables arising in the three ARDS trials. With high probabilities of class membership, participants will be assigned to their most likely phenotype, and an additional approach may be required accounting for uncertainty in class membership. Regression methods with likelihood ratio tests will be used to assess the association of classes with clinical outcomes with randomization kept intact, and extended to compare response amongst randomised treatments. Given the factorial nature of the VANISH trial, this will involve a sequence of interactions tests respecting the design.

Patient and Public involvement

A detailed plan for Patient and Public involvement embedded approach to enhance dissemination and impact of our work will be put in place during the first 6 months. To this end, we have established links with ICUsteps and with the UK Sepsis Trust. We plan to obtain PPI help with written material and presentations of our research findings to reach patient groups. We also plan to disseminate the research findings using ICUsteps - The intensive care patient support charity webpage. The PPI team's travel and subsistence expenses for meeting attendances will be provided by the study team.

Exploitation and dissemination plan

We will develop a formal exploitation and dissemination plan. This will be communicated to the EME board.

Research governance

- Bioassays

All bioassays will be run in duplicate and in the event of any discrepancies will be repeated. We estimate that we will need to complete approximately 450 ELISA plates allowing for up to 1:4 samples requiring an additional measurement at new dilution (based on previous data from HARP-2 trial preliminary samples). We anticipate that 15 plates can be reliably repeated and analysed per week, and therefore we anticipate 30 weeks for the analysis of the cytokines/ biomarkers. Several of the kits require an additional validation step and will add 2-3 weeks to this time line.

HARP-2 trial samples:

Empty sample tubes were sent out to sites pre-labelled with a barcode. Each label and every aliquot had a unique identifier. All samples were sent from sites via a speciality medical courier (Biocair). All samples were shipped on dry ice, and thus kept frozen. On no occasion did any samples arrive into the QUB laboratory not still fully frozen. The samples were sent from the local site with a sample tracking log (Appendix 2), ensuring all samples were traceable. All samples were scanned on arrival using the pre-labelled barcode so the sample location was accurately recorded in the sample database held electronically which facilitates ready access to the samples as required. Samples are stored in remotely temperature monitored (T scan) -80^{o}C freezers. The readout from the temperature monitored are stored and are available on request. If the freezer functions outside of the defined temperature range (more than -60^{o}C) all users of the freezer are sent a text and an email. If the freezer was found to be operating incorrectly, the samples were moved to a back-up freezer (again temperature monitored). This occurred both in and out of hours. The freezers are maintained in rooms locked by a numerical keypad, and only selected members of staff have access to the room. There were no occasions when samples were thawed accidentally.

As the samples are required, the samples are again scanned using the pre-labelled barcode as they are removed from the freezer. When samples are removed from long term storage (for use or when sending to collaborators) the date of disposal/transfer is noted, the destination of the sample is entered (such as ‘used in ELISA analysis May 2017’, or ‘Sent to xxx March 2016’) and the samples are shaded yellow in the sample database to highlight these as no longer being present. The HARP 2 study was audited by Queen’s university Belfast in January 2014 while the study was ongoing. This audit identified no issues with sample management.

VANISH Trial samples:

All samples were collected according to standardised operating procedures across all recruiting sites (and similar SOPs were used for LeoPARDS). Blood samples were rapidly spun, separated and divided into multiple aliquots before being frozen and stored at -80C. Samples were then shipped to Imperial in batches where they have remained frozen at -80C. The multiple aliquots allow samples to be used that have not undergone any freeze-thaw cycles.

All samples have been tracked with bar codes, stored and transported with temperature control logs. This has been overseen by the trial manager according to the ICTU SOPs and has been audited by the ICTU QI manager and the VANISH trial has been audited by the MHRA and there were no major findings. The samples are registered with the Imperial Tissue Bank. We have requested some QA time for project oversight to ensure that there is full audit trail for the project and that the samples are of sufficient standard to undertake this research.

- Statistical analysis:

Data obtained will be held on a master database by the research statistical team based at Imperial College London (CTU). Assay results will be pseudo-anonymised and linked to the specific patient by the HARP-2 study ID number and VANISH trial ID. Professor TP will be co-located with the statistician and provide statistical supervision for the statistician through regular twice-monthly meetings, and commenting on analysis plans and reports. The statistician will also engage with investigators in study management meetings and for trial specific advice. The planned research timetable for the statistician is as follows: M1-M3: Develop initial statistical analysis plans (SAP) for the two trials. M1-M5: Identify and assess robustness of variables; describe selected variables and their correlation structure; Review point with investigators. M6-M8: assemble principal modelling datasets; identify sensitivity analyses; update SAPs as an interim report, Review point with investigators; M8-M13: latent class analysis modelling; derive and determine number of sub-phenotypes; Review point with investigators; Describe sub-phenotype composition; M13-M15: validation against those published; undertake sensitivity analyses and assess influence; merge modelling datasets with existing trial outcome datasets; update SAPs; Review point with investigators; M15-M18: investigate sub-phenotypes as prognostic for trial outcome; assess heterogeneity of treatment effect and sub-phenotypes interactions; Review point with investigators; assess internal validity; investigate stability of sub-phenotypes over time; write report sections.

Project timetable

Project activity and timetables are summarized below.

Activity	**Timeline**
Database set up for HTE	November 2017 - February 2018
Preliminary analysis for HTE	March 2018 - April 2018
STUDY GROUP MEETING	First week of May 2018
Final analysis output for HTE	May 2018

STUDY GROUP MEETING	May 2018
Manuscript - HTE	July 2018
Measurement of additional markers	November 2017 – June 2018
Database set up for LCA	June 2018
Preliminary analysis for LCA	July 2018 - August 2018
STUDY GROUP MEETING	September 2018
Interim analysis output for LCA	September 2018 – October 2018
STUDY GROUP MEETING	November 2018
Final analysis output for LCA	January 2019
Manuscript - LCA	March 2019
End of project STUDY GROUP MEETING	April 2019

Project parts	Project month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
	Calender month - NEW CALENDAR	Nov-17	Dec-17	Jan-18	Feb-18	Mar-18	Apr-18	May-18	Jun-18	Jul-18	Aug-18	Sep-18	Oct-18	Nov-18	Dec-18	Jan-19	Feb-19	Mar-19	Apr-19
Part-1 = HTE analyses	Milestone-1 Database for HTE analyses	X	X	X	X														
	Milestone-2 Preliminary HTE analyses					X	X												
	Milestone-3 Study group meeting to discuss preliminary HTE anlyses						X												
	Milestone-4 Finalise HTE analyses						X												
	Milestone-5 Complete write up of HTE analyses							X	X										
	Milestone-6 Submit HTE analyses manuscript									X									
Part-2 = Sepsis and ARDS sub-Phenotypes	Milestone-7 Approvals and ethics submission	X	X																
	Milestone-8 Measurement of additional biomarkers			X	X	X	X	X	X										
	Milestone-9 Database set-up for LCA								X	X									
	Milestone-10 Preliminary LCA analyses									X	X								
	Milestone-11 Study group meeting to discuss preliminary LCA anlyses											X							
	Milestone-12 Interim LCA analyses											X	X						
	Milestone-13 Study group meeting													X					
	Milestone-14 Final output for LCA analyses														X	X			

	Milestone-14 Submit LCA analyses manuscript																X	X	
	Milestone-15 Prepare final report															X	X	X	
	Milestone-16 End of project study group meeting																		X
	Milestone-17 Submit Final report																		X

Discussions with Technology Transfer Office to ensure oversight of any relevant IP

Advice has been sought from the Guy's & St Thomas' NHS Foundation Trust Technology Transfer Office in the event of potential new IP being identified and they will have the oversight of any relevant IP. Further discussions will be held between the study teams at Imperial College London and The Queen's University of Belfast. Any IP generated would be shared by agreement.

Pilot data

Rationale: Hyper-inflammatory and hypo-inflammatory ARDS sub-phenotypes have been identified in three US-based ARDS Network clinical trials and respond differently to positive end-expiratory pressure and fluid management[5, 29]. It remains unknown if these sub-phenotypes respond differently to pharmacotherapies.

Methods: We studied 540 patients enrolled in a multicenter, placebo-controlled randomized trial of simvastatin for ARDS. Simvastatin did not improve clinical outcomes in the overall population of patients with ARDS[25].

Latent class analysis was applied to baseline data without consideration of clinical outcomes and using a smaller set of clinical and biological data than prior studies (Interleukin-6 [IL-6], soluble Tumor Necrosis Factor receptor-1 [sTNFr1], creatinine, bilirubin, age, plateau pressure, tidal volume, P/F ratio, platelets, vasopressors, sex, ARDS risk factor). Logistic regression tested for an interaction between simvastatin treatment and sub-phenotypes on mortality; Poisson regression tested for interactions for the outcomes of ventilator-free days (VFD) and organ failure-free days (OFFD).

Results:

A two class (two sub-phenotype) model was an improvement over a one-class model ($p<0.0001$), with 352 subjects (65%) in Class 1 (hypo-inflammatory) and 188 subjects (35%) in Class 2 (hyperinflammatory). Additional classes did not improve model fit.

Subjects in Class 2 had higher sTNFr1, creatinine and IL-6 and lower platelets compared to subjects in Class 1. Subjects in Class 1 had significantly better outcomes than those in Class 2 (Table).

The effect of simvastatin on VFD was significantly different in the two classes ($p=0.02$ for interaction); specifically, simvastatin-treated subjects in Class 2 had a median of 7 more VFD than placebo-treated subjects in Class 2, whereas there was no difference in VFD by simvastatin treatment in Class 1 (Table). Similarly, simvastatin-treated subjects in Class 2 had a median of 7 more OFFD than placebo-treated subjects in Class 2, whereas there was no difference in OFFD by simvastatin treatment in Class 1 ($p<0.0001$; Table). A similar

pattern was observed for 28-day survival, though this interaction was not statistically significant (p=0.14; Table).

Conclusions: This analysis confirms the presence of two distinct ARDS sub-phenotypes with differing outcomes in a non-U.S. setting, using a minimal set of clinical and biological data compared to previous studies. Patients with a hyper-inflammatory ARDS benefited from randomly assigned simvastatin treatment, while patients with a hypo-inflammatory ARDS did not. This finding supports the need for a precision medicine approach (predictive enrichment) for clinical trials in critical care.

Table. Clinical Outcomes Differ In the Hyperinflammatory Class 2 By Simvastatin Treatment

	Class 1 (n=352)	**Class 2 (n=188)**	**p-value**
VENTILATOR-FREE DAYS (Median)			
Placebo	18	0	0.02*
Simvastatin	18	7	
Overall	18	2.5	<0.001
ORGAN FAILURE-FREE DAYS (Median)			
Placebo	27	13	< .0001*
Simvastatin	27	20	
Overall	27	15	< .001
28-DAY SURVIVAL (%)			
Placebo	84%	55%	0.14*
Simvastatin	83%	68%	
Overall	83%	61%	<0.0001

* p-value for interaction tests difference in response to treatment by class

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Additional information

1. Abstracts of LeoPARDS trial
2. Abstract of VACS trial

LeoPARDS trial[27]

Gordon AC et al.

BACKGROUND

Levosimendan is a calcium-sensitizing drug with inotropic and other properties that may improve outcomes in patients with sepsis.

METHODS

We conducted a double-blind, randomized clinical trial to investigate whether levosimendan reduces the severity of organ dysfunction in adults with sepsis. Patients were randomly assigned to receive a blinded infusion of levosimendan (at a dose of 0.05 to 0.2 μg per kilogram of body weight per minute) for 24 hours or placebo in addition to standard care. The primary outcome was the mean daily Sequential Organ Failure Assessment (SOFA) score in the intensive care unit up to day 28 (scores for each of five systems range from 0 to 4, with higher scores indicating more severe dysfunction; maximum score, 20). Secondary outcomes included 28-day mortality, time to weaning from mechanical ventilation, and adverse events.

RESULTS

The trial recruited 516 patients; 259 were assigned to receive levosimendan and 257 to receive placebo. There was no significant difference in the mean (±SD) SOFA score between the levosimendan group and the placebo group (6.68±3.96 vs. 6.06±3.89; mean difference, 0.61; 95% confidence interval [CI], −0.07 to 1.29; P=0.053). Mortality at 28 days was 34.5% in the levosimendan group and 30.9% in the placebo group (absolute difference, 3.6 percentage points; 95% CI, −4.5 to 11.7; P=0.43). Among patients requiring ventilation at baseline, those in the levo- simendan group were less likely than those in the placebo group to be success- fully weaned from mechanical ventilation over the period of 28 days (hazard ratio, 0.77; 95% CI, 0.60 to 0.97; P=0.03). More patients in the levosimendan group than in the placebo group had supraventricular tachyarrhythmia (3.1% vs. 0.4%; abso- lute difference, 2.7 percentage points; 95% CI, 0.1 to 5.3; P=0.04).

CONCLUSIONS

The addition of levosimendan to standard treatment in adults with sepsis was not associated with less severe organ dysfunction or lower mortality. Levosimendan was associated with a lower likelihood of successful weaning from mechanical ventilation and a higher risk of

supraventricular tachyarrhythmia. (Funded by the NIHR Efficacy and Mechanism Evaluation Programme and others; LeoPARDS Current Controlled Trials number, ISRCTN12776039.)

VACS trial[28]

Gordon AC et al.

Objectives: Vasopressin and corticosteroids are both commonly used adjunctive therapies in septic shock. Retrospective analyses have suggested that there may be an interaction between these drugs, with higher circulating vasopressin levels and improved outcomes in patients treated with both vasopressin and cortico- steroids. We aimed to test for an interaction between vasopressin and corticosteroids in septic shock.

Design: Prospective open-label randomized controlled pilot trial. **Setting:** Four adult ICUs in London teaching hospitals. **Patients:** Sixty-one adult patients who had septic shock.

Interventions: Initial vasopressin IV infusion titrated up to 0.06U/ min and then IV hydrocortisone (50mg 6 hourly) or placebo. Plasma vasopressin levels were measured at 6–12 and 24–36 hours after hydrocortisone/placebo administration. **Measurements and Main Results:** Thirty-one patients were allocated to vasopressin + hydrocortisone and 30 patients to vasopressin + placebo. The hydrocortisone group required a shorter duration of vasopressin therapy (3.1 d; 95% CI, 1.1–5.1; shorter in hydrocor- tisone group) and required a lower total dose of vasopressin (ratio, 0.47; 95% CI, 0.32–0.71) compared with the placebo group. Plasma vasopressin levels were not higher in the hydrocortisone group com- pared with the placebo group (64 pmol/L difference at 6- to 12-hour time point; 95% CI, –32 to 160 pmol/L). Early vasopressin use was well tolerated with only one serious adverse event possibly related to study drug administration reported. There were no differences in mortality rates (23% 28-day mortality in both groups) or organ failure assessments between the two treatment groups.

Conclusions: Hydrocortisone spared vasopressin requirements, reduced duration, and reduced dose, when used together in the treatment of septic shock, but it did not alter plasma vasopres- sin levels. Further trials are needed to assess the clinical effec- tiveness of vasopressin as the initial vasopressor therapy with or without corticosteroids. (*Crit Care Med* 2014; 42:1325–1333)