VANISH

Vasopressin vs Noradrenaline as Initial therapy in Septic Shock

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This protocol describes the VANISH study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.
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<td>ACTH</td>
<td>Adrenocorticotrophin Hormone</td>
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<td>AE</td>
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<td>AKIN</td>
<td>Acute Kidney Injury Network</td>
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<td>British Medical Journal</td>
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<td>mRNA</td>
<td>Messenger Ribonucleic Acid</td>
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<td>OR</td>
<td>Odds Ratio</td>
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<td>PerLR</td>
<td>Personal Legal Representative</td>
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<td>PIS</td>
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<td>RRT</td>
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<td>SAE</td>
<td>Serious Adverse Event</td>
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<td>S$<em>{c}$O$</em>{2}$</td>
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<td>SIRS</td>
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KEYWORDS

Septic shock, vasopressin, noradrenaline, hydrocortisone, blood pressure, acute kidney injury, organ failure
1 STUDY SUMMARY

TITLE  Vasopressin vs Noradrenaline as Initial therapy in Septic Shock

DESIGN  Double-blind factorial (2x2) randomised controlled trial

AIMS  1. Does vasopressin reduce renal dysfunction compared to noradrenaline when used as the initial vasopressor in the management of adult patients who have septic shock?

2. Is there an interaction between vasopressin and corticosteroids when used in the management of septic shock?

POPULATION  Adult patients who have septic shock managed in ICU. 412 patients will be recruited for this trial.

ELIGIBILITY  Adult patient who has sepsis and requires vasopressors to maintain blood pressure despite adequate fluid resuscitation

TREATMENT  1. Vasopressin vs. Noradrenaline as initial vasopressor therapy and then

2. Intravenous hydrocortisone vs. “placebo”

DURATION  Vasopressin vs. Noradrenaline until hypotension has resolved (Maximum 28days)

Hydrocortisone vs. “placebo” for 11 days

PRIMARY OUTCOME  Renal failure free days during the 28 days after randomisation

SECONDARY OUTCOMES

• Rates and duration of renal replacement therapy

• Length of renal failure in survivors and non-survivors

• 28-day, ICU and hospital mortality rates.

• Organ failure free days in the first 28 days, assessed using the serial organ failure assessment (SOFA) score

• Organ support data assessed using the standard NHS Healthcare Resource Groups

• Blood, plasma and urinary biomarkers of renal function and inflammation (including genetic polymorphisms).
SCHEMATIC TRIAL PLAN

Adult patient who has sepsis and requires vasopressors to maintain blood pressure despite adequate fluid resuscitation

RANDOMISE

A
Vasopressin infusion (0.06U/min) titrated to BP. Continues until shock resolved

If BP still low
Hydrocortisone (50mg IV 6 hourly for 5 days then tapered to 50mg every 12 hours for days 6 to 8, 50mg every 24 hours for days 9 to 11 and then stopped.)

B
Vasopressin infusion (0.06U/min) titrated to BP. Continues until shock resolved

If BP is still low
Placebo (0.5ml 0.9% Saline IV 6 hourly for 5 days then tapered to every 12 hours for days 6 to 8, every 24 hours for days 9 to 11, and then stopped).

C
Noradrenaline infusion (0.12µg/min) titrated to BP. Continues until shock resolved

If BP is still low
Hydrocortisone (50mg IV 6 hourly for 5 days then tapered to 50mg every 12 hours for days 6 to 8, 50mg every 24 hours for days 9 to 11 and then stopped.)

D
Noradrenaline infusion (0.12µg/min) titrated to BP. Continues until shock resolved

If BP is still low
Placebo (0.5ml 0.9% Saline IV 6 hourly for 5 days then tapered to every 12 hours for days 6 to 8, every 24 hours for days 9 to 11, and then stopped).

If BP still low, catecholamine vasopressors at physician discretion

Blood and urine research sample collection on days 1, 3, 5, and 7. Daily routine clinical data collection from notes while on ICU. 28-day and hospital survival status.
2 INTRODUCTION

2.1 BACKGROUND

Severe sepsis is an increasingly common problem worldwide and is an important cause of mortality (10th most common cause of death in US). In the UK the incidence of severe sepsis has increased 68% over a nine year period, such that in 2004 there were 31,000 patients who had severe sepsis admitted to intensive care units in England, Wales and Northern Ireland.\(^1\) A substantial number of patients will also develop severe sepsis after admission to intensive care,\(^2\) so the total number of severe sepsis cases may be in excess of 45,000 per annum. As the population ages and receive more complex medical treatments, this will continue to rise.

Mortality from sepsis is 35-50%, proportional to illness severity and the number of organs failing. Patients who have sepsis consume a disproportionate amount of resources compared to other critically ill patients. The estimated annual hospital costs for treating these patients in the UK is about £700 million.\(^3\)

Septic shock, the most severe form of sepsis, is defined as hypotension in response to overwhelming infection.\(^4\) As well as appropriate antibiotic treatment one of the main treatments is cardiovascular resuscitation using intravenous fluids and catecholamines. Although usually effective at restoring blood pressure, catecholamines have important adverse effects and may even increase mortality.\(^5\)

Recently vasopressin, an endogenous stress hormone, has been proposed as an adjunct to catecholamines in the treatment of septic shock. The rationale for its use is that a relative vasopressin deficiency occurs in septic shock and exogenously administered vasopressin restores vascular tone,\(^6\) increases blood pressure and thus leads to a reduced requirement for catecholamine vasopressors.\(^7\) Furthermore, vasopressin via interaction with a family of receptors may result in differential vasoconstriction and possibly vasodilatation in different vascular beds compared to noradrenaline.\(^8\) Thus vasopressin may be a potentially useful therapy in septic shock due both to its direct beneficial effects and also reduction / avoidance of catecholamine infusions.

Similarly corticosteroids are another commonly used adjunctive therapy in septic shock. Low dose hydrocortisone has repeatedly been shown to reduce catecholamine infusion rates, lead to earlier resolution of shock but its effect on survival remains uncertain.\(^9\)\(^,\)\(^11\) Recently it has been suggested that there may be an interaction between vasopressin and corticosteroids on mortality rates in septic shock.\(^12\)\(^,\)\(^13\)

Biological rationale

Vasopressin (anti-diuretic hormone) is an endogenous nonapeptide hormone secreted from the posterior pituitary. In health, vasopressin acts primarily as anti-diuretic hormone, resulting in avid free water retention by the kidney and has little effect on arterial pressure at physiologic levels under normal conditions. However, during hypotension and hypovolaemia, vasopressin concentration increases and maintains arterial blood pressure by acting as a potent vasoconstrictor – through stimulation of V1a receptors – but vasopressin has little or no anti-diuretic effect during hypotension.

Plasma vasopressin concentration in humans is normally less than 4 pg/ml. Hypotension is the most potent stimulus to secretion of vasopressin and so circulating vasopressin concentrations increase markedly in response to hypotension. In cardiogenic shock vasopressin concentration increases to more than 20 pg/ml\(^6\) and in severe hypotensive haemorrhage vasopressin concentrations of 100-1000 pg/ml have been reported.\(^14\) In septic shock there is a relative vasopressin deficiency. In a small case series (n=19) of patients who had vasodilatory shock, Landry and colleagues discovered mean plasma vasopressin concentration of 3.1 pg/ml.\(^9\) Very low plasma vasopressin concentrations have also been found in other small studies of vasopressin infusion in established septic shock. Mean baseline plasma vasopressin concentration was 1.3 pg/ml in 24 patients in a phase II trial\(^7\) and 7.3 pg/ml in a small (n = 16) cohort of patients who had
vasodilatory shock. There was a slight increase in plasma vasopressin concentration in the early stages of septic shock (4.1 – 21 pg/ml) in a study of 62 patients, although this rise was smaller than the rise seen in other forms of shock. Furthermore, vasopressin concentrations fell significantly over time such that by 36 hours (and as quickly as 6 hours in some) most patients exhibited a relative vasopressin deficiency. In the recent VASST study vasopressin levels were measured in a convenience sample of 107 patients. The median plasma vasopressin level at baseline was 3.2 pmol/L and remained low in those patients treated with noradrenaline.

Vasopressin exerts its effects via interaction with a family of vasopressin receptors. V1a receptors are located on vascular smooth muscle cells and are responsible for the vasoconstriction. Heterogeneity of the distribution of V1a receptor could have important clinical and therapeutic implications. For example, vasopressin causes renal efferent – but not renal afferent - artery vasoconstriction thereby increasing renal perfusion pressure and glomerular filtration rate. In contrast, norepinephrine interacts with alpha-1 receptors on both renal afferent and renal efferent arterioles and so norepinephrine at high doses may decrease renal perfusion and glomerular filtration rate. Thus, in contrast to noradrenaline, there is compelling organ-specific heterogeneity in the vascular responsiveness to vasopressin. Importantly, vasopressin also binds to oxytocin receptors, which, in addition to their uterine contractile effects, mediate calcium-dependent vasodilatation via stimulation of the nitric oxide pathway in endothelial cells of pulmonary, coronary, and cerebral arteries.

Corticosteroids are powerful anti-inflammatory agents and have a number of actions that may be beneficial in septic shock including inhibition of cytokine synthesis via NF-κB suppression, inhibition of inducible nitric oxide synthase, restoration of vascular reactivity and attenuation of adrenergic receptor down regulation. Furthermore a relative adrenocortical insufficiency is often seen in septic shock.

Both vasopressin and corticosteroids increase responsiveness to endogenous and infused catecholamines. The interaction between vasopressin and corticosteroids remains controversial as there are complex interactions between the hypothalamic-pituitary- adrenal axis and the hypothalamic-posterior pituitary-vasopressin axis. Vasopressin binds to V3 receptors located in the anterior pituitary and may increase adrenocorticotrophin hormone (ACTH) production and secretion. Similarly corticosteroids may increase vasopressin messenger RNA and may reverse the hypo-responsiveness to vasopressin in endotoxaemia. However other studies have found that corticosteroids do not change vasopressin levels and others have suggested that corticosteroids may actually suppress gene expression.

Evidence from previous randomised controlled trials and meta-analyses
Vasopressin
A 2011 Cochrane review concluded “There is not sufficient evidence that any one of the investigated vasopressors is clearly superior over others”. There are six randomised controlled trials of vasopressin in adults with septic shock. The majority of these trials were small proof-of-principle studies that used physiological variables as the primary outcome and only 150 patients in total have been studied in five of these trials.

In all studies the use of vasopressin allowed a reduction in the dose of conventional catecholamines required to maintain blood pressure. In one study vasopressin also increased cardiac index, and combined vasopressin and norepinephrine infusion improved gastrointestinal perfusion as assessed by gastric tonometry. Two other studies reported a reduction in cardiac output but this was due to a reduction in heart rate rather than a change in stroke volume. Another important finding in one study was that vasopressin infusion doubled urine output and increased creatinine clearance by the end of the 4-hour study period.
The recently completed VASST study is the only randomised controlled trial of vasopressin that was powered to evaluate the effect on mortality. In this multi-centre study 779 patients who had established septic shock were randomised to receive a blinded infusion of either vasopressin or noradrenaline in addition to conventional catecholamine vasopressors. There was no significant difference in 2-day mortality between the treatment groups (35.4% vasopressin group and 39.3% noradrenaline group, p = 0.26) nor in 90-day mortality (43.9% and 49.6 % respectively; p = 0.11). Importantly the VASST study showed that vasopressin infusion in septic shock was safe. The overall serious adverse event rate was the same in the vasopressin and noradrenaline groups (10.3% and 10.5% respectively). There was no difference in any of the specific categories of serious adverse events, including coronary and mesenteric ischaemia, hyponatraemia, cardiac arrests or arrhythmias.

Although there was no difference in the primary analysis between treatment groups in the VASST study, in the pre-defined stratum of less severe shock there was a significantly lower mortality in the vasopressin group compared to the noradrenaline group (26.5% v 35.7% respectively, p=0.05). There was no difference in mortality between treatment groups in the patients who had more severe shock. In a post-hoc subgroup analysis, patients treated with vasopressin within 12 hours (n=427) had a trend to reduced mortality (33.2% v 40.5%, p=0.12) but no difference in mortality if treated with vasopressin after 12 hours. The editorial accompanying the VASST report suggested that early use of vasopressin may be needed to produce a significant improvement in survival.

In other post-hoc analyses vasopressin treatment led to lower rates of progression to renal failure (21.2% v 41.2%, p=0.02) and mortality (30.8% v 54.7%, p=0.01) in patients at “Risk” of renal failure (using RIFLE criteria) but no difference in outcomes if renal failure was already established. Furthermore serum creatinine reduced significantly more over time after vasopressin treatment compared to noradrenaline in the “Risk” group but there was no difference in serum creatinine over time according to treatment group if renal failure was already established.

A retrospective study of septic shock found first-line vasopressin therapy similar in efficacy to titrated noradrenaline or dopamine and there were no adverse effects. There has been one small randomised trial of vasopressin vs noradrenaline in early septic shock. Vasopressin infusion was associated with reduced organ dysfunction at 48 hours, mainly due to the beneficial effect on renal function, in that creatinine clearance improved in the vasopressin group.

Corticosteroids
There have been many randomised controlled trials of corticosteroids in septic shock. There were three systematic reviews of corticosteroids in sepsis in 2004. A Cochrane review (also published in part in the BMJ) considered data from 15 trials (n=2023). This review concluded that overall corticosteroids did not change mortality in severe sepsis and septic shock but there was marked heterogeneity between studies. When analysing the subgroup of studies that tested a long course (≥25 days) of low dose corticosteroids (≤300mg hydrocortisone), the relative risk of dying at day 28 was 0.80 (95%CI 0.67-0.95) and for hospital mortality 0.83 (95%CI 0.71-0.97). A second review found similar results, that a long course of low dose steroids had a relative risk of 28-day mortality of 0.80 (95%CI 0.67-0.95) but there was no beneficial effect of a short course of high dose steroids. Another meta-analysis that same year examined five trials published since 1997. This analysis found a consistent and overall improvement in survival associated with steroid use (relative survival benefit 1.23 [95%CI 1.01-1.50]).

However since these meta-analyses a large international multi-centre RCT of low dose hydrocortisone has been published. In this study 499 adult patients who had septic shock were randomised to receive 50mgs hydrocortisone intravenously 6 hourly for 5 days followed by a tapering course until day 11 or placebo. Although time to reversal of shock was reduced in the hydrocortisone group there was no difference in 28-day mortality rates. Similar results were seen for patients with or without relative adrenal insufficiency as defined using an adrenocorticotropic hormone (ACTH) stimulation test.
Two of the meta-analyses detailed above have recently been updated. The 2009 Cochrane review\textsuperscript{11} included 17 randomised controlled trials and as in previous analyses the most benefit was seen in the 12 recent trials that investigated prolonged low-dose corticosteroid treatment (RR 0.84 95%CI 0.72-0.97, p=0.02, for 28-day mortality). The other updated review in 2009 included seven new trials and found a similar beneficial effect of low dose steroids.\textsuperscript{43} However the decrease in mortality was confined to the more severely ill patients. This observation is consistent with the Surviving Sepsis Campaign suggestion that corticosteroids should only be used in septic shock after blood pressure is identified to be poorly responsive to fluid resuscitation and vasopressor therapy.\textsuperscript{14} This is the planned use of corticosteroids in this proposal.

**Evidence of an interaction between vasopressin and corticosteroids**

Despite the widespread use of both vasopressin and corticosteroids in the management of septic shock little is known about possible interactions. A recently published sub-group analysis from the VASST study\textsuperscript{12} reported that a statistically significant interaction was found between vasopressin / noradrenaline treatment and corticosteroid treatment (interaction statistic p=0.008). The combination of vasopressin and steroids led to a significantly lower mortality compared to noradrenaline plus steroids (35.9\% v 44.7\% respectively, \(p=0.03\)) and less organ dysfunction demonstrated by more days alive and free from shock, ventilation and renal failure. In contrast patients who were treated with vasopressin and had no corticosteroids had a trend towards increased mortality compared to patients who were treated with noradrenaline and no steroids (33.7\% v 21.3\% respectively, \(p=0.06\)). Interestingly patients who received steroids as well as vasopressin had higher levels of circulating vasopressin compared to patients treated with vasopressin alone.

The mechanism behind this finding remains uncertain at present. There is animal data to suggest that corticosteroids may increase the efficacy of vasopressin. In an ovine endotoxaemia model the vasopressor effect of vasopressin decreased over time but was then restored by administration of corticosteroids.\textsuperscript{27} Other animal studies have demonstrated that corticosteroids increase mRNA and V1 receptor expression.\textsuperscript{45}

This data is further supported by two retrospective studies that reported that the combination of vasopressin and corticosteroids to treat septic shock resulted in better outcomes compared to vasopressin use alone.\textsuperscript{46,47}

However, all of this data arises from either animal work or retrospective clinical studies. Prospective clinical testing is therefore required. A recent editorial by a leading investigator in the field of septic shock states that a 2 \times 2 factorial designed study is needed to clarify the risk / benefit ratio of vasopressin and corticosteroids.\textsuperscript{13}

**Safety of vasopressin and hydrocortisone in septic shock**

Although there are few randomised controlled trials of vasopressin in septic shock there are a number of case studies reporting experience of using vasopressin in the management of septic shock. In general, in non-randomized studies of septic shock it is difficult to separate adverse events due to the therapy or due to the underlying pathological process. These case studies have invariably shown the catecholamine sparing effect of vasopressin but have also reported possible safety concerns. A number of the studies have reported a fall in cardiac output,\textsuperscript{6,33,35,48,49} although an increase in cardiac output has also been recorded.\textsuperscript{32} In general the decrease of cardiac output has been related to a fall in heart rate, as stroke volume has been unchanged.\textsuperscript{33,35,49}

Other cardiac concerns have included myocardial ischemia. There was one case of myocardial ischemia in a patient without known ischemic cardiac disease induced by high dose vasopressin that resolved when vasopressin was stopped.\textsuperscript{33} Also in the retrospective case series by Holmes et al, higher doses of vasopressin (> 0.05 U/min) compared to lower doses of vasopressin infusion were associated with an increased rate of
cardiac arrest. However, a recently completed prospective study found no adverse effects of vasopressin infusion at 0.067 U/min.

Vasopressin is used for its splanchnic vasoconstrictor effects in patients with bleeding oesophageal varices. As a result, there has been concern that vasopressin could cause mesenteric ischemia. There is clinical equipoise regarding whether vasopressin induces mesenteric ischemia as there are studies showing vasopressin worsened, did not change or improved mesenteric perfusion as assessed by gastric tonometry. Vasopressin increased gastric to arterial CO₂ partial pressure gap compatible with gastric hypoperfusion in a dose-dependent fashion in one small case series of patients who had septic shock. In contrast, there was an improvement in gastro-intestinal perfusion as assessed by gastric tonometry in the randomized controlled trial by Dünser et al. This study did however report an increase in bilirubin levels and the same group reported similar findings in a prior retrospective study, along with a fall in platelet count. In a large case series of 316 patients who had vasodilatory shock, vasopressin infusion was associated with increased bilirubin levels and liver transaminases as well as decreased platelet count. Vasopressin has been shown to cause platelet aggregation and it has been suggested that this may contribute to ischemic skin lesions in vasopressin treated patients during septic shock. Interestingly in this latter report increasing dose of noradrenaline (but not vasopressin) was associated with ischaemic skin lesions.

In the only large randomised controlled trial of vasopressin (VASST) there were no safety concerns. The overall serious adverse event rate was the same in the vasopressin and norepinephrine groups (10.3% and 10.5% respectively) and there was no difference in any of the specific categories of serious adverse events. In particular, there was no significant difference in the rates of myocardial, mesenteric or digital ischemia, cardiac arrest, life-threatening arrhythmias, hyponatraemia, or cerebrovascular accident. There were fewer cases of acute mesenteric ischaemia in the vasopressin group than in the noradrenaline group (9 (2.3%) versus 13 (3.4%) respectively). There were more cases of digital ischemia in the vasopressin than the noradrenaline group (8 [2%] versus 2 [0.5%] respectively). Further post-hoc analyses of the VASST data found no difference in troponin levels or ischaemic ECG changes between patients treated with vasopressin or noradrenaline.

Corticosteroids have been reported to have a number of adverse effects when used in sepsis. There have been reports of increased rates of secondary infections particularly in early trials where high dose steroids were used to try and reduce the excessive inflammatory response. The use of low-dose steroids, used as a physiological replacement, may reduce this risk although in the recent CORTICUS study there was an increased rate of superinfection (OR 1.37 95%CI 1.05-1.79) in patients in the hydrocortisone group. However, the two recent meta-analyses showed no evidence of increased superinfection, gastro-intestinal bleeding or neuromuscular weakness. In the Cochrane review there was an increased risk of developing hyperglycaemia (51.7% v 45.6%, p <0.001).

2.2 RATIONALE FOR CURRENT STUDY

The rationale for the current study is that evidence to date suggests that vasopressin may prevent renal dysfunction in septic shock when used early, and that vasopressin may interact with corticosteroids.

In order to ensure that patients are treated as early as possible we will compare vasopressin to noradrenaline as the first line vasopressor in the management of septic shock. As international guidelines only suggest corticosteroids for cases of septic shock that are poorly responsive to fluids and vasopressors, patients will only be prescribed corticosteroids once higher doses of vasopressin or noradrenaline are required.

3 STUDY OBJECTIVES

3.1 Primary objectives
The main objectives of this trial are:

1. To test if vasopressin reduces renal dysfunction compared to noradrenaline when used as the initial vasopressor in the management of adult patients who have septic shock.
2. To test if there is an interaction between vasopressin and corticosteroids when used in the management of septic shock?

3.2 Secondary objectives
1. To assess if vasopressin improves other secondary outcomes (need for dialysis, survival rates, other organ failures and resource usage) compared to noradrenaline in the management of septic shock in adult patients.
2. To test if there are genetic polymorphisms that determine response to the study drugs.

4 STUDY DESCRIPTION

4.1 DESIGN
This is a double-blind factorial (2x2) randomised controlled trial. It will be conducted in multiple general adult ICUs within the UK. The study will recruit 412 patients.

4.2 TREATMENT REGIMEN
Patients will be randomised to receive vasopressin (0-0.06 U/min) or noradrenaline (0-12 µg/min) as the initial vasopressor therapy to maintain mean arterial blood pressure after adequate fluid resuscitation. This first study drug will continue as a continuous intravenous infusion until the septic shock has resolved and the patient no longer requires vasopressor support.

If maximum doses of the first study drug are reached (4.5mls/hr of the blinded infusion) the patient will be treated with the second study drug (hydrocortisone or placebo), before additional clinically indicated vasopressors / inotropes are prescribed.

<table>
<thead>
<tr>
<th>2nd study drug</th>
<th>1st study drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosteroids</td>
<td>103</td>
</tr>
<tr>
<td>Placebo</td>
<td>103</td>
</tr>
<tr>
<td></td>
<td>103</td>
</tr>
</tbody>
</table>

5 TRIAL METHODS

5.1 SUBJECT SELECTION

5.1.1 Pre-randomisation evaluations
Patients will only need to be assessed for the inclusion and exclusion criteria detailed below. This will require a history and clinical examination. A full blood count test or an arterial blood gas sample may be needed but would normally be collected as part of routine clinical care.

5.1.2 Inclusion Criteria
The target population is adult patients who require vasopressors for the management of sepsis despite fluid resuscitation.

Inclusion criteria will use the internationally-established consensus definitions of sepsis. In brief:

- Fulfil 2/4 of the criteria of the systemic inflammatory response syndrome (SIRS) due to known or suspected infection within the previous 24 hours. The SIRS criteria are:
(1) fever (>38°C) or hypothermia (< 36°C),
(2) tachycardia (heart rate > 90 beats per minute),
(3) tachypnoea (respiratory rate > 20 breaths per minute or PaCO₂ < 4.3 kPa) or need for mechanical ventilation,
(4) abnormal leukocyte count (> 12,000 cells/mm³, < 4000 cells/mm³, or > 10% immature [band] forms).

- Hypotension despite adequate intravenous fluid resuscitation (suggested minimum of one litre in the previous four hours, some patients may need considerably more, see treatment guideline below).

5.1.3 Exclusion Criteria

- Patient has received a continuous infusion of vasopressors previously during this ICU admission (other than vasopressors used as emergency treatment [for less than six hours] to stabilise the patient during this episode). Vasopressors include noradrenaline, adrenaline, vasopressin, dopamine, metaraminol, phenylephrine, and (intermittent) terlipressin.
- Regular systemic corticosteroid therapy within the previous three months (this does not include inhaled steroid therapy).
- Known adrenal dysfunction / insufficiency.
- End-stage renal failure (i.e. requiring long term dialysis)
- Physician and team are not committed to full active care.
- Patient is known to be pregnant.
- Patient has known acute mesenteric ischemia.
- Patient is known to have Raynaud’s phenomenon, systemic sclerosis or other vasospastic diseases.
- Patient has been enrolled in another clinical trial of an investigational medicinal product within 30 days or is enrolled in another interventional study that might interact with the study drugs.
- Patient has a history of anaphylaxis or hypersensitivity to any study drug.

5.2 PROCEDURES AND MEASUREMENTS

5.2.1 Screening

All patients who are clinically judged to have septic shock will be screened against the inclusion and exclusion criteria to be eligible for the study.

5.2.2 Informed Consent

In most cases it will not be possible to obtain prospective consent from the patient at the time of enrolment. This is due to the fact that many patients will have a reduced level of consciousness due to their illness or due to sedative medication used as part of their treatment. As all the study drugs are already routinely used in the management of septic shock there is minimal extra risk from participation in this study. As septic shock is a medical emergency and the fact that delays in administering the study drugs may affect patient outcome, the patient will be treated and samples collected in the emergency situation without prospective consent. No analysis of data or samples will occur until retrospective consent is obtained. At the first available opportunity once the clinical condition has stabilised retrospective consent should be sought. This process was used in the pilot / feasibility study (VACS, REC ref 10/H0604/35) and was found to be acceptable to clinicians, patients and their families.

- **Patient Consent**

If possible, informed consent will be obtained from the patient. The patient will be informed about the trial by the responsible clinician or a member of the research team and given a copy of the Patient Information Sheet (PIS). Informed patients will be given an adequate amount of time to consider their participation in the trial. If the patient decides to participate in the trial they will be asked to sign the Patient Consent Form which will then be counter signed by the responsible clinician. The patient will retain one copy of the signed
Confidential

• **Personal Legal Representative Consent**
  If the patient is unable to give consent, informed consent will be sought from the patient’s ‘Personal Legal Representative’ (PerLR) who may be a relative, partner or close friend. The PerLR will be informed about the trial by the responsible clinician or a member of the research team and provided with a copy of the Covering Statement for Personal Legal Representative with an attached PerLRIS and asked to give an opinion as to whether the patient would object to taking part in such medical research. The PerLR will be given up to 24 hours to consider the patient’s participation in the study. If the PerLR decides that the patient would have no objection to participating in the trial they will be asked to sign the PerLR Consent Form which will then be counter signed by the responsible clinician. The PerLR will retain a copy of the signed Consent Form. A second copy will be placed in the patients’ medical records whilst the original will be retained in the Trial Site File.

• **Professional Legal Representative Consent**
  If the patient is unable to give informed consent and no PerLR is available, a doctor who is not connected with the conduct of the trial may act as a Professional Legal Representative (ProLR). The doctor will be informed about the trial by the responsible clinician or a member of the research team and given a copy of the PIS. If the doctor decides that the patient is suitable for entry into the trial they will be asked to sign the ProLR Consent Form. The doctor will retain one copy of the signed Consent Form. A second copy will be placed in the patient’s medical records; the original will be retained in the Trial Site File.

• **Retrospective Patient Information**
  Patients for whom consent is given by a PerLR or ProLR will be informed of their participation in the trial by the responsible clinician or a member of the research team once they regain capacity to understand the details of the trial. The responsible clinician will discuss the study with the patient and the patient will be given a copy of the PIS to keep. The patient will be asked for consent to continue participation in the trial and to sign the Consent Form. The patient will retain one copy of the signed Consent Form. Another copy will be placed in the patient’s medical records whilst the original will be retained in the Trial Site File. If the patient does not want to continue participation in the study they will be given the choice of having already collected data and samples excluded from the final analysis.

• If the patient dies before consent is obtained retrospective consent should be sought from the PerLR (or ProLR, if no PerLR can be identified) for inclusion and collection of data.

The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant’s best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

5.2.3 **Randomisation**

Randomisation will be by computer generated randomised number and will use the InForm eCRF online system. Randomisation will be stratified by ICU and will occur on a 1:1 basis in permuted blocks.
VISIT SCHEDULE

<table>
<thead>
<tr>
<th>Visit</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Day 8 to 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Informed Consent (Patient consent/ PerLR/ ProLR/ Retrospective Patient Information)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Retrospective Consent (PerLR/ProLR) will be obtained at the first available opportunity. Retrospective patient consent will be obtained when the patient has recovered.</td>
</tr>
<tr>
<td>Inclusion / Exclusion criteria</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomisation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study drug administration (Study drug 1 &amp; 2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Study drug 1(Vasopressin/Noradrenaline) continued until shock resolved. If BP still low, Study drug 2 (Hydrocortisone/placebo) administered as described in this protocol.</td>
</tr>
<tr>
<td>Follow up</td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood &amp; Urine sampling</td>
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<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily collection of clinical data</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Final Visit</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>On the day of discharge from the hospital</td>
</tr>
</tbody>
</table>

5.2.4 Treatments

- **Treatment arms**

The four treatment arms will be:

1. Vasopressin + hydrocortisone;
2. Vasopressin + placebo;
3. Noradrenaline + hydrocortisone
4. Noradrenaline + placebo

Study drug 1 will be vasopressin (0 - 0.06 U/min) or noradrenaline (0 - 12 µg/min) and this will be used as the first line vasopressor for the management of septic shock. After study inclusion and randomisation study drug 1 will be given via continuous infusion through a central intravenous line and titrated by the bedside nurse to achieve and maintain a target mean arterial pressure (65-75 mmHg). The treating physician may alter the target mean arterial pressure if clinically indicated.

All study drugs will be supplied to the ICU by pharmacy as specific research study drugs and they will be stored in separate research stores (e.g. locked boxes / fridges in ICU). All drugs will be drawn up by the bedside critical care nurse.

Study drug 1 will come as three ampoules / vials; one small ampoule / vial (2ml) and two large ampoule (4mls); one size of ampoule will contain active drug (vasopressin or noradrenaline) and one will contain saline. All three ampoules (total 10mls) will be added to 40 mls of 5% dextrose into a syringe. The infusion will be started at 1 ml/hr (equal to vasopressin 0.013 U/min or noradrenaline 2.66 µg/min) and increased to a maximum of 4.5mls/hr (equal to vasopressin 0.06 U/min or noradrenaline 12µg/min), as clinically indicated to achieve / maintain the target mean arterial pressure.

If the maximum limit of study drug 1 is reached (4.5mls/hr), then study drug 2 will be administered. This will be either hydrocortisone phosphate (50mg) or placebo (0.9% saline) and 0.5ml of study drug 2 will be administered by intravenous injection 6 hourly. This study drug will be administered for 5 days and then
tapered to 0.5ml every 12 hours for days 6 to 8, 0.5ml every 24 hours for days 9 to 11, and then stopped. If the septic shock has reversed (i.e. all vasopressin and catecholamine infusion stopped) before these timescales then the physician may taper study drug 2 more quickly.

If additional vasopressors are required to maintain blood pressure after the maximum rate of study drug 1 is reached (4.5mls/hr) and the first dose of study drug 2 (hydrocortisone or 0.9% Saline) has been administered then the treating physician may then start any catecholamine vasopressor as clinically indicated (no additional vasopressin or vasopressin analogue is allowed).

The study drugs should not be started until the treating physician is confident that adequate fluid resuscitation has been achieved. Adequate fluid resuscitation should be achieved using repeated fluid challenges. Examples of appropriate targets include any or all of the following:

- Central venous pressure ≥8mmHg (≥12 mmHg in mechanically ventilated patients)
- Urine output >0.5 ml/kg/hr
- ScVO₂ ≥ 70%
- Good peripheral perfusion on clinical examination
- Other measures of cardiac output / flow

During the study drug administration and especially during the first 6 hours patients must be repeatedly reassessed to ensure adequate fluid resuscitation using any or all of the targets above. Study drug 1 infusion will continue until the septic shock has resolved. When a patient is recovering from septic shock any open-label catecholamine vasopressor infusions will be weaned first, followed by study drug 1. The rate of weaning of the drugs should be as clinically indicated to maintain the target mean arterial pressure. Once study drug 1 has stopped if vasopressors are required within 24 hours, study drug 1 should be restarted. If more than 24 hours have elapsed and vasopressors are required again these could be with any open-label vasopressors at the treating physician’s discretion.

Other management of septic shock, including use of inotropes (e.g. dobutamine) will be at the treating physician’s discretion, based on the international 'Surviving Sepsis' guidelines. High volume haemofiltration for the management of sepsis (i.e. RRT not to treat kidney failure) should not be used.

All study drugs will be prescribed on the patient drug chart as per each ICU’s policy. Pre-printed stickers or pre-set electronic prescriptions will be provided to ensure standardised prescribing, dilution and administration of the drugs.

- **Dose modifications for toxicity**

If there are any adverse events or reactions observed thought to be associated with the study drug 1 infusion (vasopressin or noradrenaline) then the infusion should be stopped for at least four hours. “Alternative” vasopressors may be administered during this time. Study drug 1 may then be restarted at the lowest dose and titrated up as per protocol above, if the adverse event has been treated, the condition reversed and the treating physician thinks the event was unrelated to the study drug 1 infusion (e.g. severe transient arrhythmia). If the adverse event should recur then study drug 1 should be discontinued permanently.

If there are any adverse events thought to be associated with study drug 2 (hydrocortisone or placebo) then continued administration should be stopped.
• **Emergency treatment**

Study drug 1 should be given as the initial vasopressor to treat the septic shock. However in an emergency situation the patient’s blood pressure should be stabilised using any clinically indicated vasopressors and / or inotropes. As soon as the clinical situation has stabilised these other vasopressors should be replaced by study drug 1 according to the study protocol and no later than six hours. Use of emergency vasopressors in this situation will not be an exclusion criteria or protocol violation.

• **Rescue Steroid Therapy**

If patients are receiving study drugs 1 and 2, and are not responding to open-label catecholamine vasopressors (i.e. life-threatening hypotension) then they may be administered IV hydrocortisone therapy as rescue therapy. This should not occur until the treating physician has reassessed fluid resuscitation status and a reasonable dose of catecholamine has been given an opportunity to increase the blood pressure (suggested ≥ 0.5 mcg/kg/min of noradrenaline or equivalent).

In this situation study drug 2 should be stopped and open-label steroid given as per treating physician’s prescription. Study drug 1 should continue.

Similarly if after inclusion, systemic steroids are required to treat another medical condition (i.e. not septic shock) then study drug 2 should be stopped and the necessary steroid prescribed. Study drug 1 should continue.

Inhaled steroids can be prescribed as clinically indicated at any time. Steroids will be contraindicated in patients treated for Chicken pox, Shingles or Measles.

• **Premedication**

All other drugs (other than vasopressors) should be prescribed as clinically indicated.

• **Interaction with other drugs**

Ganglion blocking agents may produce a marked increase in sensitivity to the pressor effect of vasopressin but they are rarely used and should be avoided in this trial.

Noradrenaline can lead to severe, prolonged hypertension in patients receiving monoamine oxidase inhibitors or tricyclic antidepressants. Caution should be exercised if treating a patient who has been taking these drugs.

Rifampicin, rifabutin, carbamazepine, phenobarbital, phenytoin, primidone, ephedrine and aminogluthethimide enhance the metabolism of corticosteroids. However, in this trial there should be no alteration of dosing of study drug 2 (hydrocortisone) if these drugs are prescribed.

5.2.5 **Follow-Up**

Participants will be followed up daily whilst on the ICU. Routinely collected clinical data (cardiovascular, respiratory and renal physiological variables as well as haematological, biochemical and microbiological blood test results) will be recorded on a daily basis during this time.

Patients will also be followed up to ascertain survival status at 28 days post recruitment and hospital discharge.
5.2.6 Lab evaluations
Blood and urine sampling (in select centres): 25mls of blood and 10mls of urine will be collected on the day of inclusion and days 3, 5 and 7. Samples will be sent to coordinating centre for storage and analysis. Samples will be kept beyond the end of the trial and stored in accordance with the Human Tissue Act.

5.3 END POINT MANAGEMENT

5.3.1 Primary Outcome
The primary outcome of the trial will be the difference in renal failure free days between treatment groups. Renal failure will be defined by the Acute Kidney Injury Network (AKIN) group stage 3 definition:\textsuperscript{59}:

- Increase in serum creatinine to ≥300% (≥3-fold) from baseline
- or serum creatinine ≥354 µmol/L with an acute rise of at least 44 µmol/L
- or initiation of renal replacement therapy (for AKI)
- or a urine output of <0.3 mL/kg/h ≥24 h
- or anuria ≥12 h

5.3.2 Secondary Outcome
Secondary outcomes will include:

- Rates and duration of renal replacement therapy
- Length of renal failure in survivors and non-survivors
- 28-day, ICU and hospital mortality rates.
- Organ failure free days in the first 28 days, assessed using the serial organ failure assessment (SOFA) score
- Organ support data assessed using the standard NHS Healthcare Resource Groups
- Blood, plasma and urinary biomarkers of renal function and inflammation (including genetic polymorphisms).

6 PHARMACOVIGILANCE

6.1 DEFINITIONS

Adverse Event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered. All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

Unexpected Adverse Reaction: an AR, the nature or severity of which is not consistent with the applicable product information (eg investigator’s brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected.
Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.

Serious Adverse Event (SAE) or Serious Adverse Reaction: any untoward medical occurrence or effect that at any dose

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of existing inpatients’ hospitalisation
- Results in persistent or significant disability or incapacity
- Is a congenital anomaly or birth defect

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

6.2 CAUSALITY

The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigator. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>There is no evidence of any causal relationship</td>
</tr>
<tr>
<td>Unlikely</td>
<td>There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant’s clinical condition, other concomitant treatment).</td>
</tr>
<tr>
<td>Possible</td>
<td>There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant’s clinical condition, other concomitant treatments).</td>
</tr>
<tr>
<td>Probable</td>
<td>There is evidence to suggest a causal relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>Definitely</td>
<td>There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>Not assessable</td>
<td>There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.</td>
</tr>
</tbody>
</table>
6.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. A flowchart is given below to aid in the reporting procedures.

- **Non serious AR/AEs**

  All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the study coordination centre within one month of the form being due.

- **Serious AR/AEs**

  Fatal or life threatening SAEs should be reported on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should assign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

  An SAE form should be completed and entered into eCRF for all SAEs within 24 hours. This will automatically send alert emails to Chief Investigator and the Project Manager. However, relapse, organ failure and death due to sepsis (see definitions below), and hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

**Clinical outcomes:** Clinical outcomes from sepsis are exempt from adverse event reporting, unless the investigator deems the event to be related to the administration of study drug. The following events will be considered clinical outcomes:

- Death related to sepsis
- Cardiovascular failure, including the need vasopressors / inotropes
- Respiratory failure, including mechanical ventilation and acute lung injury
- Hepatic failure
- Renal failure, including the need for renal replacement therapy
- Haematological / Coagulation failure, including thrombocytopenia

Clinical details about these clinical outcomes will be routinely collected in the case report form.

In relation to the study drugs in this trial the following specific serious adverse events will be recorded in the eCRF.

- Myocardial infarction / acute coronary syndrome
- Life threatening arrhythmia
- Mesenteric ischaemia
- Digital ischaemia
- Secondary infection

- **SUSARs**

  In the case of suspected unexpected serious adverse reactions, the staff at the site should:

  Complete the SAE case report form & send it immediately (within 24 hours), signed and dated to the study coordination centre together with relevant treatment forms and anonymised copies of all relevant investigations.

  Or
Contact the study coordination centre by phone and then send the completed SAE form to the study coordination centre within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Local investigators should report any SUSARs and/or SAEs as required by their Local Research Ethics Committee and/or Research & Development Office.

Safety Reporting Overview

[Diagram showing the process of reporting adverse events, serious adverse events, suspected unexpected serious adverse reactions (SUSARs), and expedited reporting.

* Unless identified in the protocol as not requiring immediate reporting

Contact details for reporting SAEs and SUSARs
Complete an SAE form & submit to the Trial Coordination Centre as soon as possible (within 24 hr)
Attention VANISH Trial Manager
Tel: XXXX

6.4 DATA MONITORING & ETHICAL COMMITTEE
An independent Data Monitoring and Ethical Committee will be set up to monitor the progress, patient safety and any ethical issues involved in this trial. They will review trial progress, recruitment rates, event
rates and safety data. A separate charter will be drawn up defining their exact remit and criteria for reporting to the trial steering committee. There will be 6-monthly meetings of the independent DMEC.

7 EARLY DISCONTINUATION OF THE STUDY OR INDIVIDUAL SUBJECTS

7.1 EARLY DISCONTINUATION OF THE STUDY

If, in the opinion of the chief investigator, clinical events indicate that it is not justifiable to continue the trial, the Trial Steering Committee may terminate the trial following consultation with the Sponsor.

7.2 EARLY DISCONTINUATION OF THE SUBJECTS

Withdrawal criteria
Patients will be free to withdraw at any stage of the study.

If the patient wishes to withdraw from the study during the treatment period the treating physician will no longer follow the trial protocol and the study drugs will be stopped and substituted for open-label drugs at the physician’s discretion. The patient’s data may or may not be included in the final analysis according to the patient’s wishes.

If the patient wishes to withdraw from the study after the treatment period no further data collection will continue. They will be offered the option of having their existing data excluded from the final analysis.

7.3 LOSS TO FOLLOW-UP

Many patients may still be hospital in-patients at day 28 and so loss to follow-up is unlikely. If the patient has been discharged home they will be contacted directly or via their GP.

8 STATISTICS AND DATA ANALYSIS

8.1 OUTCOME MEASURES

The primary outcome will be renal failure free days during the 28-days post study inclusion. Patients will be scored as being alive or dead on each calendar day after inclusion. If alive they will be scored as either having or being free from renal failure. The total number of days alive and free of renal failure during the 28-days of the study will be calculated (a higher number reflects a better outcome).

A full statistical analysis plan will be developed with the trial statistician. The data will be presented as median and interquartile range. The first analysis will compare renal failure free days between vasopressin and noradrenaline treatment groups using a Mann-Whitney U test. The second analysis will examine for an interaction between study drug 1 (vasopressin / noradrenaline) and study drug 2 (hydrocortisone / placebo) on the outcome of renal failure free days. For completeness we will also compare renal failure free days between the hydrocortisone and placebo treatment groups.

The primary analyses will be carried out on an intention-to-treat basis. However as not all patients will require study drug 2 the analyses will be repeated on an “as treated” basis.

8.2 POWER CALCULATIONS

Due to the non-parametric nature of the primary outcome data power calculations have been carried out using a series of simulations. This has been based on data from previous septic shock trials and data from Imperial College NHS Trust’s ICU database. A study size of 400 patients will have at least 80% power to detect a 20-25% relative reduction of developing renal failure if treated with vasopressin compared to noradrenaline, assuming an overall incidence of acute renal failure of anywhere between 30% and 50%.
Assuming the vasopressin / steroid interaction is twice the overall vasopressin effect (as seen in VASST) the interaction test will have the same power as the overall treatment effect.

In view of the emergency nature of the intervention and that the majority of patients will be unable to consent at the time of enrolment due to an altered mental status as part of the disease process or sedative drugs, delayed consent to study participation will be sought. In line with previous critical care studies within the UK, a 3% withdrawal of consent would be expected and therefore we will recruit a total of 412 patients.

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.

9 TREATMENT

9.1 INVESTIGATIONAL MEDICINAL PRODUCT DETAILS

Bilcare Limited will supply the study drugs for this trial.

<table>
<thead>
<tr>
<th>Drug Name</th>
<th>Dosage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study Drug 1</td>
<td>Vasopressin: 0 - 0.06 U/min</td>
<td>Supplied as 3 ampoules (2ml &amp; 2x 4ml) to contain Vasopressin (40U)/Noradrenaline (8mg) and Placebo (saline). 9 vials in 3 packs to be supplied for the first 3 days per patient and then 9 vial/packs to be topped up as required.</td>
</tr>
<tr>
<td></td>
<td>Noradrenaline: 0 - 12 µg/min</td>
<td></td>
</tr>
<tr>
<td>Study Drug 2</td>
<td>Hydrocortisone: 50mg</td>
<td>Supplied as 29 ampoules (1ml) per pack per patient to contain hydrocortisone phosphate (100mg) or 0.9% saline.</td>
</tr>
<tr>
<td></td>
<td>Placebo: 0.9% saline</td>
<td></td>
</tr>
</tbody>
</table>

9.2 LABELLING, STORAGE AND DISPENSING

Bilcare Limited will be responsible for assuring that the quality of all IMPs are adequate for the duration of the trial and in compliance with the Good Manufacturing Practice (GMP) standards. Vasopressin will be imported and all other drugs are sourced from UK. All drugs will be packaged, labelled to meet the MHRA requirements and distributed to sites by Bilcare Limited. The Trial Coordination Centre will keep accurate records of supply to trial centres and destruction of unused IMP at the end of the trial.

It is each trial centre’s responsibility to ensure that accurate records of IMPs dispensed and returned are maintained and reported to the Trial Coordination Centre. It is the Trial Investigator’s responsibility to ensure that accurate records of IMPs prescriptions are maintained. The Trial Coordination Centre will track supplies of IMPs via information from Bilcare Limited and site IMP tracking documents. At the completion of the trial, the Trial Coordination Centre, via the monitor, will ensure the destruction of all returned dispensed IMPs (after close out and before archiving).

9.3 ACCOUNTABILITY

Hospital pharmacies will be responsible for recording study drugs dispensed to the ICU. Preparation of all drug infusions will be recorded on the Nursing Staff Drug Accountability Form and drug administration on the patient’s prescription chart. The study drug stores will include a sheet on which the fate of all ampoules will be recorded (infused, opened but not infused, unused). At the end of the study any remaining unused drug will be returned to the hospital pharmacy for recording.
9.4 UNBLINDING
It is unlikely that there would be a need to unblind the study drugs. The vasopressor infusions have very short half-lives and therefore if there was an adverse event the drug could be stopped and any effects would wear off in minutes. However, emergency envelopes will be supplied to each hospital pharmacy to allow emergency unblinding if needed.
The local investigators should aim to discuss the need for unblinding with the trial coordinator or Chief Investigator beforehand.

10 REGULATORY, ETHICAL AND LEGAL ISSUES

10.1 DECLARATION OF HELSINKI
This trial will be conducted in full conformity with the Declaration of Helsinki according to its 1996 version.

10.2 GOOD CLINICAL PRACTICE
The study will be conducted in accordance with the protocol, Good Clinical Practice (ICH GCP E6 guidelines), Imperial Clinical Trials Unit Standard Operating Procedures (ICTU SOPs) and national regulatory requirements and the provisions of relevant ethics committees.

10.3 INDEPENDENT ETHICS COMMITTEE APPROVAL

10.3.1 Initial Approval
Prior to the shipment of IMPs and the enrolment of subjects, the IEC must provide written approval of the conduct of the study at named sites, the protocol and any amendments, the Patient Information Sheet and Consent Form, any other written information that will be provided to the subjects, any advertisements that will be used and details of any subject compensation.

10.3.2 Approval of Amendments
Proposed amendments to the protocol and aforementioned documents must be submitted to the IEC for approval as instructed by the Sponsor. Amendments requiring IEC approval may be implemented only after a copy of the IEC’s approval letter has been obtained.
Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving Sponsor or IEC approval. However, in this case, approval must be obtained as soon as possible after implementation.

10.3.3 Annual Progress Reports and End of Trial Notification
The regulatory authorities and IEC will be offered annual progress reports and informed about the end of trial, within the required timelines.

10.4 REGULATORY AUTHORITY APPROVAL
The study will be performed in compliance with UK clinical trial regulations. Clinical Trial Authorisation from the Medicines and Healthcare products Regulatory Authority (MHRA) will be obtained prior to the start of the study. In addition, the Regulatory Authority must approve amendments (as instructed by the Sponsor), receive SUSAR reports and annual safety updates, and be notified of the end of the trial.

10.5 TRIAL REGISTRATION
The VANISH trial is registered with the European Clinical Trial Database with the EudraCT number: 2011-005363-24. Trial is registered with the “International Standard Randomised Controlled Trial Number Register” database with the ISRCTN number: ISRCTN20769191.
10.6 INSURANCE & INDEMNITY
Imperial College London, the Sponsor of the trial has civil liability insurance, which covers this study in all participating centres. Imperial College London also holds negligent harm and non-negligent harm insurance policies which apply to this study.

10.7 SUBJECT CONFIDENTIALITY
Participants’ identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

The investigator must ensure that the subject’s privacy is maintained. On the CRF or other documents submitted to the Sponsors, subjects will be identified by a subject ID number only. Documents that are not submitted to the Sponsor (e.g. signed informed consent form) should be kept in a strictly confidential file by the investigator.

The investigator shall permit direct access to subjects’ records and source document for the purposes of monitoring, auditing, or inspection by the Sponsor, authorised representatives of the Sponsor, Regulatory Authorities and IECs.

10.8 DATA PROTECTION
All personnel involved in the study will observe or work within the confines of the local data protection guidelines.

10.9 END OF TRIAL
This study will end when the specified number of patients have been recruited and the last patient has reached day 28 post enrolment or at hospital discharge, whichever is later.

10.10 STUDY DOCUMENTATION AND DATA STORAGE
The investigator will retain essential documents until notified by the Sponsor, and at least for ten years after study completion, as per Sponsor’s SOPs. Subject files and other source data (including copies of protocols, CRFs, original reports of test results, correspondence, records of informed consent, and other documents pertaining to the conduct of the study) must be kept for the maximum period of time permitted by the institution. Documents will be stored in such a way that they can be accessed/data retrieved at a later date. Consideration should be given to security and environmental risks.

No study document will be destroyed without prior written agreement between the Sponsor and the investigator. Should the investigator wish to assign the study records to another party or move them to another location, written agreement must be obtained from the Sponsor.

11 ADMINISTRATIVE MATTERS

11.1 SOURCE DATA
Source documents include original documents related to the trial, to medical treatment and to the history of the participant, and adequate source documentation must be maintained to allow reliable verification and validation of the trial data. What constitutes the source data for this trial will be outlined in the Source Data agreement.
11.2 ELECTRONIC DATA CAPTURE

Electronic CRF: the principal means of data collection from participant visits will be Electronic Data Capture (EDC) via the internet. Data is entered into the EDC system via site personnel. All source data recorded in the CRF will be signed by the Investigator or his/her appropriate designee. All changes made following the electronic signing will have an electronic audit trail with a signature and date. Specific instructions and further details will be outlined in the CRF manual.

11.3 TRIAL MANAGEMENT

11.3.1 Trial Steering Committee
A Trial Steering committee (TSC) with an independent Chair will be appointed and will be responsible for overseeing the progress of the trial. A TSC Charter will be devised to list the roles and responsibilities of the TSC members. TSC will be convened biannually either in person or by teleconference.

11.3.2 Trial Management Group
The Trial Management Group will be set up by the Chief Investigator. TMG will convene on a monthly basis and will discuss on the recruitment, and other practical aspects of the trial.

The day-to-day management of the trial will be co-ordinated through the Imperial Clinical Trials Unit and the Chief Investigator.

11.3.3 Monitoring
A monitoring plan will be devised based on risk analysis and described in detail in the monitoring manual by the project manager. Trial Monitors will visit all sites and facilities where the trial will take place to ensure compliance with the protocol, GCP and local regulatory compliance. Monitoring visits will be performed by trained monitors before, during and after the trial as required by the protocol and trial procedures according to the monitoring manual to ensure patient safety, accurate data collection and reporting. Monitoring visits will also be dependent on rates of and numbers of participant recruitment per site. Communication with sites by telephone, mail and email will also be made as necessary. Training sessions will be organised for the investigators and all site staff at the beginning of the trial and then as appropriate. Initiation visits will be completed at all trial centres prior to the recruitment of participants, and will consist of review of protocol and trial documents, training with respect to trial procedures (informed consent, SAE reporting, inclusion and exclusion criteria), review of recruitment strategy, review of site facilities and equipment, essential document receipt, collection and filing, and archiving and inspection. Copies of the trial specific procedure manuals and related documents will be given to the investigators. The approved version of the protocol should be followed at all times, and any significant protocol deviations will be documented in a Protocol Violation Form and submitted to the Trial coordination centre as soon as possible. The investigators will allow the monitors to:

- inspect the site, the facilities, IMP management and materials used for the trial
- meet all members of the team involved in the trial, and ensure all staff working on the trial are experienced and appropriately trained, and have access to review all of the documents relevant to the trial
- have access to the electronic case report forms and source data
- discuss with the investigator and site staff trial progress and any issues on a regular basis

The monitor will ensure that:
- all participant records will be inspected for confirmation of existence, eligibility and informed consent
- there is adherence to the protocol, including consistency with inclusion/exclusion criteria
- there is GCP and regulatory compliance
• Trial Documentation is complete and up to date (e.g. correct versions of documents being used, source data captured) and relevant documents are collected for the Trial Master File (TMF)
• The eCRFs have been completed correctly and accurately, and all entries correspond to data captured in source documents
• The IMP accountability records are in order (receipt, dispensing and destruction), storage is under appropriate conditions and secure, expiry dates are being checked and adhered to, and dispensing is according to the protocol and trial procedures.
All information dealt with during such visits will be treated as strictly confidential. At the end of the trial, close out visits will be performed by the monitor after the final participant visit has been completed and prior to database lock. During this visit the monitor will verify that all trial close out activities are completed – all queries resolved, missing data completed, monitoring completed, archiving arrangements in place, IMP accountability complete and all used and unused IMP destroyed, ISF completed and TMF documents collected, and end of trial notification. Each investigator will also be notified that an audit or inspection may be carried out - by the sponsor, sponsor’s representatives or the host institution, or regulatory authorities - at any time, before, during or after the end of the trial. The investigator must allow the representatives of the audit or inspection team:
• to inspect the site, facilities and material used for the trial,
• to meet all members of his/her team involved in the trial,
• to have direct access to trial data and source documents, to consult all of the documents relevant to the trial. If an Investigator is informed of an impending audit or inspection, the trial coordination centre should be notified immediately.

11.4 QUALITY CONTROL AND QUALITY ASSURANCE
Quality Control will be performed according to Imperial Clinical Trials Unit internal procedures. The study may be audited by a Quality Assurance representative of the Sponsor. All necessary data and documents will be made available for inspection.

11.5 AUDITS AND INSPECTIONS
The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

11.6 PUBLICATION POLICY
All publications and presentations relating to the study will be authorised by the Trial Management Group. Authorship will be determined according to the internationally agreed criteria for authorship (www.icmje.org). Authorship of parallel studies initiated outside of the Trial Management Group will be according to the individuals involved in the project but must acknowledge the contribution of the Trial Management Group and the Study Coordination Centre.
12 REFERENCES


Imperial Clinical Trials Unit

VANISH

Vasopressin vs Noradrenaline as Initial therapy in Septic Shock

Chief Investigator: Dr Anthony Gordon

EudraCT NUMBER: 2011-005363-24
SPONSOR: Imperial College London
FUNDER: National Institute for Health Research
DEVELOPMENT PHASE: PHASE IV
STUDY COORDINATION CENTRE: Imperial Clinical Trial Unit
SAP Version: Version 1
Date: 15th April, 2014

STATISTICAL ANALYSIS PLAN

Non-confidential

Prepared by Alexina Mason (Trial Statistician)

This is a SAP for a full report to the TSC
not all the analysis is intended for publication
**Approvals**

This SAP is approved by:

<table>
<thead>
<tr>
<th>Version</th>
<th>Name</th>
<th>Signature</th>
<th>Role</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dr Anthony Gordon</td>
<td></td>
<td>Chief Investigator</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Prof Deborah Ashby</td>
<td></td>
<td>Senior Statistician</td>
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</tr>
<tr>
<td></td>
<td>Dr Alexina Mason</td>
<td></td>
<td>Trial Statistician</td>
<td></td>
</tr>
</tbody>
</table>

Approved by the TSC on 15 April 2014.
1 Background

1.1 Introduction

Severe sepsis is an increasingly common problem worldwide and is an important cause of mortality. Septic shock, the most severe form of sepsis, is defined as hypotension in response to overwhelming infection (ACCP/SCCM, 1992). As well as appropriate antibiotic treatment one of the main treatments is cardiovascular resuscitation using intravenous fluids and catecholamines. Although usually effective at restoring blood pressure, catecholamines have important adverse effects and may even increase mortality (Hayes et al., 1994).

Recently vasopressin, an endogenous stress hormone, has been proposed as an adjunct to catecholamines in the treatment of septic shock. The rationale for its use is that a relative vasopressin deficiency occurs in septic shock and exogenously administered vasopressin restores vascular tone (Landry et al., 1997), increases blood pressure and thus leads to a reduced requirement for catecholamine vasopressors (Patel et al., 2002). Furthermore, vasopressin via interaction with a family of receptors may result in differential vasoconstriction and possibly vasodilatation in different vascular beds compared to noradrenaline (Barrett et al., 2007). Thus vasopressin may be a potentially useful therapy in septic shock due both to its direct beneficial effects and also reduction / avoidance of catecholamine infusions.

Similarly corticosteroids are another commonly used adjunctive therapy in septic shock. Low dose hydrocortisone has repeatedly been shown to reduce catecholamine infusion rates, lead to earlier resolution of shock but its effect on survival remains uncertain (Annane et al., 2002; Sprung et al., 2008; Annane et al., 2009).

Recently it has been suggested that there may be an interaction between vasopressin and corticosteroids on mortality rates in septic shock (Russell et al., 2009). In VASST, the combination of vasopressin and steroids led to a lower mortality compared to noradrenaline plus steroids (35.9% v 44.7% respectively, difference -8.8%, 95%CI -16.7 to -0.9). In contrast, patients who were treated with vasopressin and did not receive corticosteroids had an increased mortality compared to patients who received noradrenaline and no corticosteroids (33.7% v 21.3%, difference 12.3%, 95%CI -0.2 to 24.9).

The rationale for the current study is that evidence to date suggests that vasopressin may prevent renal dysfunction in septic shock when used early, and that vasopressin may interact with corticosteroids.

1.2 Study objectives

The primary objectives of this trial are:

1. To test whether vasopressin reduces renal dysfunction compared to noradrenaline when used as the initial vasopressor in the management of adult patients who have septic shock.

2. To test whether there is an interaction between vasopressin and corticosteroids when used in the management of septic shock.

The first of these primary objectives is considered the more important.
Secondary objectives are:
1. To assess whether vasopressin improves other secondary outcomes (need for dialysis, survival rates, other organ failures and resource usage) compared to noradrenaline in the management of septic shock in adult patients.
2. To test whether there are genetic polymorphisms that determine response to the study drugs.

1.3 Study population

Adult patients in an Intensive Care Unit (ICU) who require vasopressors for the management of septic shock despite adequate fluid resuscitation.

1.4 Study design

This is a double-blind factorial (2x2) randomised controlled trial, conducted in multiple general adult ICUs within the UK. The four treatment arms are:
1. vasopressin + hydrocortisone;
2. vasopressin + placebo;
3. noradrenaline + hydrocortisone;
4. noradrenaline + placebo

All patients will be treated with the first study drug (vasopressin or noradrenaline) after adequate fluid resuscitation, but will only receive the second study drug (hydrocortisone or placebo) if the maximum doses of the first study drug are reached. The study will recruit 103 patients to each of the treatment arms, giving 412 patients in total. Randomisation and treatment are summarised in Figure 1.

1.5 Primary outcome

The primary outcome will be the total number of days alive and free of renal failure during the 28 days after randomisation.

For the main analysis to answer the first primary objective, the primary outcome will be compared for subjects receiving vasopressin (vasopressin + hydrocortisone and vasopressin + placebo arms combined) with the subjects receiving noradrenaline (noradrenaline + hydrocortisone and noradrenaline + placebo arms combined).

Other analyses of the primary outcome will compare all four treatment arms. Provided the interaction between the first and second study drugs is not too strong, we will also compare subjects receiving hydrocortisone with subjects receiving placebo for completeness.

1.6 Secondary outcomes

Secondary outcomes will include:
• Rates and duration of renal replacement therapy
Figure 1: Schematic Trial Plan

Adult patient who has sepsis and requires vasopressors to maintain blood pressure despite adequate fluid resuscitation

RANDOMISE

Vasopressin infusion (0.0-0.06U/min) titrated to BP. Continues until shock resolved

If BP still low Hydrocortisone (50mg IV 6 hourly for 5 days then tapered to 50mg every 12 hours for days 6-8, 50mg every 24 hours for days 9-11, and then stopped.)

If BP still low Placebo (0.5ml 0.9% Saline IV 6 hourly for 5 days then tapered to 0.5ml every 12 hours for days 6-8, 0.5ml every 24 hours for days 9-11, and then stopped.)

Noradrenaline infusion (0-12µg/min) titrated to BP. Continues until shock resolved

If BP still low Hydrocortisone (50mg IV 6 hourly for 5 days then tapered to 50mg every 12 hours for days 6-8, 50mg every 24 hours for days 9-11, and then stopped.)

If BP still low Placebo (0.5ml 0.9% Saline IV 6 hourly for 5 days then tapered to 0.5ml every 12 hours for days 6-8, 0.5ml every 24 hours for days 9-11, and then stopped.)

If BP still low, catecholamine vasopressors at physician discretion

Blood and urine research sample collection on days 1, 3, 5 and 7. Daily routine clinical data collection from notes while on ICU. 28-day and hospital survival status.

- Length of renal failure in survivors and non-survivors
- 28-day, ICU and hospital mortality rates
- Organ failure free days in the first 28 days, assessed using the serial organ failure assessment (SOFA) score
- Organ support data assessed using the standard NHS Healthcare Resource Groups
- Blood, plasma and urinary biomarkers of renal function and inflammation (including genetic polymorphisms).

1.7 Sample size

The original power calculations were carried out using a series of simulations, based on a non-parametric approach to analysing the primary outcome that used a Mann-Whitney U test. These were underpinned by data from previous septic shock trials and data from Imperial College NHS
Trust’s ICU database. They showed that a study size of 400 patients would have at least 80% power to detect a 20-25% relative reduction of risk of developing renal failure if treated with vasopressin compared to noradrenaline, assuming an overall incidence of acute renal failure of anywhere between 30% and 50% and a significance level of 0.05. Accordingly 412 patients will be recruited, which allows for a 3% withdrawal of consent in line with previous critical care studies within the UK.

These power calculations were revisited during the development of the SAP, taking account of the now available data from the pilot study, VACS. New scenarios were developed using a slightly more sophisticated algorithm, but similar assumptions, to generate more realistic simulated data, and these suggest about 75% power. However, simulating power is an inexact science and the estimated power is sensitive to the precise assumptions underpinning these calculations. Based on current knowledge 70-80% is a plausible range for the power of this study. Alternative endpoints were also investigated and these are likely to have similar power.

### 1.8 Randomisation

Randomisation will be by pre-prepared computer generated randomised number and will use the InForm eCRF online system. Randomisation will be stratified by ICU and will occur on a 1:1 basis in permuted blocks.

The randomisation lists were generated by Dr Jane Warwick. Details such as the block size are kept confidential and are held separately by the trials unit.

### 1.9 Schedule of time and events

The trial started on 4 February 2013 and is expected to recruit for two years and three months. There will be six-monthly meetings of the independent Data Monitoring and Ethical Committee (DMEC).

As can be seen from Table 1, participants will be followed up daily whilst on the ICU with routinely collected clinical data (cardiovascular, respiratory and renal physiological variables as well as haematological, biochemical and microbiological blood test results) being recorded. In addition, blood and urine samples will be collected (in select centres) on the day of inclusion and days 3, 5 and 7. Patients will also be followed up to ascertain survival status at 28 days post recruitment and hospital discharge.

<table>
<thead>
<tr>
<th></th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 6</th>
<th>Day 7</th>
<th>Days 8-28*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood &amp; Urine sampling</td>
<td>✔</td>
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<td>✔</td>
<td>✔</td>
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</tr>
<tr>
<td>Daily clinical data</td>
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<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>

* if discharged before day 28, until day of discharge from ICU

Table 1: Data collection schedule

VANISH: Statistical Analysis Plan
2 General Considerations

2.1 Analysis strategy

The primary analyses will be carried out on an intention-to-treat basis. However as not all patients will require study drug 2 the analyses will be repeated on an ‘as treated’ basis. The choice of models has been informed by analysis of data from the pilot study, VACS.

VANISH has been designed as a factorial randomised controlled trial with four treatments groups to meet two primary objectives. The more common use of a $2 \times 2$ factorial trial is to test a) the effect of treatment A and b) the effect of treatment B, where treatments A and B do not have the potential for substantive interaction (McAlister et al., 2003; Montgomery et al., 2003), and in this case an ‘at the margins’ analysis is appropriate. However, in VANISH the aim is to test a) the effect of vasopressin on renal dysfunction and b) the effect of the interaction between vasopressin and corticosteroids. If there is an interaction, an ‘at the margins’ analysis is no longer appropriate and an ‘inside the table’ analysis is required (McAlister et al., 2003).

However, the first of these (the vasopressin question) is considered the more important and the study has been powered to answer this question using the Mann-Whitney U test. For this primary analysis the vasopressin + hydrocortisone and vasopressin + placebo groups will be combined and the noradrenaline + hydrocortisone and noradrenaline + placebo groups combined, for an ‘at the margins’ analysis. Corticosteroid use should be approximately balanced between the two groups through the randomisation, but any interaction effect will be ignored.

For an explanatory analysis to provide insight into both primary objectives, we will set up an appropriate regression model, choosing the noradrenaline + placebo arm as the baseline group and including indicator variables for vasopressin, corticosteroids and vasopressin $\times$ corticosteroids. These indicator variables will be set as shown in Table 2. We will then answer the question posed by objective 1, taking account of any interaction, using the parameter associated with the vasopressin indicator, and the question posed by objective 2 using the parameter associated with the vasopressin $\times$ corticosteroids indicator.

<table>
<thead>
<tr>
<th></th>
<th>vasopressin</th>
<th>corticosteroids</th>
<th>vasopressin $\times$ corticosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>vasopressin + hydrocortisone</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>vasopressin + placebo</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>noradrenaline + hydrocortisone</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>noradrenaline + placebo</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Alternatively we could set the indicator variables by group, but this would not give direct answers to the questions posed by the two objectives.

In general, the analysis of the secondary outcomes will compare the four treatment groups. Hence, these indicator variables will also be incorporated into appropriate regression models to compare treatment effects on the secondary outcomes. Comparisons of vasopressin v noradrenaline and corticosteroids v placebo will also be presented, provided the strength of any interaction does not
render these ‘at the margins’ analyses meaningless.

Details of the proposed models are given in Section 5 and Appendix D.1. As a subsidiary analysis, the statistical models will be extended to incorporate ICU random effects and adjust for important baseline covariates, in particular severity of illness.

Prior to performing any statistical tests or fitting statistical models, an exploratory analysis of the baseline variables and outcome measures will be completed. This will include producing summary tables, and exploring variable ranges and distributions using graphical methods. As a further check on the data, site specific tables and plots will also be generated. The validity of the underlying assumptions for the proposed models will be checked and alternatives sought if necessary. Standard checks of model fit will also be carried out, and again the model specifications will be adjusted if necessary. The reasons for any changes will be fully documented.

2.2 Definition of datasets for analysis

2.2.1 Recruitment, baseline characteristic and safety analysis

The recruitment, baseline characteristic and safety analysis will be carried out on an intention-to-treat basis (using all patients who have been randomised and consent obtained).

2.2.2 Clinical outcome analysis

There will be two analyses of the clinical outcomes: one on a slightly modified intention-to-treat basis (the main analysis) and one on a treatment received basis (subsidiary analysis). Patients who are randomised, but did not receive any study drug and no consent obtained will be excluded from both analyses. The reason is that this is an emergency medicine trial and patients should be recruited as quickly as possible. As the situation is rapidly evolving new information may become apparent that excludes the patient from the trial. As the patients have not provided consent at this point we cannot include them in the final analysis. However, these patients will be included in the CONSORT diagram.

The protocol allows for a rescue steroid therapy in which hydrocortisone can be administered to patients, if they are not responding to study drugs and catecholamine vasopressors. This may give rise to ‘cross-overs’ with subjects switching from the placebo arms, to the arms with hydrocortisone as their drug 2. There should be very few other ‘cross-overs’ (see Section 5.7). If any do occur, they will be dealt with in the analysis in a consistent way to the rescue therapy ‘cross-overs’.

The main analyses of the clinical outcomes will be carried out using a dataset in which patients are allocated to treatment arm on an intention-to-treat basis. A second dataset formed by allocating patients to treatment arm ‘as treated’ will be used for a sensitivity analysis. The ‘as treated’ dataset will differ from the ‘intention-to-treat’ dataset in two respects. Firstly some ‘cross-overs’ will switch group. Secondly, patients who do not receive the maximum dose of the first study drug, and therefore do not receive any of the second study drug, will be moved if their second drug would have been hydrocortisone to the equivalent placebo group. The differences between the two datasets will be described.
2.3 Data management

The trial data will be collected and managed using InForm, which is an electronic data capture system built around an Oracle database. The InForm system includes validation rules for data entry to help ensure data accuracy, and has a full audit trail of data entry and changes.

The monitoring plan suggested by the QA Manager specified:
- 100% monitoring for consent, AE/SAE/SUSAR, eligibility;
- 50% monitoring for existence, drug delivery to patients, endpoints.

In line with this, 100% monitoring will be carried out for all data points for the first two patients at all sites to check that the protocol and procedures are being followed correctly. Then the 50% monitoring will be performed for randomly selected patients at all sites.

If problems or fundamental issues become apparent in the on-going checking that forms part of the statistical analysis, the trial statistician will raise these with a senior statistician who will consult with the appropriate individuals. Any such action and subsequent decisions will be documented.

2.4 Missing data

Daily data is recorded as clinically indicated for up to 28 days while the patient is in ICU. A decision not to take a measurement usually reflects the clinical judgement that there has been no change in that variable or the patient is getting better. As far as possible, other reasons for non-entry will be collected using the InForm comment facilities. Every effort will be made to minimise missing baseline and outcome data in this trial.

Before starting the data analysis, the level and pattern of the missing data in the baseline variables and outcomes will be established by forming appropriate tables. The likely causes of any missingness will be investigated. Details for the primary outcome can be found in Section 5.8.2. Similar tables will be produced for other variables. This information will be used to determine whether the level and type of missing data has the potential to introduce bias into the analysis results for the proposed statistical methods, or substantially reduce the precision of estimates related to treatment effects. If necessary, these issues will be dealt with using multiple imputation (Sterne et al., 2009; Kenward and Carpenter, 2007) or Bayesian methods for missing data (Daniels and Hogan, 2008) as appropriate. The choice of missing data methods will also be informed by the analysis of the secondary outcomes in the VACS trial.

3 Outcomes and Variables of Interest

3.1 Data recorded on InForm

Routinely collected clinical data is recorded and stored on paper charts or electronically for each patient on the ICU. The details required for the analysis of this trial are periodically entered into the appropriate electronic case report form within the InForm system. The data that are stored in InForm includes randomisation details, baseline evaluation, daily evaluations, safety data, other outcomes, data from the Critical Care Minimum Data Set (CCMDS) and protocol deviations. These are summarised in Appendix B.
3.2 Data from blood and urine samples

In addition to the routinely collected data held in InForm, blood samples (25mls) and urine samples (10mls) will be collected on the day of inclusion and days 3, 5 and 7 and sent for laboratory analysis.

3.3 Derived variables

The required derived variables are described below, and the code used for their calculation can be found in Appendix E.

**APACHE II score**

The APACHE II score is an established severity of disease classification system based on 12 routine physiologic measurements, age and previous health status (Knaus et al., 1985). It can take values in the range 0-71, with higher scores indicating a greater disease severity. Points are added to the score for abnormally low or abnormally high physiologic measurements, based on the most abnormal physiologic value over a 24 hour period, with each variable contributing up to 4 points.

We will calculate the APACHE II score for each patient using data collected during the baseline evaluation. Details of the criteria for assigning points for each element of this score are given in Appendix C.1.

**AKI score**

A number of systems for classifying acute kidney injury/failure have been proposed. We will calculate an acute kidney injury (AKI) stage (1, 2 or 3) for each patient for each day they are in an ICU, according to Mehta et al. (2007), using measurements recorded in the daily data (Section B.3). Details are given in Appendix C.2.

**SOFA score**

The SOFA (Serial Organ Failure Assessment) score is the sum of scores relating to six organs. It comprises respiration, coagulation, liver, cardiovascular, renal and central nervous system components, each of which can take values from 0 (normal) to 4 (most abnormal), based on the most extreme values recorded each day (Vincent et al., 1996). Details of the criteria for assigning values are provided in Appendix C.3. Although the SOFA score has six components, we are only interested in five of these, and will exclude the central nervous system from our analysis.

We will calculate the SOFA score component attributable to the respiration, coagulation, liver, cardiovascular and renal components for each patient for each day they are in an ICU, using measurements recorded in the daily data (Appendix B.3). We will also calculate an overall patient SOFA score for each day by adding the five components together.

**Mean SOFA score**

The mean SOFA score while in ICU has been shown to be closely correlated with death (Ferreira et al., 2001), and is the primary end-point for LeoPARDS. We will calculate this variable for each patient as the sum of all their daily SOFA scores divided by the number of days in ICU.
Organ failure free days

The number of organ failure free days in the first 28 days will be calculated for the five organs of interest for this study. Patients will be scored as being alive or dead on each calendar day after randomisation, and if alive they will be scored as either having or being free from organ failure using the daily data. Renal failure will be defined as having AKI stage 3, while the other organ failures (respiration, coagulation, liver and cardiovascular) will be defined as a SOFA score of 3 or 4. Further detail on the calculation of renal failure free days is given in Section 3.4 as this is the primary outcome. Subjects who have been discharged alive from ICU before day 28, are assumed to be free of organ failure for the remaining days in the 28 day period, except renal failure where the need for ongoing dialysis (RRT) treatment will be collected post ICU discharge (see Section B.5.1). (In this definition, subjects who die at some point in the 28 days may still be scored as having organ failure free days while they are alive. Hence on this measure a subject who dies may have more organ failure free days than one who does not.)

MAKE28

MAKE28 will be analysed as an additional secondary endpoint. MAKE (major acute kidney event) at a certain time point e.g. Day 28 or 90 is a recently suggested alternative RF outcome (Palevsky et al., 2012), that was not around at the inception of VANISH. We will use the version based on 28 days, in line with the daily data collection and other endpoints, and compare its performance with the primary endpoint analysis to provide information on endpoint selection for future trials involving RF outcomes.

As with number of days alive and free of renal failure, MAKE28 is a composite endpoint and contains similar elements. However, it may be an easier endpoint to work with, as it is a simple proportion rather than continuous measure with an awkward distribution requiring a non-parametric analysis. For VANISH, a patient will be defined as having MAKE28 if they experience any of the following in the 28 days following randomisation:

- death;
- need for renal replacement therapy (RRT);
- sustained loss of kidney function (prolonged RF).

Prolonged RF will be defined as AKI stage 2 or 3 at day 28, or on ICU discharge if discharged before day 28.

3.4 Definition of primary outcome

The primary outcome will be the total number of days alive and free of renal failure during the 28 days after randomisation (a higher number reflects a better outcome). Patients will be scored as being alive or dead on each calendar day after inclusion, and if alive they will be scored as either having or being free from renal failure.

Renal failure will be defined by the Acute Kidney Injury Network (AKIN) group stage 3 definition (Mehta et al., 2007):

- increase in serum creatinine to > 300% (>3-fold) from baseline
- or serum creatinine ≥ 354 µmol/L with an acute rise of at least 44 µmol/L
- or initiation of renal replacement therapy (for AKI)
- or a urine output of < 0.3 mL/kg/h for ≥ 24 hours
• or anuria for $\geq$ 12 hours

4 Interim Analysis

There will be no interim analysis.

5 Final Analysis

5.1 Recruitment details

Details about patient enrolment, treatment allocation, follow-up and inclusion in analysis will be provided using a patient flow diagram as recommended by the CONSORT statement (Schulz et al., 2010).

Recruitment will be summarised by specific ICU (Appendix A, Table A1), including the numbers of major and minor protocol deviations. A breakdown of the reasons for exclusion will also be provided in tabular form. Additionally, listings and summaries of the major and minor protocol deviations will be produced. A protocol deviation will be classified as major if it significantly affects patient safety or the scientific value of the trial (recorded as serious in the protocol deviations eCRF).

5.2 Baseline characteristics

Baseline characteristics of all randomised subjects will be summarised by treatment group using appropriate descriptive statistics (Appendix A, Tables A2 and A3).

5.3 Safety data analysis

Adverse events will be summarised by seriousness, relationship to study medication and treatment group (Appendix A, Table A4). Using the description of each adverse event recorded on the adverse event eCRF, each serious adverse event will be assigned to one of the following categories:

- Myocardial infarction / acute coronary syndrome
- Life threatening arrhythmia
- Mesenteric ischaemia
- Digital ischaemia
- Secondary infection
- Other.

These categorised data will be tabulated by treatment group (Appendix A, Table A5). In addition, a listing of serious adverse events by patient will be produced.
5.4 Blood pressure and other daily data

We will check whether similar blood pressures were maintained across treatment groups throughout the study period by plotting median blood pressure, with interquartile range, in each group over time. Similar plots will be produced for other daily data measures which are not part of the SOFA score to check for differences between treatment groups (see Appendix B.3). These measures will include:

- total intravenous fluid administered for each 24 hour period,
- total fluid balance over each 24 hour period,
- highest daily lactate levels,
- highest heart rate,
- ScvO$_2$,
- cardiac output,
- troponin.

Any differences in these measures will be investigated. As a first step, we will look at whether the baseline distributions differ between groups using box plots. Using regression models as appropriate, we will then check whether there are any differences in the rate of change in the measure between groups after taking account of baseline levels.

5.5 Other vasoactive drugs

The number of subjects receiving other vasoactive drugs will be summarised by treatment group and ICU, along with the level received, and differences investigated.

5.6 Etomidate usage

The number of subjects receiving etomidate will be summarised by treatment group and ICU. (Etomidate is recorded on the Event Dates eCRF, see Appendix B.2.)

5.7 Rescue therapy

As noted in Section 2.2.2, the protocol allows the administration of hydrocortisone for a rescue steroid therapy if patients are not responding to study drugs and catecholamine vasopressors. It is also possible that vasopressin will be given as rescue therapy, although the numbers should be very small as this is a protocol deviation. To assess the extent of the use of both hydrocortisone and vasopressin in this way, a summary table providing the frequency of the use of both drugs by treatment arm will be produced. As further detail, listings of what happened will also be provided.
5.8 Primary outcome analysis

5.8.1 Exploratory analysis of the primary outcome

The primary outcome is days alive and free of renal failure (DAF RF) and will be calculated using the AKIN criteria (see Section 3.4). As a data check for outliers, we will produce a series of longitudinal plots (one for each centre) of AKI score for each patient, differentiating between treatment arm. Histograms of DAF RF for each treatment will also be formed.

5.8.2 Missing data checks for the primary outcome

The level and pattern of any missingness in the AKI scores will be assessed. Initially the completeness of the AKI data will be summarised on both a subject and a score level by ICU and treatment arm (Table A6). Data for calculating the AKI score are only collected while the patient is in ICU. Scores for days after discharge from ICU are not expected and will not be counted as missing. Reasons for any major differences in the level of missingness between treatment arms or ICUs will be investigated. For subjects with missing data we will also look at the number of values that were typically missing and the pattern of occurrence. As AKI is calculated from serum creatinine, urine output and renal replacement therapy criteria, a summary of whether all or only some of the elements required for its calculation are missing will be provided.

In the pilot study, VACS, only a small percentage (3%) of AKI values had one or more of the required measurements missing (usually creatinine only), and a similar level of missingness is expected for VANISH. For VACS, last observation carried forward was used to impute any missing element as it was reasonable to assume that the data were not collected because no change was expected by the clinician. The resulting imputed AKI values were then checked for consistency against the patient’s other daily scores. If the level of missingness is similar for VANISH, then we will take the same approach. However, if the level of missingness is substantially higher or the likely cause of the missingness differs and is thought to have the potential for introducing bias into the results, then multiple imputation or Bayesian methods will be used as appropriate to fully account for the associated uncertainty.

5.8.3 Main analysis of the primary outcome

For the primary analysis, a Mann-Whitney U test will be used to answer the first stated primary objective, whether vasopressin reduces renal dysfunction compared to noradrenaline when used as the initial vasopressor in the management of adult patients who have septic shock. This non-parametric test will compare the distributions of DAF RF of the subjects receiving vasopressin (vasopressin + hydrocortisone and vasopressin + placebo arms) with the subjects receiving noradrenaline (noradrenaline + hydrocortisone and noradrenaline + placebo arms).

Interpretation and reporting

The non-parametric test is required because DAF RF data are not normally distributed, as shown by the histogram of the data from the VACS trial (Figure 2). There is a large spike at 28 days, the point at which the measure is censored. A smaller spike is expected at 0 days, although this is not apparent in the small VACS sample. Consequently, no single summary measure can adequately
describe DAF RF.

Figure 2: Distribution of days alive and free of renal failure for VACS

Data from the two arms, vasopressin + hydrocortisone and vasopressin + placebo, have been combined.

Instead we will summarise DAF RF using two measures:

1. the proportion of patients who survive and have no RF during the 28 days after randomisation (% no RF);
2. the median DAF RF for patients who do not survive and/or experience some RF during the 28 days after randomisation (median some RF).

Hence if using the Mann-Whitney U test we reject the hypothesis that the DAF RF for the vasopressin group and the noradrenaline group come from the same distribution, then this will be the result of a change in % no RF and/or a change in median some RF (if the changes are in opposite directions, then one of these will dominate). A priori we will consider changes in both measures to be of equal importance.

Dependent upon result, the reporting of the primary analysis will take the form:

Using a Mann-Whitney U test, we found that the distribution of the DAF RF was shifted to the right for patients treated with vasopressin compared with those treated with noradrenaline (p=x.xx). This was the result of a combination of a xx% increase in the proportion of patients who survive and have no RF during the 28 days after randomisation (yy% v zz%) and an increase of x in the median DAF RF for patients who do not survive and/or experience some RF during the 28 days after randomisation (y v z).

5.8.4 Explanatory analysis of the primary outcome

This primary analysis uses a composite endpoint on combined groups, providing a top level answer to the trial’s main objective. It provides no insight into the possible interaction between vasopressin and corticosteroids (the second primary objective), and no information on how much of any increase
in DAF RF can be attributed to preventing RF and how much to preventing death. To investigate the story behind the changes in DAF RF, we will fit multi-state models as an exploratory analysis. Details of these are given in Appendix D.1.

5.8.5 Sensitivity analysis of the primary outcome

For the primary analysis, renal failure has been defined using a criteria which has three components: a) serum creatinine, b) urine output and c) renal replacement therapy. We will recalculate renal failure using 1) the serum creatinine component only and 2) the urine output component only, repeat the main analysis and compare the results. This is motivated by recent research undertaken by Myburgh \textit{et al.} (2012), who found that their results differed depending on which component was used for the renal function scoring.

5.9 Secondary outcome analysis

In general, the secondary outcomes will be compared across the four treatment arms, to allow for any interaction between vasopressin and corticosteroids. Comparisons of vasopressin v noradrenaline and corticosteroids v placebo will also be presented, provided the strength of any interaction does not render them meaningless.

5.9.1 Rates and duration of renal replacement therapy

As a descriptive analysis we will tabulate the proportion of subjects in each treatment arm who receive renal replacement therapy on one or more days during the 28 days after randomisation. We will use a logistic regression model, incorporating the treatment indicators described in Section 2.1, to compare the treatment effects.

Regarding duration, we will calculate the number of days each subject receives renal replacement therapy. The analysis of these data is complicated by death, so an initial analysis will describe these data for survivors and non-survivors separately. Histograms and a table of appropriate summary statistics will be produced for each treatment arm. Appropriate regression analysis will also be carried out to compare the four groups, with and without adjustment for ICU effects and severity of illness, taking account of the competing risk of death.

5.9.2 Renal failure in survivors and non-survivors

This DAF RF secondary outcome will be analysed in a similar way to the primary outcome (Section 5.8.3). To assess whether the treatment effects on DAF RF are similar in survivors and non-survivors, a survival indicator will be incorporated as an explanatory variable into the multi-state models (Appendix D.1).
5.9.3 Mortality rates

Mortality after 28 days, on ICU discharge and on hospital discharge will be summarised. Kaplan-Meier survival curves of time to death will also be produced for each treatment group (truncated at 28 days). Cox regression will be used to compare treatment effects across the four groups, with and without adjustment for ICU effects and severity of illness.

5.9.4 Organ failure

We will summarise the following measures of organ failure/injury:
- organ failure free days in the first 28 days for each organ;
- total SOFA score (respiration, coagulation, liver, cardiovascular and renal components);
- SOFA score for each of the five components;

The SOFA scores per patient will be calculated for each of the 28 days.

We will analyse respiration, coagulation, liver and cardiovascular organ failure using the derived variables described in the organ failure free days part of Section 3.3. For each organ we will produce histograms showing the distribution of the number of failure free days by treatment and a table providing summary statistics. Analysis will look at onset of new organ dysfunction, and organ failure free days for subjects who had some organ failure.

For the total SOFA score (see sofa score part of Section 3.3), we will produce:
- a summary table;
- boxplots of SOFA score by day;
- histograms of SOFA score by treatment for selected days;
- plot of mean SOFA score over time by treatment (mean calculated using all patients);
- plot of mean SOFA score over time by treatment (mean calculated using only patients who were still alive after 28 days).

This analysis will be repeated on each of the five components of the SOFA score.

We will also calculate the mean SOFA score over time while in ICU after randomisation and summarise this measure. The mean SOFA score will be compared between treatment groups using boxplots and regression techniques.

5.9.5 Organ support data

Information on resource use in terms of the number of organ support days is being collected while the patient is in ICU (see Appendix B.5.5 for a list). Histograms and boxplots will be produced as an exploratory analysis of these data, and appropriate summary statistics tabulated by group.

5.9.6 MAKE28

We will tabulate the proportion of subjects with MAKE28 in each of the four treatment arms, and use logistic regression, incorporating the treatment indicators described in Section 2.1, to compare the treatment effects.
Further, to provide a comparison with the results of the Mann-Whitney U test on DAF RF, we will compare subjects receiving vasopressin with the subjects receiving noradrenaline. This will entail tabulating the proportions of subjects in each group with MAKE28, and calculating the difference and associated 95% confidence interval. We will also test the hypothesis that there is no difference between the proportions from the vasopressin and noradrenaline groups.

6 Sub-studies

Sub-studies will analyse blood, plasma and urinary biomarkers.
References


VANISH: Statistical Analysis Plan


