TITLE OF THE TRIAL

Acetazolamide as a chloride sparing Diuretic in patients Admitted with Heart Failure: a pilot and exploratory study.

SHORT STUDY TITLE

ADA-HF

This protocol has regard for the HRA guidance and order of content

RESEARCH REFERENCE NUMBERS

IRAS project ID:1005718

EudraCT Number: 2022-001566-34

ISRCTN Number / Clinical trials.gov ID:

PROTOCOL VERSION: V2, 27.01.2023

Sponsor ID: R2806

SPONSOR: Hull University Teaching Hospitals NHS Trust

Hull University Teaching Hospitals NHS Trust

SIGNATURE PAGE

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and 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 any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's SOPs, and other regulatory requirements as amended.

I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor

I also confirm that I will make the findings of the study publically available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the study will be given; and that any discrepancies from the study as planned in this protocol will be explained.

For and on behalf of the Study Sponsor:

Signature: DocuSigned by: James Illingworth 3DDA0F96873F430... Date: 29/09/2022

Name (please print): James Illingworth

Position: R&D Manager

Principal Investigator:

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Name: (please print): Dr Joseph James Cuthbert

Statistician:

Signature: DocuSigned by: Professor Alan Rigby 6DBDA9B96755440... Date: 29/09/2022

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Position: Trial Statistician

KEY TRIAL CONTACTS

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Sponsor	Hull University Teaching Hospitals NHS Trust
Funder(s) details	British Heart Foundation Clinical Research Collaborative Research Development Fund
Key Protocol Contributors	Dr Joe Cuthbert Professor Andrew Clark HUTH Research and Development Department British Society of Heart Failure Research Committee PPI obtained on primary outcomes, methods, schedule of events.

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TRIAL SUMMARY

Trial Title	Acetazolamide as a chloride sparing Diuretic in patients Admitted with Heart Failure: a pilot study
Internal ref. no. (or short title)	ADA-HF
Clinical Phase	Phase II
Trial Design	Open label, randomised, controlled, pilot and exploratory study.
Trial Participants	Patients admitted to Castle Hill Hospital with venous congestion due to heart failure who are deemed to require a continuous intravenous infusion (IVI) of furosemide at (10mg/hr) – standard of care for severe venous congestion.
Planned Sample Size	40 (maximum recruitment of 50)
Treatment duration	4 days
Follow up duration	180 days
Planned Trial Period	30 weeks
Primary Objective	1) Difference in net fluid loss daily and over a 4 day period 2) Difference in serum chloride between baseline and day 4
Secondary Objective	Differences between the two groups during the 4 day treatment period in: 1) daily urine output 2) daily weight 3) serum electrolyte levels (sodium, bicarbonate and potassium) 4) incidence of electrolyte abnormalities; hyponatraemia (<135 mmol/L), hypochloraemia (<96 mmol/L), hypokalaemia (<3.5 mmol/L), high (>30 mmol/L) or low (<22 mmol/L) serum bicarbonate. 5) change in renal function measured by serum creatinine (µmol/L) and estimated glomerular filtration rate (eGFR) (ml/min/1.72m2) 6) change in urinary electrolyte levels after 48 and 96 hours of treatment; sodium, chloride, potassium. 7) clinical assessment of congestion 8) ultrasound measures of congestion period; inferior vena cava (IVC) diameter. 9) change in breathlessness score using the Likert scale. 10) time to clinical euvolaemia and end of intravenous therapy 11) length of stay 12) rate of adverse events related to acetazolamide – treatment arm only 13) rate of adverse events

	14) rate of recruitment to study 15) time taken to screen & recruit 16) rate of drop-out after randomisation – cause specific 17) Cause specific hospitalisation or mortality after 30 days and 180 days follow up.
Investigational Medicinal Product(s)	Acetazolamide
Formulation, Dose, Route of Administration	Tablet. 250mg twice daily. Oral.
Main inclusion/exclusion criteria	<u>Inclusion</u> 1) Aged ≥18 of any gender 2) Able to give informed written consent to participate in the trial 3) All women of child bearing potential (see appendix 3 for definition) must have a negative pregnancy test regardless of contraceptive use. The only form of contraception that will be allowed during the trial is abstinence. 4) Heart failure of any aetiology 5) Admitted to hospital with a primary diagnosis of peripheral oedema caused by heart failure and deemed by treating clinicians to require treatment with intravenous diuretic. 6) Patients are considered eligible as long as they are deemed to require standard of care. 7) Patients whose medications have been discontinued for other reasons ≥48 hours previously may be considered eligible. These medications include; high dose aspirin (≥500mg / day), methotrexate, lithium, Sando-K®, sodium bicarbonate, other sodium tablets, oral steroids, or sodium valproate. <u>Exclusion</u> 1) Unable to give informed written consent 2) Allergy or contraindication to carbonic anhydrase inhibitors, or are taking another medication (other than loop diuretic) that has a diuretic effect such as bendroflumethiazide, metolazone or sodium-glucose linked transporter-2 inhibitors. 3) Patient thought to be at end-of-life 4) Concurrently taking thiazide (or thiazide-like) diuretic or sodium-glucose linked transporter-2 inhibitor

	5) Concurrently taking high dose aspirin (>500mg / day), methotrexate, lithium, or sodium valproate – risk of drug interactions with ACZ 6) Concurrently taking Sando-K®, oral sodium bicarbonate, or other sodium tablets – confounding electrolyte analysis 7) Concurrently taking oral steroids – confounding diuretic analysis 8) Peripheral oedema due to heart failure that has been triggered by an underlying illness such as **severe** anaemia (haemoglobin ≤8 g/DL) or concurrent **severe** infection (requiring intravenous antibiotics). 9) SBP ≤80 mmHg at randomisation 8) Serum sodium (severe hyponatraemia) <130 mmol/L at randomisation 9) Serum potassium (hypokalaemia) <3.5 mmol/L at randomisation 10) Serum chloride (severe hyperchloraemia) >110 mmol/L at randomisation 11) Severe renal dysfunction with an eGFR (estimate glomerular filtration rate) result of ≤30 ml/min calculated by the Cockcroft-Gault formula 12) Pregnant or breast feeding, or intends to become pregnant whilst taking part in the trial
Investigations performed	**As part of routine practice:** Admission and daily blood tests including serum electrolytes, renal function and NTproBNP; chest x-ray; echocardiogram; daily weight; daily urine output; fluid balance (volume in minus volume out). **As part of study:** daily weight; daily urine output; daily fluid balance; daily urine for electrolytes on days 2 and 4; daily clinical assessment of congestion as detailed above; daily ultrasound assessment of congestion as detailed above; daily patient-rated symptom severity using Likert scale; daily diet sheet to record daily salt and fluid intake.
Biological samples to be taken from patients	Urine and serum.
Planned trial sites	Ward 39 at Hull Royal Infirmary, Hull, HU3 2JZ and wards 26 & 28 and Cardiac Monitoring Unit at Castle Hill Hospital, Cottingham, East Yorkshire, HU16 5JQ

LIST OF ABBREVIATIONS

Define all unusual or 'technical' terms related to the trial. Add or delete as appropriate to your trial. Maintain alphabetical order for ease of reference.

ACZ	Acetazolamide
AE	Adverse Event
AHF	Acute heart failure
AR	Adverse Reaction
CA	Competent Authority
CI	Chief Investigator
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
DSUR	Development Safety Update Report
EC	European Commission
EMEA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
GCP	Good Clinical Practice
HF	Heart failure
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of technical requirements for registration of pharmaceuticals for human use.
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File
ISRCTN	International Standard Randomised Controlled Trials Number
MA	Marketing Authorisation
MHRA	Medicines and Healthcare products Regulatory Agency
MS	Member State
NHS R&D	National Health Service Research & Development
NIMP	Non-Investigational Medicinal Product
NTproBNP	N-terminal pro-B-type natriuretic peptide
PI	Principal Investigator
PIS	Participant Information Sheet

QA	Quality Assurance
QC	Quality Control
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
SmPC	Summary of Product Characteristics
SSI	Site Specific Information
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
TMF	Trial Master File
IVC	Inferior Vena Cava

KEY WORDS:

Acetazolamide, Heart Failure, Chloride, Hypochloraemia, Diuretics, In-patient

CONTENTS LIST

ROLES AND RESPONSIBILITIES OF TRIAL MANAGEMENT COMMITEES/GROUPS & INDIVIDUALS

Trial Management Group

Dr Joe Cuthbert – PI

Dr Oliver Brown – SI

Professor Andrew Clark – SI and Supervisor to PI

Jeanne Bulemfu – Research Nurse

Karen Dobbs – Research Nurse

HUTH RnD Staff

Pharmacy

Team meetings will occur on a weekly basis and that these will be recorded in terms of minutes.

1 BACKGROUND

The cornerstone of treatment for congestion in patients with heart failure is loop diuretics such as furosemide or bumetanide,[1] but failure to respond or worsening despite increasing dose of diuretic - diuretic resistance - is common.[2] Current guidelines recommend the addition of a thiazide (or thiazide-like) diuretic (e.g. bendroflumethiazide or metolazone) when a patient has become 'resistant' to loop diuretics.[4] However, only a few small studies have tested the effects of thiazide and thiazide-like diuretics in patients with heart failure with inconsistent results.[3,4,5,6]

Loop and thiazide diuretics prevent sodium reabsorption in the loop of Henle and distal tubule but the majority of renal sodium reabsorption (~70%) occurs in the proximal tubule.[7] Reabsorption of sodium in the proximal tubule depends predominantly on the sodium-hydrogen (Na^+/H^+) antiporter.[8] Carbonic anhydrase catalyses the interconversion between carbon dioxide and water on the one hand, and hydrogen (H^+) and bicarbonate ions on the other. Increased intracellular H^+ concentration promotes sodium reabsorption in the proximal tubule by increased activity of Na^+/H^+ antiporters. [9,10] Thus carbonic anhydrase inhibitors (such as acetazolamide) reduce sodium reabsorption in the proximal tubule and act as a diuretics.

Early studies of acetazolamide in the 1950-60s in patients with heart failure, before the advent of loop diuretics, found that high dose acetazolamide (>1000mg per day) used alone was less effective than standard of care at the time (intramuscular mercurial diuretics).[11,12,13,14,15,16,17,18] However, more recent studies have found that acetazolamide at doses between 250-750mg daily may have both natriuretic and diuretic effects when used in combination with loop diuretics or spironolactone,[19,20,21,22,23] and may have a greater diuretic effect than thiazide diuretics.[18,19]

The recent ADVOR study of 500mg intravenous acetazolamide or placebo alongside intravenous furosemide found that acetazolamide was associated with greater diuresis and recovery from decompensated heart failure.[24] However, the median dose of furosemide given in the study was 120mg per day, which is less than half the mean dose used in the high-dose arm of the DOSE trial,[25] a fraction of what was permitted in the pharmacological treatment arm of the CARESS-HF trial,[26] and half of what might be considered standard of care in the UK. Furthermore, only a third of patients had oedema above the knee. Given the low doses of loop diuretic used and the fact two thirds had only moderate oedema, the majority of patients in the study may not have needed hospitalization at all. How acetazolamide effects diuresis in patients given larger doses of intravenous loop diuretic in patients with severe congestion, the majority of patients admitted to hospital with heart failure. remains unanswered by the ADVOR trial.

An additional benefit of acetazolamide may be increased renal chloride reabsorption and thus increased serum levels during diuresis.[11,27,28] Hypochloraemia is an important marker of a poor prognosis in patients with HF but whether it causes a poor prognosis is unclear.[29,30] There are mechanisms by which hypochloraemia may drive worsening heart failure and diuretic resistance. Prevention or correction of hypochloraemia may be a worthwhile therapeutic goal in patients with HF. The effect of ACZ on chloride homeostasis in patients with HF is untested in a prospective randomised controlled trial (RCT): whether acetazolamide may act as a "chloride-sparing diuretic" is unknown.

2 RATIONALE

Symptoms of venous congestion due to heart failure is the leading cause of admission to hospital for patients aged ≥65 and causes or complicates 5% of all hospital admissions in the UK.[31] In the last 5 years, hospitalisation rates have increased by a third, more so than for any other medical condition.[32] The median length of stay in hospital for patients with heart failure is 9 days and tends to be longer if the patient is under the care of a heart failure specialist,[33] reflecting the time taken to adequately treat the venous congestion – "decongestion".

High dose diuretic prescriptions and long hospital stays are both associated with greater risk of adverse outcome in patients with heart failure compared to patients requiring lower doses or shorter periods of admission.[30,34] Furthermore, failure to respond or worsening symptoms despite increasing dose of diuretic - diuretic resistance - is common.[35]

One possible reason for the association between high dose diuretic and adverse outcome is diuretic-induced electrolyte abnormalities: hyponatraemia and hypokalaemia are well recognised complications of diuretic treatment,[36] and adverse prognostic markers in admitted with heart failure.[37,38] Hypochloraemia (<96 mmol/L) is common in patients with HF and is associated with high dose diuretic treatment and a higher risk of mortality, independent of prognostic markers such as amino-terminal pro-B-type natriuretic peptide (NTproBNP) *and* other electrolytes including sodium and potassium.[39,40,41]

While hyponatraemia and hypokalaemia have been therapeutic targets in some recent trials,[42,43] data on pharmacological manipulation of chloride levels is scarce. Amongst patients admitted with heart failure, those with hypochloraemia that resolves by the time of discharge appear to have a similar post-discharge prognosis as patients with normal chloride levels throughout admission.[44] Conversely, incident hypochloraemia is associated with an increased risk of adverse outcome post-discharge.[44] Some small trials of acetazolamide have reported increased chloride levels alongside increased diuresis in patients admitted with heart failure.[19,25] However, doses of loop diuretic and other medications in these studies was variable and data on daily changes to serum and urinary electrolytes lacking.

Whether acetazolamide has a significant diuretic effect in patients also taking standard treatment in the UK (240mg / 24 hours IV furosemide infusion) is unknown. Furthermore, how it affects renal chloride excretion and whether it can correct hypochloraemia or prevent incident hypochloraemia due to high dose loop diuretic is unknown.

There is a pressing need to clarify whether or not acetazolamide can increase diuresis and / or favourably affect renal chloride excretion. Our pilot and exploratory study will provide data to estimate the effect of acetazolamide on diuresis and serum chloride concentrations during treatment with IVI of furosemide at 10mg/hr (standard of care in the UK for patients admitted with severe venous congestion).

It will also provide data on the possible tolerability of acetazolamide and recruitment rate to a study of acetazolamide in patients hospitalised with heart failure in the UK.

Should the data suggest a favourable effect on either diuresis and / or chloride homeostasis during diuresis then the results of study will be used to design a large multi-centre randomised placebo controlled trial of acetazolamide combined with high dose loop diuretics to prevent or correct hypochloraemia during in-patient diuresis.

2.1 Dosing and study duration

Acetazolamide is currently licenced to treat glaucoma and epilepsy, and is used in the prophylaxis of high altitude pulmonary oedema. The British National Formulary recommends doses between 250-1000mg per day in divided doses. We chose a dose of 250mg twice daily based on dosing in previous contemporary trials which appeared to show some diuretic effect with acetazolamide,[22,25] and the relatively low rate of side-effects reported in much earlier studies with doses <500mg per day.[10-17]

The oral route was chosen as other adjunctive diuretics such as bendroflumethiazide or metolozone are also given orally.[1] A study period of 4 days was chosen to represent approximately half the median duration of in-patient stay in the UK over which time we would expect to see some biochemical and clinical improvements in response to standard of care.

2.2 Assessment and management of risk

The British National Formulary lists the common or very common side effects of acetazolamide to be: ataxia, depression, diarrhoea, dizziness, fatigue, flushing, irritability, headache, loss of appetite, nausea, vomiting, taste disturbance, parasthesiae, reduced libido, polyuria, and thirst. Notable uncommon side effects include: metabolic acidosis, electrolyte disturbances, blood disorders, skin rashes and symptoms consistent with renal calculi.

The early studies suggested that side effects such as drowsiness and paraesthesia are seen in 14-38% of patients with heart failure at doses >1000mg per day, although side-effects were less common at doses ≤500mg per day (5-10%).[10-17] Modern day studies did not report any adverse events but it is not explicitly stated that this is due to the medication being well tolerated.

We judge the above side effects to be of low clinical risk to the patient however it is possible a patient experiencing any one of the those side effects after starting acetazolamide may wish to end their participation. This, in itself, would be a useful endpoint. One of the many unknowns about acetazolamide in contemporaneous studies is the tolerability used either in isolation or in conjunction with high dose loop diuretic.

There are potential risks to the patient from the combination of acetazolamide and high-dose loop diuretic treatment, however we are unable to estimate their expected frequency: 1) increased risk of hyponatraemia – as both medications increase urinary sodium excretion; 2) increased risk of symptomatic hypotension due to increased intravascular volume loss and 3) increased risk of renal dysfunction. Daily assessment of urine and serum electrolytes and the patient will detect any changes that might be associated with increased risk to the patient and these will be discussed with the patient regarding continuation. Again, this would be a useful endpoint in itself regarding tolerability of the combination of diuretics. Furthermore, worsening electrolyte abnormalities, symptomatic hypotension and worsening renal function would be expected in the standard care arm also.

Ultimately we would expect all treatment related adverse events to resolve upon withdrawal of the study treatment should the patient wish to do so or if the risk to the patient was felt too great to allow their continuation in the study.

Aside from the IMP, we anticipate no increased risk to either the patient or study staff by their participation in the study. All data will be recorded on paper CRFs and stored in a locked filing cabinet in an office with a locked door on NHS property accessible only via swipe-card. It will then be transferred to a password-protected Excel spreadsheet on a password protected NHS server behind an NHS firewall.

3 OBJECTIVES AND OUTCOME MEASURES/ENDPOINTS

3.1 Research Question

What are the effects of acetazolamide 250mg BD by mouth (PO) in conjunction with 240mg/24 hours furosemide IVI on diuresis and chloride homeostasis compared to 240mg/24 hours IV furosemide infusion alone (standard of care).

3.2 Primary objective

Primary hypothesis

The addition of 250mg acetazolamide orally twice-daily to 240mg / 24hr IV furosemide infusion will increase diuresis while also reducing the amount by which serum chloride concentrations fall during diuresis in a patient admitted to hospital for venous congestion due to heart failure compared to furosemide infusion alone.

Null hypothesis

The addition of 250mg acetazolamide orally twice-daily to 240mg / 24hr IV furosemide infusion will have no effect on diuresis or serum chloride concentrations in a patient admitted to hospital for venous congestion due to heart failure compared to furosemide infusion alone

3.3 Secondary objectives

Secondary hypothesis

Acetazolamide is well tolerated in patients admitted to hospital with heart failure and neither the time taken to screen and recruit patients and collect data, or the rate of recruitment will be prohibitive to a larger study of acetazolamide in the same patient population.

3.4 Primary endpoint/outcome

Difference in mean net fluid loss daily and over a 4 day period

- Total volume intake in millilitres (mL) – total volume passed as urine in mL

Difference in serum chloride concentrations between day 1 and day 4

- Serum chloride day 4 – serum chloride day 1

3.5 Secondary endpoints/outcomes

All secondary endpoints are exploratory designed to inform future research.

Differences between the two groups during the 4 day treatment period in:

1) daily weight

2) serum electrolyte levels (sodium, bicarbonate and potassium)

3) incidence of electrolyte abnormalities; hyponatraemia (<135 mmol/L), hypochloraemia (<96 mmol/L), hypokalaemia (<3.5 mmol/L), high (>30 mmol/L) or low (<22 mmol/L) serum bicarbonate.

4) change in renal function measured by serum creatinine (µmol/L) and estimated glomerular filtration rate (eGFR) (ml/min/1.72m2)

5) change in urinary electrolyte levels after 48 and 96 hours of treatment; sodium, chloride, potassium.

6) clinical assessment of congestion

7) ultrasound measures of congestion period; inferior vena cava (IVC) diameter

8) change in breathlessness score using the Likert scale.

9) time to clinical euvolaemia and end of intravenous therapy

10) length of stay

11) rate of adverse reactions, serious adverse reactions and suspected unexpected serious adverse reactions – treatment arm

12) rate of adverse events and serious adverse events – both arms

13) rate of recruitment to study

14) time taken to screen & recruit

15) rate of drop-out after randomisation – cause specific

16) Cause specific hospitalisation or mortality after 30 days and 180 days follow up.

4 TRIAL DESIGN

This will be an open label, randomised, controlled pilot and exploratory study which will be powered to detect superiority of the addition of acetazolamide 250mg BD by mouth (PO) in conjunction with 240mg/24 hours furosemide IVI on diuresis and chloride homeostasis compared to 240mg/24 hours IV furosemide infusion alone (standard of care) both primary endpoints with the primary aim of gathering data on the feasibility and tolerability of the intervention which will inform a larger study.

5 STUDY SETTING

The study will be run at a single site: Castle Hill Hospital (CHH), Cottingham, Kingston upon Hull, East Yorkshire. The site has been chosen as it is the base of the primary and chief investigators and is a tertiary cardiology centre treating patients with heart failure from across East Yorkshire and Northern Lincolnshire.

Most patients who will be invited to take part will be admitted first under the care of General Medicine at a different site ~5 miles away – Hull Royal Infirmary (HRI). Upon referral to the cardiology team at HRI patients are assessed for their clinical suitability for transfer to the specialist unit at CHH. Only patients in who the primary reason for admission is venous congestion due to heart failure are transferred to CHH which has three cardiology wards – ward 26, 28 and the cardiac monitoring unit (CMU). A patient who has heart failure due to severe anaemia or chest infection will remain at HRI under the care of the team responsible for treatment the primary problem.

Patients will be identified by daily ward rounds by the study doctors (JJC, OB, SD, AD) on the acute cardiology wards (ward 26, ward 28 and the cardiac monitoring unit at CHH). They will be assessed against inclusion/exclusion criteria and introduced to the nature and purpose of the study. Each eligible participant will be informed of the aims, methods, anticipated benefits and potential hazards of the study and their right to withdraw consent at any stage, using both the patient information sheet (PIS) and verbally. Patients will have the opportunity to ask any and all questions before giving written consent. Confirmation of the approach for study participation, outcome (participation or not) and confirmation of written consent will be documented in the medical notes by the study doctor.

6 ELIGIBILITY CRITERIA

Inclusion criteria

1) Aged >18 of any gender and able to give informed consent (All women of child bearing potential (see appendix 3 for definition) must have a negative pregnancy test regardless of contraceptive use. The only form of contraception that will be allowed during the trial is abstinence.)
2) Heart failure of any aetiology (new or established diagnosis)
3) Admitted to hospital with a primary diagnosis of peripheral oedema caused by heart failure and deemed by treating clinicians to require treatment with intravenous diuretic.
4) Deemed to require standard of care – 10mg/hr continuous furosemide infusion.
5) Patients whose medications have been discontinued for other reasons ≥48 hours previously may be considered eligible. These medications include; sodium glucose co-transporter 2 inhibitors, thiazide or thiazide-like diuretics, high dose aspirin (≥500mg / day), methotrexate, lithium, Sando-K®, sodium bicarbonate, other sodium tablets, oral steroids, or sodium valproate.
Exclusion criteria
1) Unable to give informed written consent
2) Allergy or contraindication to carbonic anhydrase inhibitors
3) Patient thought to be at end-of-life
4) Concurrently taking thiazide (or thiazide-like) diuretic or sodium-glucose linked transporter-2 inhibitor, high dose aspirin (>500mg / day), methotrexate, lithium, or sodium valproate, oral steroids Sando-K®, oral sodium bicarbonate, or other sodium tablets
5) Severe anaemia (haemoglobin ≤8 g/DL); or severe infection (investigator opinion).
6) SBP ≤80 mmHg at randomisation
7) Serum sodium (severe hyponatraemia) <130 mmol/L at randomisation
8) Serum potassium (hypokalaemia) <3.5 mmol/L at randomisation
9) Serum chloride (severe hyperchloraemia) >110 mmol/L at randomisation
10) Severe renal dysfunction with an eGFR (estimate glomerular filtration rate) result of ≤20 ml/min calculated by the Cockcroft-Gault formula
12) Pregnant or breast feeding, or intends to become pregnant whilst taking part in the trial

7 TRIAL PROCEDURES

7.1 Recruitment

Patients will be identified as detailed below on cardiology wards at CHH and Hull Royal Infirmary (HRI) and screened for eligibility with consent by the study team. Data on patients age, gender, and eligibility status will be recorded anonymously to help define the generalisability of the results. We estimate the recruitment period will be for 30 weeks but can be extended to 52 weeks. We aim to recruit a minimum of 40 patients with a maximum of 50 during that time frame.

7.1.1 Patient identification

Potentially eligible patients with HF will be identified by the treating team who will inform the study team. The study team will then approach each patient for consent to screen the medical notes

(electronic and paper) for inclusion / exclusion criteria, the approach and the outcome will be documented in the medical notes. If consent is given the study doctor will use all electronic and paper medical records alongside taking a detailed medical history from the patient to decipher whether or not a patient is eligible.

Identification of potentially eligible patients will be done by the treating team but confirmation of eligibility will be done by the study team in order to streamline the process from identification to consent and enrollment. Given the busy nature of clinical work we feel it would be too great a burden to place on the treating team that they assess each patient against all eligibility criteria and recruitment may suffer as a result.

7.1.2 Screening

Screening will involve detailed history from the patient alongside reviewing all electronic and medical records. All data required for screening is collected as part of routine care.

Patients who fail initial screening may subsequently become eligible for inclusion. A patient who fails initial screening will be informed of this and verbal consent obtained for re-screening at 48 hour intervals (done remotely using the electronic record), and for the treating team to inform the study team should the reason for the screen failure resolve (IE low systolic blood pressure). There is a maximum limit of three re-screens per patient however; this will be limited by the duration of in-patient stay.

7.2 Informed Consent

After the confirmation of eligibility and reconfirmation that the patient wishes to proceed, each potential participant will be informed of the aims, methods, nature of participation, anticipated benefits and potential hazards of the study, the alternatives to taking part and their right to withdraw consent at any stage without giving a reason and without prejudicing further treatment. The person taking consent will assume each participant to have capacity to give consent unless concerns are raised by the treating team or during the screening process (e.g. history of dementia or confusion on admission, nursing or medical concerns regarding cognition).

If concerns are raised, each participant will be assessed against the 2 stage capacity assessment defined by the Mental Capacity Act: 1) Is the patient able to make a particular decision? and 2) Is the person able to understand, retain and use information given to come to a decision and are they able to communicate this decision? If a person is deemed unable to give valid consent they are ineligible for the study.

The information will be provided to patient verbally and via the patient information sheet (PIS) which will be reviewed and approved by the Regional Ethics Committee (REC) and in accordance with GCP. Contact details for the study team will be provided. Patients will have the opportunity to ask any and all questions during and after the discussion before giving written consent.

The Chief Investigator (CI) will be jointly responsible for obtaining informed consent of all participants according to the approved protocol, principles of Good Clinical Practice and Declaration of Helsinki.

No trial procedure will be undertaken before informed consent is given. Data from procedures performed as part of routine care will not be collected before informed consent is given.

Should a patient lose capacity during the study attempts will be made to replace the patient in the recruitment total but data will be retained. In such an event, the results of symptom questionnaires and diet sheets will be recorded but not used in the final analysis.

Should a patient require re-consenting due to a significant alteration to the trial protocol or new information regarding the study, the IMP or the condition become available it is the joint responsibility of the PI and CI to inform the participants in a timely manner.

The geographical location of the study site is such that most participants are likely to be elderly, frail and English speaking. The PI and CI take joint responsibility for ensuring the safety of such vulnerable patients and ensuring that they give valid consent free from coercion. Patients with cognitive impairment are eligible as long as they are deemed to be able to give valid consent. Patients who are unable to read the provided PIS for whatever reason can invite a family member or member of the study team to read it to them. If a participant is unable to sign the consent form this may be done by a witness who is not a member of the study team. If a patient is able to produce a legible signature but unable to complete the date the consent form may be dated by a member of the study team.

There will be no PIS or consent forms *printed* in foreign languages as the proportion of patients who require a non-English PIS will be low: 94% of people resident in Yorkshire and the Humber region are English speaking – this will represent 38 of our planned recruitment of 40 patients, however may be more if the recruitment target is extended.

7.3 The randomisation scheme

Randomisation will be simple randomisation via a web-based system.

7.3.1 Method of implementing the allocation sequence

Randomisation will be performed by the Simple Randomisation Service offered by Sealed Envelope ™ (https://www.sealedenvelope.com). Patients will be randomised 1:1 to either A – acetazolamide or B – standard of care in a random permuted block of up to 50 patients (maximum recruitment). Randomisation will be accessed by the study doctors and the sponsors but randomisation will be performed by the study doctors at the point of consent. Sealed Envelope confirms allocation via email to the person performing the randomisation, a copy of which will also be sent to a member of the Research and Development team who will print the email and include in the CRF. The CI (JJC) can be contacted in an emergency via phone.

7.4 Blinding

The trial will be an open label investigation without blinding for two reasons 1) we aim to collect pilot data to inform a larger blinded study 2) the primary endpoints and most secondary endpoints will not be susceptible to bias from unblinded treatment such as urine volumes and serum and urine electrolytes.

7.5 Unblinding

No unblinding required.

7.6 Baseline data

- Medical history including age (years), sex, co-morbidities, and aetiology of heart failure
- Bedside observations including body weight, systolic and diastolic blood pressure, heart rate and rhythm.
- Patient symptoms including: investigator assessed New York Heart Association class, peripheral oedema severity (none = 0, trace = 1, ankles = 2, below knees = 3, above knees = 4, sacral = 5), pulmonary congestion severity (lung crackles) (absent = 0, bibasal = 1, midzone = 2, whole lung = 3), jugular venous pressure severity (not visible = 0, 1-4cm above the sternal notch = 1, >4cm above the sternal notch = 2, up to the earlobes = 3), patient assessed symptoms using the Likert breathlessness scale (patients will be asked to rate their breathlessness severity on a 5-point scale of: 1=not short of breath, 2=mildly short of breath, 3=moderately short of breath, 4=severely short of breath, 5=very severely short of breath)

- Echocardiographic data including: left ventricular ejection fraction either calculated by Simpson's biplane method or qualitatively on transthoracic echocardiogram performed either as routine practice or by the investigator, presence of moderate or worse valve disease, inferior vena cava diameter
- Biochemical data including: NTproBNP, haemoglobin (g/dL), haematocrit (fraction), sodium (mmol/L), chloride (mmol/L), potassium (mmol/L), bicarbonate (mmol/L), creatinine (µmol/L), urea (mmol/L).
- Data on pre-admission medical and device therapy including: angiotensin converting enzyme inhibitor, angiotensin receptor blocker, sacubitril valsartan, beta-blocker, mineralocorticoid receptor antagonist, oral loop diuretic dose, presence of cardiac resynchronisation therapy device, presence of implantable cardioverter defibrillator, cumulative dose of in-patient loop diuretic (mg / day)

7.7 Schedule of Events

Procedures	On Admission	Visits							
		Visit 1 Screening	**Visit 2** Baseline (Day 1 – 0hrs)	**Visit 3** Day 2 – 24hrs	**Visit 4** Day 3 - 48hrs	**Visit 5** Day 4 – 72hrs	**Visit 6** Day 5 – 96hrs	**Visit 7** 30 Day Follow Up	**Visit 8** 180 Day Follow Up
Informed consent		X							
Demographics		X							
Medical history	X (RC)	X							
Physical examination	X (RC)	X	X	X	X	X	X		
Vital signs									
Height			X						
Weight*			X	X	X	X	X		
BMI			X						
BP			X	X	X	X			
HR			X	X	X	X			
Concomitant medications		X							
Ward round assessments by treating teams **	X	X	X	X	X	X	X		
ECG	X (RC)	X							
Chest X-ray	X (RC)								
Laboratory tests									
FBC	X (RC)		X	X	X	X			
Urea & Electrolytes	X (RC)		X	X	X	X			

NTproBNP	X (RC)		X (if not performed on admission)						
Eligibility assessment		X							
Randomisation		X							
Intravenous Furosemide infusion at 10mg/hr (standard of care)***	X		X	X****	X****	X			
Dispensing of trial drugs (Acetazolamide arm only)		X							
Acetazolamide given to the patient			X	X	X	X			
Compliance with medication and diet sheet				X (previous day)	X (previous day)	X (previous day)	X (previous day)		
Oral intake – mL				X (previous day)	X (previous day)	X (previous day)	X (previous day)		
Urine output		X	X	X (previous day)	X (previous day)	X (previous day)	X (previous day)		
Urine electrolytes					X (previous day)		X (previous day)		
Likert breathlessness scale			X	X	X	X	X		
Diet Sheet		X (x4 given to the patient)	X	X (previous day)	X (previous day)	X (previous day)	X (previous day)		
Catheter sited (to measure urine output / jug used)		X							
Clinical assessment of congestion			X	X	X	X	X		
Ultrasound assessment of congestion			X				X		
Adverse event / reaction assessments				X	X	X	X		

Time to clinical euvolaemia*****				X	X	X	X	X	
Length of stay								X	
Cause specific re-hospitalisation								X	X
Cause specific mortality								X	X

RC – Routine care

* Only weight assessment conducted daily

** Includes assessment of vital signs, ongoing medical history, clinical examination, treatment plans, imaging procedures, involvement of allied healthcare professionals such as physiotherapist.

*** Patients may not immediately be started on the 'gold standard' treatment does (240mg/24hr infusion) but maybe changed on an infusion after consultant or specialist review

**** If the patient is deemed to not require standard of care by day 2 and has their diuretic treatment de-escalated efforts will be made to replace the patient

***** Defined as the absence of venous congestion or transition to oral diuretic therapy assessed by either the treating team or study team and documented in medical notes

7.8 Trial Visits

On admission

- Chest X-ray, blood tests (full blood count, biochemistry profile, NTproBNP), electrocardiogram, medical history and clinical examination – all part of routine care
- Started on intravenous furosemide (standard care). Patients may not immediately be started on the "gold standard" treatment dose (240mg/24hr infusion) but may be changed onto an infusion after consultant or specialist review.

Routine care consists of;

- Ward round assessments by treating teams
- Biochemical profile at least every 48 hours
- Echocardiography
- Daily assessment of weight and urine output
- Ongoing clinical input from treating teams depending on patient requirements.

Visit 1 - Screening

- Clinical examination and medical history to assess eligibility
- Informed Consent
- Demographics
- Randomisation
- Routine care as detailed above

Plus:

In the acetazolamide arm only:

- Acetazolamide will be prescribed on electronic drug card (ePMA) and dispensed by pharmacy clinical trials on a non-stock request for the patient and taken to drug cupboard store on ward by study doctor (250mg BD for 4 days taken in the morning and again 6 hours later - 8x 250mg tablets – 2x per day for 4 days per patient).

In both treatment arms:

- Diet sheet x4 given
- Catheter sited if patient consents and one is not already as part of routine care. If catheter not being used to measure urine output then a urine jug will be used

Visit 2 - Day 1 – 0 hours

Visit 2 can occur on the same day as screening if randomisation occurs before 12PM and the first dose of acetazolamide is given before 12PM (ACZ arm only).

- Furosemide infusion to continue
- Vital signs measured by nursing staff
- Bloods taken
  - Urea and electrolytes
  - Full blood count
- Acetazolamide 250mg to be given by ward staff in the morning and again 6 hours later as prescribed on the electronic prescription (in ACZ group only)
- Ongoing input from treating team: ward round and any input that stems from that, regular observations from nursing staff, input from other health professionals if indicated (for example dieticians, physiotherapists, occupational therapists)
- Study doctor visit at +/- 2 hours from first dose of acetazolamide / randomisation (SoC arm)
  - Clinical examination

- NTproBNP added to blood tests taken in the morning if not performed on admission
- Weight measured using the same set of scales
- Ultrasound assessment of congestion
  - Inferior vena cava diameter
- Patient assessed breathlessness using 5-point Likert breathlessness scale
- Diet sheet to be completed at 1800 (after evening meal)
- Patient provided with 24hr urine electrolyte bottle if not catheterised and instructed to begin collection at 24hrs from the first dose of acetazolamide the following day
  - Nursing staff to be informed

Visit 3 - Day 2 – 24 hours

- Furosemide infusion to continue if clinically indicated
  - If patient deemed to not require standard of care by day 2 and has their diuretic treatment de-escalated efforts will be made to replace patient. All data collected will be retained but patient will have "completed" the study early.
- Vital signs measured by nursing staff
- Bloods taken
  - Urea and electrolytes
  - Full blood count
- Acetazolamide 250mg to be given by ward staff in the morning (24 hours after first dose) and 6 hours later (in ACZ group only) as per electronic prescription
- Ongoing input from treating team: ward round and any input that stems from that, regular observations from nursing staff, input from other health professionals if indicated (for example dieticians, physiotherapists, occupational therapists)
- Study doctor visit (24 hours +/- 2 hours from first dose of ACZ (ACZ arm) or randomisation (SoC arm)
  - Clinical examination and medical history
  - Diet sheet collected from previous day
  - Fluid balance and urine output
  - 24 hour urine collection to begin 24 hours from first dose of ACZ or randomisation
    - Patient asked to empty bladder or nursing staff to empty catheter bag and then begin urine collection
  - Patient assessed breathlessness using 5-point Likert breathlessness scale

Visit 4 - Day 3 – 48 hours

- Furosemide infusion to continue if clinically indicated
  - If patient deemed to not require standard of care by day 3 and has their diuretic treatment de-escalated efforts will be made to replace patient. All data collected will be retained but patient will be deemed to have completed the study and further study-related investigations not carried out.
- Body weight, blood pressure and heart rate measured by nursing staff
- Bloods taken
  - Urea and electrolytes
  - Full blood count
- Acetazolamide 250mg to be given by ward staff in the morning (48 hours after first dose) and 6 hours later (in ACZ group only) as per electronic prescription
- Ongoing input from treating team: ward round and any input that stems from that, regular observations from nursing staff, input from other health professionals if indicated (for example dieticians, physiotherapists, occupational therapists)
- Study doctor visit (48 hours +/- 2 hours from first dose of ACZ (ACZ arm) or randomisation (SoC arm)

- Clinical examination and medical history
- Diet sheet collected from previous day
- Fluid balance and urine output
- Patient assessed breathlessness using 5-point Likert breathlessness scale
- Urine collection to stop at 48 hours after first dose of ACZ or randomisation
- Patient provided with 24hr urine electrolyte bottle if not catheterised and instructed to begin collection at 72hrs from the first dose of acetazolamide the following day
  - Nursing staff to be informed

Visit 5 - Day 4 – 72 hours

- Furosemide infusion to continue if clinically indicated
  - If patient deemed to not require standard of care by day 4 and has their diuretic treatment de-escalated the patient will be deemed to have completed the study and further study-related investigations not carried out.
- Body weight, blood pressure and heart rate measured by nursing staff
- Bloods taken
  - Urea and electrolytes
  - Full blood count
- Acetazolamide 250mg to be given by ward staff in the morning (72 hours after first dose) and 6 hours later (in ACZ group only) as per electronic prescription
- Ongoing input from treating team: ward round and any input that stems from that, regular observations from nursing staff, input from other health professionals if indicated (for example dieticians, physiotherapists, occupational therapists)
- Study doctor visit (72 hours +/- 2 hours from first dose of ACZ (ACZ arm) or randomisation (SoC arm)
  - Clinical examination and medical history
  - Diet sheet collected from previous day
  - Fluid balance and urine output
  - 24 hour urine collection to begin 72 hours from first dose of ACZ or randomisation
    - Patient asked to empty bladder or nursing staff to empty catheter bag and then begin urine collection
  - Patient assessed breathlessness using 5-point Likert breathlessness scale

Visit 6 - Day 5 – 96 hours

- Ongoing input from treating team: ward round and any input that stems from that, regular observations from nursing staff, input from other health professionals if indicated (for example dieticians, physiotherapists, occupational therapists)
- Study doctor visit (96 hours +/- 2 hours from first dose of ACZ (ACZ arm) or randomisation (SoC arm)
  - Clinical examination and medical history
  - Diet sheet collected from previous day
  - Fluid balance and urine output
  - Patient assessed breathlessness using 5-point Likert breathlessness scale
  - Urine collection to stop at 96 hours after first dose of ACZ or randomisation
  - Ultrasound assessment of congestion
    - IVC diameter

Visit 7 - 30 day Telephone Follow Up (from point of randomisation)

- Discuss any concerns or queries regarding the trial and treatment
- Invite participants to any and all presentations of the data at local or national events
- Outcome data from electronic records (from point of baseline visit)

- Any hospitalisations or death

Visit 8 - 180 day Follow up (from point of baseline visit)

- Outcome data from electronic records
- Any hospitalisations or death

7.9 Withdrawal criteria

Patients can be withdrawn from the trial if:

- They withdraw their consent at any point during the study **for any reason**. This can happen at any time after consent and during follow up. In such an event no additional data will be collected from the patient and there will be no follow up. The study doctor will ascertain whether the patient wishes the withdrawal of consent to apply retrospectively to the withdrawal (i.e. can we use data already collected?). The outcome of any and all discussions regarding the withdrawal of consent will be documented in the medical notes and case report form.
- A patient's condition deteriorates due to heart failure or another pathology to the extent that they are expected to die within the next 24 to 48 hours.
- The study doctors deems the risk of continuation due to serious drug reactions or adverse events is too great to the patient. This will be fully discussed with the patient and the content and outcome of all discussions will be documented in the medical notes and CRF.
    - Worsening symptoms of heart failure*
    - Hyponatraemia (<135 mmol/L)
        - Asymptomatic*
        - Severe (<130 mmol/L)*
        - Symptomatic (confusion, drowsiness, nausea or vomiting)**
    - Hypotension
        - Asymptomatic*
        - Symptomatic with no sequelae*
        - Symptomatic with significant sequelae i.e. fall due to postural hypotension**
    - Hyperchloraemia
        - Mild 107 – 110 mmol/L*
        - Severe >110mmol/L**
    - Worsening renal function
        - >30% increase in serum creatinine from admission. *
        - >50% increase in serum creatinine from admission.*
        - >100% increase in serum creatinine from admission.**
    - Side effects of acetazolamide: haemorrhage, ataxia, depression, diarrhoea, dizziness, fatigue, flushing, irritability, headache, loss of appetite, nausea, vomiting, taste disturbance, paraesthesia,
        - Tolerable to the patient.*
        - Intolerable to the patient.**

* - Clinically relevant safety endpoint that would not automatically lead to the discontinuation of the study drug. Withdrawal would be at patient's request or the study doctor's discretion if the risk of continuation was deemed too high.
** - Occurrence would lead to withdrawal from the study if it occurred in the acetazolamide arm

We will attempt to replace patients that withdraw from the trial prior to completing day 4 assessments. The reason(s) for withdrawal will be carefully monitored for trends in patient opinions of participation in the study, adverse drug reactions or adverse events.

7.10 Storage and analysis of samples

All blood and urine samples will be collected as per protocols developed by the Pathology Laboratories at Hull University Teaching Hospitals Trust, tested in the Castle Hill Hospital or Hull Royal Infirmary laboratories and destroyed thereafter in accordance with local protocols.

As all serum sampling for the trial will be done as part of routine care, venepuncture can be performed by any member of the medical, nursing or study team as long as they are trained and competent to do so. The volume of blood and type of tubes used for the full blood count, biochemical profile and NTproBNP will be as per local protocols. All blood tests will be sent to the lab and processed there the same day as per local protocols. Results will be published on the patient's electronic record when available and data entered into the CRF from there. The 24 hour urine collections that lie outside of the tests performed as part of routine care will be collected by nursing staff and/or the study doctor on the wards the samples will be sent to the local laboratory the same day as per local protocol.

Destruction of all samples will occur after analysis as per local protocols whether collected as part of routine care (blood samples) or part of the study (24 hour urine samples). Destruction of the study specific urine samples will be recorded by the local laboratory.

It is the responsibility of the Principle Investigator and Research Team to ensure that samples are appropriately labelled in accordance with the trial procedures to comply with the 1998 Data Protection Act. Biological samples collected from participants as part of this trial will be transported, stored, accessed and processed in accordance with national legislation relating to the use and storage of human tissue for research purposes and such activities shall at least meet the requirements as set out in the 2004 Human Tissue Act and the 2006 Human Tissue (Scotland) Act.

7.11 End of trial

An end of trial declaration form will be submitted to the MHRA, REC and HUTH R&D **within 90 days** from completion of the trial and **within 15 days** if the trial is discontinued prematurely. A summary of the trial final report/publication will be submitted to the MHRA, REC and HUTH R&D **within 1 year** of the end of trial. HUTH R&D will be notified immediately of any reason to halt the trial. The Principal investigator and HUTH R&D as sponsor will decide if the trial should be halted temporarily. The MHRA, REC and HUTH R&D will be notified **within 15 days** of a decision to temporarily halt the trial by submitting a substantial amendment notification.

8 TRIAL MEDICATION

Acetazolamide – 250mg tablets, immediate release.

Medicinal product	Route of administration	Dose	Regulatory status	Storage
Acetazolamide	By mouth (oral)	250mg	Approved for treatment of: Glaucoma: Abnormal retention of fluids. Epilepsy	Manufacturer advises to do not store above 25°C.

Furosemide – 10mg / ml, solution for injection

Medicinal product	Route of administration	Dose	Regulatory status	Storage
Furosemide	Intravenous	10mg/ml	Approved for treatment of: Oedema and/or ascites caused by cardiac or hepatic diseases Oedema caused by renal diseases Pulmonary oedema Hypertensive crisis	Do not store above 25°C. Do not refrigerate. Keep the ampoules in the outer carton in order to protect from light.

8.1 Missed Doses

If dose is missed, the missed dose should be taken as soon as remembered. However, if this is within two hours of the next dose then skip the missed tablet and carry on taking the rest of the tablets as usual. Do not take a double dose to make up for a forgotten dose. Following this patients can then resume their regular dosing schedule. If intravenous access is lost at any time during the study period the patient will not have been receiving the active comparator (IV furosemide). We will record the amount of time without IV furosemide in the CRF and if a patient has >6 hours without receiving IV furosemide we will attempt to replace the patient data.

8.2 Summary of Product Characteristics (SmPC)

The Electronic Medicines Compendium has an SmPC for acetazolamide and furosemide solution for infusion which have been used to write the protocol.

8.3 Drug storage and supply

The trial drug and active comparator will be available from the pharmacy at CHH from normal hospital stock. All acetazolamide tablets for the entire study will be dispensed on the day of randomisation from the pharmacy upon receipt of a suitably signed trial specific electronic prescription. The medications will be stored in labelled boxes securely on the ward and made available to the patient at during the morning and afternoon drug rounds by the nursing staff on the ward. The study doctor visit will be around the time of the first dose to monitor adherence and address any concerns. Any tablets that are not taken for whatever reason will be returned to the hospital pharmacy for destruction. There will be no arrangements for post-trial access to acetazolamide. The active comparator will be prescribed by the patient's clinical team and given by nursing staff on the ward from ward stock.

8.4 Preparation and labelling of Investigational Medicinal Product (IMP)

Eight acetazolamide 250mg tablets will be dispensed into a suitable container and labelled according to Annexe 13.

8.5 Dosage schedules

The acetazolamide will be taken orally as it has good oral bioavailability (70-90%) reaching peak concentrations after 2 hours with a plasma half life of 4 hours. All other adjunctive diuretics are routinely given orally in the UK – e.g. Bendroflumethiazide, metolazone, dapagliflozin, empagliflozin.

ACZ will be given in the morning and again 6 hours later so that the maximal diuretic effect occurs in the daytime to reduce the chance of the patient waking in the night. The maximum daily dose is 500mg in maximum split doses of 250mg. There will be no individualised dosing. Duration of treatment is 4 days. Furosemide infusions will be continuous as long as is clinically indicated and replenished as and when required.

8.6 Dosage modifications

There are no planned dose modifications.

8.7 Known drug reactions and interaction with other therapies

Acetazolamide is currently licenced to treat glaucoma and epilepsy, and is used in the prophylaxis of high altitude pulmonary oedema.

Side Effects of Acetazolamide Taken from The British National Formulary

Common or Very Common	Uncommon	Rare or Very Rare	Unknown frequency
Haemorrhage	Bone marrow disorders	Anaphylaxis	Agranulocytosis
Metabolic acidosis	Depression	Loss of appetite	Drowsiness
Paraesthesia	Dizziness	Confusion / Irritability	Myopia
Nephrolithiasis	Electrolyte disturbance	Fatigue	Polyuria
	Hepatic disorders	Diarrhoea	Suicidal behaviour
	Hearing impairment / tinnitus	Fever / Flushing	Altered taste
	Leucopoenia	Headache	Thirst
	Nausea / Vomiting	Decreased libido	Agitation
	Symptoms of nephrolithiasis	Paralysis	
	Rash	Seizure	
	Thrombocytopenia	Ataxia	
	Osteomalacia	Hyperglycaemia	
		Hypoglycaemia	
		Renal tubular necrosis	

The early studies suggested that side effects such as drowsiness and paraesthesia are seen in 14-38% of patients with heart failure at doses >1000mg per day, although side-effects were less common at doses ≤500mg per day (5-10%).[10-17] Modern day studies did not report any adverse events but it is not explicitly stated that this is due to the medication being well tolerated.

Side Effects of Furosemide Taken from The British National Formulary

Common or Very Common	Uncommon	Rare or Very Rare	Unknown frequency
Dizziness;	Diarrhoea	Bone marrow depression	Deafness
Electrolyte imbalance		Photosensitivity reaction	Leucopoenia
Fatigue			Paraesthesia
Headache;			Rash
metabolic alkalosis;			Thrombocytopenia
Muscle spasm			Tinnitus
Nausea			Vomiting

8.8 Concomitant medication

Due to possible drug interactions with acetazolamide patients taking high dose aspirin (>500mg / day), methotrexate, lithium, or sodium valproate will be ineligible for the study unless these medications have been discontinued >48 hours previously patients concurrently taking these medications will be excluded. Patients taking other drugs that have a notable diuretic effect thiazide and thiazide-like diuretics, and sodium-glucose co-transporter 2 inhibitors (SGLT2I) will be excluded.

Loop diuretics are commonly given to patients with heart failure, as a general rule there are no concomitant medications that would restrict the use of intravenous furosemide in patients who have severe oedema.

8.9 Trial restrictions

There are no dietary requirements or restrictions however patients will be required to complete diet sheets detailing all oral intake during the study. Starting sodium-glucose co-transporter 2 inhibitors or thiazide and thiazide-like diuretics will be contraindicated during the 4 days of treatment with ACZ unless the patient is deemed euvolaemic and ready for discharge in which case it may be clinically appropriate to start an SGLT2I for morbidity and mortality benefit if the patient has left ventricular systolic dysfunction – this decision would be taken by the treating team and not the study team.

Patients with COVID-19 infection which is **not** thought to be contributing to their symptoms – incidental diagnosis – are still eligible for the trial. Similarly, if a patient becomes COVID-19 positive during the study they can continue.

8.10 Assessment of compliance

The study will rely on patient reported compliance however, pill taking during drug rounds in hospitals is done under nurse supervision and any concerns regarding non-adherence can be reported to the study doctors. All prescriptions and taking of medicines is recorded in the electronic health record.

8.11 Non-Investigational Medicinal Product (NIMP)

None

9 PHARMACOVIGILANCE

9.1 Definitions

Term	Definition
Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • results in death • is life-threatening • requires inpatient hospitalisation or prolongation of existing hospitalisation • results in persistent or significant disability/incapacity • consists of a congenital anomaly or birth defect Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant 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.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the summary of product characteristics MHRA approved (SmPC) for that product • in the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question

9.2 Operational definitions for (S)AEs

Due to the pilot and exploratory nature of this study **all** adverse events and adverse reactions will be recorded. All serious adverse events serious adverse reactions, and suspected unexpected serious adverse reactions will be reported.. This will enable collection on safety data for the use of ACZ concurrently with high dose loop diuretics as well as data on the tolerability of treatment for a future trial.

Adverse Events	Asymptomatic hypotension (systolic blood pressure (SBP) <90 mmHg) Symptomatic hypotension (SBP <90 mmHg **and** symptoms of: dizziness or presyncope) Asymptomatic hyponatraemia (serum sodium concentrations <130 mmol/L) Symptomatic hyponatraemia (serum sodium concentrations <130 mmol/L **and** symptoms of fatigue, nausea, vomiting, or headache not promoting further treatment or investigation)† Flare of gout requiring oral treatment and analgesia Decrease in estimated glomerular filtration rate >50% of baseline value † Treatment of headache with analgesia not included in this definition however treatment of nausea or vomiting with antiemetic is.
Serious Adverse Events	Symptomatic hypotension with sequelae (SBP <90 mmHg **and** symptoms of: dizziness or presyncope **and** fall or injury as a direct result) Symptomatic hyponatraemia with sequelae (serum sodium concentrations <130 mmol/L **and** symptoms of fatigue, nausea, vomiting, headache which prompt further treatment or investigation,† or confusion or seizure deemed secondary to hyponatraemia). Decrease in eGFR >100% of baseline value or serum creatinine >221µmol/L Deterioration in condition during trial defined as: - Deemed to need escalating doses of loop diuretic or the addition of a second diuretic agent such as thiazide diuretic. - Deemed to need inotropic support - New diagnosis of infection - New diagnosis of another concurrent illness that will prolong hospital stay - Transition to end-of-life care - Death Overdose of ACZ or furosemide infusion defined as 1) >500mg given per day of ACZ or 2) >240mg IV furosemide per day.
Adverse Reactions	Metabolic acidosis bicarbonate <23 mmol/L Hyperchloraemia 107-110 mmol/L

	Any of the following possible side effects of acetazolamide that are tolerable to the patient: haemorrhage, ataxia, depression, diarrhoea, dizziness, fatigue, flushing, irritability, headache, loss of appetite, nausea, vomiting, taste disturbance, paraesthesia.
Serious Adverse Reactions	Severe hyperchloraemia 107-110 mmol/L Severe metabolic acidosis bicarbonate <20 mmol/L Any of the possible side effects of acetazolamide listed in the MHRA approved SPC that are **intolerable** to the patient and lead to discontinuation of the study drug, and / or lead to the further investigations.
Suspected Unexpected Serious Adverse Reactions	Any SAR that is not expected based on the MHRA SPC for acetazolamide in relation to the nature and severity of the SAR will be reported as SUSAR. Due to the lack of data on the safety and tolerability of acetazolamide in modern patients with heart failure treated with high dose loop diuretic we will consider any SAR that occurs in >5% of patients as a SUSAR..

9.3 Reporting Process

All reportable adverse events (serious and non-serious) will be recorded on the Adverse Event Form at the back of the Case Report Form (CRF). All AEs will be followed-up by research staff until the event has resolved or a decision has been taken for no further follow-up. If a clinically significant abnormal laboratory value occurs, this abnormality will be recorded as an adverse event/reaction.

9.4 Reporting of SAEs AND SUSARs

All SAEs, SARS and SUSARs (except those defined as not requiring expedited reporting) will be reported to the Sponsor using the study specific SAE report form, within 24 hours of the research staff becoming aware of the event.

For each reported SAE the following information will be collected:

- full details in medical terms and case description
- event duration (start and end dates, if applicable, and if admitted, admission and discharge dates, with specialty noted)
- action taken
- outcome
- seriousness criteria
  - causality (i.e. relatedness to trial drug / investigation), in the opinion of the investigator
- whether the event would be considered expected or unexpected.

Assessment of severity, seriousness and causality will be made by the PI or another authorised study doctor. The PI or authorised study doctor will need to decide whether the serious event is an SAE or SAR by assessing whether the event is either unrelated or possibly related to the IMP (causality). If the event is possibly related then it is a SAR and an assessment needs to be made by the Sponsor as to whether the event is expected or not for the IMP. If the SAR is listed in Section 4.8 of the MHRA approved Summary of Product Characteristics then it is expected.

In addition to the PI, the Sponsor will also be required to assess causality and document their assessment on the SAE form. The Sponsor must assess causality after the PI and must confirm that they

have not influenced the PI. The PI's assessment of causality must not be downgraded by the Sponsor.

If an authorised study doctor is unavailable, initial reports without the causality assessment will be submitted to the Sponsor by a healthcare professional within 24hours of becoming aware of the SAE, but will be followed-up by medical assessment as soon as possible thereafter. The PI must always review the SAE form and sign to confirm the contents of the report are accurate and complete and that he/she has also assessed the severity, seriousness and causality of the SAE.

Any change of condition or other follow-up information should be reported to the Sponsor using the study specific SAE follow-up form, as soon as it becomes available. Events will be followed up until the event has resolved or a final outcome has been reached. The PI is required to assess causality again on the follow-up form. If the PI has a change of opinion on causality after considering the additional follow-up information, the Sponsor will be required to reassess causality and if a SAR, then to assess expectedness.

All SAEs assigned by the PI or authorised study doctor as possibly related to IMP-treatment and those the Sponsor has assessed as unexpected will be classified as SUSARs and will be subject to expedited reporting to the MHRA. The Sponsor will inform the MHRA and the REC of SUSARs within the required expedited reporting timescales.

Additionally, due to the lack of data on the safety and tolerability of acetazolamide in modern patients with heart failure treated with high dose loop diuretic we will consider any SAR that occurs in >5% of patients, and any AR that occurs in >10% of patients as a SUSAR.

9.5 Reporting Period

Study doctors will assess for adverse events or reactions at each visit and can be alerted by email or telephone by the ward staff, the patient will be assessed as soon as is practical and within 12 hours of the alert. The reporting period for AE and SAEs starts from the date and time of consent, and the reporting period for AR, SAR, and SUSARs starts from the first IMP dose regardless of treatment allocation (ACZ or SoC). The monitoring period for events will be from 5 days from baseline but data on all adverse events will be collected up to the point of discharge from the written and electronic medical record.

9.6 Responsibilities

The responsibilities regarding AE and AR reporting as follows:

Principal Investigator (PI):
1. Checking for AEs and ARs when participants undergo retreatment and at follow-up.
2. Using medical judgement in assigning severity, seriousness and causality.
3. Ensuring that all SAEs and SARs are recorded and reported in line with the requirements of the protocol and SUSARs are recorded and reported to the Sponsor within 24 hours of becoming aware of the event and provide further follow-up information as soon as available.
Ensuring that SAEs and SARs (including SUSARs) are chased with Sponsor if a record of receipt is not received within 2 working days of initial reporting.
4. Ensuring that AEs and ARs are recorded and reported in line with the requirements of the protocol.
5. Preparing the clinical sections and final sign off of the Development Safety Update Report (DSUR).

Sponsor:
A medical R&D Director will assess causality and expectedness of behalf of the Sponsor:

1. Clinical oversight of the safety of patients participating in the trial, including an ongoing review of the risk/benefit.
2. Using medical judgement in assessing, causality of SAEs.
3. In order to assess expectedness, the R&D Director will need to check if the SAR is listed in the Reference Safety Information (RSI). If the SAR is listed in the RSI then it is expected. If the event is not listed in the RSI then it is unexpected and is a SUSAR and subject to expedited reporting to the MHRA and REC.
4. Immediate (within 24 hours) review of all SUSARs.
5. Review of specific SAEs and SARs in accordance with the trial risk assessment and protocol as detailed in the Trial Monitoring Plan.
6. Central data collection and verification of AEs, ARs, SAEs, SARs and SUSARs according to the trial protocol.
7. Reporting safety information to the Sponsor Oversight Group for the ongoing assessment of the risk / benefit according to the Trial Monitoring Plan.
8. Expedited reporting of SUSARs to the Competent Authority (MHRA in UK) and REC within required timelines.
9. Notifying Investigators of SUSARs that occur within the trial.
10. Checking for (annually) and notifying the PI of updates to the Reference Safety Information for the trial.
11. Preparing standard tables and other relevant information for the DSUR in collaboration with the PI and ensuring timely submission to the MHRA and REC.

Table: Serious adverse event report

Severity Assessed by the Co-I and PI or just PI	The assessment of severity of an SAE will be based on the investigator's clinical judgement using the following definitions: **Mild:** An event that is easily tolerated by the trial subject, causing minimal discomfort, and not interfering with everyday activities. **Moderate:** An event that is sufficiently discomforting to interfere with normal everyday activities. **Severe:** An event that prevents normal everyday activities.
Seriousness Assessed by the Co-I and PI or just PI	An event is considered serious if it meets one or more of the following criteria: (a) results in death (b)life-threatening (c) requires hospitalisation or prolongation of existing hospitalisation (d) results in persistent or significant disability or incapacity (e) consists of a congenital anomaly or birth defect. Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the patient or may require intervention to prevent one of the outcomes listed in the definition above should also be considered serious.

Causality Assessed by the Co-I and PI or just PI	The investigator will assess whether the SAE is likely to be related to the IMP according to the following definitions: • **Unrelated**: Where an event is not considered to be related to the IMP. • **Possibly related**: The nature of the event, the underlying medical condition, concomitant medication, or temporal relationship make it possible that the SAE has a causal relationship to the study drug.
Expectedness Assessed by the R&D Director (on behalf of the Sponsor) for single-site trials	To assess expectedness, the R&D Director will need to check if the SAR is listed in the Reference Safety Information (RSI). The RSI for IMPs with a marketing authorization (MA) is section 4.8 (Undesirable Effects) of the MHRA approved Summary of Product Characteristics (SmPC) or for IMPs without an MA, the RSI is the relevant section in the Investigator Brochure (IB). If the SAR is listed in the RSI then it is expected. If the event is not listed in the RSI then it is unexpected and is a SUSAR and subject to expedited reporting to the MHRA and REC.

9.7 Notification of deaths

The average in-patient mortality rate for patients admitted to hospital with heart failure is 10%, the 30-day mortality of those surviving to discharge is 25%. Thus we expect up to 4 patients to die in hospital and a further 5 to die in the month following discharge. We may expect further deaths up to the 180 day follow up point. We will report all deaths to the sponsor, all in-patient deaths will be reported within 24 hours, all out-patient deaths will be reported at 30 and 180 days.

9.8 Pregnancy reporting

If a study patient, or the partner of a study patient, falls pregnant when participating in a clinical trial, the study patient should be withdrawn from the trial unless the Principal Investigator decides that the risk to the patient is not clinically significant.
The patient, or the partner of the study patient, should be followed up by monthly or two monthly visits/telephone contacts during pregnancy and at birth and at 3 months after the birth of the baby. Whether the visits are every month or two months will depend on clinical judgement and will be agreed with R&D and documented in the TMF.
Pregnancy is not expected to occur or be diagnosed in a patient recruited to the study during the active phase of the trial. Pregnancies that occur during follow up will not be reported as they are unrelated to the endpoints.

9.9 Overdose

Any overdose of ACZ or furosemide will be reported as an SAE and recorded in the medical notes, CRF and deviation log as soon as the study doctor is alerted. The sponsor will be alerted within 24 hours of a suspected serious breach. Overdoses will be observed from the electronic prescribing record. A patient receiving an overdose will be removed from analysis and replaced. The incident will also be reported using the Hull University Teaching Hospital Trust incident reporting system – Datix.

9.10 Reporting urgent safety measures

If any urgent safety measures are taken the PI/Sponsor shall immediately and in any event no later than 3 days from the date the measures are taken, give written notice to the MHRA and the relevant REC of the measures taken and the circumstances giving rise to those measures. Fatal SUSARs will be reported within 7 days, non-fatal SUSARs reported within 14 days.
https://www.gov.uk/guidance/clinical-trials-for-medicines-manage-your-authorisation-report-safety-issues#report-an-urgent-safety-issue

9.11 The type and duration of the follow-up of subjects after adverse events.

Follow up for any AE, SAE, AR or SAR will be until the event has resolved or 30 days after baseline. This follow up will be via telephone consultation and the written and electronic record. Any SUSAR related to the IMP will need to be reported to the Sponsor irrespective of how long after IMP administration the reaction has occurred.

9.12 Development safety update reports (DSUR)

The PI will provide (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the MHRA in the UK, Ethics Committee, Host NHS Trust and Sponsor.

The report will be submitted within 60 days of the MHRA clinical trial authorisation of the trial each year until the trial is declared ended.

10 STATISTICS AND DATA ANALYSIS

All statistical analysis will be carried out using SPSS or R software provided by the University of Hull.

10.1 Sample size calculation

The study is designed as a pilot and exploratory study but we have performed a power calculation in order to ensure the results form a reliable basis to inform future work and to gauge an appropriate timeline for recruitment (and thus will influence any future funding). The calculation may appear crude as we are limited by the lack of reliable data on net fluid loss and serum chloride concentrations in patients receiving high dose diuretics (table 1 and table 2).

Net fluid loss

Current guidelines recommend a target of ~1000ml net fluid loss per day during in-patient diuresis, which generally occurs over a period of up to 9 days (median length of stay for a patient hospitalised with heart failure in the UK). We estimate that a clinically significant difference in daily net fluid loss is 500-1000 ml – a minimum of 4.5L over a 9 day period and 2L over the 4 day period of our study. Such an increase in net fluid loss would indicate a significant increase in diuresis that may shorten hospital admission.

Using the available data on urine output (appendix 7), we expect an average daily fluid loss of 1000ml per day with a standard deviation ranging from 500-1000ml. Patients admitted with heart failure are often breathless on very modest exertion thus we anticipate a drop-out rate of 10% in the control arm. Furthermore, given the lack of reliable clinical data on the tolerability of acetazolamide we have anticipated a higher drop-out rate of 20% in the acetazolamide arm. Thus, to detect a difference of

500-1000ml in net fluid loss per day with statistical power of 80% and two-tailed significance of 5% we would need to recruit a minimum of 35 patients (17 in the control arm and 18 in the acetazolamide arm).

Serum chloride concentrations

Low serum chloride is associated with adverse outcome in patients with HF. We estimate a clinically meaningful change in chloride to be -0.9 mmol/L per day over the course of an admission (9 days) and -3.6 mmol/L over the four day period of our study (appendix 7). To detect such a difference with 80% power and 5% two-tailed significant with the above anticipated drop out rate we would need to recruit a minimum of 14 patients in the control arm and 17 patients in the ACZ arm.

We expect two potential confounding factors for the co-primary endpoints: 1) daily salt intake and 2) loss of IV access reducing the amount of IV furosemide given. We will collect data on both potential confounders using a daily dietary sheet (on which the patient will record their meals for the day and from which the daily salt intake will be estimated) and recording the “number of hours of furosemide” per day. We do not expect any patient to have excessive salt intake (defined by the European Society of Cardiology Heart Failure Guidelines 2021 as >5g per day). Any patient with >5g salt intake per day or <20 hours of furosemide per day will be removed from the analysis. Due to the exploratory nature of the study we will conduct an intention to treat analysis and a per-protocol analysis in which only patients who completed the study without deviating from the protocol will be included. Thus, we will recruit a maximum of 50 patients – 23 in the control arm and 27 in the ACZ arm with anticipated drop out of 10% and 20% respectively. The extra patients recruited will also act as a contingency should any patient need to be removed from the analyses for reasons of excess salt intake, prolonged loss of IV access, or for the per-protocol analysis.

10.2 Planned recruitment rate

There are, on average, 10 patients with heart failure on the Cardiology wards at CHH in any given week, each with a median stay of 9 days. Entry criteria are broad and exclusion criteria minimal with the intent that enrolment is as representative a population as possible. It is expected to be able to recruit at least 2 patients per week. This estimate may seem conservative however after enrolment, the patient will have daily visits from the study doctor with collection of multiple data points and the associated paper work to complete. Thus we estimate recruitment to take 20-30 weeks.

10.3 Statistical analysis plan

Baseline data

Primary outcomes

Differences in daily net fluid loss and total change in serum chloride (day 1 to day 4) will be compared using independent samples T test. If a difference in number of hours of furosemide per day or salt intake per day is different between the groups then the change in the primary outcomes will be stratified sub groups of hours of furosemide per day and grams of salt per day (cut-offs for group membership will be identified post-hoc).

Secondary outcomes

Associations with outcome (heart failure hospitalisation, any-cause hospitalisation, cardiovascular mortality, all-cause mortality, and new diagnosis of HF (analysis 2 only)) will be assessed by time-to-event analysis using Kaplan-Meier estimates and uni- and multivariable Cox regression. We plan two separate outcome analyses; firstly, adjusting for all variables listed in the baseline table (appendix 1 – table 5) and secondly, adjusting only for age, sex and BMI to avoid statistical over-fitting. Variables

that are associated with outcome with P>0.1 (an arbitrary cut-off) will not be included in multivariable analysis.

Data will be presented graphically using Kaplan-Meier curves and using hazard ratios with 95% confidence intervals and two-sided P values. Direct comparisons of the number of consultations with HF-related symptoms will be made with Mann-Witney U tests, and uni- and multivariable forward step-wise hazard regression. Two-tailed statistical significance will be set as P<0.05.

10.3.1 Summary of baseline data and flow of patients

Baseline characteristics (Table 2) will be described as median (25^{th} - 75^{th} centiles) for continuous data and total number (percentage of population) for categorical data. Normality of distribution will be assessed by plotting bell-curves for each continuous variable.

Patient entry into the study will be described using a consort flow diagram.

Table 2: Baseline table

	Acetazolamide N = X	Control N = X	P
Age – years			
Sex – male (%)			
IHD – N (%)			
Diabetes – N (%)			
Hypertension – N (%)			
COPD – N (%)			
Atrial fibrillation – N (%)			
Cancer – N (%)			
“De novo” HF – N (%)			
Ischaemic cardiomyopathy – N (%)			
DCM – N (%)			
LVEF - %			
LVEF <40% - N (%)			
Valve disease – N (%)			
Mod or worse AS – N (%)			
Mod or worse MR – N (%)			
Weight – kg			
BMI – kg/m^2			
SBP – mmHg			
Heart rate – bpm			
NYHA class IV			
Likert breathlessness scale			

Peripheral oedema affecting up to: *ankles – N (%)* *knees – N (%)* *sacrum – N (%)*			
Lung crackles *None – N (%)* *Bibasal – N (%)* *Mid-zone – N (%)* *Whole lung – N (%)*			
Jugular venous pulse *Not visible – N (%)* *Raised 1-4m – N (%)* *>4cm – N (%)* *Level of Earlobes – N (%)*			
NTproBNP – ng/L			
Haemoglobin – g/dL			
Haematocrit – fraction			
Sodium – mmol/L			
Chloride – mmol/L			
Potassium – mmol/L			
Bicarbonate – mmol/L			
Urea – mmol/L			
Creatinine - µmol/L			
Estimated Glomerular Filtration Rate – ml/min/1.73m^2			
Albumin – g/dL			
Inferior vena cava diameter – cm			
ACEI/ARB/ARNI – N (%) *ACEI or ARB – N (%)* *ARNI – N (%)*			
Beta-blocker – N (%)			
MRA – N (%)			
Median oral loop diuretic dose prior to admission (mg / day)			
Median intravenous loop diuretic dose received as an in-patient prior to randomisation (mg / day)			

10.3.2 Primary outcome analysis

Primary outcomes

We will calculate the median net fluid loss each day and from baseline to day 4 for patients in the ACZ arm and the control arm. We will assess distribution of daily and total fluid loss in both arms, if there is normal distribution we will compare the median net fluid loss using independent samples T-test, if the distribution is not normal we will use the Mann-Witney-U test. A P value <0.05 will indicate a statistically significant difference between the two arms. We will also perform linear regression with total net fluid loss from day 1 to day 4 as the dependent variable and treatment assignment, median daily salt intake and median number of hours of furosemide per day as the independent variables.

We will calculate the median change in serum chloride concentrations between the two groups between baseline and day 4. We will compare the median change in serum chloride concentration using independent samples T test. We will also perform linear regression with total change in serum chloride levels day 1 to day 4 as the dependent variable and treatment assignment, median daily salt intake, and median number of hours of furosemide per day as the independent variables.

The independent samples T test assumes that both net fluid loss (daily and total) and change in serum chloride concentrations between day 1 and day 4 are normally distributed in both groups; this will be tested using distribution curves. It also assumes that there is equal variance in both net fluid loss (daily and total) and change in serum chloride concentrations between day 1 and day 4; this will be tested using Levene's test, the samples will be assumed to have equal variance if Levene's test is P>0.05.

We do not expect any missing data, however any patient with missing data for each of the co-primary endpoints will be excluded from the analysis.

We expect two potential confounding factors for the co-primary endpoints: 1) daily salt intake and 2) loss of IV access reducing the amount of IV furosemide given. Any patient with >5g salt intake per day or <20 hours of furosemide per day will be removed from the analysis.

10.3.3 Secondary outcome analysis

Secondary outcomes are exploratory and none are powered for statistical significance. Secondary outcomes that are measured as continuous variables (difference in daily urine output; difference in weight from baseline to day 4; difference in change in serum sodium potassium, bicarbonate, urea, creatinine, and estimated glomerular filtration rate between baseline and day 4; urinary electrolyte concentrations at day 2 and day 4; inferior vena cava diameter; change in breathlessness score (Likert scale); time to clinical euvolaemia; and length of stay) will be assessed using independent samples t-test, and unadjusted linear regression (with treatment assignment as the independent variable), and linear regression adjusted for daily salt intake and median number of hours of furosemide per day.

Secondary outcomes that are measured as categorical variables (incidence of hyponatraemia, hypochloraemia, hypokalaemia, metabolic alkalosis (serum bicarbonate concentrations >29 mmol/L) or metabolic alkalosis (serum bicarbonate concentrations <23 mmol/L); clinical assessment of congestion; proportion of patients with IVC diameter >2cm; proportion of patients with JVD ratio <4; rate of adverse and serious adverse events; rate of drop-out after randomisation will be assessed using chi-squared tests, un-adjusted logistic regression (with treatment assignment as the independent variable), and logistic regression adjusted for daily salt intake and median number of hours of furosemide per day.

Survival analysis (time to cause specific hospitalisation and time to cause specific mortality) will be conducted with a time-to-first event analysis using Kaplan-Meier and logrank tests, un-adjusted Cox proportional-hazard outcome models.

10.4 Subgroup analyses

Subgroup analyses for all endpoints on sex and age (above and below median value).

10.5 Interim analysis

No planned interim analysis

10.6 Subject population

As this is a pilot and exploratory study, we will perform both an intention to treat analysis on all patients completing the study so that we can most accurate describe the clinical effects of ACZ including tolerability and side effects and a per-protocol analysis so we can most accurately assess the primary endpoints.

10.7 Procedure(s) to account for missing or spurious data

We will attempt to replace any patient with data missing for either primary endpoint (i.e. those who did not complete 4 days of treatment, those with data on net fluid loss missing on any day, and those with data on serum chloride missing on baseline or day 4). However, we expect missing data to be a minor problem as data collection for net fluid loss will be done by the study team during each visit and data on chloride concentrations will be collected as part of routine care. Reasons for missing data will be recorded in a dedicated section within the CRF for each study visit. We will not perform any imputation of missing data.

10.8 Other statistical considerations.

The sponsor will be notified in writing of any deviation from the statistical plan with appropriate justification with statistical advice and references attached. Any changes to the statistical plan will be acknowledged in the final reports and during dissemination of the results.

11 DATA HANDLING

11.1 Data collection tools and source document identification

All source data will be entered into Case Report Forms (CRF). The CRF will be a paper booklet that will contain data fields for all data collected for primary and secondary outcomes from baseline to day 5, including follow up data to day 180. The CRF will be kept by the trial team in a secure location accessible only by the trial team on the Sponsor's premises with the relevant participant consent form.

Source Data	**Source**
Primary endpoints	
Net fluid loss	Patient diet sheet and nursing record of urine output recorded in CRF
Serum chloride concentrations	Electronic medical record (Lorenzo) recorded in CRF
Secondary endpoints	
Weight	Measured by study doctor recorded in CRF
Serum electrolyte concentrations	Electronic medical record (Lorenzo) recorded in CRF
Incidence of electrolyte abnormalities	Electronic medical record (Lorenzo) recorded in CRF
Change in renal function	Electronic medical record (Lorenzo) recorded in CRF
Change in urinary electrolyte levels	Electronic medical record (Lorenzo) recorded in CRF
Clinical congestion	Clinical examination by study doctor recorded in CRF
Ultrasound measures of congestion	Ultrasound assessment by study doctor using handheld ultrasound device. Measurements will be taken from the live images, there will be no facility to save the images. Recorded in CRF
Breathlessness score	Recorded using the Likert scale and documented in CRF
Time to clinical euvolaemia	Clinical examination recorded in CRF
Length of stay	Electronic medical record (Lorenzo) recorded in CRF

Rate of AR, SAR, SUSARs	Patient interview, clinical assessment, recorded in CRF
Rate of AE or SAEs	Patient interview, clinical assessment, recorded in CRF
Rate of recruitment	Separate Microsoft ® Excel database
Time taken to screen	Separate Microsoft ® Excel database
Rate of drop-out after randomisation	Separate Microsoft ® Excel database
Cause specific hospitalisation and mortality	Electronic medical record (Lorenzo)

The CRF will be considered as source documentation for the following items: Demography, symptom directed physical examination, vital signs, body weight and height, dates and times of visits and assessments. All other evaluations that are reported in the CRF must be supported by documentation in hospital notes or electronic medical record. CRFs are provided for each participant, and all data related to the trial will be recorded in these CRFs. The CRFs are to be completed at the time of the participant's visit so that they always reflect the latest observations on the participants. The investigator must verify that all data entries in the CRFs are accurate and correct by signing the relevant pages. If certain information is not available, not applicable, not done, or unknown, the research staff or investigator will enter the relevant abbreviation, i.e. N/A to confirm that the data field has not been overlooked. This also applies to participants who fail to complete the trial. If a participant withdraws from the trial, the reason must be noted on the CRF. If a participant is withdrawn from the trial because of an adverse event, a thorough effort should be made clearly to document the outcome. All forms should be completed using a black ballpoint pen and must be legible. All entries, corrections and alterations are to be made by the responsible investigator or her/his designee. Except for obvious mistakes, the corrections need to be commented. Corrections should be made in such a way that the original entry is not obscured. The corrected data should be entered, dated, and initialled by the investigator or his designee. On all trial-specific documents, other than the signed consent form and prescription, the participant will be referred to by the trial participant ID number, not by name.

Each contact with the participant will also be documented in the medical notes. As a minimum, the following information will be recorded in patient's medical records from trial visits or telephone contacts:

- Clearly written date of visit or contact, trial acronym and visit number.
- Date participant given participant information sheet
- Date consent form signed
- Date of screening
- Medical history, concomitant diseases and medication including trial medication, and any changes in concomitant diseases and medication at subsequent visits.
- Anything which is relevant to the ongoing care of the subject;
  - Relevant results and study medic's assessment of these results.
  - Brief description of any AEs with start & stop times/dates and any significant test results or a medical summary of events if more appropriate.
- Any other relevant information.

11.2 Data handling and record keeping

Data will be collected from the source and recorded in the CRF by the study doctors and study nurse. The CRF will be stored in an archiving box on the Sponsor's premises in a lockable office. Only members of the study team and Sponsor will have the necessary access. Data should be transferred from the CRF to the electronic database at the end of each study visit. The electronic database will be a Microsoft Excel ® file. Transfer of data from the CRF to the electronic database will be checked with

proof-reading by the study doctors. The electronic database will be a password protected document, stored on the Y: drive servers of the Hull University Teaching Hospitals network. The servers are backed up to disk media each night. The disks run on a 4 week cycle. Files stay on the server unless deleted by accident or deliberately. Anything deleted more than 4 weeks previously is therefore lost. Additional 'archive' backups are taken for archived data, so data should not be lost from this type of system. Disks are stored in a fireproof safe. Data will not be transferred from the servers. Prior to opening the database in the statistical analysis software package the data will be anonymised by assigning each patient an enrolment number in the electronic database which, alongside the participant's date of birth, will be used as identifiers in the anonymised database. The PI and statistician (Professor Alan Rigby) will be responsible for data analysis.

Data will be collected and retained in accordance with General Data Protection Regulation (GDPR)- https://gdpr.eu/.

11.3 Access to data

Direct access will be required to patients medical records and CRFs at site by authorised representatives from the Sponsor, and the regulatory authorities to permit trial-related monitoring, audits and inspections.

Trial documents (paper and electronic) will be retained in a secure location during and after the trial has finished.
Anonymised data will be made available to outside institutions on reasonable request with appropriate data security in place.

Access to the final trial dataset
The database will be 'locked' to obtain the final dataset after:

- trial completion (usually last patient, last visit)
- completion of coding and data entry
- all data queries resolved
- trial team notified of date of lock

A copy of the final trial dataset will be send to HUTH R&D as Sponsor prior to the statistical analysis.

For single or double-blind randomised trials, HUTH R&D will be sent a copy of the final trial dataset and end of trial notification before the randomisation list will be released by the organisation in charge of randomisation.

11.4 End of Trial and Discontinuation

The end of the trial is defined as the date the last patient has completed their last study visit. An end of the trial declaration form will be submitted to the MHRA, REC and HUTH R&D within 90 days from completion of the trial and within 15 days if the trial is discontinued prematurely. HUTH R&D will be notified immediately of any reason to halt the trial. The Principal Investigator and HUTH R&D as Sponsor will decide if the trial should be halted temporarily. The MHRA, REC and HUTH R&D will be notified within 15 days of a decision to temporarily halt the trial by submitting a substantial amendment notification. A summary of the trial final report will be submitted to the MHRA, REC and HUTH R&D within 1 year of the end of the study.

Upon completion of the trial, the following activities, when applicable, must be conducted by the Investigator and by the Sponsor's Monitor, as appropriate: data clarifications and/or resolutions; review of site study records for completeness. In addition, the Sponsor, the Funder, and the Principal Investigators reserve the right to temporarily suspend or prematurely discontinue this study for any reason. If such action is taken, the Sponsor will discuss this with the Funder and the Investigator (including the reasons for taking such action) at that time. If the trial is terminated for safety reasons, the sponsor will promptly inform the regulatory authorities of the suspension or termination of the study and the reason(s) for the action. The Investigator must inform the Ethics Committee promptly and provide the reason for the suspension or termination. After such a decision, the Investigator must call in all participating patients within a reasonable time period. At this visit all Medical Files and Case Report Forms must be completed as far as possible.

11.5 Archiving

All essential trial documents including source documents will be archived by the Sponsor for a minimum period of 5 years after study completion. Destruction of essential documents will require authorisation from the Sponsor.

12 MONITORING, AUDIT & INSPECTION

The trial will be monitored in accordance with Hull University Teaching Hospitals R&D department's standard operating procedures to ensure compliance with UK Clinical Trial Regulations and ICH GCP. All trial related documents will be made available upon request for monitoring by R&D monitors and for inspection by the MHRA.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Research Ethics Committee (REC) review& reports

Approval from the Regional Ethics Committee will be in place before the commencement of the trial. The study team will apply for REC approval via the IRAS platform. The REC will be asked to approve the trial protocol and informed consent documents. Any proposed substantial amendments to the protocol will be approved by the REC before implementation alongside other approvals needed for substantial amendments. All correspondence with the REC will be downloaded from the IRAS platform and stored in the Master File. The REC will receive an annual progress report, written by the CI, within 30 days of the anniversary of REC approval. The REC will receive notification of completion of the study from the CI. Finally, the CI will send a final report including any present or planned publications or presentations of the data to the REC within 1 year of completion of the study.

13.2 Peer review

This protocol has been developed with and the final version reviewed by an international expert on heart failure with an excellent and long-established record for high quality research which has led to multiple publications in high-impact peer reviewed journals. The study plan has been reviewed and approved by the British Society of Heart Failure Research Committee – a multidisciplinary committee of heart failure specialists involving pharmacists, nurses, clinicians, and trialists. The statistical analysis plan has been reviewed and approved by an international expert in medical statistics currently employed by the University of Hull.

13.3 Public and Patient Involvement

The project has been presented to and reviewed by Involve Hull, a network of patients and carers in the East Riding of Yorkshire (https://www.hull.ac.uk/work-with-us/research/institutes/institute-for-clinical-and-applied-health-research/public-and-patient-involvement). The group provided positive and

constructive feedback which is detailed in the appendix. A common theme in the responses was the need to ease the burden of the trial on patients, thus all questionnaires will be completed by the study doctor based on answers given by the patient and the ultrasound measures of congestion will occur on day 1 and day 4 rather than every day. All patients involved in the trial will be invited to local and national presentations of the data.

13.4 Regulatory Compliance

The trial will not start until a Clinical Trial Authorisation (CTA) is obtained from the MHRA. Trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004 and any relevant amendments. All members of the study must have in-date Good Clinical Practice compliance and maintain membership of their professional organisations and have clinical practice indemnity cover in place.

13.5 Protocol Compliance

Prospective, pre-meditated, deviations from the protocol are not permitted. Any accidental deviation from the protocol will be documented in the CRF and medical notes, and reported to the CI and Sponsor. The participant will receive a full and frank explanation. Frequent deviations from the protocol will be promptly investigated by the CI, a remedy put in place, and reported to the Sponsor to consider classification as a serious breach of protocol.

13.6 Notification of Serious Breaches to GCP and/or the protocol

The sponsor will be made aware immediately of any potential serious breach of the protocol where “serious breach” is defined as a breach that effects the safety or physical or mental integrity of the subjects of the trial or the scientific value of the trial. The Sponsor will notify the MHRA of any serious breach of the protocol within 7 days of being alerted.

13.7 Data protection and patient confidentiality

Data entered into the source documents will be de-identified at source – patients will be allocated an enrolment number and entered into the database. The patient identifiable data will be kept with the corresponding enrolment number on a separate Microsoft Excel ® file which will be password protected and stored on the HUTH Y: drive behind an NHS firewall in a lockable office on the Sponsor’s premises. The password for the trial database and the patient list will be different. Both will contain characters with at least one capital letter, numbers, and at least one special character. Access to the offices and the databases will be available only to the study research team and relevant members of the HUTH R and D department. The data will be stored for a minimum of 5 years from completion of the trial. The data custodian is the PI.

13.8 Indemnity

This is an NHS-sponsored research trial. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS indemnity covers NHS staff and medical academic staff with honorary contracts only when the trial has been approved by the Trust R&D department. NHS indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm. Where the Principal investigator is employed by the University of Hull, the University has an insurance policy that includes cover for no-fault compensation in respect of accidental injury to a research subject.

13.9 Amendments

The PI and Sponsor will make decisions regarding the need for substantial amendments. Substantial amendments will be submitted in writing (email) to the MHRA and REC. The correspondence will be retained in the Master file. A tracked changes document of the protocol (and any other trial document that is changed as a result of the substantial amendment) will be saved including the relevant changes in the Master file.

13.10 Post trial care

Patients will receive a follow up phone call from a member of the study team within 30 days of discharge. Most participants enrolled in the trial will live in the region served by Castle Hill Hospital thus will continue to receive emergency and routine secondary and tertiary healthcare as and when needed. The study is not designed to detect a treatment benefit, as such patients will not have access to acetazolamide as a treatment for heart failure after the trial is completed.

14 DISSEMINIATION POLICY

14.1 Dissemination policy

The PI and Sponsor will own the data collected by the trial and will prepare a final study report on completion. Participating investigators will be able to publish from the data after reasonable request to the PI. The funding body will be acknowledged in any publication. We intend to publish the trial protocol in a “design and rationale” paper, full study report focussing on the primary endpoints, and secondary analyses of secondary endpoints in peer-reviewed journals, and statistical code will be made available on GitHub. There will be no time-limit on publications of the trial outcomes however the protocol will be published within 1 year of the trial commencing. During the consent process participants will be asked if they wish to know of any publication or presentation of the data. Those that do will notified of publications or presentations via letters written by the PI.

14.2 Authorship eligibility guidelines

The PI will be first author or co-first author if another author contributes to >50% of the manuscript for the protocol and primary endpoint publications. The PI will be senior author if another member of the study team wishes to publish a manuscript based on secondary endpoint data. All other members of the study team will be given authorship on any publications relating to the study.

15 Sponsor Standard Operating Procedures

All relevant Sponsor organisation SOPs will be followed to ensure that this TRIAL complies with all relevant legislation and guidelines.

Declaration of Helsinki

The Investigator will ensure that this trial is conducted in full conformity with the current revision of the Declaration of Helsinki (last amended October 2000, with additional footnotes added 2002 and 2004).

ICH Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in full conformity with relevant regulations and with the ICH Guidelines for Good Clinical Practice (CPMP/ICH/135/95) July 1996.

Approvals

Once Sponsor authorisation has been confirmed, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), regulatory authorities (MHRA in the UK), HRA approval and host institution(s) for written approval.

Study cannot commence until sponsor green light is given. Once Sponsor authorisation has been confirmed, the Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

Centres

Ward 26 & 28 and Cardiac Monitoring Unit at Castle Hill Hospital.

Ward 39 at Hull Royal Infirmary

16 REFERENCES

See endnotes.

16. APPENDICIES

Appendix 1-Risk

Risks associated with trial interventions

☒ LOW ≡ Comparable to the risk of standard medical care

☐ MODERATE ≡ Somewhat higher than the risk of standard medical care

☐ HIGH ≡ Markedly higher than the risk of standard medical care

Justification: Briefly justify the risk category selected and your conclusions below (where the table is completed in detail the detail need not be repeated, however a summary should be given):

There is little data on which to draw to estimate how well acetazolamide will be tolerated in a contemporary cohort of patients admitted with heart failure. As the treatment period is short, we do not anticipate any long term harm from any known side effect of acetazolamide. It is possible that the combination of acetazolamide and furosemide infusion may have an additional diuretic effect compared to furosemide alone which might increase the risk of worsening hyponatraemia, symptomatic hypotension or worsening renal function. We expect that discontinuation of the study drug will negate the increased risk of combination treatment.

What are the key risks related to therapeutic interventions you plan to monitor in this trial?		How will these risks be minimised?		
IMP/Intervention	Body system/Hazard	Activity	Frequency	Comments
Acetazolamide	Side effects of treatment as listed by BNF	Examination and history	Daily	
	Serum electrolytes - hyponatraemia	Blood tests	Daily	Done as part of routine care and would expect to see a proportion of patients with electrolyte abnormalities in the control arm also
	Symptomatic hypotension	Bedside BP monitoring	Daily	Done as part of routine care and would expect to see a proportion of patients with symptomatic hypotension in the control arm also
	Worsening renal function	Blood tests	Daily	Done as part of routine care and would expect to see a proportion of patients with worsening renal function in the control arm also. Transient impairment in renal function may be associated with better outcomes than those whose renal function remains unchanged or improves during admission.[45]

Appendix 2 – Data to support power calculations

Net Fluid Loss

Of the recently published trials of diuretics in patients admitted with heart failure (table 1) the DOSE trials provides the most reliable data on which we can base a power calculation: a similar patient population was recruited, patients taking diuretics other than loop diuretics were ineligible and patients in the high-dose arm received an average of 260mg IV furosemide per day. Net fluid loss over a 3-day period was 4899 ±3479 mL in the high-dose arm equating to 1633 ±1160 mL per day.

The other comparable data come from the control arm of the ATHENA study (table 1) in which patients received an average of 120mg IV furosemide per day. Median net fluid loss over a 4-day period was 5584 (2924-8132).

Table 1 – Net fluid loss in recent trials involving high dose intravenous loop diuretic

Trial	N (trial arm)	Diuretic dose per day (mean)	Time period over which UO was measured	Net fluid loss (ml)
DOSE	151 (low dose)	120mg	72 hours	3575 ±2635
	157 (high dose)	260mg	72 hours	4899 ±3479
TACTICS-HF	128 (placebo)	140mg	24 hours – Day 1 24 hours – Day 2 24 hours – Day 3	1541 ±1525 1419 ±1379 1401 ±1387
ATHENA-HF†	178 (standard care)	120mg	24 hours 48 hours 72 hours 96 hours	1183 (510-1955) 2282 (1155-4135) 3810 (2011-5565) 5584 (2924-8132)
UNLOAD	100 (standard care)	180mg	48 hours	3300 ±2610
CARESS-HF	94 (standard care)	NR	96 hours	7082 ±4183

Legend
NR – not reported. Results presented as mean ± standard deviation apart from † where results reported as median (interquartile range).

Serum Chloride Change

Data on clinically significant changes in serum chloride levels are lacking. Furthermore, there is conflicting data on the prognostic significance of reductions in serum chloride during in-patient diuresis.[39-41,44] However, low serum chloride (<96 mmol/L – hypochloraemia) is strongly and independently associated with adverse outcome in both in- and out-patients with HF. In the PROTECT study patients with hypochloraemia on admission which had corrected by the time of discharge (day

14) were at no greater risk of mortality after discharge than those who had never had hypochloraemia.[41] The median change in chloride between day 1 and day 14 for patients in that group was 5.5 mmol/L (interquartile range (IQR) 4 – 9mmol) which equates to a difference of 0.4 (0.3 – 0.6) mmol/L per day. Conversely, those with normal chloride levels on admission who had developed hypochloraemia by day 14 had a three-fold greater risk of mortality compared to those with normal chloride levels at day 1 and day 14. The median change in chloride between day 1 and 14 for this group was -7 mmol/L (95% CI -10 – 5) equating to -0.5 (-0.7 – 0.4) mmol/L per day.

Data from our local population found similar results:[44] those with hypochloraemia on admission who had normal chloride by the time of discharge were at no greater risk of mortality compared to those who had normal chloride levels on admission and discharge. The median change in chloride levels in this group was 6 (5 – 8) mmol/L over a median in-patient stay of 12 days; 0.5 (0.4 – 0.7) mmol/L per day. Conversely, those with normal chloride levels on admission who had hypochloraemia at discharge were at 50% increased risk of adverse outcome. The median change in chloride levels in this group was -8 (-12 – -5) mmol/L over a median in-patient stay of 13 days; -0.6 (-0.9 – -0.4) mmol/L per day. The median chloride on admission in patients with normal chloride levels on admission was 100 (98 – 103) with a standard deviation of 3 mmol/L thus a 8 mmol/L reduction in serum chloride concentrations would cause hypochloraemia in the majority of patients; similar to the median change in chloride in patients with normal chloride levels on admission who develop hypochloraemia by discharge (table 2).

Table 2 – Available Data on Changes in Serum Chloride Levels During In-patient Diuresis

Study		*Ter Maarten et al (2016)*	*Cuthbert et al (2022)*
Study Design		Retrospective cohort of patients enrolled in the PROTECT trial	Retrospective cohort of unselected patients admitted with HF
Daily IV Diuretic Dose		80mg	Not reported
Patients with normal Cl^- on admission who develop hypochloraemia on discharge	**Median (IQR) Cl^- on admission – mmol/L**	Not reported	100 (98 – 103)
	SD – mmol/L	Not reported	3
	Median (IQR) ΔCl^- during admission – mmol/L	-7.0 (-10.0 – 5.0)†	-8 (-12 – -5)
	Length of stay - days	14	12
	Median (IQR) Daily ΔCl^- - mmol/L	-0.5 (-0.7 – 0.4)†	-0.5 (-0.7 – -0.4)
Patients with hypochloraemia on admission who develop normal Cl^- on discharge	**Median (IQR) Cl^- on admission – mmol/L**	Not reported	6 (5 – 8)
	SD – mmol/L	Not reported	3
	Median (IQR) ΔCl^- during admission – mmol/L	5.5 (4.0 – 9.0)	6 (5 – 8)
	Length of stay - days	14	13

	Median (IQR) Daily ΔCl^- - mmol/L	0.4 (0.3 – 0.6) mmol/L	0.5 (0.4 – 0.6)

Legend

† - this data must be erroneous as patients who have developed hypochloraemia since admission cannot have increases in serum chloride levels; we included it as it is the only published data available from which we can estimate clinically meaningful changes in serum chloride levels. Abbreviations used: IV – intravenous; HF – heart failure; Cl^- - serum chloride concentrations; SD – standard deviation.

Appendix 3

The definition of women of child-bearing potential is taken from the Clinical Trials Facillitation Group guidance: fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

End Notes

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