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The efficacy and optimal dose of Acetic Acid to treat colonised burns wounds: protocol for a pilot randomized controlled trial

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Title

The efficacy and optimal dose of Acetic Acid to treat colonised burns wounds: protocol for a pilot randomized controlled trial

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Abstract

Introduction and Article Summary

Despite of recent advancement in the burns wound management, burn wound infection (BWI) is still one of the major cause of burns mortality. Patients who survive their burns injury still suffers from BWI related complication like delayed wound healing and poor scarring. BWI has been treated by application of topical antimicrobial agents or systemic antibiotics. Due to the global risk of developing systemic antibiotics resistance, medical research focuses on identifying single topical agent which has effective antimicrobial activity, easily available and cost effective. One such agent is Acetic acid (AA). AA has been used as a topical antibacterial agent for the treatment of burns wounds for many years and has shown to have activity against Gram-negative organisms including *Pseudomonas aeruginosa*. So far there has been no consensus on optimal concentration that's has effective antimicrobial activity, frequency of application, duration of treatment and most importantly good patient's tolerability. A randomised control study is required to answer all these questions.

Objective

To investigate the efficacy and tolerability of 0.5% and 2% of acetic acid when applied to colonised burns wounds for 3 days after admittance to the Queen Elizabeth Hospital Birmingham.

Methods and analysis

This is a double-blinded, prospective, randomised, controlled, single-centre trial. Patients will be screened for eligibility in the inpatient area and those who are found to be eligible will be randomly assigned to one of two treatment groups:

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3 GROUP 1: 0.5% acetic acid (10 patients)
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6 GROUP 2: 2% acetic acid (10 patients)
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9 The two concentration strengths of acetic acid will be assessed for the
10 antimicrobial activity and pain tolerability. Antimicrobial activity will be assessed
11 using repeated measures methods to analyse the number of colony forming units.
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15 **Article Summary**

16 **Strengths and limitations of this trial**

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21 1. There is no consensus about the optimal concentration of acetic acid to treat
22 colonised burn wounds in the way of antimicrobial efficacy and causing the least
23 pain to the patient. The trial should be a strong evidence to answer this question
24 being a randomised, controlled, double blinded trial.
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30 2. This trial will contribute to a better understanding of the role of acetic acid as a
31 feasible, topical, antibacterial agent for burn wounds colonized with *Pseudomonas*
32 *spp.* and other microorganisms.
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37 3. Although acetic acid has been used for a long duration in burns practice, this
38 trial is the first of its kind as a double blinded, randomised control trial comparing
39 two strengths of acetic acid regarding antimicrobial efficacy and tolerability.
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44 4. The results of this trial will be employed to develop a dressing carrier with
45 acetic acid as the active antibacterial agent.
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49 5. Difficulties in recruitment are anticipated as the number of patients with
50 colonised burn wounds who are expected to stay as inpatients for the trial duration
51 and not undergoing for surgical intervention may be limited.
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Introduction

Burns wound infection (BWI) is a serious complication following burn injury. It is reported that BWI accounts approximately 9%-17% of all burn injury related complications (1)(2). Pruitt *et al.* reported that invasive wound infection represents 5% of all the infections that occurred in patients admitted with severe burn injuries(3). It is very concerning that morbidity and mortality of burn patients are highly correlated to the incidence of wound infection and its sequelae (4)(5). Other complications include delayed wound healing, poor scarring. Hence, the medical community aim to effectively manage BWI to improve patient's prognosis.

Invasive BWI are classically treated with systemic antibiotics. However, excessive use of antibiotics has been associated with antimicrobial resistance. Alternative antimicrobial regimes are currently needed to minimise the antimicrobial resistance, as per WHO recommendations (6). A wide range of topical treatments to manage BWI are available. This includes but not limited to, silver nitrate, povidone-iodine and acetic acid. To date, there is no consensus in regards to effectiveness and efficiency of various topical management regimes for BWI (7).

Ideal topical regime for treating BWI needs to have potent antimicrobial properties, readily available and cost effective. One such treatment is topical acetic acid (vinegar). The antimicrobial properties of acetic acid (AA) have been well-known for centuries. AA is included on the WHO list of essential medicines published in 2019, a list of the safest and most effective medicines (7).

AA has been used a topical agent in burn care for decades (8)(9). It has been used for wound management in WW1 when Taylor observed the elimination of *Bacillus Pyocyaneus* upon using 1% acetic acid(AA) solution (10). It has been shown to be effective against Multi Drug Resistant organisms (MDRO) and biofilms (11)(12)

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7 Since then, a number of studies has been conducted to assess the effectiveness and
8 efficacy of acetic acid in management of BWI(13)(14).
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11 *In vitro* studies, diluted AA (1-5%) has shown potential to reduce or eradicate
12 bacterial load, specifically *Pseudomonas aeruginosa* (15)(16). Minimum inhibitory
13 concentrations (MIC) of acetic acid has been studied in vitro both before and after
14 evaporation and exposure to gauze. These showed that the methicillin-susceptible
15 strain of *Staphylococcus aureus* (MSSA) had an MIC of 0.312% and a methicillin-
16 resistant strain (MRSA) was less susceptible to MIC of 0.625%. Strains of
17 *Acinetobacter baumannii* also had a MIC of 0.312% and all strains of
18 *Pseudomonas aeruginosa* were susceptible to MIC of 0.166%(11).
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30 Different concentrations of acetic acid has been studied to treat BWI (17)(18)(19).
31 Patient's tolerability to topical agent is also very important. Patients usually
32 complain of stinging and pain on application of acetic acid to wounds, particularly
33 at higher concentrations, e.g. strength of 5% or more (13)(14). In another study 3%
34 concentration was used with better pain tolerance and less itching (16). In a
35 recently published survey of burn centres in UK, 6 centres (32%), routinely use
36 AA topically as gauze soaked with 2.5-3.0%. This high concentration was reported
37 to be well tolerated (20).
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46 Despite of all the previous studies, question still remains to find a good balance
47 between AA concentration which has efficient antimicrobial activity, low toxicity
48 and better patient's tolerability. Hence, A randomised controlled clinical trial is
49 warranted to answer all these questions.
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Methods and analysis

Trial design

The is a double-blinded, prospective, controlled, and randomised pilot trial, where 20 patients will be randomised to receive treatment with either 0.5% or 2% acetic acid (10 patients in each treatment arm) to treat the bacterial load in colonised burn wounds. The burn wounds will be required to be colonised with a specifically identifiable bacteria (Table 1), this wound will then be treated twice daily with acetic acid dressings for 2 consecutive days then once on the third day of treatment. Initially, the trial treatment period was 5 days, with twice a day dressing change on day 1,2,4,5 and once a day dressing changes on day 3. However, due to the COVID-19 pandemic and the changes in the standard care pathways, the treatment period was reduced to 3 days to shorten the period of hospital stay. Now patient will get twice a day dressing change on day1,2 and once a day on day3. On day 1 and 2, the trial focuses on comparing the effectiveness of acetic acid 0.5% and acetic acid 2% in reducing bacterial load and evaluating patient's tolerability to justify a larger scale, randomised, controlled trial. The anticipated sample size is small as this is a pilot trial with no placebo arm. The trial will be double blinded to minimise bias in the assessment of the outcome measures.

The acetic acid concentrations chosen in this trial (0.5% and 2%) were selected based on the in vitro findings of Halstead *et al.* showing efficacy of acetic acid at lower dilutions than previously thought or used in clinical practice (21)(22).

This is a single site trial, in which the cohort will be generated from patients admitted to the Burns Centre or Critical Care Unit at the Queen Elizabeth Hospital

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3 Birmingham, United Kingdom (QEHB) with a colonised burn wound. The target
4 population is patients with burns of $\geq 1\%$ TBSA.
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9 Table 1: BWI Identifiable Bacteria
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|----------------------------|
| 11 Pseudomonas aeruginosa |
| 12 Acinetobacter baumannii |
| 13 Escherichia coli |
| 14 Proteus mirabilis |
| 15 Staphylococcus aureus |
| 16 Klebisella Pneumoniae |
| 17 ESBL E. coli |
| 18 Enterobacter cloacae |

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31 Conduct of Trial

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35 Inclusion and Exclusion criteria:

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39 The trial will aim to recruit adult patients (age ≥ 16 years old) who sustained a $\geq 1\%$
40 TBSA burn injury. Prior to enrolment, the targeted burn wound has to be colonised
41 with specifically identifiable bacteria. The recruited patients are anticipated to
42 remain as inpatients for the trial duration (3 days).
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46 The study design is very simple with very broad inclusive criteria so the results can
47 have worldwide potential impact. Only patients with severe burn injury or burn
48 which require surgery will be excluded. The reason for choosing $>1\%$ TBSA burn
49 is potentially the patient will have inpatient care. There was no contraindication to
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include pregnant patients or who are breast feeding as the IP has no systemic effects.

Patients with burn TBSA <1% and burns solely to the face and/or genital area will be excluded in addition to patients who have received acetic acid as part of standard therapy upon admission for this burn injury (Table 2). Recruitment eligibility and unsuitability will be checked by the trial investigators.

Table 2: Summary of inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|--|-------------------------------------|
| Age \geq 16 years | Paediatric patients |
| \geq 1% TBSA burn injury | <1% TBSA burn injury |
| Colonised wound with a specifically identifiable bacterium | Burns to face or genitalia |
| Anticipated hospital stay for at least for 3 days | Previous treatment with acetic acid |

Screening and consenting:

Patients who meet eligibility criteria will be approached by a member of the research team and asked whether they would be willing for an additional microbiology swab to be alongside their routine swab for analysis, to confirm eligibility. If the patient is unconscious, then a Personal Legal Representative or Professional Legal Representative will be approached on behalf of the patient to request the swab be taken. If the appropriate patient or legal representative agrees to this, they will be asked to sign the trial consent form for retrieval of this swab.

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3 Once a positive microbiology test result has come back and the patient is found to
4 be eligible according to the inclusion and exclusion criteria, the patient or their
5 legal representative will be approached by a member of the research team for
6 enrolment treatment with acetic acid dressings.
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11 12 13 **Randomisation and treatment allocation** 14

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17 Randomisation and treatment will only begin once eligibility is confirmed by a
18 positive microbiology test to take part in the trial. Patients will be randomised to a
19 treatment arm in order to start the morning treatment procedure of day 1 within 48
20 hours of the positive microbiology result.
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27 Patients will be randomised to one of two treatment groups:

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29 GROUP 1: 0.5% acetic acid (10 patients)

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31 GROUP 2: 2% acetic acid (10 patients)
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36 Enrolment and randomisation of the patient will be completed on the electronic the
37 Clinical Research Tool (CREST) system (an in-house bespoke, electronic Remote
38 Data Capture system (eRDC) developed by QEHB), on which patients' trial
39 number and treatment pack numbers will be allocated and randomisation
40 confirmation emails will be sent to relevant trial team members. The CREST
41 system is also trial database system.
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53 **Blinding:** 54 55 56 57

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3 The treatment group allocation will be concealed from the patient and clinical staff
4 throughout the trial period. Each treatment box will be numbered according to a
5 randomisation list generated on a randomisation wizard by biostatistician. The
6 randomisation list will be provided to the manufacturer, Stockport
7 Pharmaceuticals, who will in turn package and label the treatment boxes.
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15 **Un-blinding process:**

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17 As both arms of the trial are the same active treatment, in cases of emergency they
18 will likely be dealt in the same way. As a result, there is unlikely to be a
19 requirement for emergency un-blinding. If the need does arise, the local pharmacy
20 will hold the randomisation list that can provide details of what treatment each
21 patient has been randomised to. If un-blinding needs to be carried out then a full
22 record of the procedure will need to be recorded and maintained i.e. reasons for un-
23 blinding, by whom etc.
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33 **Trial intervention:**

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35 The burn wounds will be required to be colonised with a specifically identifiable
36 bacteria, this wound will then be treated twice daily for the first two days with
37 acetic acid dressings. This will allow determination of whether the acetic acid is
38 still active after 12 hours of being in contact with a colonised burn wound. In order
39 to ascertain if the acetic acid is still effective after 24 hours the dressing will be
40 changed once on day 3. The antimicrobial activity of acetic acid extracted from the
41 dressings, will be conducted by determining its minimum inhibitory concentration
42 (MIC) (7).
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51 A microbiological swab of the burn wound will be collected once daily during each
52 morning dressing change and sent to the microbiology lab where it will be
53 analysed to determine the microbial load.
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3 The swabbing procedure will be carried out prior to burn wound cleaning. The
4 10x10cm² blue gauze will be removed from the burn wound and over this area a
5 swab moistened in normal saline will be applied and whilst twisting the tip the area
6 will be swept from left to right at 1cm intervals. The swab will be transferred to a
7 neutralising agent (containing 30g/L Tween 80, 3g/L lecithin, 1g/L histidine, 5g/L
8 sodium thiosulphate, 8.5g/L sodium chloride and 1g/L tryptone) to nullify carry
9 over of antimicrobial activity. Serial dilutions using diluent containing 8.5g/L
10 sodium chloride and 1g/L tryptone will be made, and number of CFU/mL
11 determined.
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22 The trial will assess tolerability of the two different strengths of acetic acid, by
23 assessing the patient's pain score at time of application of acetic acid and in the
24 following hour, using the Visual Analogue Scale (VAS). Please find the Trial
25 Schema (Figure 1) and Schedule of Events (Figure 2).
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37 **AE reporting and analysis**

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39 The reporting period for Adverse Events (AEs) starts from the time of application
40 of the first dressing and continues until the day 3 dressing has been removed on
41 day 4. Before COVID-19 pandemic, the reporting period for AEs started from the
42 time of application of the first dressing and continued until the day 5 dressing had
43 been removed on day 6. All Serious Adverse Events (SAEs) and adverse reactions
44 will be evaluated by the investigators and recorded. The National Cancer
45 Institute's common terminology criteria for AEs (CTCAE, V.4.02, 2010) will be
46 used to grade each AE. The coordinating trial office (CRCTU, Birmingham) will
47 keep detailed records of all AEs reported (nature, onset, duration, severity,
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outcome) and perform an evaluation with respect to severity, causality and expectedness.

Data handling, quality assurance, record keeping and retention

The trial sponsor (UHBFT) and patients are recruited from one of the sponsors hospitals (QEHB). The sponsor worked in collaboration with CRCTU, University of Birmingham (UoB), Some Sponsor responsibilities were delegated to the clinical trials unit the division of key responsibilities is detailed in (Table 3).

Table 3: Summary of responsibilities

| Responsibility | Institution |
|---|-------------|
| Provision of Investigational Medicinal Medicine (IMP) – Acetic Acid | UHBFT |
| Provision of Electronic Remote Data Capture System (eRDC-CREST) | UHBFT |
| On site monitoring | UHBFT |
| Regulatory submission, Trial Management, Data Storage & Analysis, | CRCTU |

The sponsor and CRCTU are fully compliant with the Data Protection Act 1998. Applicable regulations and laws associated with testing and development of Investigational Medicinal Products (IMPS) for human use and Good Clinical Practice (GCP). The sponsor is responsible for monitoring the trial. Confidentiality will be maintained throughout the trial and thereafter. On completion of the trial,

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3 data will be transferred to a secure archiving facility at the UoB, where data will be
4 held for a minimum of 15 years and then destroyed.
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8 9 **Case report forms**

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12 Case Report Forms (CRFs) included medical history and concomitant medications
13 in the trial's electronic database, CREST. Other CRFs incorporated in the
14 electronic database included: pain scores and burn injury examinations recorded
15 from day 1 through to day3; microbiology results; AE reporting and end of
16 treatment forms. The data will be collected on paper CRFs as well as electronic
17 remote data capture eRDC. CREST was not available at the beginning of the study.
18 This data was then later transcribed to CREST when it was available.
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29 **Statistical justification and outcome analysis**

30 **Sample size and justification:**

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35 This trial is not powered to the primary endpoint. Rather, the sample size for
36 AceticA has been selected based on what is feasible to be recruited at a single
37 centre in a reasonable timeframe for this phase of clinical trial. It was thought that
38 10 evaluable patients per treatment arm, analysed using repeated measures
39 methods, would provide the information needed to inform a larger, randomised
40 controlled trial.
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48 **Outcome measures and statistical analysis**

49 **Primary outcome**

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52 Efficacy will be assessed by measuring the bacterial load from microbiology
53 wound swabs, these will be taken daily from recruitment for 3 consecutive days.
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Secondary outcomes

Secondary outcome measures include; (1) the antimicrobial activity of acetic acid will be measured by extracting fluid from removed burns dressings and assessing the minimum inhibitory concentrations (MIC) to establish if active acetic acid is still present; (2) tolerance will be assessed by measuring a patient's pain scores with a VAS if the patient has capacity to provide scores; (3) time to 95% wound healing of the treated area of interest will be obtained from patients' records; (4) perceived treatment allocation will be assessed by asking patients after treatment completion which treatment they believed they received if they have capacity to do so.

Analysis of outcome measures

Full details will be specified in the Statistical Analysis Plan; however, an outline is given here. Analysis will use all patients whom are deemed to be evaluable.

Patients are evaluable if all 5 dressing changes from the first 3 days of treatment are completed. Patients will be analysed in the groups that reflect the treatment they actually received. This is because the samples size is small, and the aim of a pilot trial is to inform a subsequent pivotal trial. Where frequentist tests are used, and unless specified otherwise, a significance level of 5% will be used to designate significance.

It is possible that many wounds could be assessed within patients. If this happens, hierarchical models will be used for wound specific outcome measures to reflect the wound-within-patient structure of the data.

Analysis of Primary Outcome Measure

Efficacy:

Burn wound swabs will be taken periodically from baseline and bacterial load will be quantified by microbiology as the number of colonies forming units. To maximise information, repeated measures methods will be used. The model assuming fixed effects for the mean bacterial load, the mean change in bacterial load from baseline, and the additional mean change that is associated with receiving 2% instead of 0.5% acetic acid, with random effects adjusting for the mean bacterial load at baseline for each patient, will be compared to the analogous model without the adjustment for treatment received. A likelihood-ratio test of the nested models would yield inference on the treatment effects through time. As detailed above, hierarchical structures will be considered as necessitated by the observed data.

The dependent variable could be extremely fat-tailed so appropriate transforms (e.g. log) will be considered. This model could also be re-specified to use fewer parameters if load is found to be well approximated by a smooth function of time, potentially using transformations or restricted cubic splines. In this case, equivalent adaptations would be made to each model so that the method of testing nested models to isolate treatment effect is valid.

The parameters in the full model will be reported with means and standard errors.

There may be particular interest in the presence or absence of a particular set of bacteria. If this is the case, these will be identified in the statistical analysis plan. The expectation is that the lower concentration will be non-inferior in terms of efficacy. This suggests a one-tailed comparison.

Analysis of Secondary Outcome Measures

Tolerability:

Pain scores will be collected at many points throughout the trial via the verbal pain intensity scale. Zor *et al.*(23) collect scores in a similar manner and analyse them as numerical data, i.e. they assume the scores to reflect order and scale. We initially propose to also analyse the pain scores as numerical variables. Explanatory variables will be included to reflect treatment allocation and we will present evidence on the extent to which reported pain is associated with treatment allocation.

The assumption that pain scores are numerical and not ordinal is potentially controversial. Supporting analysis may be provided that treats the scores as ordinal levels. Hierarchical structure similar to that described in the primary outcome would be used. Provision of this analysis is at least partially contingent on patients in the overwhelming majority of cases using the provided levels and not providing scores between levels. For instance, if patients frequently score pain experienced as 3.5 to convey “between 3 and 4”, then that would diminish the suitability of the described ordinal variable analysis. In that case, a re-codification of the ordinal levels, or reliance only on the analysis of the continuous data could be indicated.

Antimicrobial activity:

The antimicrobial activity of acetic acid will be measured by extracting fluid from removed burns dressings. The minimum inhibitory concentrations (MIC) will be estimated by successively halving the concentration of retrieved acid and testing whether microbial growth occurs. MICs could be modelled as numerical (after appropriate transform) and/or ordinal data. Furthermore, group structure could be

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3 required as described above. Details of this are given in the Statistical Analysis
4 Plan (SAP).
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8 **Time to 95% wound healing:**

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10 Time from randomisation to 95% wound healing of the treated area will be
11 presented using reverse Kaplan-Meier curves. In presentation of these curves,
12 patients will be appropriately censored at the point they withdraw from or
13 complete the trial. Presentation of these curves will account for the nested data
14 structure as necessary.
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21 The time to wound healing will be assessed using hierarchical (also referred to as
22 multi-level) cox models. The hierarchical structure will be included, if necessary,
23 to reflect the nesting of wound through patient.
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28 **Perceived treatment allocation:**

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30 After completion of protocol treatment, patients will be asked to identify the
31 treatment arm to which they believe they were randomised. For each patient,
32 identification of treatment arm will either be: correct; incorrect; or missing. Counts
33 will be reported by arm. Association of treatment arm and identification of
34 treatment arm could be assessed by chi-squared test.
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42 **End of trial**

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44 The end of trial will be the date of completion of treatment for the last patient. The
45 Trials Office will notify the MHRA and REC that the trial has ended and will
46 provide them with a summary of the clinical trial report within 12 months of the
47 end of trial.
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54 **Patient and Public Involvement**

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3 Development of the research question, outcome measures and trial design were
4 informed by meetings held with the Trial Steering Committee, which included a
5 Patient and Public Involvement (PPI) Representative. The PPI reviewed the trial
6 documentations and considered the overall burden of trial participation during the
7 design process specifically the practicality of twice daily dressing of an infected
8 burn wound.
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16 **Trial Status**

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18 Recruitment into the AceticA trial began in February 2018 and it is still open to
19 recruitment.
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23 **Confidentiality and Data Protection**

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26 Personal data recorded on all documents will be regarded as strictly confidential
27 and will be handled and stored in accordance with the General Data Protection
28 Regulation and Data Protection Act 2018. UHB NHSFT, as the sponsor for the
29 AceticA trial, will be using information from patient medical records in order to
30 undertake this trial and will act as the data controller for the study. The computers
31 on this network have restricted physical access; data are stored under coded
32 filenames and the local network has secure password access restricted to a limited
33 set of people.
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43 Anonymised data will be provided to UoB for data analysis and will only be
44 accessible by authorised personnel. All AceticA participants provided specific
45 written consent at trial entry to enable data with UoB. Otherwise, confidentiality
46 was maintained throughout the trial and thereafter.
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3 All the complied and analysed results will be presented at national and
4 international conferences concerning. Results will also be submitted for peer
5 review and publication in the subject journals/literature.
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10 The trial results will be reported and submitted for publication in peer-reviewed
11 journals and presentation at appropriate national and international academic
12 meetings. Trial participants will be sent a summary of the final results, including
13 references to full papers. Trial data may be made available to external groups
14 wishing to undertake original analysis, subject to approval from the Trial
15 Management Group.
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26 **Discussion**

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28 BWI is correlated with higher mortality and morbidity following burn injury
29 (4).Hence, one of the most important aims in burns wound management is to
30 prevent infection to prevent invasive burn wound infection and sepsis. Multiple
31 studies has shown effectiveness of Acetic acid in managing BWI (8)(14)(10). It is
32 also known as an effective agent against biofilm producing microorganisms which
33 are notoriously difficult to decolonise, due to limited anti-microbial penetration
34 and deactivation by biofilm matrix(22). In addition, as a weak acid with a pKa
35 close to its pH, acetic acid can kill bacteria without being toxic to human cells, a
36 key consideration in wound healing(24). It is also very cost effective and easily
37 available agent(14). But there is not enough data to standardise its usage like
38 effective strength, frequency, duration of treatment.
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3 This is a prospective interventional controlled trial with a very simple trial design.
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5 The results will have worldwide impact because of its generic inclusion criteria.
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7 This study will show the outcome in BWI treated with two different strengths of
8
9 Acetic acid. 0.5 and 2%. It will help in establishing a balance between the effective
10
11 strength of Acetic acid against BWI, frequency and duration of treatment and
12
13 patient's tolerability. As Acetic acid is very cost effective and easily available
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15 agent. The results of the study will have effects in both developed and developing
16
17 countries with limited medical resources. This trial has a potential to have
18
19 significant impact on development of future studies on burns wound management
20
21 and treatment of burn wound infection by incorporating acetic acid in a dressing
22
23 carrier as the active antibacterial agent.
24

25 26 27 **Acknowledgements**

28
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40
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44

45 **Conflict of Interests:**

46
47
48 The authors have no conflict of interests to declare.
49

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1
2
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12

13 **Author Contributions**

14

15
16 The concept of the AceticA trial was developed by NM (Chief investigator) and
17 BO, TH, RI, KP(Co-investigator). NM, KB, DB designed/developed the initial
18 AceticA trial protocol. NM, DB, VH, GS developed, wrote, reviewed subsequent
19 protocol versions. AC designed the proforma used for pre-screening,
20 microbiological lab manual and protocol procedure documentations. GS, DB, TH
21 submitted all REC, MHRA and local R&D applications. KB and VH devised the
22 statistical plan. GS, DB, TH and AC wrote/designed the patient information sheets,
23 external trial information and patient CRFs. RI and TH wrote the manuscript and
24 all authors reviewed the final version. All authors are guarantors.
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35 **Ethics and dissemination**

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37
38 This trial has been approved by the Medicines and Healthcare products Regulatory
39 Agency on 22 November 2017. CTA reference number - 16719/0232/001-0001
40 and National Research Ethics Service (West Midlands - Edgbaston Research
41 Ethics Committee on 21 December 2017 – REC 17/WM/0407; IRAS 234132.
42
43 In addition, the trial has been registered on the publicly available ISRCTN registry
44 (ISRCTN11636684).
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51 The analysed results will be presented at national and international conferences
52 related to management of burn patients. The generated articles based on the trial
53 results will be submitted to peer review journals for publication.
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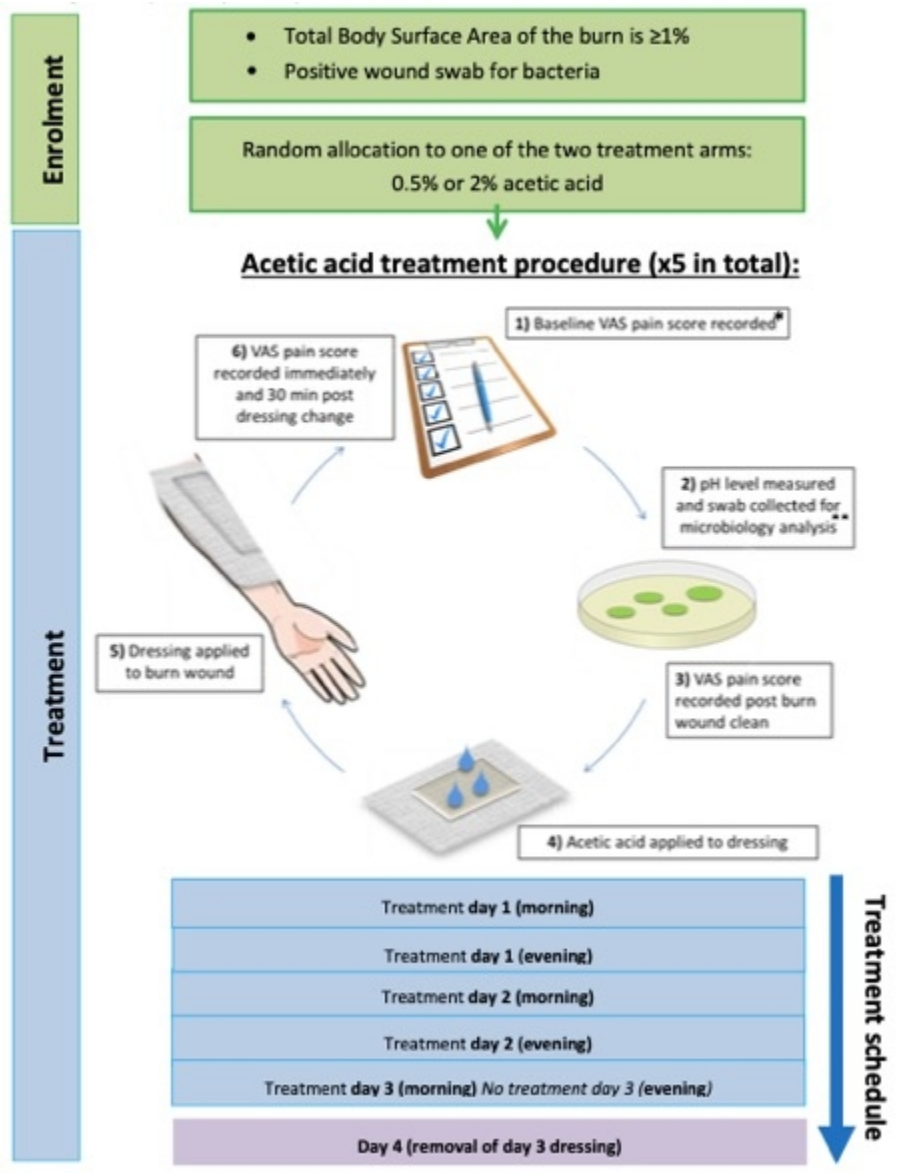
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For peer review only

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159x206mm (72 x 72 DPI)

| | Burns Centre | | Burns Centre | | | | | |
|---|------------------------------|--|--------------|----|-------|----|---------------|---------------|
| | Upon admission to burns ward | Day 0: up to 48 hours prior to Day 1 AM treatment (following positive microbiology swab results) | Day 1 | | Day 2 | | Day 3 AM only | Day 4 AM only |
| | | | AM | PM | AM | PM | | |
| Screening (to include TBSA calculation) | x | | | | | | | |
| Consent | x | | | | | | | |
| Randomisation | | x | | | | | | |
| AZ/SAE collection | | | x | | x | | x | |
| Vital signs ¹ | | | x | x | x | x | x | |
| Burn wound examination | | | x | x | x | x | x | |
| Bacterial load swab | x ² | | x | | x | | x | x |
| Burn wound surface pH | | | x | x | x | x | x | |
| Visual Analogue Scale ³ | | | x | x | x | x | x | |
| IMP application; Dressing Change | | | x | x | x | x | x | |
| Gauze fluid sample collection | | | x | x | x | x | x | x |
| Dressing Removal | | | | x | x | x | x | x |

181x103mm (72 x 72 DPI)

BMJ Open

The efficacy and optimal dose of Acetic Acid to treat colonised burns wounds: protocol for a pilot randomized controlled trial

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| | |

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Title

The efficacy and optimal dose of Acetic Acid to treat colonised burns wounds:
protocol for a pilot randomized controlled trial

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22 **Keywords**

23
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25 Burns; Acetic acid; randomised control trial; colonised wound; infected wound;
26 pain
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33 **Word Count**

34
35 4335 words
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Abstract

Introduction and Article Summary

Despite of recent advancement in the burns wound management, burn wound infection (BWI) is still one of the major cause of burns mortality. Patients who survive their burns injury still suffers from BWI related complication like delayed wound healing and poor scarring. BWI has been treated by application of topical antimicrobial agents or systemic antibiotics. Due to the global risk of developing systemic antibiotics resistance, medical research focuses on identifying single topical agent which has effective antimicrobial activity, easily available and cost effective. One such agent is Acetic acid (AA). AA has been used as a topical antibacterial agent for the treatment of burns wounds for many years and has shown to have activity against Gram-negative organisms including *Pseudomonas aeruginosa*. So far there has been no consensus on optimal concentration that's has effective antimicrobial activity, frequency of application, duration of treatment and most importantly good patient's tolerability. A randomised control study is required to answer all these questions.

Objective

To investigate the efficacy and tolerability of 0.5% and 2% of acetic acid when applied to colonised burns wounds for 3 days after admittance to the Queen Elizabeth Hospital Birmingham.

Methods and analysis

This is a double-blinded, prospective, randomised, controlled, single-centre trial. Patients will be screened for eligibility in the inpatient area and those who are found to be eligible will be randomly assigned to one of two treatment groups:

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3 GROUP 1: 0.5% acetic acid (10 patients)
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6 GROUP 2: 2% acetic acid (10 patients)
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9 Total Number: 20 patients
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11 The two concentration strengths of acetic acid will be assessed for the
12 antimicrobial activity and pain tolerability. Antimicrobial activity will be assessed
13 using repeated measures methods to analyse the number of colony forming units.
14
15

16 17 18 **Ethics and dissemination**

19 AceticA trial protocol was approved by the National Research Ethics Service
20 (West Midlands - Edgbaston Research Ethics Committee; 17/WM/0407; IRAS
21 234132).
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28 The analysed results will be presented at national and international conferences
29 related to management of burn patients. The generated articles based on the trial
30 results will be submitted to peer review journals for publication.
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35 **Article Summary**

36 37 **Strengths and limitations of this trial**

- 38
39 • It is a double blinded, randomised control trial comparing two different
40 strengths of Acetic acid.
- 41
42 • The study design is very simple and feasible for patients with the trial
43 duration of only 3 days with no extra outpatient follow ups.
- 44
45 • Two different strengths of acetic acid will be used to compare the
46 antimicrobial efficacy, tolerability and feasibility in patients.
- 47
48 • The study does not interfere with the standard burns care of the patient.
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- Difficulties in recruitment are anticipated as the number of patients with colonised burn wounds who are expected to stay as inpatients for the trial duration and not undergoing for surgical intervention may be limited.

For peer review only

Background

Burns wound infection (BWI) is a serious complication following burn injury. It is reported that BWI accounts approximately 9%-17% of all burn injury related complications (1)(2). Pruitt *et al.* reported that invasive wound infection represents 5% of all the infections that occurred in patients admitted with severe burn injuries(3). It is very concerning that morbidity and mortality of burn patients are highly correlated to the incidence of wound infection and its sequelae (4)(5). Other complications include delayed wound healing, poor scarring. Hence, the medical community aim to effectively manage BWI to improve patient's prognosis.

Invasive BWI are classically treated with systemic antibiotics. However, excessive use of antibiotics has been associated with antimicrobial resistance. Alternative antimicrobial regimes are currently needed to minimise the antimicrobial resistance, as per WHO recommendations (6). A wide range of topical treatments to manage BWI are available. This includes but not limited to, silver nitrate, povidone-iodine and acetic acid. To date, there is no consensus in regards to effectiveness and efficiency of various topical management regimes for BWI (7).

Ideal topical regime for treating BWI needs to have potent antimicrobial properties, readily available and cost effective. One such treatment is topical acetic acid (vinegar). The antimicrobial properties of acetic acid (AA) have been well-known for centuries. AA is included on the WHO list of essential medicines published in 2019, a list of the safest and most effective medicines (7).

AA has been used a topical agent in burn care for decades (8)(9). It has been used for wound management in WW1 when Taylor observed the elimination of *Bacillus Pyocyaneus* upon using 1% acetic acid(AA) solution (10). It has been shown to be effective against Multi Drug Resistant organisms (MDRO) and biofilms (11)(12)

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7 Since then, a number of studies has been conducted to assess the effectiveness and
8 efficacy of acetic acid in management of BWI(13)(14).
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11 *In vitro* studies, diluted AA (1-5%) has shown potential to reduce or eradicate
12 bacterial load, specifically *Pseudomonas aeruginosa* (15)(16). Minimum inhibitory
13 concentrations (MIC) of acetic acid has been studied in vitro both before and after
14 evaporation and exposure to gauze. These showed that the methicillin-susceptible
15 strain of *Staphylococcus aureus* (MSSA) had an MIC of 0.312% and a methicillin-
16 resistant strain (MRSA) was less susceptible to MIC of 0.625%. Strains of
17 *Acinetobacter baumannii* also had a MIC of 0.312% and all strains of
18 *Pseudomonas aeruginosa* were susceptible to MIC of 0.166%(11).
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30 Different concentrations of acetic acid has been studied to treat BWI (17)(18)(19).
31 Patient's tolerability to topical agent is also very important. Patients usually
32 complain of stinging and pain on application of acetic acid to wounds, particularly
33 at higher concentrations, e.g. strength of 5% or more (13)(14). In another study 3%
34 concentration was used with better pain tolerance and less itching (16). In a
35 recently published survey of burn centres in UK, 6 centres (32%), routinely use
36 AA topically as gauze soaked with 2.5-3.0%. This high concentration was reported
37 to be well tolerated (20).
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46 Despite of all the previous studies, question still remains to find a good balance
47 between AA concentration which has efficient antimicrobial activity, low toxicity
48 and better patient's tolerability. Hence, A randomised controlled clinical trial is
49 warranted to answer all these questions.
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Methods and analysis

Trial design

The is a double-blinded, prospective, controlled, and randomised pilot trial, where 20 patients will be randomised to receive treatment with either 0.5% or 2% acetic acid (10 patients in each treatment arm) to treat the bacterial load in colonised burn wounds. The burn wounds will be required to be colonised with a specifically identifiable bacteria (Table 1), this wound will then be treated twice daily with acetic acid dressings for 2 consecutive days then once on the third day of treatment. Initially, the trial treatment period was 5 days, with twice a day dressing change on day 1,2,4,5 and once a day dressing changes on day 3. However, due to the COVID-19 pandemic and the changes in the standard care pathways to minimise the exposure by reducing the inpatient stay, the treatment period was reduced to 3 days to avoid impact on recruitment process. This proposed change of the study design was also supported by interim analysis of 11 subjects as there was an increase in bacterial colonisation on day 4 am, compared to day 2 and 3. Now patient will get twice a day dressing change on day1,2 and once a day on day3. On day 1 and 2, the trial focuses on comparing the effectiveness of acetic acid 0.5% and acetic acid 2% in reducing bacterial load and evaluating patient's tolerability to justify a larger scale, randomised, controlled trial. The anticipated sample size is small as this is a pilot trial with no placebo arm. The trial will be double blinded to minimise bias in the assessment of the outcome measures.

The acetic acid concentrations chosen in this trial (0.5% and 2%) were selected based on the in vitro findings of Halstead *et al.* showing efficacy of acetic acid at lower dilutions than previously thought or used in clinical practice (21)(22).

This is a single site trial, in which the cohort will be generated from patients admitted to the Burns Centre or Critical Care Unit at the Queen Elizabeth Hospital Birmingham, United Kingdom (QEHB) with a colonised burn wound. The target population is patients with burns of $\geq 1\%$ TBSA. The expected start and end dates of the study are as follows (Table 2).

| | |
|-------------------------|------------------------------------|
| Pseudomonas aeruginosa | Table 1: BWI Identifiable Bacteria |
| Acinetobacter baumannii | |
| Escherichia coli | |
| Proteus mirabilis | |
| Staphylococcus aureus | |
| Klebisella Pneumoniae | |
| ESBL E. coli | |
| Enterobacter cloacae | |

Table 2: Start and End dates

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|-------------------------------------|--------------------------------|
| First site open | 20 th February 2018 |
| First patient recruited | 23 rd March 2018 |
| Last Patient Last Visit | 8 th October 2021 |
| End of Study Declaration submission | 5 th January 2022 |

Conduct of Trial

Inclusion and Exclusion criteria:

The trial will aim to recruit adult patients (age ≥ 16 years old) who sustained a $\geq 1\%$ TBSA burn injury. Prior to enrolment, the targeted burn wound has to be colonised with specifically identifiable bacteria. The recruited patients are anticipated to remain as inpatients for the trial duration (3 days).

The study design is very simple with very broad inclusive criteria so the results can have worldwide potential impact. Only patients with severe burn injury or burn which require surgery will be excluded. The reason for choosing $>1\%$ TBSA burn is potentially the patient will have inpatient care. There was no contraindication to include pregnant patients or who are breast feeding as the IP has no systemic effects.

Patients with burn TBSA $<1\%$ and burns solely to the face and/or genital area will be excluded in addition to patients who have received acetic acid as part of standard therapy upon admission for this burn injury (Table 3). Recruitment eligibility and unsuitability will be checked by the trial investigators.

Table 3: Summary of inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|--|----------------------------|
| Age ≥ 16 years | Paediatric patients |
| $\geq 1\%$ TBSA burn injury | $<1\%$ TBSA burn injury |
| Colonised wound with a specifically identifiable bacterium | Burns to face or genitalia |

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|--|-------------------------------------|
| Anticipated hospital stay for at least for 3 days | Previous treatment with acetic acid |
|--|-------------------------------------|

Screening and consenting:

Patients who meet eligibility criteria will be approached by a member of the research team and asked whether they would be willing for an additional microbiology swab to be alongside their routine swab for analysis, to confirm eligibility. If the patient is unconscious, then a Personal Legal Representative or Professional Legal Representative will be approached on behalf of the patient to request the swab be taken. If the appropriate patient or legal representative agrees to this, they will be asked to sign the trial consent form for retrieval of this swab. Once a positive microbiology test result has come back and the patient is found to be eligible according to the inclusion and exclusion criteria, the patient or their legal representative will be approached by a member of the research team for enrolment treatment with acetic acid dressings.

Randomisation and treatment allocation

Randomisation and treatment will only begin once eligibility is confirmed by a positive microbiology test to take part in the trial. Patients will be randomised to a treatment arm in order to start the morning treatment procedure of day 1 within 48 hours of the positive microbiology result.

Patients will be randomised to one of two treatment groups:

GROUP 1: 0.5% acetic acid (10 patients)

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3 GROUP 2: 2% acetic acid (10 patients)
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5 Total number: 20 patients
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7 Enrolment and randomisation of the patient will be completed on the electronic the
8 Clinical Research Tool (CREST) system (an in-house bespoke, electronic Remote
9 Data Capture system (eRDC) developed by QEHB), on which patients' trial
10 number and treatment pack numbers will be allocated and randomisation
11 confirmation emails will be sent to relevant trial team members. The CREST
12 system is also trial database system.
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25 **Blinding:**

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27 The treatment group allocation will be concealed from the patient and clinical staff
28 throughout the trial period. Each treatment box will be numbered according to a
29 randomisation list generated on a randomisation wizard by biostatistician. The
30 randomisation list will be provided to the manufacturer, Stockport
31 Pharmaceuticals, who will in turn package and label the treatment boxes.
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40 **Un-blinding process:**

41 As both arms of the trial are the same active treatment, in cases of emergency they
42 will likely be dealt in the same way. As a result, there is unlikely to be a
43 requirement for emergency un-blinding. If the need does arise, the local pharmacy
44 will hold the randomisation list that can provide details of what treatment each
45 patient has been randomised to. If un-blinding needs to be carried out then a full
46 record of the procedure will need to be recorded and maintained i.e. reasons for un-
47 blinding, by whom etc.
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Trial intervention:

The burn wounds will be required to be colonised with a specifically identifiable bacteria, this wound will then be treated twice daily for the first two days with acetic acid dressings. This will allow determination of whether the acetic acid is still active after 12 hours of being in contact with a colonised burn wound. In order to ascertain if the acetic acid is still effective after 24 hours the dressing will be changed once on day 3. The antimicrobial activity of acetic acid extracted from the dressings, will be conducted by determining its minimum inhibitory concentration (MIC) (7).

A microbiological swab of the burn wound will be collected once daily during each morning dressing change and sent to the microbiology lab where it will be analysed to determine the microbial load.

The swabbing procedure will be carried out prior to burn wound cleaning. The 10x10cm² blue gauze will be removed from the burn wound and over this area a swab moistened in normal saline will be applied and whilst twisting the tip the area will be swept from left to right at 1cm intervals. The swab will be transferred to a neutralising agent (containing 30g/L Tween 80, 3g/L lecithin, 1g/L histidine, 5g/L sodium thiosulphate, 8.5g/L sodium chloride and 1g/L tryptone) to nullify carry over of antimicrobial activity. Serial dilutions using diluent containing 8.5g/L sodium chloride and 1g/L tryptone will be made, and number of CFU/mL determined.

The trial will assess tolerability of the two different strengths of acetic acid, by assessing the patient's pain score at time of application of acetic acid and in the following hour, using the Visual Analogue Scale (VAS). Please find the Trial Schema (Supplementary file 1) and Schedule of Events (Supplementary file 2).

AE reporting and analysis

The reporting period for Adverse Events (AEs) starts from the time of application of the first dressing and continues until the day 3 dressing has been removed on day 4. Before COVID-19 pandemic, the reporting period for AEs started from the time of application of the first dressing and continued until the day 5 dressing had been removed on day 6. All Serious Adverse Events (SAEs) and adverse reactions will be evaluated by the investigators and recorded. The National Cancer Institute's common terminology criteria for AEs (CTCAE, V.4.02, 2010) will be used to grade each AE. The coordinating trial office (CRCTU, Birmingham) will keep detailed records of all AEs reported (nature, onset, duration, severity, outcome) and perform an evaluation with respect to severity, causality and expectedness.

Data handling, quality assurance, record keeping and retention

The trial sponsor (UHBFT) and patients are recruited from one of the sponsors hospitals (QEHB). The sponsor worked in collaboration with CRCTU, University of Birmingham (UoB), Some Sponsor responsibilities were delegated to the clinical trials unit the division of key responsibilities is detailed in (Table 4).

Table 4: Summary of responsibilities

| Responsibility | Institution |
|--|-------------|
| Provision of Investigational Medicinal Medicine (IMP) – Acetic Acid | UHBFT |

| | |
|---|-------|
| Provision of Electronic Remote Data Capture System (eRDC-CREST) | UHBFT |
| On site monitoring | UHBFT |
| Regulatory submission, Trial Management, Data Storage & Analysis, | CRCTU |

The sponsor and CRCTU are fully compliant with the Data Protection Act 1998. Applicable regulations and laws associated with testing and development of Investigational Medicinal Products (IMPS) for human use and Good Clinical Practice (GCP). The sponsor is responsible for monitoring the trial. Confidentiality will be maintained throughout the trial and thereafter. On completion of the trial, data will be transferred to a secure archiving facility at the UoB, where data will be held for a minimum of 15 years and then destroyed.

Case report forms

Case Report Forms (CRFs) included medical history and concomitant medications in the trial's electronic database, CREST. Other CRFs incorporated in the electronic database included: pain scores and burn injury examinations recorded from day 1 through to day3; microbiology results; AE reporting and end of treatment forms. The data will be collected on paper CRFs as well as electronic remote data capture eRDC. CREST was not available at the beginning of the study. This data was then later transcribed to CREST when it was available.

Statistical justification and outcome analysis

Sample size and justification:

This trial is not powered to the primary endpoint. Rather, the sample size for AceticA has been selected based on what is feasible to be recruited at a single centre in a reasonable timeframe for this phase of clinical trial. It was thought that 10 evaluable patients per treatment arm, analysed using repeated measures methods, would provide the information needed to inform a larger, randomised controlled trial.

Outcome measures and statistical analysis

Primary outcome

Efficacy will be assessed by measuring the bacterial load from microbiology wound swabs, these will be taken daily from recruitment for 3 consecutive days.

Secondary outcomes

Secondary outcome measures include; (1) the antimicrobial activity of acetic acid will be measured by extracting fluid from removed burns dressings and assessing the minimum inhibitory concentrations (MIC) to establish if active acetic acid is still present; (2) tolerance will be assessed by measuring a patient's pain scores with a VAS if the patient has capacity to provide scores; (3) time to 95% wound healing of the treated area of interest will be obtained from patients' records; (4) perceived treatment allocation will be assessed by asking patients after treatment completion which treatment they believed they received if they have capacity to do so.

Analysis of outcome measures

Full details will be specified in the Statistical Analysis Plan; however, an outline is given here. Analysis will use all patients whom are deemed to be evaluable.

Patients are evaluable if all 5 dressing changes from the first 3 days of treatment are completed. Patients will be analysed in the groups that reflect the treatment they actually received. This is because the samples size is small, and the aim of a pilot trial is to inform a subsequent pivotal trial. Where frequentist tests are used, and unless specified otherwise, a significance level of 5% will be used to designate significance.

It is possible that many wounds could be assessed within patients. If this happens, hierarchical models will be used for wound specific outcome measures to reflect the wound-within-patient structure of the data.

Analysis of Primary Outcome Measure

Efficacy:

Burn wound swabs will be taken periodically from baseline and bacterial load will be quantified by microbiology as the number of colonies forming units. To maximise information, repeated measures methods will be used. The model assuming fixed effects for the mean bacterial load, the mean change in bacterial load from baseline, and the additional mean change that is associated with receiving 2% instead of 0.5% acetic acid, with random effects adjusting for the mean bacterial load at baseline for each patient, will be compared to the analogous model without the adjustment for treatment received. A likelihood-ratio test of the nested models would yield inference on the treatment effects through time. As

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2
3 detailed above, hierarchical structures will be considered as necessitated by the
4 observed data.
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8 The dependent variable could be extremely fat-tailed so appropriate transforms
9 (e.g. log) will be considered. This model could also be re-specified to use fewer
10 parameters if load is found to be well approximated by a smooth function of time,
11 potentially using transformations or restricted cubic splines. In this case, equivalent
12 adaptations would be made to each model so that the method of testing nested
13 models to isolate treatment effect is valid.
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19 The parameters in the full model will be reported with means and standard errors.
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27 There may be particular interest in the presence or absence of a particular set of
28 bacteria. If this is the case, these will be identified in the statistical analysis plan.
29 The expectation is that the lower concentration will be non-inferior in terms of
30 efficacy. This suggests a one-tailed comparison.
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35 **Analysis of Secondary Outcome Measures**

36 **Tolerability:**

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39 Pain scores will be collected at many points throughout the trial via the verbal pain
40 intensity scale. Zor *et al.*(23) collect scores in a similar manner and analyse them as
41 numerical data, i.e. they assume the scores to reflect order and scale. We initially
42 propose to also analyse the pain scores as numerical variables. Explanatory
43 variables will be included to reflect treatment allocation and we will present
44 evidence on the extent to which reported pain is associated with treatment
45 allocation.
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3 The assumption that pain scores are numerical and not ordinal is potentially
4 controversial. Supporting analysis may be provided that treats the scores as ordinal
5 levels. Hierarchical structure similar to that described in the primary outcome
6 would be used. Provision of this analysis is at least partially contingent on patients
7 in the overwhelming majority of cases using the provided levels and not providing
8 scores between levels. For instance, if patients frequently score pain experienced as
9 3.5 to convey “between 3 and 4”, then that would diminish the suitability of the
10 described ordinal variable analysis. In that case, a re-codification of the ordinal
11 levels, or reliance only on the analysis of the continuous data could be indicated.
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22 **Antimicrobial activity:**

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24 The antimicrobial activity of acetic acid will be measured by extracting fluid from
25 removed burns dressings. The minimum inhibitory concentrations (MIC) will be
26 estimated by successively halving the concentration of retrieved acid and testing
27 whether microbial growth occurs. MICs could be modelled as numerical (after
28 appropriate transform) and/or ordinal data. Furthermore, group structure could be
29 required as described above. Details of this are given in the Statistical Analysis
30 Plan (SAP).
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40 **Time to 95% wound healing:**

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42 Time from randomisation to 95% wound healing of the treated area will be
43 presented using reverse Kaplan-Meier curves. In presentation of these curves,
44 patients will be appropriately censored at the point they withdraw from or
45 complete the trial. Presentation of these curves will account for the nested data
46 structure as necessary.
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3 The time to wound healing will be assessed using hierarchical (also referred to as
4 multi-level) cox models. The hierarchical structure will be included, if necessary,
5 to reflect the nesting of wound through patient.
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9 10 **Perceived treatment allocation:**

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12 After completion of protocol treatment, patients will be asked to identify the
13 treatment arm to which they believe they were randomised. For each patient,
14 identification of treatment arm will either be: correct; incorrect; or missing. Counts
15 will be reported by arm. Association of treatment arm and identification of
16 treatment arm could be assessed by chi-squared test.
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23 24 **End of trial**

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26 The end of trial will be the date of completion of treatment for the last patient. The
27 Trials Office will notify the MHRA and REC that the trial has ended and will
28 provide them with a summary of the clinical trial report within 12 months of the
29 end of trial.
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35 36 **Patient and Public Involvement**

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38 Development of the research question, outcome measures and trial design were
39 informed by meetings held with the Trial Steering Committee, which included a
40 Patient and Public Involvement (PPI) Representative. The PPI reviewed the trial
41 documentations and considered the overall burden of trial participation during the
42 design process specifically the practicality of twice daily dressing of an infected
43 burn wound.
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51 52 **Trial Status**

53 Recruitment into the AceticA trial began in February 2018 and it is still open to
54 recruitment.
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Confidentiality and Data Protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation and Data Protection Act 2018. UHB NHSFT, as the sponsor for the AceticA trial, will be using information from patient medical records in order to undertake this trial and will act as the data controller for the study. The computers on this network have restricted physical access; data are stored under coded filenames and the local network has secure password access restricted to a limited set of people.

Anonymised data will be provided to UoB for data analysis and will only be accessible by authorised personnel. All AceticA participants provided specific written consent at trial entry to enable data with UoB. Otherwise, confidentiality was maintained throughout the trial and thereafter.

All the complied and analysed results will be presented at national and international conferences concerning. Results will also be submitted for peer review and publication in the subject journals/literature.

The trial results will be reported and submitted for publication in peer-reviewed journals and presentation at appropriate national and international academic meetings. Trial participants will be sent a summary of the final results, including references to full papers. Trial data may be made available to external groups wishing to undertake original analysis, subject to approval from the Trial Management Group.

Discussion

BWI is correlated with higher mortality and morbidity following burn injury (4). Hence, one of the most important aims in burns wound management is to prevent infection to prevent invasive burn wound infection and sepsis. Multiple studies has shown effectiveness of Acetic acid in managing BWI (8)(14)(10). It is also known as an effective agent against biofilm producing microorganisms which are notoriously difficult to decolonise, due to limited anti-microbial penetration and deactivation by biofilm matrix(22). In addition, as a weak acid with a pKa close to its pH, acetic acid can kill bacteria without being toxic to human cells, a key consideration in wound healing(24). It is also very cost effective and easily available agent(14). But there is not enough data to standardise its usage like effective strength, frequency, duration of treatment.

This is a prospective interventional controlled trial with a very simple trial design. The results will have worldwide impact because of its generic inclusion criteria. This study will show the outcome in BWI treated with two different strengths of Acetic acid. 0.5 and 2%. It will help in establishing a balance between the effective strength of Acetic acid against BWI, frequency and duration of treatment and patient's tolerability. As Acetic acid is very cost effective and easily available agent. The results of the study will have effects in both developed and developing countries with limited medical resources. This trial has a potential to have significant impact on development of future studies on burns wound management and treatment of burn wound infection by incorporating acetic acid in a dressing carrier as the active antibacterial agent.

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1
2
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19 **Conflict of Interests:**

20 The authors have no conflict of interests to declare.
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27 Microbiology Research Centre (NIHR SRMRC).
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36

37 **Author Contributions**

38 The concept of the Acetica trial was developed by NM (Chief investigator) and
39 BO, TH, RI, KP, KA (Co-investigator). NM, KB, DB designed/developed the
40 initial Acetica trial protocol. NM, DB, VH, GS developed, wrote, reviewed
41 subsequent protocol versions. AC designed the proforma used for pre-screening,
42 microbiological lab manual and protocol procedure documentations. GS, DB, TH
43 submitted all REC, MHRA and local R&D applications. KB and VH devised the
44 statistical plan. GS, DB, TH and AC wrote/designed the patient information sheets,
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external trial information and patient CRFs. RI and TH wrote the manuscript and all authors reviewed the final version. All authors are guarantors.

Ethics and dissemination

This trial has been approved by the Medicines and Healthcare products Regulatory Agency on 22 November 2017. CTA reference number - 16719/0232/001-0001 and National Research Ethics Service (West Midlands - Edgbaston Research Ethics Committee on 21 December 2017 – REC 17/WM/0407; IRAS 234132. In addition, the trial has been registered on the publicly available ISRCTN registry (ISRCTN11636684).

The analysed results will be presented at national and international conferences related to management of burn patients. The generated articles based on the trial results will be submitted to peer review journals for publication.

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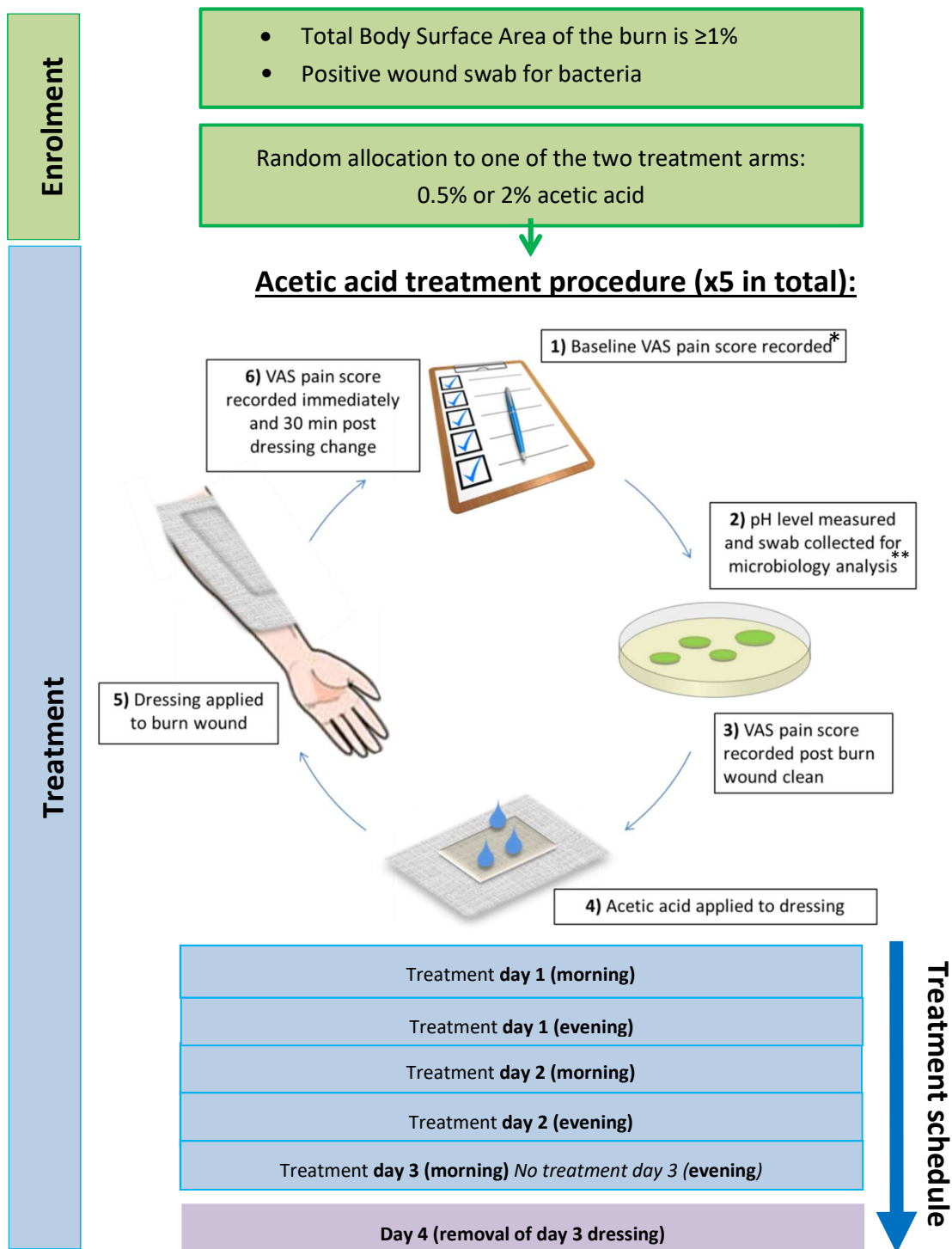
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AceticA

TRIAL SCHEMA

Figure 1: Diagram of patient pathway



* Visual Analogue Scale; collected if possible

** Burn injury swab only collected once daily (in the AM).

AceticA

SCHEDULE OF EVENTS

| | Burns Centre | Day 0: up to 48 hours prior to Day 1 AM treatment (following positive microbiology swab results) | Burns Centre | | | | | |
|---|------------------------------|--|--------------|----|-------|----|---------------|---------------|
| | Upon admission to burns ward | | Day 1 | | Day 2 | | Day 3 AM only | Day 4 AM only |
| | | | AM | PM | AM | PM | | |
| Screening (to include TBSA calculation) | x | | | | | | | |
| Consent | X | | | | | | | |
| Randomisation | | X | | | | | | |
| AE/SAE collection | | | x | | x | | x | |
| Vital signs ¹ | | | x | x | x | x | x | |
| Burn wound examination | | | x | x | x | x | x | |
| Bacterial load swab | X ² | | x | | x | | x | |
| Burn wound surface pH | | | x | x | x | x | x | |
| Visual Analogue Scale ³ | | | x | x | x | x | x | |
| IMP application; Dressing Change | | | x | x | x | x | x | |
| Gauze fluid sample collection | | | x | x | x | x | x | |
| Dressing Removal | | | | x | x | x | x | |

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5 ¹Vital Signs to be carried out up to 1 hour prior to dressing change during treatment days (to include heart rate, blood pressure, temperature,
6 oxygen saturation and respiration rate)

7 ² Two microbiology swabs will be taken upon screening (1 routine and 1 trial-specific)

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9 ³ Visual Analogue Scale to be carried out at the following 4 time-points around each AM/PM session (The scale can only to be completed if
10 patients have the capacity to do so):

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- 13 - Pre-treatment change analgesia
- 14 - Immediately post burn wound cleaning
- 15 - Immediately post treatment
- 16 - 30 minutes post treatment
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| 21. | Informed consent material..... | Attached as supplemental material |
| 22. | Participant Time line..... | Attached as supplemental material |
| 23. | Figures..... | Attached as supplemental material |

BMJ Open

The efficacy and optimal dose of Acetic Acid to treat colonised burns wounds: protocol for a pilot randomized controlled trial

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| Date Submitted by the Author: | 22-Apr-2022 |
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| Secondary Subject Heading: | Surgery |
| Keywords: | PLASTIC & RECONSTRUCTIVE SURGERY, WOUND MANAGEMENT, SURGERY, Plastic & reconstructive surgery < SURGERY |
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SCHOLARONE™
Manuscripts

Title

The efficacy and optimal dose of Acetic Acid to treat colonised burns wounds:
protocol for a pilot randomized controlled trial

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22 **Keywords**

23
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25 Burns; Acetic acid; randomised control trial; colonised wound; infected wound;
26 pain
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33 **Word Count**

34
35 4665 words
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Abstract

Introduction

Despite of recent advancement in the burns wound management, burn wound infection (BWI) is still one of the major cause of burns mortality. Patients who survive their burns injury still suffers from BWI related complication like delayed wound healing and poor scarring. BWI has been treated by application of topical antimicrobial agents or systemic antibiotics. Due to the global risk of developing systemic antibiotics resistance, medical research focuses on identifying single topical agent which has effective antimicrobial activity, easily available and cost effective. One such agent is Acetic acid (AA). AA has been used as a topical antibacterial agent for the treatment of burns wounds for many years and has shown to have activity against Gram-negative organisms including *Pseudomonas aeruginosa*. So far there has been no consensus on optimal concentration that's has effective antimicrobial activity, frequency of application, duration of treatment and most importantly good patient's tolerability. A randomised control study is required to answer all these questions.

Objective

To investigate the efficacy and tolerability of 0.5% and 2% of acetic acid when applied to colonised burns wounds for 3 days after admittance to the Queen Elizabeth Hospital Birmingham.

Methods and analysis

This is a double-blinded, prospective, randomised, controlled, single-centre trial. Patients will be screened for eligibility in the inpatient area and those who are found to be eligible will be randomly assigned to one of two treatment groups:

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3 GROUP 1: 0.5% acetic acid (10 patients)
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6 GROUP 2: 2% acetic acid (10 patients)
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9 Total Number: 20 patients
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11 **Outcome measures**

12 **Primary outcome**

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15 (1) Efficacy will be assessed by measuring the bacterial load from microbiology
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wound swabs for 3 consecutive days.

22 **Secondary outcomes**

25 (1) The assessment of antimicrobial activity of acetic acid and the minimum
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inhibitory concentrations (MIC).

(2) Patient's tolerance by assessing VAS pain score.

(3) Time to 95% wound healing of treatment area.

(4) Patient's perceived treatment allocation.

41 **Ethics and dissemination**

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45 AceticA trial protocol was approved by the National Research Ethics Service
46
47 (West Midlands - Edgbaston Research Ethics Committee; 17/WM/0407; IRAS
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49 234132). This article refers to protocol V5.0 dated 06th July 2020
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3 The analysed results will be presented at national and international conferences
4 related to management of burn patients. The generated articles based on the trial
5 results will be submitted to peer review journals for publication.
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10 **Strengths and limitations of this trial**

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- 13 • It is a double blinded, randomised control trial comparing two different
14 strengths of Acetic acid.
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- 16 • The study design is very simple and feasible for patients with the trial
17 duration of only 3 days with no extra outpatient follow ups.
18
- 19 • Two different strengths of acetic acid will be used to compare the
20 antimicrobial efficacy, tolerability and feasibility in patients.
21
- 22 • The study does not interfere with the standard burns care of the patient.
23
- 24 • Difficulties in recruitment are anticipated as the number of patients with
25 colonised burn wounds who are expected to stay as inpatients for the trial
26 duration and not undergoing for surgical intervention may be limited.
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Background

Burns wound infection (BWI) is a serious complication following burn injury. It is reported that BWI accounts approximately 9%-17% of all burn injury related complications (1)(2). Pruitt *et al.* reported that invasive wound infection represents 5% of all the infections that occurred in patients admitted with severe burn injuries(3). It is very concerning that morbidity and mortality of burn patients are highly correlated to the incidence of wound infection and its sequelae (4)(5). Other complications include delayed wound healing, poor scarring. Hence, the medical community aim to effectively manage BWI to improve patient's prognosis.

Invasive BWI are classically treated with systemic antibiotics. However, excessive use of antibiotics has been associated with antimicrobial resistance. Alternative antimicrobial regimes are currently needed to minimise the antimicrobial resistance, as per WHO recommendations (6). A wide range of topical treatments to manage BWI are available. This includes but not limited to, silver nitrate, povidone-iodine and acetic acid. To date, there is no consensus in regards to effectiveness and efficiency of various topical management regimes for BWI (7).

Ideal topical regime for treating BWI needs to have potent antimicrobial properties, readily available and cost effective. One such treatment is topical acetic acid (vinegar). The antimicrobial properties of acetic acid (AA) have been well-known for centuries. AA is included on the WHO list of essential medicines published in 2019, a list of the safest and most effective medicines (7).

AA has been used a topical agent in burn care for decades (8)(9). It has been used for wound management in WW1 when Taylor observed the elimination of *Bacillus Pyocyaneus* upon using 1% acetic acid(AA) solution (10). It has been shown to be effective against Multi Drug Resistant organisms (MDRO) and biofilms (11)(12)

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7 Since then, a number of studies has been conducted to assess the effectiveness and
8 efficacy of acetic acid in management of BWI(13)(14).
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11 *In vitro* studies, diluted AA (1-5%) has shown potential to reduce or eradicate
12 bacterial load, specifically *Pseudomonas aeruginosa* (15)(16). Minimum inhibitory
13 concentrations (MIC) of acetic acid has been studied in vitro both before and after
14 evaporation and exposure to gauze. These showed that the methicillin-susceptible
15 strain of *Staphylococcus aureus* (MSSA) had an MIC of 0.312% and a methicillin-
16 resistant strain (MRSA) was less susceptible to MIC of 0.625%. Strains of
17 *Acinetobacter baumannii* also had a MIC of 0.312% and all strains of
18 *Pseudomonas aeruginosa* were susceptible to MIC of 0.166%(11).
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31 Different concentrations of acetic acid has been studied to treat BWI (17)(18)(19).
32 Patient's tolerability to topical agent is also very important. Patients usually
33 complain of stinging and pain on application of acetic acid to wounds, particularly
34 at higher concentrations, e.g. strength of 5% or more (13)(14). In another study 3%
35 concentration was used with better pain tolerance and less itching (16). In a
36 recently published survey of burn centres in UK, 6 centres (32%), routinely use
37 AA topically as gauze soaked with 2.5-3.0%. This high concentration was reported
38 to be well tolerated (20).
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48 Despite of all the previous studies, question still remains to find a good balance
49 between AA concentration which has efficient antimicrobial activity, low toxicity
50 and better patient's tolerability. Hence, A randomised controlled clinical trial is
51 warranted to answer all these questions.
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Methods and analysis

Trial design

The is a double-blinded, prospective, controlled, and randomised pilot trial, where 20 patients will be randomised to receive treatment with either 0.5% or 2% acetic acid (10 patients in each treatment arm) to treat the bacterial load in colonised burn wounds. The burn wounds will be required to be colonised with a specifically identifiable bacteria (Table 1), this wound will then be treated twice daily with acetic acid dressings for 2 consecutive days then once on the third day of treatment. On day 1 and 2, the trial focuses on comparing the effectiveness of acetic acid 0.5% and acetic acid 2% in reducing bacterial load and evaluating patient's tolerability to justify a larger scale, randomised, controlled trial. The anticipated sample size is small as this is a pilot trial with no placebo arm. The trial will be double blinded to minimise bias in the assessment of the outcome measures.

The acetic acid concentrations chosen in this trial (0.5% and 2%) were selected based on the in vitro findings of Halstead *et al.* showing efficacy of acetic acid at lower dilutions than previously thought or used in clinical practice (21)(22). This is a single site trial, in which the cohort will be generated from patients admitted to the Burns Centre or Critical Care Unit at the Queen Elizabeth Hospital Birmingham, United Kingdom (QEHB) with a colonised burn wound. The target population is patients with burns of $\geq 1\%$ TBSA.

Table 1: BWI Identifiable Bacteria

| |
|-------------------------|
| Pseudomonas aeruginosa |
| Acinetobacter baumannii |
| Escherichia coli |
| Proteus mirabilis |
| Staphylococcus aureus |
| Klebisella Pneumoniae |
| ESBL E. coli |
| Enterobacter cloacae |

Conduct of Trial

Inclusion and Exclusion criteria:

The trial will aim to recruit adult patients (age ≥ 16 years old) who sustained a $\geq 1\%$ TBSA burn injury. At the start, the inclusion criteria was aged 18 years which was changed to 16 years, as in UK these patients are legally considered adults and this may increase the patient pool.

There was an upper limit of TBSA 10% which has been removed and changed to minimum limit of burn injury TBSA $\geq 1\%$. These changes were made to cover wider spectrum of patients and severity of injury. However, the total burn wound to be treated with AA will remain $< 10\%$.

Initially, the patients who have capacity to give informed consent were included in the trial. During the screening process the clinical team discovered that a large

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3 number of otherwise eligible patients could not be approached and hence recruited
4 as they were admitted to ITU and so lacked capacity to give informed consent.
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6 Changes were made in protocol to included patients who lacked capacity to give
7 informed consent. The consent for these patients can be sought from a Professional
8 or Personal Legal Representative.
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14 Prior to enrolment, the targeted burn wound has to be colonised with specifically
15 identifiable bacteria. The recruited patients are anticipated to remain as inpatients
16 for the trial duration (3 days). At first, the trial treatment period was 5 days, with
17 twice a day dressing change on day 1,2,4,5 and once a day dressing changes on day
18 3. However, due to the COVID-19 pandemic and the changes in the standard care
19 pathways to minimise the exposure by reducing the inpatient stay, the treatment
20 period was reduced to 3 days to avoid impact on recruitment process. This
21 proposed change of the study design was also supported by interim analysis of 11
22 subjects as there was an increase in bacterial colonisation on day 4, compared to
23 day 2 and 3. Now patients will get twice a day dressing change on day 1, 2 and
24 once a day on day 3.
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38 Patients receiving systemic antibiotics for cellulitis were excluded initially. The
39 clinical team suggested that these are the patients who may benefit most from
40 topical antimicrobial treatment. In fact, acetic acid (usually of higher
41 concentrations) is part of routine therapy for these patients. Antibiotics are
42 prescribed to control the systemic spread of infection and do not interfere with the
43 wound microbial burden. Therefore, this exclusion point was removed to allow
44 these patients the opportunity to receive 0.5% or 2% acetic acid solution to treat
45 their wounds. This should not affect the outcome of determining the difference in
46 the microbial burden of the two different concentrations.
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Because of all the above changes the study design is very simple with very broad inclusive criteria so the results can have worldwide potential impact. Only patients with severe burn injury or burn which require surgery will be excluded. There was no contraindication to include pregnant patients or who are breast feeding as the IP has no systemic effects.

Patients with burn TBSA <1% and burns solely to the face and/or genital area will be excluded in addition to patients who have received acetic acid as part of standard therapy upon admission for this burn injury (Table 2). Recruitment eligibility and unsuitability will be checked by the trial investigators.

Table 2: Summary of inclusion and exclusion criteria.

| Inclusion criteria | Exclusion criteria |
|--|-------------------------------------|
| Age ≥ 16 years | Paediatric patients |
| $\geq 1\%$ TBSA burn injury | <1% TBSA burn injury |
| Colonised wound with a specifically identifiable bacterium | Burns to face or genitalia |
| Anticipated hospital stay for at least for 3 days | Previous treatment with acetic acid |

Screening and consenting:

Patients who meet eligibility criteria will be approached by a member of the research team and asked whether they would be willing for an additional microbiology swab to be alongside their routine swab for analysis, to confirm eligibility. If the patient is unconscious, then a Personal Legal Representative or

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3 Professional Legal Representative will be approached on behalf of the patient to
4 request the swab be taken. If the appropriate patient or legal representative agrees
5 to this, they will be asked to sign the trial consent form for retrieval of this swab.
6
7 Once a positive microbiology test result has come back and the patient is found to
8 be eligible according to the inclusion and exclusion criteria, the patient or their
9 legal representative will be approached by a member of the research team for
10 enrolment treatment with acetic acid dressings.
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19 At first, there were two consent forms for the study, pre-screening consent form for
20 initial microbiology swab to indicate eligibility. If patient is eligible for treatment,
21 then informed consent form will be taken prior to start the treatment. To make
22 consent process easier, single consent form was introduced later on for screening
23 and treatment phase of the trial.
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31 **Randomisation and treatment allocation**

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35 Randomisation and treatment will only begin once eligibility is confirmed by a
36 positive microbiology test to take part in the trial. Patients will be randomised to a
37 treatment arm in order to start the morning treatment procedure of day 1 within 48
38 hours of the positive microbiology result.
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45 Patients will be randomised to one of two treatment groups:

46 GROUP 1: 0.5% acetic acid (10 patients)

47 GROUP 2: 2% acetic acid (10 patients)

48 Total number: 20 patients

49 Enrolment and randomisation of the patient will be completed on the electronic the
50 Clinical Research Tool (CREST) system (an in-house bespoke, electronic Remote
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3 Data Capture system (eRDC) developed by QEHB), on which patients' trial
4 number and treatment pack numbers will be allocated and randomisation
5 confirmation emails will be sent to relevant trial team members. The CREST
6 system is also trial database system.
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11 12 13 **Blinding:** 14

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17 The treatment group allocation will be concealed from the patient and clinical staff
18 throughout the trial period. Each treatment box will be numbered according to a
19 randomisation list generated on a randomisation wizard by biostatistician. The
20 randomisation list will be provided to the manufacturer, Stockport
21 Pharmaceuticals, who will in turn package and label the treatment boxes.
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28 29 **Un-blinding process:** 30

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33 As both arms of the trial are the same active treatment, in cases of emergency they
34 will likely be dealt in the same way. As a result, there is unlikely to be a
35 requirement for emergency un-blinding. If the need does arise, the local pharmacy
36 will hold the randomisation list that can provide details of what treatment each
37 patient has been randomised to. If un-blinding needs to be carried out then a full
38 record of the procedure will need to be recorded and maintained i.e. reasons for un-
39 blinding, by whom etc.
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49 **Trial intervention:** 50

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53 The burn wounds will be required to be colonised with a specifically identifiable
54 bacteria, this wound will then be treated twice daily for the first two days with
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3 acetic acid dressings. This will allow determination of whether the acetic acid is
4 still active after 12 hours of being in contact with a colonised burn wound. In order
5 to ascertain if the acetic acid is still effective after 24 hours the dressing will be
6 changed once on day 3. The antimicrobial activity of acetic acid extracted from the
7 dressings, will be conducted by determining its minimum inhibitory concentration
8 (MIC) (7).

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11 A microbiological swab of the burn wound will be collected once daily during each
12 morning dressing change and sent to the microbiology lab where it will be
13 analysed to determine the microbial load.
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17 The swabbing procedure will be carried out prior to burn wound cleaning. The
18 10x10cm² blue gauze will be removed from the burn wound and over this area a
19 swab moistened in normal saline will be applied and whilst twisting the tip the area
20 will be swept from left to right at 1cm intervals. The swab will be transferred to a
21 neutralising agent (containing 30g/L Tween 80, 3g/L lecithin, 1g/L histidine, 5g/L
22 sodium thiosulphate, 8.5g/L sodium chloride and 1g/L tryptone) to nullify carry
23 over of antimicrobial activity. Serial dilutions using diluent containing 8.5g/L
24 sodium chloride and 1g/L tryptone will be made, and number of CFU/mL
25 determined.
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30 The trial will assess tolerability of the two different strengths of acetic acid, by
31 assessing the patient's pain score at time of application of acetic acid and in the
32 following hour, using the Visual Analogue Scale (VAS). Please find the Trial
33 Schema (Supplementary file 1) and Schedule of Events (Supplementary file 2).
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AE reporting and analysis

The reporting period for Adverse Events (AEs) starts from the time of application of the first dressing and continues until the day 3 dressing has been removed on day 4. Before COVID-19 pandemic, the reporting period for AEs started from the time of application of the first dressing and continued until the day 5 dressing had been removed on day 6. All Serious Adverse Events (SAEs) and adverse reactions will be evaluated by the investigators and recorded. The National Cancer Institute's common terminology criteria for AEs (CTCAE, V.4.02, 2010) will be used to grade each AE. The coordinating trial office (CRCTU, Birmingham) will keep detailed records of all AEs reported (nature, onset, duration, severity, outcome) and perform an evaluation with respect to severity, causality and expectedness.

Data handling, quality assurance, record keeping and retention

The trial sponsor (UHBFT) and patients are recruited from one of the sponsors hospitals (QEHB). The sponsor worked in collaboration with CRCTU, University of Birmingham (UoB), Some Sponsor responsibilities were delegated to the clinical trials unit the division of key responsibilities is detailed in (Table 3).

Table 3: Summary of responsibilities

| Responsibility | Institution |
|---|-------------|
| Provision of Investigational Medicinal Medicine (IMP) – Acetic Acid | UHBFT |
| Provision of Electronic Remote Data Capture System (eRDC-CREST) | UHBFT |
| On site monitoring | UHBFT |

| | |
|---|-------|
| Regulatory submission, Trial Management, Data Storage & Analysis, | CRCTU |
|---|-------|

The sponsor and CRCTU are fully compliant with the Data Protection Act 1998. Applicable regulations and laws associated with testing and development of Investigational Medicinal Products (IMPS) for human use and Good Clinical Practice (GCP). The sponsor is responsible for monitoring the trial. Confidentiality will be maintained throughout the trial and thereafter. On completion of the trial, data will be transferred to a secure archiving facility at the UoB, where data will be held for a minimum of 15 years and then destroyed.

Case report forms

Case Report Forms (CRFs) included medical history and concomitant medications in the trial's electronic database, CREST. Other CRFs incorporated in the electronic database included: pain scores and burn injury examinations recorded from day 1 through to day3; microbiology results; AE reporting and end of treatment forms. The data will be collected on paper CRFs as well as electronic remote data capture eRDC. CREST was not available at the beginning of the study. This data was then later transcribed to CREST when it was available.

Statistical justification and outcome analysis

Sample size and justification:

This trial is not powered to the primary endpoint. Rather, the sample size for AceticA has been selected based on what is feasible to be recruited at a single

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3 centre in a reasonable timeframe for this phase of clinical trial. It was thought that
4
5 10 evaluable patients per treatment arm, analysed using repeated measures
6
7 methods, would provide the information needed to inform a larger, randomised
8
9 controlled trial.
10

11 **Outcome measures and statistical analysis**

12 **Primary outcome**

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17 Efficacy will be assessed by measuring the bacterial load from microbiology
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19 wound swabs, these will be taken daily from recruitment for 3 consecutive days.
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22 **Secondary outcomes**

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25 Secondary outcome measures include; (1) the antimicrobial activity of acetic acid
26
27 will be measured by extracting fluid from removed burns dressings and assessing
28
29 the minimum inhibitory concentrations (MIC) to establish if active acetic acid is
30
31 still present; (2) tolerance will be assessed by measuring a patient's pain scores
32
33 with a VAS if the patient has capacity to provide scores; (3) time to 95% wound
34
35 healing of the treated area of interest will be obtained from patients' records; (4)
36
37 perceived treatment allocation will be assessed by asking patients after treatment
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39 completion which treatment they believed they received if they have capacity to do
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41 so.
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45 Patient's tolerability was changed from primary to secondary outcome anticipating
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47 few patients might be sedated and ventilated and unable to respond to visual
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49 analogue pain score. Secondly, 'percentage of wound healed at day 21' was
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51 changed to 'time to 95% wound healing'. This amendment was done as some
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53 patients develop colonised burns wound and became eligible for recruitment after
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3 few weeks of injury. This measurement will be collected as part of burns patients'
4 standard care and can be obtained retrospectively from the patients' medical notes.
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8 **Analysis of outcome measures**

10 Full details will be specified in the Statistical Analysis Plan; however, an outline is
11 given here. Analysis will use all patients whom are deemed to be evaluable.
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14 Patients are evaluable if all 5 dressing changes from the first 3 days of treatment
15 are completed. Patients will be analysed in the groups that reflect the treatment
16 they actually received. This is because the samples size is small, and the aim of a
17 pilot trial is to inform a subsequent pivotal trial. Where frequentist tests are used,
18 and unless specified otherwise, a significance level of 5% will be used to designate
19 significance.
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27 It is possible that many wounds could be assessed within patients. If this happens,
28 hierarchical models will be used for wound specific outcome measures to reflect
29 the wound-within-patient structure of the data.
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35 **Analysis of Primary Outcome Measure**

36 **Efficacy:**

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38 Burn wound swabs will be taken periodically from baseline and bacterial load will
39 be quantified by microbiology as the number of colonies forming units. To
40 maximise information, repeated measures methods will be used. The model
41 assuming fixed effects for the mean bacterial load, the mean change in bacterial
42 load from baseline, and the additional mean change that is associated with
43 receiving 2% instead of 0.5% acetic acid, with random effects adjusting for the
44 mean bacterial load at baseline for each patient, will be compared to the analogous
45 model without the adjustment for treatment received. A likelihood-ratio test of the
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3 nested models would yield inference on the treatment effects through time. As
4 detailed above, hierarchical structures will be considered as necessitated by the
5 observed data.
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10 The dependent variable could be extremely fat-tailed so appropriate transforms
11 (e.g. log) will be considered. This model could also be re-specified to use fewer
12 parameters if load is found to be well approximated by a smooth function of time,
13 potentially using transformations or restricted cubic splines. In this case, equivalent
14 adaptations would be made to each model so that the method of testing nested
15 models to isolate treatment effect is valid.
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23 The parameters in the full model will be reported with means and standard errors.
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26 There may be particular interest in the presence or absence of a particular set of
27 bacteria. If this is the case, these will be identified in the statistical analysis plan.
28 The expectation is that the lower concentration will be non-inferior in terms of
29 efficacy. This suggests a one-tailed comparison.
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35 **Analysis of Secondary Outcome Measures**

36 **Tolerability:**

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40 Pain scores will be collected at many points throughout the trial via the verbal pain
41 intensity scale. Zor *et al.*(23) collect scores in a similar manner and analyse them as
42 numerical data, i.e. they assume the scores to reflect order and scale. We initially
43 propose to also analyse the pain scores as numerical variables. Explanatory
44 variables will be included to reflect treatment allocation and we will present
45 evidence on the extent to which reported pain is associated with treatment
46 allocation.
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3 The assumption that pain scores are numerical and not ordinal is potentially
4 controversial. Supporting analysis may be provided that treats the scores as ordinal
5 levels. Hierarchical structure similar to that described in the primary outcome
6 would be used. Provision of this analysis is at least partially contingent on patients
7 in the overwhelming majority of cases using the provided levels and not providing
8 scores between levels. For instance, if patients frequently score pain experienced as
9 3.5 to convey “between 3 and 4”, then that would diminish the suitability of the
10 described ordinal variable analysis. In that case, a re-codification of the ordinal
11 levels, or reliance only on the analysis of the continuous data could be indicated.
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22 **Antimicrobial activity:**

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24 The antimicrobial activity of acetic acid will be measured by extracting fluid from
25 removed burns dressings. The minimum inhibitory concentrations (MIC) will be
26 estimated by successively halving the concentration of retrieved acid and testing
27 whether microbial growth occurs. MICs could be modelled as numerical (after
28 appropriate transform) and/or ordinal data. Furthermore, group structure could be
29 required as described above. Details of this are given in the Statistical Analysis
30 Plan (SAP).
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40 **Time to 95% wound healing:**

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42 Time from randomisation to 95% wound healing of the treated area will be
43 presented using reverse Kaplan-Meier curves. In presentation of these curves,
44 patients will be appropriately censored at the point they withdraw from or
45 complete the trial. Presentation of these curves will account for the nested data
46 structure as necessary.
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3 The time to wound healing will be assessed using hierarchical (also referred to as
4 multi-level) cox models. The hierarchical structure will be included, if necessary,
5 to reflect the nesting of wound through patient.
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9 10 **Perceived treatment allocation:**

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12 After completion of protocol treatment, patients will be asked to identify the
13 treatment arm to which they believe they were randomised. For each patient,
14 identification of treatment arm will either be: correct; incorrect; or missing. Counts
15 will be reported by arm. Association of treatment arm and identification of
16 treatment arm could be assessed by chi-squared test.
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23 24 **End of trial**

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26 The end of trial will be the date of completion of treatment for the last patient. The
27 Trials Office will notify the MHRA and REC that the trial has ended and will
28 provide them with a summary of the clinical trial report within 12 months of the
29 end of trial.
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35 36 **Patient and Public Involvement**

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38 Development of the research question, outcome measures and trial design were
39 informed by meetings held with the Trial Steering Committee, which included a
40 Patient and Public Involvement (PPI) Representative. The PPI reviewed the trial
41 documentations and considered the overall burden of trial participation during the
42 design process specifically the practicality of twice daily dressing of an infected
43 burn wound.
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Trial Status

Recruitment into the AceticA trial began in February 2018 and currently the recruitment has been completed. End of trial report will be submitted in January, 2023 (Table 4).

Table 4: Start and End dates

| | |
|-------------------------------------|--------------------------------|
| First site open | 20 th February 2018 |
| First patient recruited | 23 rd March 2018 |
| Last Patient Last Visit | 8 th October 2021 |
| End of Study Declaration submission | 5 th January 2022 |
| End of trial report submission | January, 2023 |

Confidentiality and Data Protection

Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the General Data Protection Regulation and Data Protection Act 2018. UHB NHSFT, as the sponsor for the AceticA trial, will be using information from patient medical records in order to undertake this trial and will act as the data controller for the study. The computers on this network have restricted physical access; data are stored under coded filenames and the local network has secure password access restricted to a limited set of people.

Anonymised data will be provided to UoB for data analysis and will only be accessible by authorised personnel. All AceticA participants provided specific

1
2
3 written consent at trial entry to enable data with UoB. Otherwise, confidentiality
4 was maintained throughout the trial and thereafter.
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8 All the complied and analysed results will be presented at national and
9 international conferences concerning. Results will also be submitted for peer
10 review and publication in the subject journals/literature.
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15 The trial results will be reported and submitted for publication in peer-reviewed
16 journals and presentation at appropriate national and international academic
17 meetings. Trial participants will be sent a summary of the final results, including
18 references to full papers. Trial data may be made available to external groups
19 wishing to undertake original analysis, subject to approval from the Trial
20 Management Group.
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28 **Discussion**

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30 BWI is correlated with higher mortality and morbidity following burn injury
31 (4). Hence, one of the most important aims in burns wound management is to
32 prevent infection to prevent invasive burn wound infection and sepsis. Multiple
33 studies has shown effectiveness of Acetic acid in managing BWI (8)(14)(10). It is
34 also known as an effective agent against biofilm producing microorganisms which
35 are notoriously difficult to decolonise, due to limited anti-microbial penetration
36 and deactivation by biofilm matrix(22). In addition, as a weak acid with a pKa
37 close to its pH, acetic acid can kill bacteria without being toxic to human cells, a
38 key consideration in wound healing(24). It is also very cost effective and easily
39 available agent(14). But there is not enough data to standardise its usage like
40 effective strength, frequency, duration of treatment.
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3 This is a prospective interventional controlled trial with a very simple trial design.
4
5 The results will have worldwide impact because of its generic inclusion criteria.
6
7 This study will show the outcome in BWI treated with two different strengths of
8
9 Acetic acid. 0.5 and 2%. It will help in establishing a balance between the effective
10
11 strength of Acetic acid against BWI, frequency and duration of treatment and
12
13 patient's tolerability. As Acetic acid is very cost effective and easily available
14
15 agent. The results of the study will have effects in both developed and developing
16
17 countries with limited medical resources. This trial has a potential to have
18
19 significant impact on development of future studies on burns wound management
20
21 and treatment of burn wound infection by incorporating acetic acid in a dressing
22
23 carrier as the active antibacterial agent.
24
25

26 **Acknowledgements**

27
28
29 The Acetica trial would like to thank the TSC consisting of Mr Nadeem Khwaja
30
31 (Independent Clinical Chair), Dr Brian Jones (Independent Clinician), Mr Timothy
32
33 Pickles (Independent Statistician), Mr David Udale (Patient and Public
34
35 Involvement Representative), National Institute for Health Research, Surgical
36
37 Reconstruction and Microbiology Research Centre (NIHR SRMRC) clinical team
38
39 (Clinicians, Research nurses, data managers) for their time and input.
40
41

42 **Conflict of Interests:**

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44
45 The authors have no conflict of interests to declare.
46
47

48 **Funding:**

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51 This is a clinician-initiated and clinician-led trial funded by the Scar Free
52
53 Foundation and National Institute for Health Surgical Reconstruction &
54
55 Microbiology Research Centre (NIHR SRMRC).
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3 Grant Number: Not applicable
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6 **Author Contributions**

7

8
9 The concept of the AceticA trial was developed by NM (Chief investigator) and
10 BO, TH, RI, KP, KA (Co-investigator). NM, KB, DB designed/developed the
11 initial AceticA trial protocol. NM, DB, VH, GS developed, wrote, reviewed
12 subsequent protocol versions. AC designed the proforma used for pre-screening,
13 microbiological lab manual and protocol procedure documentations. GS, DB, TH
14 submitted all REC, MHRA and local R&D applications. KB and VH devised the
15 statistical plan. GS, DB, TH and AC wrote/designed the patient information sheets,
16 external trial information and patient CRFs. RI and TH wrote the manuscript and
17 all authors reviewed the final version. All authors are guarantors.
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28 **Ethics and dissemination**

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30
31 This trial has been approved by the Medicines and Healthcare products Regulatory
32 Agency on 22 November 2017. CTA reference number - 16719/0232/001-0001
33 and National Research Ethics Service (West Midlands - Edgbaston Research
34 Ethics Committee on 21 December 2017 – REC 17/WM/0407; IRAS 234132.
35 In addition, the trial has been registered on the publicly available ISRCTN registry
36 (ISRCTN11636684).
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44 The analysed results will be presented at national and international conferences
45 related to management of burn patients. The generated articles based on the trial
46 results will be submitted to peer review journals for publication.
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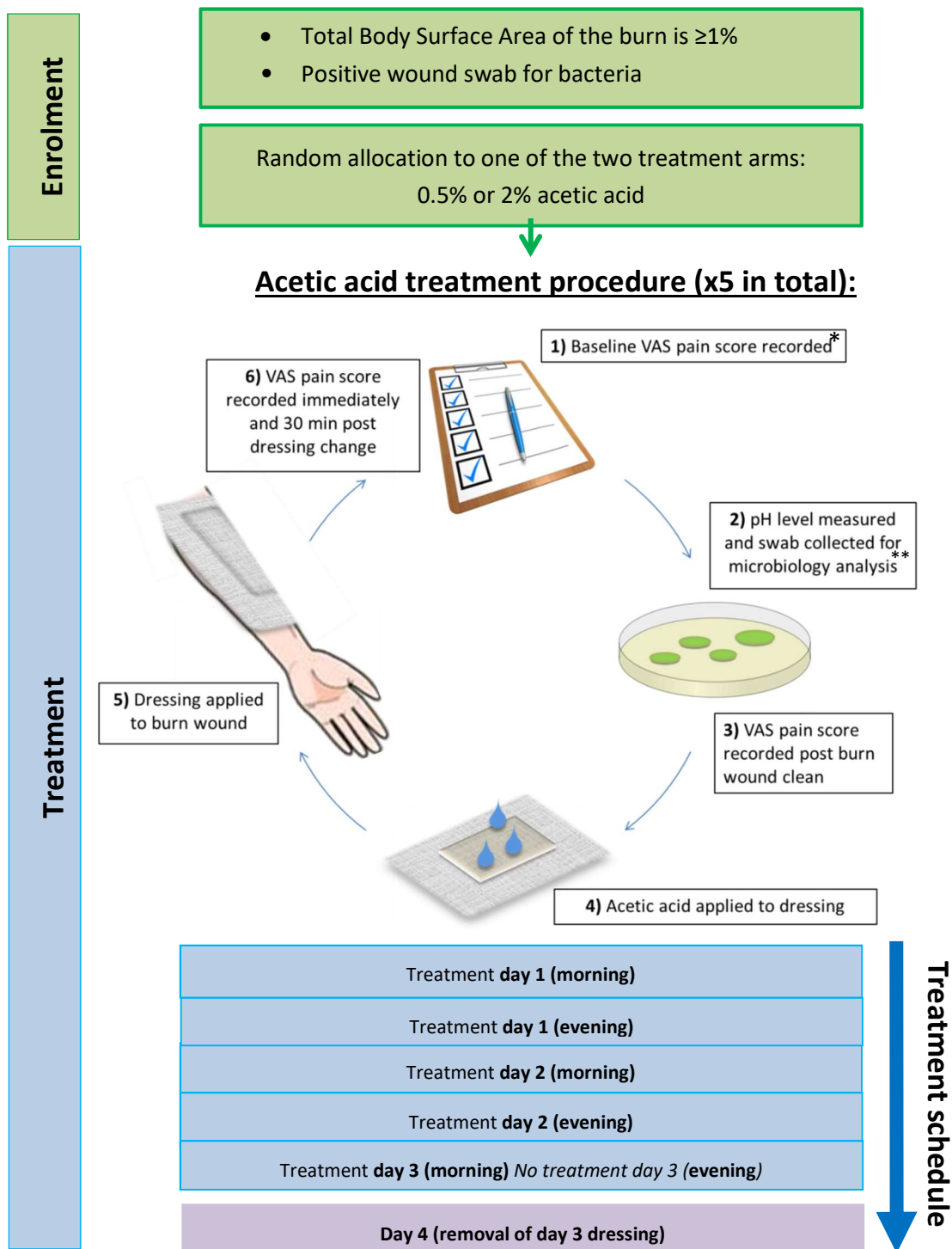
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For peer review only

AceticA

TRIAL SCHEMA

Figure 1: Diagram of patient pathway



* Visual Analogue Scale; collected if possible

** Burn injury swab only collected once daily (in the AM).

AceticA

SCHEDULE OF EVENTS

| | Burns Centre | Day 0: up to 48 hours prior to Day 1 AM treatment (following positive microbiology swab results) | Burns Centre | | | | | |
|---|------------------------------|--|--------------|----|-------|----|---------------|---------------|
| | Upon admission to burns ward | | Day 1 | | Day 2 | | Day 3 AM only | Day 4 AM only |
| | | | AM | PM | AM | PM | | |
| Screening (to include TBSA calculation) | x | | | | | | | |
| Consent | X | | | | | | | |
| Randomisation | | X | | | | | | |
| AE/SAE collection | | | x | | x | | x | |
| Vital signs ¹ | | | x | x | x | x | x | |
| Burn wound examination | | | x | x | x | x | x | |
| Bacterial load swab | X ² | | x | | x | | x | |
| Burn wound surface pH | | | x | x | x | x | x | |
| Visual Analogue Scale ³ | | | x | x | x | x | x | |
| IMP application; Dressing Change | | | x | x | x | x | x | |
| Gauze fluid sample collection | | | x | x | x | x | x | |
| Dressing Removal | | | | x | x | x | x | |

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5 ¹Vital Signs to be carried out up to 1 hour prior to dressing change during treatment days (to include heart rate, blood pressure, temperature,
6 oxygen saturation and respiration rate)

7 ² Two microbiology swabs will be taken upon screening (1 routine and 1 trial-specific)

8
9 ³ Visual Analogue Scale to be carried out at the following 4 time-points around each AM/PM session (The scale can only to be completed if
10 patients have the capacity to do so):

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- 13 - Pre-treatment change analgesia
- 14 - Immediately post burn wound cleaning
- 15 - Immediately post treatment
- 16 - 30 minutes post treatment
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SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Page No |
|-----------------------------------|---------|--|---------|
| Administrative information | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 01 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 25 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | n/a |
| Protocol version | 3 | Date and version identifier | 04 |
| Funding | 4 | Sources and types of financial, material, and other support | 24 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 15 |
| | 5b | Name and contact information for the trial sponsor | 15 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 15,16 |
| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 24 |
| Introduction | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 06 |
| | 6b | Explanation for choice of comparators | 06,08 |
| Objectives | 7 | Specific objectives or hypotheses | 03 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 08 |

Methods: Participants, interventions, and outcomes

1 SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and
 2 related documents*
 3

| | | | | |
|----|----------------------|-----|--|-----|
| 4 | Study setting | 9 | Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 08 |
| 5 | | | | |
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| 8 | Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 11 |
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| 13 | Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 13 |
| 14 | | | | |
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| 16 | | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | n/a |
| 17 | | | | |
| 18 | | | | |
| 19 | | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | n/a |
| 20 | | | | |
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| 23 | | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | n/a |
| 24 | | | | |
| 25 | Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 17 |
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| 32 | | | | |
| 33 | Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 14 |
| 34 | | | | |
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| 36 | | | | |
| 37 | Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 16 |
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| 41 | Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 11 |
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43 **Methods: Assignment of interventions (for controlled trials)**

44 Allocation:

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| 47 | Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 12 |
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1 SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and
 2 related documents*
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| 4 | Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | n/a |
| 8 | Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 12 |
| 11 | Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 13 |
| 15 | | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 13 |

21 **Methods: Data collection, management, and analysis**

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| 23 | Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 15,16 |
| 31 | | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | n/a |
| 36 | Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 22 |
| 42 | Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 16-20 |
| 47 | | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 18,19 |
| 50 | | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | n/a |

54 **Methods: Monitoring**

1 SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and
 2 related documents*
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| | | | | |
|----|---------------------------------|-----|---|-------------|
| 4 | Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | n/a |
| 5 | | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | n/a |
| 6 | Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 15 |
| 7 | Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | n/a |
| 8 | Ethics and dissemination | | | |
| 9 | Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 25 |
| 10 | Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 09,10,12,17 |
| 11 | Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 11 |
| 12 | | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | n/a |
| 13 | Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 22 |
| 14 | Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 24 |
| 15 | Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 22 |
| 16 | Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | n/a |

1 SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and
 2 related documents*

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| 4 | Dissemination | 31a | Plans for investigators and sponsor to communicate trial results | 25 |
| 5 | policy | | to participants, healthcare professionals, the public, and other | |
| 6 | | | relevant groups (eg, via publication, reporting in results | |
| 7 | | | databases, or other data sharing arrangements), including any | |
| 8 | | | publication restrictions | |
| 9 | | 31b | Authorship eligibility guidelines and any intended use of | 25 |
| 10 | | | professional writers | |
| 11 | | 31c | Plans, if any, for granting public access to the full protocol, | 22 |
| 12 | | | participant-level dataset, and statistical code | |
| 13 | | | | |
| 14 | | | | |

15 **Appendices**

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| | | | | |
|----|------------------|----|---|-----|
| 17 | Informed consent | 32 | Model consent form and other related documentation given | n/a |
| 18 | materials | | to participants and authorised surrogates | |
| 19 | Biological | 33 | Plans for collection, laboratory evaluation, and storage of | 13 |
| 20 | specimens | | biological specimens for genetic or molecular analysis in | |
| 21 | | | the current trial and for future use in ancillary studies, | |
| 22 | | | if applicable | |
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