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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:

GROUP 1: 0.5% acetic acid (10 patients)

GROUP 2: 2% acetic acid (10 patients)

The two concentration strengths of acetic acid will be assessed for the antimicrobial activity and pain tolerability. Antimicrobial activity will be assessed using repeated measures methods to analyse the number of colony forming units.

Article Summary

Strengths and limitations of this trial

1.There is no consensus about the optimal concentration of acetic acid to treat colonised burn wounds in the way of antimicrobial efficacy and causing the least pain to the patient. The trial should be a strong evidence to answer this question being a randomised, controlled, double blinded trial.

2. This trial will contribute to a better understanding of the role of acetic acid as a feasible, topical, antibacterial agent for burn wounds colonized with Pseudomonas spp. and other microorganisms.

3. Although acetic acid has been used for a long duration in burns practice, this trial is the first of its kind as a double blinded, randomised control trial comparing two strengths of acetic acid regarding antimicrobial efficacy and tolerability.

4. The results of this trial will be employed to develop a dressing carrier with acetic acid as the active antibacterial agent.

5. 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.

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)

Since then, a number of studies has been conducted to assess the effectiveness and efficacy of acetic acid in management of BWI(13)(14).

I*n vitro* studies, diluted AA (1-5%) has shown potential to reduce or eradicate bacterial load, specifically Pseudomonas aeruginosa (15)(16). Minimum inhibitory concentrations (MIC) of acetic acid has been studied in vitro both before and after evaporation and exposure to gauze. These showed that the methicillin-susceptible strain of Staphylococcus aureus (MSSA) had an MIC of 0.312% and a methicillin-resistant strain (MRSA) was less susceptible to MIC of 0.625%. Strains of Acinetobacter baumannii also had a MIC of 0.312% and all strains of Pseudomonas aeruginosa were susceptible to MIC of 0.166%(11).

Different concentrations of acetic acid has been studied to treat BWI (17)(18)(19). Patient's tolerability to topical agent is also very important. Patients usually complain of stinging and pain on application of acetic acid to wounds, particularly at higher concentrations, e.g. strength of 5% or more (13)(14). In another study 3% concentration was used with better pain tolerance and less itching (16). In a recently published survey of burn centres in UK, 6 centres (32%), routinely use AA topically as gauze soaked with 2.5-3.0%. This high concentration was reported to be well tolerated (20).

Despite of all the previous studies, question still remains to find a good balance between AA concentration which has efficient antimicrobial activity, low toxicity and better patient's tolerability. Hence, A randomised controlled clinical trial is warranted to answer all these questions.

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

Birmingham, United Kingdom (QEHB) with a colonised burn wound. The target population is patients with burns of ≥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 ≥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 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
≥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.

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

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

Blinding:

The treatment group allocation will be concealed from the patient and clinical staff throughout the trial period. Each treatment box will be numbered according to a randomisation list generated on a randomisation wizard by biostatistician. The randomisation list will be provided to the manufacturer, Stockport Pharmaceuticals, who will in turn package and label the treatment boxes.

Un-blinding process:

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

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 10x10cm^2 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 (Figure 1) and Schedule of Events (Figure 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 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 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 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

required as described above. Details of this are given in the Statistical Analysis Plan (SAP).

Time to 95% wound healing:

Time from randomisation to 95% wound healing of the treated area will be presented using reverse Kaplan-Meier curves. In presentation of these curves, patients will be appropriately censored at the point they withdraw from or complete the trial. Presentation of these curves will account for the nested data structure as necessary.

The time to wound healing will be assessed using hierarchical (also referred to as multi-level) cox models. The hierarchical structure will be included, if necessary, to reflect the nesting of wound through patient.

Perceived treatment allocation:

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

End of trial

The end of trial will be the date of completion of treatment for the last patient. The Trials Office will notify the MHRA and REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

Patient and Public Involvement

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

Trial Status

Recruitment into the AceticA trial began in February 2018 and it is still open to recruitment.

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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Conflict of Interests:

The authors have no conflict of interests to declare.

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Author Contributions

The concept of the AceticA trial was developed by NM (Chief investigator) and BO, TH, RI, KP(Co-investigator). NM, KB, DB designed/developed the initial AceticA trial protocol. NM, DB, VH, GS developed, wrote, reviewed subsequent protocol versions. AC designed the proforma used for pre-screening, microbiological lab manual and protocol procedure documentations. GS, DB, TH submitted all REC, MHRA and local R&D applications. KB and VH devised the statistical plan. GS, DB, TH and AC wrote/designed the patient information sheets, 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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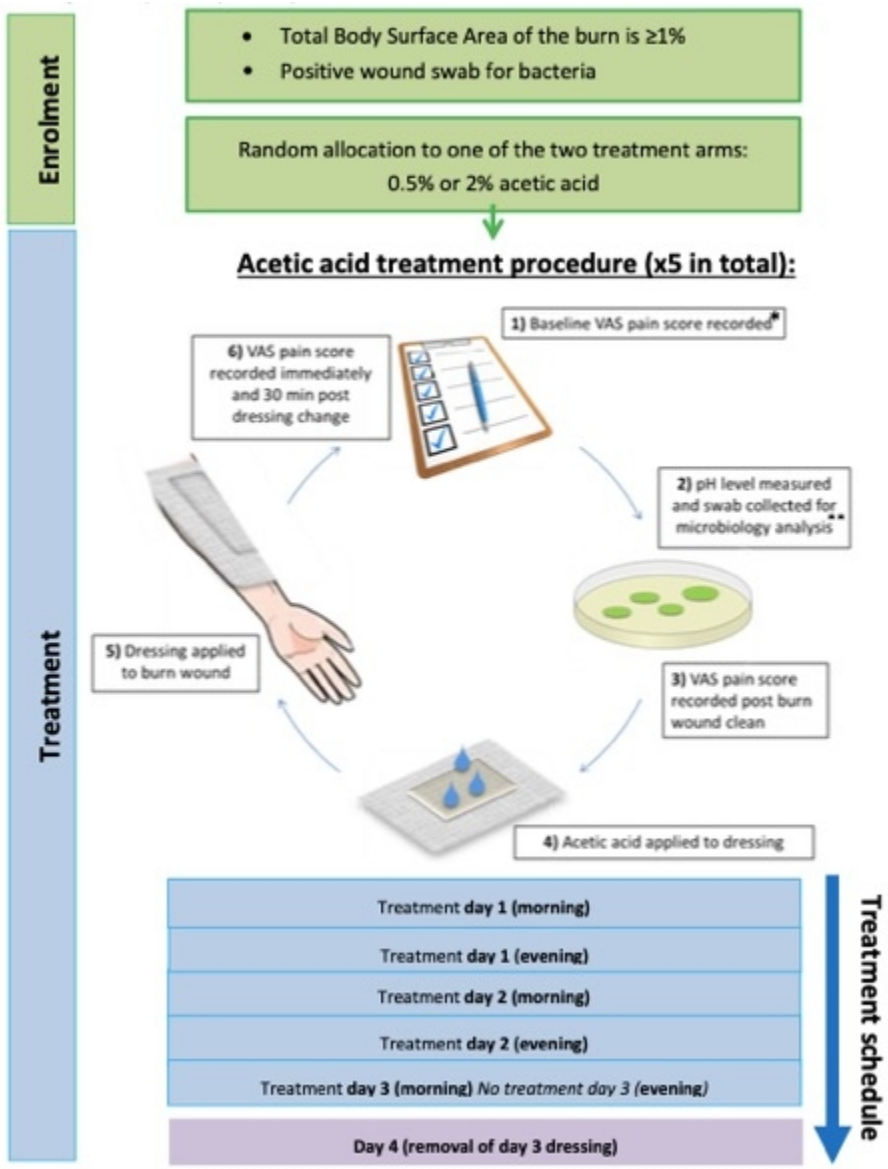

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	Burns Centre	Day 0: up to 48 hours prior to Day 1 AM treatment (following positive microbiology swab results)	Burns Centre					
			Day 1		Day 2		Day 3 AM only	Day 4 AM only
	Upon admission to burns ward		AM	PM	AM	PM		
Screening (to include TBSA calculation)	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	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

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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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Burns; Acetic acid; randomised control trial; colonised wound; infected wound; pain

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4335 words

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:

GROUP 1: 0.5% acetic acid (10 patients)

GROUP 2: 2% acetic acid (10 patients)

Total Number: 20 patients

The two concentration strengths of acetic acid will be assessed for the antimicrobial activity and pain tolerability. Antimicrobial activity will be assessed using repeated measures methods to analyse the number of colony forming units.

Ethics and dissemination

AceticA trial protocol was approved by the National Research Ethics Service (West Midlands - Edgbaston Research Ethics Committee; 17/WM/0407; IRAS 234132).

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.

Article Summary

Strengths and limitations of this trial

- It is a double blinded, randomised control trial comparing two different strengths of Acetic acid.
- The study design is very simple and feasible for patients with the trial duration of only 3 days with no extra outpatient follow ups.
- Two different strengths of acetic acid will be used to compare the antimicrobial efficacy, tolerability and feasibility in patients.
- The study does not interfere with the standard burns care of the patient.

- 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.

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)

Since then, a number of studies has been conducted to assess the effectiveness and efficacy of acetic acid in management of BWI(13)(14).

I*n vitro* studies, diluted AA (1-5%) has shown potential to reduce or eradicate bacterial load, specifically Pseudomonas aeruginosa (15)(16). Minimum inhibitory concentrations (MIC) of acetic acid has been studied in vitro both before and after evaporation and exposure to gauze. These showed that the methicillin-susceptible strain of Staphylococcus aureus (MSSA) had an MIC of 0.312% and a methicillin-resistant strain (MRSA) was less susceptible to MIC of 0.625%. Strains of Acinetobacter baumannii also had a MIC of 0.312% and all strains of Pseudomonas aeruginosa were susceptible to MIC of 0.166%(11).

Different concentrations of acetic acid has been studied to treat BWI (17)(18)(19). Patient's tolerability to topical agent is also very important. Patients usually complain of stinging and pain on application of acetic acid to wounds, particularly at higher concentrations, e.g. strength of 5% or more (13)(14). In another study 3% concentration was used with better pain tolerance and less itching (16). In a recently published survey of burn centres in UK, 6 centres (32%), routinely use AA topically as gauze soaked with 2.5-3.0%. This high concentration was reported to be well tolerated (20).

Despite of all the previous studies, question still remains to find a good balance between AA concentration which has efficient antimicrobial activity, low toxicity and better patient's tolerability. Hence, A randomised controlled clinical trial is warranted to answer all these questions.

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 ≥1% TBSA. The expected start and end dates of the study are as follows (Table 2).

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

Table 1: BWI Identifiable Bacteria

Table 2: Start and End dates

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

Conduct of Trial

Inclusion and Exclusion criteria:

The trial will aim to recruit adult patients (age ≥16 years old) who sustained a ≥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
≥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. 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)

GROUP 2: 2% acetic acid (10 patients)

Total number: 20 patients

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

Blinding:

The treatment group allocation will be concealed from the patient and clinical staff throughout the trial period. Each treatment box will be numbered according to a randomisation list generated on a randomisation wizard by biostatistician. The randomisation list will be provided to the manufacturer, Stockport Pharmaceuticals, who will in turn package and label the treatment boxes.

Un-blinding process:

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

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 10x10cm^2 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 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 required as described above. Details of this are given in the Statistical Analysis Plan (SAP).

Time to 95% wound healing:

Time from randomisation to 95% wound healing of the treated area will be presented using reverse Kaplan-Meier curves. In presentation of these curves, patients will be appropriately censored at the point they withdraw from or complete the trial. Presentation of these curves will account for the nested data structure as necessary.

The time to wound healing will be assessed using hierarchical (also referred to as multi-level) cox models. The hierarchical structure will be included, if necessary, to reflect the nesting of wound through patient.

Perceived treatment allocation:

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

End of trial

The end of trial will be the date of completion of treatment for the last patient. The Trials Office will notify the MHRA and REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

Patient and Public Involvement

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

Trial Status

Recruitment into the AceticA trial began in February 2018 and it is still open to recruitment.

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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Conflict of Interests:

The authors have no conflict of interests to declare.

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Grant Number: Not applicable

Author Contributions

The concept of the AceticA trial was developed by NM (Chief investigator) and BO, TH, RI, KP, KA (Co-investigator). NM, KB, DB designed/developed the initial AceticA trial protocol. NM, DB, VH, GS developed, wrote, reviewed subsequent protocol versions. AC designed the proforma used for pre-screening, microbiological lab manual and protocol procedure documentations. GS, DB, TH submitted all REC, MHRA and local R&D applications. KB and VH devised the statistical plan. GS, DB, TH and AC wrote/designed the patient information sheets,

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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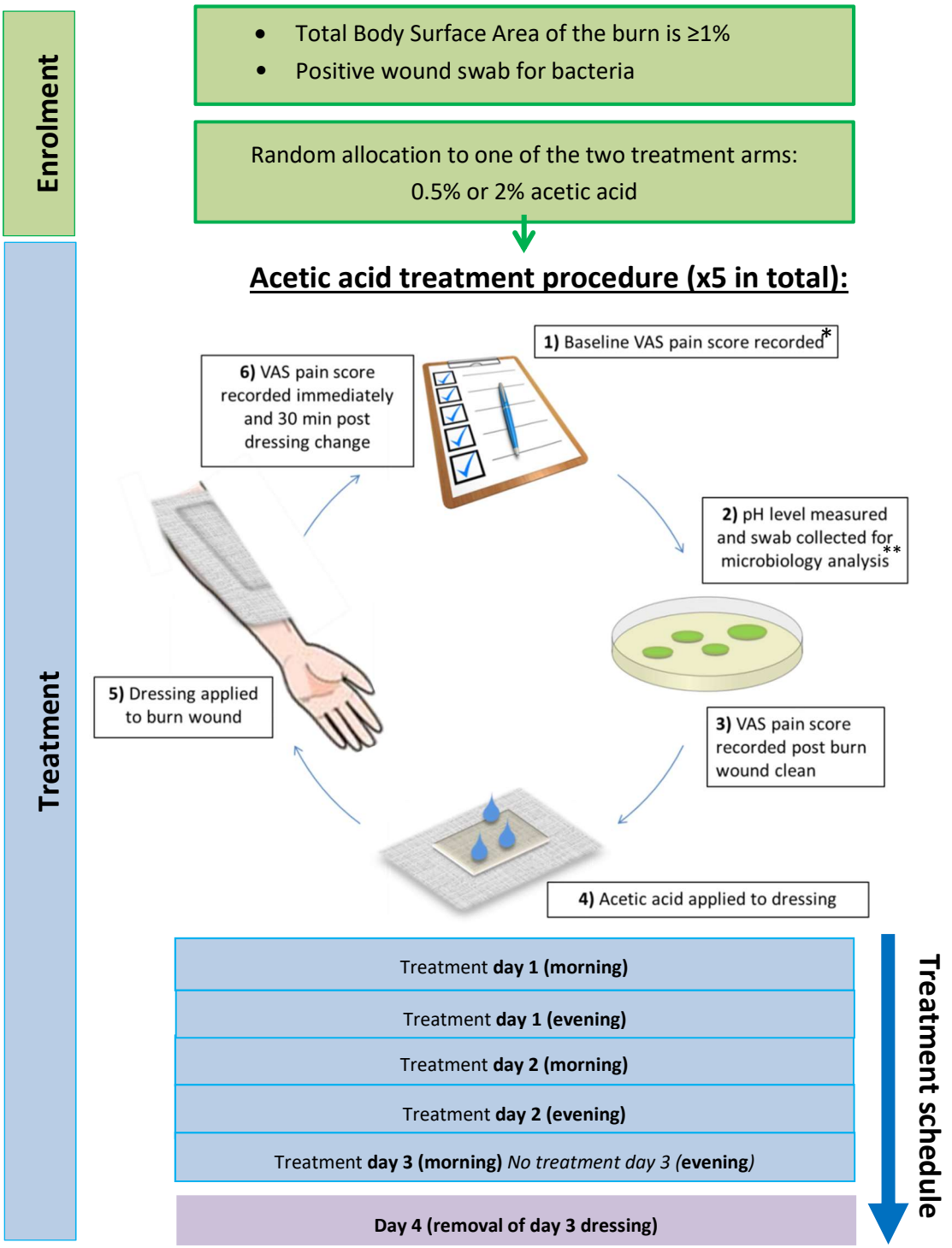
AceticA

TRIAL SCHEMA

Figure 1: Diagram of patient pathway



* Visual Analogue Scale; collected if possible

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

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	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

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

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

[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 patients have the capacity to do so):

- Pre-treatment change analgesia
- Immediately post burn wound cleaning
- Immediately post treatment
- 30 minutes post treatment

TABLE OF CONTENTS

CHAPTER 1

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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Primary Subject Heading:	Surgery
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Keywords:	PLASTIC & RECONSTRUCTIVE SURGERY, WOUND MANAGEMENT, SURGERY, Plastic & reconstructive surgery < SURGERY

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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Keywords

Burns; Acetic acid; randomised control trial; colonised wound; infected wound; pain

Word Count

4665 words

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:

GROUP 1: 0.5% acetic acid (10 patients)

GROUP 2: 2% acetic acid (10 patients)

Total Number: 20 patients

Outcome measures

Primary outcome

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

Secondary outcomes

(1) The assessment of antimicrobial activity of acetic acid and the minimum 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.

Ethics and dissemination

AceticA trial protocol was approved by the National Research Ethics Service (West Midlands - Edgbaston Research Ethics Committee; 17/WM/0407; IRAS 234132). This article refers to protocol V5.0 dated 06th July 2020

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.

Strengths and limitations of this trial

- It is a double blinded, randomised control trial comparing two different strengths of Acetic acid.
- The study design is very simple and feasible for patients with the trial duration of only 3 days with no extra outpatient follow ups.
- Two different strengths of acetic acid will be used to compare the antimicrobial efficacy, tolerability and feasibility in patients.
- The study does not interfere with the standard burns care of the patient.
- 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.

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)

Since then, a number of studies has been conducted to assess the effectiveness and efficacy of acetic acid in management of BWI(13)(14).

I*n vitro* studies, diluted AA (1-5%) has shown potential to reduce or eradicate bacterial load, specifically Pseudomonas aeruginosa (15)(16). Minimum inhibitory concentrations (MIC) of acetic acid has been studied in vitro both before and after evaporation and exposure to gauze. These showed that the methicillin-susceptible strain of Staphylococcus aureus (MSSA) had an MIC of 0.312% and a methicillin-resistant strain (MRSA) was less susceptible to MIC of 0.625%. Strains of Acinetobacter baumannii also had a MIC of 0.312% and all strains of Pseudomonas aeruginosa were susceptible to MIC of 0.166%(11).

Different concentrations of acetic acid has been studied to treat BWI (17)(18)(19). Patient's tolerability to topical agent is also very important. Patients usually complain of stinging and pain on application of acetic acid to wounds, particularly at higher concentrations, e.g. strength of 5% or more (13)(14). In another study 3% concentration was used with better pain tolerance and less itching (16). In a recently published survey of burn centres in UK, 6 centres (32%), routinely use AA topically as gauze soaked with 2.5-3.0%. This high concentration was reported to be well tolerated (20).

Despite of all the previous studies, question still remains to find a good balance between AA concentration which has efficient antimicrobial activity, low toxicity and better patient's tolerability. Hence, A randomised controlled clinical trial is warranted to answer all these questions.

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 ≥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 ≥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 ≥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

number of otherwise eligible patients could not be approached and hence recruited as they were admitted to ITU and so lacked capacity to give informed consent. Changes were made in protocol to included patients who lacked capacity to give informed consent. The consent for these patients can be sought from a Professional or Personal Legal Representative.

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). At first, 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, compared to day 2 and 3. Now patients will get twice a day dressing change on day 1, 2 and once a day on day 3.

Patients receiving systemic antibiotics for cellulitis were excluded initially. The clinical team suggested that these are the patients who may benefit most from topical antimicrobial treatment. In fact, acetic acid (usually of higher concentrations) is part of routine therapy for these patients. Antibiotics are prescribed to control the systemic spread of infection and do not interfere with the wound microbial burden. Therefore, this exclusion point was removed to allow these patients the opportunity to receive 0.5% or 2% acetic acid solution to treat their wounds. This should not affect the outcome of determining the difference in the microbial burden of the two different concentrations.

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
≥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. 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.

At first, there were two consent forms for the study, pre-screening consent form for initial microbiology swab to indicate eligibility. If patient is eligible for treatment, then informed consent form will be taken prior to start the treatment. To make consent process easier, single consent form was introduced later on for screening and treatment phase of the trial.

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)
GROUP 2: 2% acetic acid (10 patients)
Total number: 20 patients
Enrolment and randomisation of the patient will be completed on the electronic the Clinical Research Tool (CREST) system (an in-house bespoke, electronic Remote

Data Capture system (eRDC) developed by QEHB), on which patients' trial number and treatment pack numbers will be allocated and randomisation confirmation emails will be sent to relevant trial team members. The CREST system is also trial database system.

Blinding:

The treatment group allocation will be concealed from the patient and clinical staff throughout the trial period. Each treatment box will be numbered according to a randomisation list generated on a randomisation wizard by biostatistician. The randomisation list will be provided to the manufacturer, Stockport Pharmaceuticals, who will in turn package and label the treatment boxes.

Un-blinding process:

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

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 10x10cm^2 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 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

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 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.

Patient's tolerability was changed from primary to secondary outcome anticipating few patients might be sedated and ventilated and unable to respond to visual analogue pain score. Secondly, 'percentage of wound healed at day 21' was changed to 'time to 95% wound healing'. This amendment was done as some patients develop colonised burns wound and became eligible for recruitment after

few weeks of injury. This measurement will be collected as part of burns patients' standard care and can be obtained retrospectively from the patients' medical notes.

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 required as described above. Details of this are given in the Statistical Analysis Plan (SAP).

Time to 95% wound healing:

Time from randomisation to 95% wound healing of the treated area will be presented using reverse Kaplan-Meier curves. In presentation of these curves, patients will be appropriately censored at the point they withdraw from or complete the trial. Presentation of these curves will account for the nested data structure as necessary.

The time to wound healing will be assessed using hierarchical (also referred to as multi-level) cox models. The hierarchical structure will be included, if necessary, to reflect the nesting of wound through patient.

Perceived treatment allocation:

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

End of trial

The end of trial will be the date of completion of treatment for the last patient. The Trials Office will notify the MHRA and REC that the trial has ended and will provide them with a summary of the clinical trial report within 12 months of the end of trial.

Patient and Public Involvement

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

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	20th February 2018
First patient recruited	23rd March 2018
Last Patient Last Visit	8th October 2021
End of Study Declaration submission	5th 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

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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Conflict of Interests:

The authors have no conflict of interests to declare.

Funding:

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Grant Number: Not applicable

Author Contributions

The concept of the AceticA trial was developed by NM (Chief investigator) and BO, TH, RI, KP, KA (Co-investigator). NM, KB, DB designed/developed the initial AceticA trial protocol. NM, DB, VH, GS developed, wrote, reviewed subsequent protocol versions. AC designed the proforma used for pre-screening, microbiological lab manual and protocol procedure documentations. GS, DB, TH submitted all REC, MHRA and local R&D applications. KB and VH devised the statistical plan. GS, DB, TH and AC wrote/designed the patient information sheets, 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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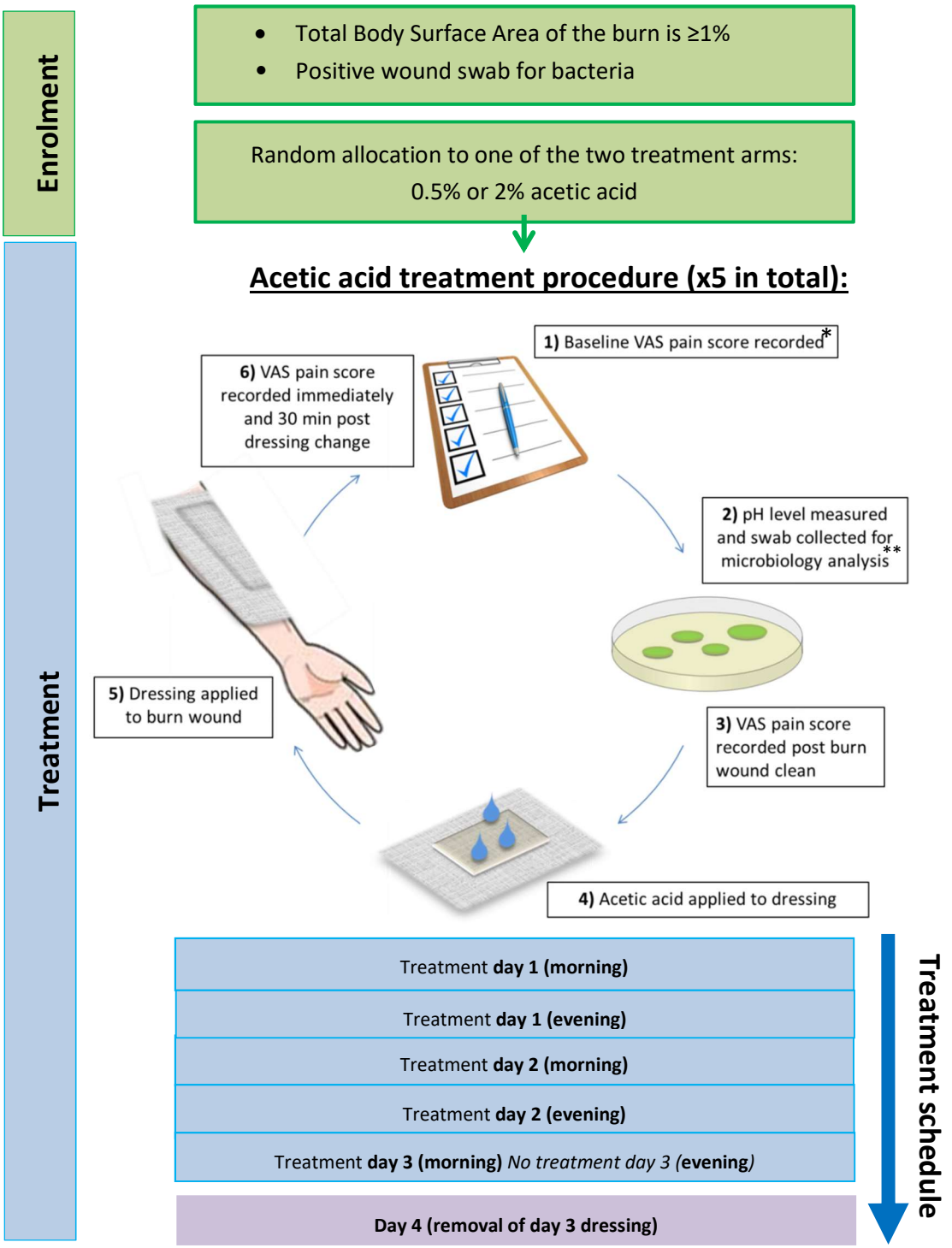
AceticA

TRIAL SCHEMA

Figure 1: Diagram of patient pathway



* Visual Analogue Scale; collected if possible

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

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	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

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

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

[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 patients have the capacity to do so):

- Pre-treatment change analgesia
- Immediately post burn wound cleaning
- Immediately post treatment
- 30 minutes post treatment

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			

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

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
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
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	13
	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
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	n/a
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	n/a
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
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
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
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	11
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
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

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

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
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	12
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	13
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	13
Methods: Data collection, management, and analysis			
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
	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
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
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
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	18,19
	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
Methods: Monitoring			

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

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
	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
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
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
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	25
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
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	11
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	n/a
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
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
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
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

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

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	25
	31b	Authorship eligibility guidelines and any intended use of professional writers	25
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	22
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	n/a
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	13