Is the mechanism of action of hypertonic saline and/or carbocisteine in the treatment of patients with acute respiratory failure due to an increase in mucus hydration?

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1. SUMMARY

Background

Patients with acute respiratory failure (ARF) experience an increased risk of respiratory tract secretion retention due to altered secretion rheology and impaired mucociliary clearance. The use of carbocisteine and hypertonic saline are now being evaluated in patients with ARF as part of the MARCH clinical trial and in this study we will investigate the mechanistic effects of these two mucoactive agents.

Hypothesis

Treatment of critically ill patients with ARF with carbocisteine, hypertonic saline, or both, will lead to increased mucus hydration and changes in sputum viscosity and elasticity.

Aim

To determine if critically ill patients with ARF treated with carbocisteine, hypertonic saline, or both, experience increased hydration of airway mucus (reflected as a reduced percentage mucus solid content[1]).

Objectives

1. To measure percentage mucus solid content (dry-to-wet weight ratio) of sputum following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual care airway clearance management alone (no mucoactive)
2. To measure the yield stress (Tc) (derived from sputum viscosity (G') and elasticity (G'')) following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone (no mucoactive)
3. To measure the inflammatory mediators, IL-6, IL-8 and 8-isoprostane following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone (no mucoactive)

Methods

Sputum samples will be collected from patients recruited to the MARCH clinical trial (https://www.fundingawards.nihr.ac.uk/award/NIHR130454). Sputum samples will be collected at 3 time-points during the study; baseline (at randomisation), Day 3, and Day 7. The solids concentration of mucus will be measured and dynamic rheology measurements will be recorded including G' and G" of samples from which the Tc value will be obtained. Inflammatory mediators will be measured by ELISA.

Anticipated project timelines

The MARCH clinical trial is currently actively recruiting patients. Ethical approval is in place for collection of sputum samples from patients enrolled into the study. The current study will commence in May 2022, with a timeframe of 3 months for set-up, and 27 months for collection and analysis of sputum for mucus solid measurements, rheometry, and inflammatory mediators. A further 6 months will allow for data linking with main trial outcomes and final reporting.

Impact and Dissemination

The positive effect of carbocisteine and/or hypertonic saline to changes in sputum hydration, sputum viscosity and elasticity, and inflammation may lead to the development of even more effective drugs for the treatment of ARF. The findings of this work will be disseminated through conferences, peer reviewed publications, and through patient and public representatives. The outputs of this work will also be aligned with the outputs of the main MARCH trial.

2. BACKGROUND

MARCH: Mucoactives for Acute Respiratory failure: Carbocisteine and Hypertonic saline

Acute respiratory failure (ARF) accounts for the majority of patient admissions to UK intensive care units (ICU)[2-5], with the majority of patients requiring invasive mechanical ventilation[2]. However, invasive mechanical ventilation increases the risk of respiratory tract secretion retention due to altered secretion rheology and impaired mucociliary clearance[6]. Usual airway clearance management may include suctioning, heated humidification, and respiratory physiotherapy techniques, supplemented with mucoactive drugs[7]. However, use of mucoactive drugs is common but highly empirical, with wide variation in prescribing across ICUs[8]. Typically, the major clinical feature prompting their use in patients with ARF is the presence of thick, difficult to clear, secretions[7 9]. Two of the most commonly used mucoactives in critically ill patients with ARF are carbocisteine and hypertonic saline[9]. However, there is little evidence to support their effectiveness in UK practice[10 11].

The MARCH clinical trial led by Dr Bronwen Connolly and Professor Danny McAuley, is currently recruiting across UK ICUs. The MARCH trial is a 2x2 factorial, open label, randomised controlled trial. Eligible patients are allocated to one of four treatment groups: i) carbocisteine plus usual airway clearance management; ii) hypertonic saline plus usual airway clearance management; iii) carbocisteine and hypertonic saline plus usual airway clearance management; iv) usual airway clearance management alone. Randomisation is on a 1:1:1:1 ratio using a central randomisation system. The primary outcome for the MARCH trial is duration of mechanical ventilation. The trial PICO is as follows:

Population:	Adult, critically ill patients admitted to the ICU with ARF and requiring invasive mechanical ventilation, with secretions that are difficult to clear with usual airway clearance management (as assessed by the treating clinical team)
Intervention:	Mucoactives (carbocisteine, or hypertonic saline, or both) in conjunction with usual airway clearance management
Comparator:	Usual airway clearance management alone, including suctioning, heated humidification (either active heated humidification devices, or passive heat and moisture exchangers), and respiratory physiotherapy; isotonic saline may also be used depending on clinician preference
Outcomes:	Primary – Duration of mechanical ventilation Secondary – Range of clinical and safety outcomes at 60 days and 6 months, cost effectiveness at 6 months

Grant funding for the MARCH trial is from the NIHR Health Technology Assessment Programme (https://www.fundingawards.nihr.ac.uk/award/NIHR130454). Recruitment commenced on 17th February 2022 and will cease on 31st October 2024. Ethical approval and governance for collection of sputum samples is integrated into the main approvals process (IRAS 293630, REC 21/YH/0234).

Why is this research needed now?

In 2019/20, approximately 146,000 patients were admitted to ICUs across England, Wales, and Northern Ireland, of whom 40% required advanced respiratory support including mechanical ventilation[12], with median duration reported as around 7 days[5 13]. Mucoactive prescription practice is empirical, and usually based on local availability of agents, personal preference, and prior experience. No national guidelines exist to direct practice which is widely variable and not evidence-based. Management of airway secretions features heavily in the patient experience of critical illness. Establishing evidence for the clinical- and cost-effectiveness of mucoactives in critical care will ensure that they are delivered to the most appropriate patients, where applicable, thus minimising the potential for harm and unnecessary expense. Our Patient and Family Advisory Group (PFAG) described

recollections of requiring significant airway clearance management to help clear thick, difficult to clear, secretions and are strong proponents of this research to investigate this topic to improve patient outcomes.

Evidence for the effectiveness of mucoactive drugs in reducing duration of mechanical ventilation in critically ill patients with ARF is minimal and low quality[10], despite the high prevalence of their empirical use[9]. Focus groups with specialist critical care physiotherapists highlighted the use of mucoactive drugs in these patients as adjunctive to usual airway clearance management to facilitate clearance of thick, difficult to clear, secretions[7]. Surveys of UK ICUs and other critical care clinicians to ascertain current practice also showed this was the primary indication for their use, as well as confirming carbocisteine and hypertonic saline as the two most commonly used mucoactives. These surveys highlighted that more than 80% of UK ICUs use mucoactives, and at any given time, approximately one-third of critically ill patients receiving invasive mechanical ventilation are prescribed at least one mucoactive drug [9].

The MARCH clinical trial will generate high-quality evidence of clinical and cost effectiveness of mucoactive drugs to inform patient care. However, there are also no available mechanistic data demonstrating the action of mucoactive drugs in patients with ARF. Therefore, a unique opportunity now exists to explore the mechanism of action of carbocisteine and hypertonic saline by investigating sputum samples obtained from patients enrolled into the MARCH trial, to determine differences in mucus hydration as well as changes in sputum viscosity and elasticity and inflammatory mediators.

By exploring the effect of carbocisteine and hypertonic saline on mucus hydration and rheology we will provide important information regarding the mechanistic effect of both drugs in the treatment of ARF and importantly, how this may relate to positive patient outcomes such as a reduced duration of mechanical ventilation. If effective, understanding the mechanistic effect of carbocisteine and hypertonic saline may facilitate more precise dosing regimes of these drugs in critically ill patients, as well as guide the development of future clinical trials of airway secretion management.

What is the knowledge gap this research will address?

Altered secretion rheology in critically ill patients within ARF causes impaired mucus clearance and potentially, persistence of bacteria within the lung leading to increased risk of infection. A similar pathophysiological scenario occurs in other chronic respiratory conditions such as Cystic Fibrosis and Bronchiectasis. The resulting inflammatory process results in loss of epithelial cells and ineffective ciliary function, destruction of the surfactant layer by airway phospholipases, and alteration of the biophysical properties of the mucus[14] [15]. In addition, by-products accumulated during the inflammatory process include neutrophil-derived DNA and filamentous actin (F-actin), dead/apoptotic cells, bacteria and cell debris. Collectively, these factors contribute to mucus purulence, and when expectorated, this mucus is termed sputum[16].

There are very few studies evaluating mucus solids in lung disease samples. Donaldson and colleagues have demonstrated mucus percent solids in the sputum of individuals with bronchiectasis are threefold higher than in healthy controls[17]. The same group of investigators have also demonstrated increased percent solids in the sputum of chronic obstructive pulmonary disease and adult cystic fibrosis patients compared to healthy controls[1] [18]. Our searches did not identify any published studies evaluating mucus solids in lung disease patients treated with carbocisteine or hypertonic saline.

In rheometry studies, the direct evaluation of carbocisteine or hypertonic saline *in vivo* is limited but with an increasing number of published studies, particularly with hypertonic saline, over the past 2 years. One study showed that the inhalation of hypertonic saline produced a sustained acceleration of mucus clearance and improved lung function[19]. Long-term inhalation of hypertonic saline without

pretreatment with amiloride (i.e., with placebo pretreatment) resulted in a sustained (> or =8 hours) increase in 1-hour rates of mucus clearance, as compared with those with amiloride pretreatment (14.0+/-2.0 vs. 7.0+/-1.5 percent, respectively; P=0.02) and increased 24-hour rates of mucus clearance over baseline[19]. Furthermore, inhalation of hypertonic saline with placebo improved the forced expiratory volume in one second (FEV1) between the baseline period and the treatment period (mean difference, 6.62 percent; 95 percent confidence interval, 1.6 to 11.7; P=0.02), whereas hypertonic saline with amiloride did not improve FEV1 (mean difference, 2.9 percent; 95 percent confidence interval, -2.2 to 8.0; P=0.23)[19]. However, direct measurement of sputum elasticity and viscosity to explain this result was not carried out in this study. Other studies have shown improved clearance of sputum in cystic fibrosis using hypertonic saline but did not evaluate the mechanistic reasons for this improved clearance[20 21]. Administration of nebulised hypertonic saline to female asthma patients resulted in an improvement in mucociliary clearance in these patients[22]. Similarly, nebulisation of hypertonic saline to a paediatric group with non-cystic fibrosis bronchiectasis showed an improvement in lung function and reduced exacerbations in this group compared to children receiving conventional treatment only[23]. Administration of hypertonic saline to patients with chronic bronchitis did not show any improvements in mucociliary clearance or spirometry in this patient group[24]. Hypertonic saline given to children with cystic fibrosis resulted in a significant improvement in whole lung clearance after 4 weeks of therapy. Improvements in spirometry with hypertonic saline did not reach statistical significance but correlated with mucociliary clearance changes[25]. Another study in sepsis found that hypertonic saline did not improve survival in patients compared to isotonic saline[26]. In another study, it was found that giving patients with moderate-to-severe acute respiratory distress syndrome a bolus of intravenous hypertonic saline reduced the lung injury score in these patients by 1 point and resulted in a decrease in length of mechanical ventilation and hospital length of stay in the hypertonic saline group[27].

For carbocisteine, clinical trials appear to have only been carried out in patients with chronic obstructive pulmonary disease. Paone and colleagues demonstrated that the efficacy of long-term administration of a single daily dose of carbocysteine lysine resulted in a statistically significant reduction of the average number of exacerbations vs. the number observed in the previous year (from 1.97±0.10 to 1.03±0.11; p<0.01), irrespective of treatment with or without inhaled steroids[28]. Another study showed that carbocisteine was effective and well-tolerated in the treatment of chronic obstructive pulmonary disease with a small percentage of reported mild adverse reactions and with a significant improvement of quality of life[29]. Esposito and colleagues have demonstrated that administration of carbocisteine for 12 months in addition to the bronchodilator therapy reduced exacerbation frequency in chronic obstructive pulmonary disease[30]. A similar study by Zheng et al showed that the numbers of exacerbations per patient per year declined significantly in chronic obstructive pulmonary disease patients treated with carbocisteine for 12 months compared to the placebo group[31].

These studies provide proof of concept data that an improvement in mucus hydration or reduced viscoelasticity may be a mechanism by which these drugs facilitate airway secretion clearance in critically ill patients with ARF[32 33].

Although there are currently no studies demonstrating a direct effect of mucoactive drugs on mucus hydration, there are a number of studies that strongly suggest that an increase in mucus hydration as result of mucoactive therapy. One recent case study has demonstrated that administration of the mucoactive drug, N-acetyl-cysteine, to a patient with pneumonia with a solid tracheal mucus plug caused sufficient mucolysis to enable removal of the thick mucus plug, enabling ventilation and gas exchange[34]. Other studies are also indicative of increased mucus hydration. One study showed that administration of hypertonic saline to patients with cystic fibrosis resulted in an acceleration of mucus clearance and improved lung function[19]. Other studies in patients with cystic fibrosis confirmed an

improved clearance of sputum in patients using hypertonic saline[20 21]. In addition, administration of nebulised hypertonic saline to female asthma patients resulted in an improvement in mucociliary clearance in these patients[22]. Furthermore, other studies demonstrate improvements in lung function and reduction in exacerbation in various patient groups, and may also be suggestive of an increase in mucus hydration[23-25 27-31].

Therefore**, although some evidence does exist regarding improved mucus clearance in chronic infective airways disease using hypertonic saline, there is a clear knowledge gap regarding the underlying mechanism of action of carbocisteine and hypertonic saline and their impact on mucus hydration, viscoelasticity, and inflammation in ARF.**

In this study**, we will directly address the *in vivo* mechanism of action of carbocisteine and hypertonic saline in ARF by focusing primarily on improvements in mucus hydration with a secondary focus on changes in sputum viscoelasticity and inflammatory mediators. A unique opportunity now exists to explore the mechanism of action of carbocisteine and hypertonic saline by obtaining sputum samples from patients currently being recruited to the MARCH trial.**

3. AIMS AND OBJECTIVES

Aim

The aim of this study is to determine if critically ill patients with ARF treated with carbocisteine, hypertonic saline, or both, experience increased hydration of airway mucus (reflected as a reduced percentage mucus solid content).

Hypothesis

Treatment of critically ill patients with ARF with carbocisteine, hypertonic saline, or both will lead to increased mucus hydration and changes in mucus viscosity and elasticity.

Primary objective

To measure percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 0 and Day 3 in critically ill patients with ARF following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone.

Secondary objectives

1. Measurement of percentage mucus solid content (dry to weight ratio) at Day 0 and Day 7 following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone
2. Measurement of yield stress (T_c) (derived from sputum viscosity (G') and elasticity (G'')) at Day 0 and Day 3 following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone
3. Measurement of sputum levels of IL-6, IL-8 and 8-isoprostane at Day 0 and Day 3 following commencement of treatment with carbocisteine, hypertonic saline, or both or usual airway clearance management alone (no mucoactive)
4. Measurement of yield stress (T_c) (derived from sputum viscosity (G') and elasticity (G'')) at Day 0 and Day 7 following commencement of treatment with carbocisteine, hypertonic saline, or both, or usual airway clearance management alone
5. Measurement of sputum levels of IL-6, IL-8 and 8-isoprostane at Day 0 and Day 7 following commencement of treatment with carbocisteine, hypertonic saline, or both or usual airway clearance management alone (no mucoactive)

4. PROJECT DESIGN AND METHODS

Study design

The proposed study is a multi-centre, exploratory mechanistic, observational cohort study linked to the MARCH trial that is sponsored by the Belfast Health and Social Care Trust. Sixty ICUs across all four nations of the UK are currently registered to participate in the MARCH trial, that are geographically spread across the UK, and represent ICUs of varying size and specialty, serving various regions of differing socioeconomic, cultural, and ethnic characteristics. The main MARCH trial has funding for translation of patient and family-facing materials (into up to 10 commonly used languages) to facilitate enrolment of patients for whom English is not their first language. These strategies will support enrolment of as diverse, inclusive, and representative patient population as possible. Eligibility criteria for enrolment into the main MARCH trial are specifically designed to facilitate enrolment of a broad and generalisable population of critically ill patients who may benefit from the therapeutic intervention, while excluding patients who may be more likely to experience an adverse reaction.

Patient Selection and treatment allocation

Patients enrolled in the MARCH trial are aged ≥16 years, with acute respiratory failure, receiving invasive mechanical ventilation for at least 48 hours or more, and with presence of secretions that are difficult to clear with usual airway clearance management (including suctioning, heated humidification, respiratory physiotherapy; isotonic saline).

The proposed study will involve collection of sputum samples from patients randomised to each of the four trial groups:

1. Carbocisteine plus usual airway clearance management
2. Hypertonic saline plus usual airway clearance management
3. Carbocisteine and hypertonic saline plus usual airway clearance management
4. Usual airway clearance management alone

Sample collection

Sputum (endotracheal secretions) samples will be collected at 3 time-points during the study; Day 0 (baseline - at randomisation), Day 3 and Day 7. Each sputum sample will be frozen at -80°C at each trial site in HTA-monitored freezers prior to shipment to the experimental site in the Wellcome-Wolfson Institute of Experimental Medicine, Queen's University Belfast. Once in the laboratory, samples will be divided into separate aliquots to undertake the analyses of mucus solids and rheometry. Research staff carrying out sputum lab analysis (mucus solids and rheometry) will be blinded to the treatment allocation (carbocisteine, hypertonic saline, a combination of carbocisteine and hypertonic saline, and usual airway clearance management alone) of each sample.

Analyses

1. *Mucus solids measurement*

The solids concentration of mucus will be measured by aliquoting between 100 and 200 µL of sputum on a pre-weighted piece of foil and recording the final mass of the sample and foil. The sample will then be placed in an 80°C oven overnight. The final mass of the dried foil and sample will be recorded and calculated as percent solids (wt%) as assessed by measurement of pre- and post-desiccation weights of sputum aliquots[1]. Percentage mucus solid content is an index of hydration. Therefore, a positive outcome from this part of the study would be for carbocisteine and/or hypertonic saline-treated sputum samples to exhibit lower percentage mucus solid content i.e. more hydrated.

2. *Rheometry*

Sputum samples will be thawed and dynamic rheology measurements recorded using the Rheomuco instrument (Rheonova). This instrument will be hired directly from Rheonova for the duration of the study. Sputum samples will be squeezed between 2 rough plates (geometries). Oscillatory rotation will be applied to the samples. Sputum is characterized by its elastic (G') and viscous (G") moduli, signatures of its cross-linked microstructure, and raw data will be collected for each sputum sample in relation to its elastic (G') and viscous (G'') values. Sputa behaves as a soft viscoelastic solid, with G' > G" both constant. Beyond a critical strain (> 1000%), the mucus becomes more fluid-like as G' decreases below G" and starts to flow; the onset is defined by the yield stress (T_c) that corresponds to the actual stress when G' = G". Yield stress thus characterizes the ability to set mucus in motion over long distances. A high Tc, indicates a greater amount of obstruction in the sample and a lower Tc, indicates a decreased amount of obstruction in the sample. Therefore, a positive outcome from this part of the study would be for carbocisteine and/or hypertonic saline-treated sputum samples to exhibit lower Tc values compared to sputum samples from patients receiving usual airway clearance management only.

3. Inflammatory marker analysis

IL-6 and IL-8 are significant markers of inflammation in the airways and reductions in these cytokines with carbocisteine and hypertonic saline treatment may be indicative of less inflamed lung environment. Likewise, a reduction in 8-isoprostane, a marker of oxidative stress, may also reflect a less oxidant-rich environment. Four (4) times the volume of 10% sputolysin solution will be added to the 3rd frozen sputum aliquot, which will be homogenized for 15 minutes on a rocker at room temperature. Subsequently, the same volume of PBS will be added, samples were filtered through 100 µm and 40 µm cell strainers and centrifuged at 300 g and 4°C for 10 minutes. Cell pellets will be resuspended in PBS and cell counts were determined using trypan blue (Sigma, St Louis, MO, USA). The sputum supernatants will be treated with protease inhibitors (Roche Diagnostics, Rotkreuz, Switzerland) and supernatants analysed for IL-6, IL-8 and 8-isoprostane by ELISA (R&D Systems).

Sample size

We plan to analyse sputum samples collected from 360 patients enrolled in the MARCH trial from across participating ICUs. All consecutive patients recruited to MARCH will be approached to participate in the current study. The main MARCH clinical trial is recruiting a target of 1956 patients across the four arms of the study. Therefore, the proposed sample size for this study (n=360) should be easily achieved given the anticipated recruitment for the MARCH study.

The sample size has been determined with statistical input from the Northern Ireland Clinical Trials Unit to generate significant differences in the primary outcome of percentage mucus solids in sputum samples of MARCH patients. A sample size of 85 in each of our treatment groups will have 90% power to detect a difference in means of 1.15% assuming that the common standard deviation is 2.3% using a two-group t-test with a 0.05 two-sided significance level. This is equivalent to an effect size of 0.5. Assuming approximately 5% dropout we will collect sputum samples from 360 patients recruited to MARCH trial (90 per arm). Although there are no examples of mucus solids measurements in patients with ARF, the sample size generated above is based on mucus solid measurements evaluated in patients with cystic fibrosis and chronic obstructive pulmonary disease[1], the closest disease examples to ARF we could find in the literature.

Importantly, there are likely to be missing samples on day 7 due to extubation, discharge, or death. However, we intend analysing these samples as they will still provide important mechanistic information regarding the effect of both drugs on this day.

Statistical analysis

The primary analysis will be on the per-protocol population as we want to determine differences between groups of patients and at a significance level of 0.05 unless adjustment for multiple testing is needed.

Primary objective

The primary objective is to measure percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 0 and Day 3 following commencement of treatment with carbocisteine and/or hypertonic saline.

Analysis

Percent solids (wt%) of sputum will be assessed by measurement of pre- and post-desiccation weights of sputum aliquots. Higher percentage weight indicates greater mucus solid content and is associated with disease[10] and lower percentage mucus solid content i.e. more hydrated indicates lower solid content which is associated with a more normal phenotype. We will assess the sputum percent solids in all 4 patient cohorts on the MARCH trial at day 3 and compare to sample measurements at baseline (before commencement of treatment). Comparisons will be made between the groups using Analysis of Covariance (ANCOVA) (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.

Secondary objectives

The secondary outcome measure post commencement of treatment will be to measure:

1. Percentage mucus solid content (dry-to-wet weight ratio) of sputum at Day 7 following commencement of treatment with hypertonic saline and/or carbocisteine
2. Sputum elasticity (G') and viscosity (G'') (and yield stress, T_c) at Day 3
3. Sputum IL-6, IL-8 and 8-isoprostane levels at Day 3
4. Sputum elasticity (G') and viscosity (G'') (and yield stress, T_c) at Day 7
5. Sputum IL-6, IL-8 and 8-isoprostane levels at Day 7

Analysis

Percent solids (wt%) of sputum will be assessed at day 7, as for the primary objective analysis, and compared to sample measurements at baseline (before commencement of treatment). Comparisons will be made between the groups using Analysis of Covariance (ANCOVA) (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values. Sputum will also be characterized by its elastic (G') and viscous (G") moduli in order to determine the yield stress value, T_c, a marker of sputum motion. A high T_c, indicates a greater amount of obstruction in the sample and a lower T_c, indicates a decreased amount of obstruction in the sample. We will assess the sputum elasticity, viscosity and yield stress in sputum samples obtained from all 4 patient cohorts on the MARCH trial at Day 3 and day 7 and compare to sample measurements at baseline (before commencement of treatment). We will also measure sputum levels of IL-6, IL-8 and 8-isoprostane in sputum samples from all 4 patient cohorts on the MARCH trial at Days 3 and 7 and compare to sample measurements at baseline (before commencement of treatment). Comparisons will be made between the groups using Analysis of Covariance (ANCOVA) (and post-hoc tests if significant differences are identified) will be used to detect difference in means adjusting for baseline values.

5. ETHICAL CONSIDERATIONS

The study will be conducted in accordance with the ethical principles originating in the Declaration of Helsinki and those in Good Clinical Practice. Ethical approval and governance for collection of sputum samples is integrated into the secured approvals for the main MARCH trial (IRAS 293630, REC 21/YH/0234). Information regarding sputum sample acquisition has been included in all information sheets and consent forms to accompany the MARCH trial, and offers participants the choice to indicate if consent for sample acquisition is given. A separate ethical approval for the MARCH EME mechanistic study for the analysis of anonymised samples with donor consent for future use will be sought from QUB's Faculty of Medicine, Health and Life Sciences Research Ethics Committee.

6. BENEFITS OF THE STUDY

The results of this trial will have significant impact in three main ways:

1. Patient benefit: If the use of mucoactive drugs is clinically effective, this therapy can be used more appropriately and efficiently, to improve patient outcomes as well as inform more precise application of these mucoactives in clinical practice.

2. Change in practice: Demonstrating that reduced time on mechanical ventilation or decreased hospital stay is associated with an improvement in mucus hydration or rheology, will greatly strengthen the MARCH trial intervention. This has the potential to improve compliance with this approach, aiding clinicians in future decision-making regarding the use of carbocisteine and/or hypertonic saline in critically ill patients with ARF. This could have significant benefits in clinical outcomes and cost savings for the NHS.

3. Future trials: Drugs such as carbocisteine and hypertonic saline have shown promise in other lung diseases where their use has been associated with improvements in lung function, reductions in exacerbations, and reductions in inflammation[19 22-25 36-37]. However, the mechanism of action of both drugs is unknown. Elucidation of this mechanism forms the basis of this study. As such, positive findings from the study related to changes in mucus hydration and rheometry could potentially be employed in future near-patient testing strategies as a way to monitor efficacy in patients receiving these drugs. In this way the current study has the potential to benefit not just populations of critically ill patients with ARF but also wider populations of patients with chronic lung diseases such as cystic fibrosis, asthma, and chronic obstructive pulmonary disease.

7. INFORMATION ON RESOURCES AND COSTS

The project is funded by an NIHR EME award (NIHR134567) of £333,387.82 (R4095CEM).

Staff costs
Costs have been awarded for a Postdoctoral Research Assistant (PDRA, 100% FTE) who will carry out the mucus solids and rheometric, measurements on the collected sputum samples. The PDRA will have experience in sputum handling as well as mucus solids and rheometric analysis. The PDRA will also work with the Lead Investigator (Taggart) to coordinate delivery of sputum samples from the various recruitment sites. Taggart (Chair of Respiratory Cell and Molecular Biology, 10% FTE) will have oversight of the project and fulfil the duties of Chief Investigator, leading, supervising and coordinating the project. Connolly (Lead applicant and co-chief investigator for MARCH), McAuley (co-chief investigator for MARCH) and Bradley (Director of the Clinical Research Facility) have been costed at 2% FTE to offer the necessary clinical trial leadership and CTIMP regulatory assurance. The Northern Ireland Clinical Trials Unit (NICTU) will provide a core team of staff including Biostatisticians, a Clinical Trial Co-ordinator, and a Trial Manager to set up, co-ordinate, monitor and support the study, including data collection and analysis throughout its duration. NICTU co-applicant Campbell (Senior Biostatistician) will provide statistical support related to the sub-study findings but also analysis and correlation of findings to the MARCH trial. Other directly incurred costs within the CTU include costings for administration, PPI costs and for travel. Travel costs have been awarded for the PDRA to present study findings at an international and national conference. Clinicians at the recruitment sites have agreed to take sputum samples from the patients on the MARCH trial to be utilised in this substudy. Importantly, they agreed to waive costs for their time as part of the sub-study as this would incur substantial costs that would impact on the budget of the sub-study. In return, they have been offered, and have agreed to, co-authorship of resulting presentations and publications emanating from the sub-study.

Other directly incurred costs
Other directly incurred costs awarded relate to the collection and measurement of biological samples as well as leasing of rheology equipment for 18 months from Rheonova. Central to this project is the acquisition of sputum samples from multiple trial sites. Ensuring stability of samples by transporting samples will ensure that high quality data is generated. Therefore, trusted and monitored shipment of sputum samples on dry ice from recruitment sites is required. Costs are based on quotes obtained from leading courier companies. We have also been awarded costs for general chemical laboratory reagents and chemicals and to disseminate the findings of this work through publication. Schedule of Events Cost Attribution Template (SoECAT) research costs were included and calculated in line with 'Attributing the cost of health and social Research and Development (AcoRD)' guidance from the Department of Health.

NHS costs
NHS costs have been greatly reduced by embedding this project within the existing MARCH trial. NHS costs have been allocated in line with AcoRD guidance from the Department of Health and a SoECAT has been approved. Costs cover the additional time for research nurses to collect sputum, freeze the samples and subsequently package them for the courier.

8. ROLES AND RESPONSIBILITIES

Funder

The National Institute for Health Research (NIHR) Health Technology Assessment (HTA) Programme is providing the research costs to the MARCH trial (Reference NIHR134567). Further details can be found at https://fundingawards.nihr.ac.uk/award/NIHR134567.

The funder has no role in the study design, data acquisition, analysis and interpretation, or manuscript preparation.

Sponsor

The Belfast Health and Social Care Trust (BHSCT) will act as Sponsor for the main MARCH trial and the Chief Investigator (CI) will take overall responsibility for the conduct of the trial. Separate agreements will be put in place between the Sponsor and each organisation undertaking Sponsor-delegated duties in relation to the management of the study. The Sponsor will have no role in the collection, analysis, and interpretation of data, writing of the report, and the decision to submit the report for publication. The agreements will cover the transfer of samples to QUB where a separate ethical approval will be sought for the analysis of samples. QUB will be responsible for the MARCH EME mechanistic study.

Trial Management Group

The Chief Investigator for the study is Professor Cliff Taggart. The study will be overseen by the Trial Management Group convened for the main MARCH clinical trial (CI Connolly), to which this study is linked. The TMG comprises the CI and the Co-CI, representatives from the Clinical Trials Unit (CTU), and any other co-investigators who provide trial specific expertise as required at the time. The TMG meet face to face or by teleconference on a monthly basis, and will communicate between times via telephone and email as needed. The roles and responsibilities of the TMG are detailed in a TMG Charter. Meetings are formally minuted and a list of actions recorded and stored in the Trial Master File (TMF).

Trial Steering Committee

The Trial Steering Committee for the main MARCH clinical trial will also oversee this linked mechanistic study. This TSC provides oversight with respect to the conduct of the study on behalf of the Funder and Sponsor. An independent chair leads the TSC, with at least 75% independent membership. The TSC also includes the CI, two Patient and Public Involvement and Engagement (PPIE) representatives, and a group of experienced critical care clinicians and trialists. The TSC meet at least annually, but more frequent meetings can be scheduled as required. Membership and roles of the TSC are listed in the TSC Charter. The TSC will take responsibility for monitoring and guiding overall progress, scientific standards, operational delivery and protecting the rights and safety of trial participants. Meetings will be formally minuted and stored in the Trial Master File. On occasion, observers may be invited and in attendance at TSC meetings.

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