

Online data supplement

Methods

Participants

Diagnosis was based on a combination of clinical characteristics in combination with at least one of the following; a dysfunction in ciliary motility observed by high-speed videomicroscopy, a ciliary ultrastructural defect observed by electron microscopy or a known genetic defect. Naturally, ciliary motility was not allowed to be normal, but was allowed to be unknown in case a genetic defect or ultrastructural defect was described.

Women with a current or intended pregnancy or who were breastfeeding, cigarette smokers and persons with a known quinine sulphate allergy were excluded, as those who had used hypertonic saline, rhDNAse, N-acetylcysteine or non-routine antibiotics in the previous 4 weeks. The forced expiratory volume in one second (FEV1) had to be at least 40% of the predicted value for height, age and sex and within 10% of the best value obtained during the previous six months. Participants whose oxygen saturation fell under 90% or whose FEV1 fell more than 15% compared to its prebronchodilator value 15 minutes after inhalation of a test solution with hypertonic saline and taste-masking agent, were not eligible to proceed in the trial.

Randomization

At the first study visit, all patients were seen by the investigator and checked for eligibility.

Upon inclusion participants received a subject identification code coupled to the

randomization list. The randomization list was generated with 2 equal blocks for 24 eligible patients, accounting for a drop out of 4 patients during the study.

Outcomes and procedures

Each visit consisted of a clinical evaluation, quality of life questionnaires, spirometry, blood sampling, sputum collection following sputum induction and safety checks. Spirometry was performed according to European Respiratory Society standard criteria(E1). Sputum samples for culture and susceptibility testing were collected at baseline and at week 12,16 and 28.

Quality of life questionnaires

Health-related quality of life was measured by two questionnaires. The SGRQ is a self-administered questionnaire designed to measure impact of chest disease on quality of life and has been validated for use in bronchiectasis(E2). It has 50 items with 76 weighted responses divided into 3 categories (symptoms, activity, impact) and requires 8-15 minutes to fill out. The categories are scored separately and can be added to provide a total score ranging from 0 to 100, with 0 indicating no impairment of health-related quality of life. The QoL-B is the first disease specific health related quality of life measure for non-CF bronchiectasis patients(E3). It includes 37 items on 8 scales, Respiratory Symptoms, Physical, Role, Emotional and Social Functioning, Vitality, Health Perception and Treatment Burden. The scores range from 0-100, with 0 indicating maximum impairment of health related quality of life. Minimal clinically important differences range from 7-10 for the different domains.

Lower respiratory tract infection symptoms

Symptoms were measured using a modified lower respiratory tract infection visual analog scale (LRTI-VAS) which has been previously described(E4). Four of five symptom domains were scored similar to the LRTI-VAS: dyspnea, fatigue, cough, chest pain. Only sputum color was replaced by ease of sputum expectoration, as this was a more valuable item in the context of this study.

Sputum induction

A bronchodilator was used before each sputum induction. Sputum induction was performed by nebulizing a solution of NaCl 3% using the UltraNeb 3000 Ultrasonic Nebuliser (DeVilbiss Healthcare)(E5). After 5 minutes of inhalation participants were instructed to expectorate sputum into a container following huffing exercises. This process was repeated twice. If patients experienced dyspnea, chest tightness or a drop of > 10% was observed in their oxygen saturation, the procedure was paused and spirometry was repeated. If the FEV1 fell by >20%, inhalation was discontinued.

Sputum processing

Sputum samples were processed by the whole-sample technique and liquified using 10 mM dithiothreitol (DTT) as described previously(E6). Supernatants were stored at -80°C till batch-wise analyses. Sputum cell differentiation was performed as described previously(E7). IL-1 β , IL-6, IL-8, IL-10, IFN- α , IFN- β and TNF- α were analysed using eBioScience reagents for luminex. Dilutions of samples were performed with 2% (w/v) bovine serum albumin in

phosphate-buffered saline, pH 7.2. As DTT treatment of sputum may interfere with antibody-based analyses like luminex, we performed both serial dilutions as well as spike recoveries (recoveries were between 80-120% of expected values). DTT treatment did not bias the detection of the measured mediators, apart from those for IFN- α and IFN- β , the values of which should be regarded as indicative. Enzyme-Linked Immuno Sorbent Assay (ELISA) was used to measure myeloperoxidase (MPO) and neutrophil elastase (NE) as previously described(E8).

Pulmonary exacerbations

Pulmonary exacerbation was defined as an acute and significant change in one or more of the common symptoms of bronchiectasis (increase in sputum volume or purulence, worsening dyspnoea, increased cough, declining lung function, increased fatigue/malaise) or the appearance of new symptoms (fever, pleurisy, haemoptysis, requirement for antibiotic treatment), as described by the British Thoracic Society Guideline for non-CF bronchiectasis(E9). Diary cards with daily report of change in symptoms, uncheduled visits to a clinician and received antibiotics were handed to the investigator at week 12,16,22 and 28. If there was a pulmonary exacerbation patients received care as usual by their attending physician, who was blinded to the patients's treatment allocation. If possible, study medication was continued during an exacerbation.

Statistical analysis

Per protocol analysis

As this is the first study in PCD patients exploring the effect of hypertonic saline, we performed a per-protocol analysis on data from subjects that completed the entire study and received at least 80% of the study medication, in addition to the intention-to-treat analysis.

References online data supplement

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