Title: The timing of hypertonic saline (HTS) and airway clearance techniques (ACT) in adults with Cystic Fibrosis (CF) during pulmonary exacerbation: Pilot data from a randomised crossover study.

Authors: Katherine O'Neill[1], Fidelma Moran[2], Michael M. Tunney[3], J. Stuart Elborn[1] Ian Bradbury[4], Damian G. Downey[5], Jackie Rendall[5], Judy M. Bradley[6].

[1]Queen's University Belfast, Centre for Experimental Medicine, Belfast, United Kingdom, [2]School of Health Sciences, Ulster University, United Kingdom, [3]Queen's University Belfast, School of Pharmacy, Belfast, United Kingdom, [4]Frontier Science (Scotland) Ltd, United Kingdom, [5]Belfast Health and Social Care Trust, United Kingdom & [6]Queen's University Belfast, Clinical Research Facility, Belfast, United Kingdom.

Corresponding author:
Dr Katherine O'Neill
Clinical Research Fellow,
Centre for Experimental Medicine,
3rd Floor, Medical Biology Centre,
Queen's University Belfast.
k.oneill@qub.ac.uk. Tel: 02890972082

Word count: 2648

ABSTRACT

Background: Streamlining the timing of treatments in Cystic Fibrosis (CF) is important to optimise adherence whilst ensuring efficacy. The optimal timing of treatment with hypertonic saline (HTS) and airway clearance techniques (ACT) is unknown.

Objectives: This study hypothesised that HTS before ACT would be more effective than HTS during ACT as measured by lung clearance index (LCI).

Methods: Adults with CF providing written informed consent were randomised to a crossover trial of HTS before ACT or HTS during ACT on consecutive days. ACT treatment consisted of Acapella® Duet. Patients completed LCI and spirometry at baseline and 90 mins post treatment. Sputum was collected immediately post treatment. Patient perception of ease of clearance and satisfaction with treatment was recorded. Wilcoxon test was used and $p<0.05$ was considered significant.

Results: Fourteen subjects were recruited and 13 completed the study (mean [SD] age 33 [12] years, FEV_1% predicted 51% [22], LCI (no. turnovers) 14 [4]). Comparing the two treatments (HTS before ACT vs HTS during ACT), the change from baseline to 90 mins post treatment in LCI ($p=0.70$) and FEV_1% predicted ($p=0.97$) was not significant. There was no difference in sputum weight ($p=0.31$), patient perceived ease of clearance ($p=0.56$) or satisfaction ($p=0.48$). The time taken for HTS during ACT was significantly shorter ($p=0.002$).

Conclusions: In this pilot study, HTS before ACT was no more effective than HTS during ACT as measured by lung clearance index (LCI).

Introduction

There is clear evidence that airway clearance techniques (ACT) to improve mucus clearance should form part of treatment in Cystic Fibrosis (CF)(1-4) and emerging evidence that some forms of ACT may be more effective in the long-term(5). Quality of evidence in this area is variable highlighting the need for high quality trials in the future to provide a more robust evidence base for treatment. Often, technique choice remains dependent on patient preference and convenience as well as age and stage of disease(5-7). Recent research strategy has shifted from examining the comparative efficacy of different ACT to the study of ways to optimise the application of techniques(6). Finding the optimal treatment for a patient at any specific time requires consideration of available research evidence on efficacy amongst a range of other factors including coordination with inhaled therapies(8). Some forms of ACT now offer the possibility to deliver inhaled therapies during treatment and whilst these devices are attractive in terms of reducing the time burden associated with treatment it is unclear how the timing of inhaled therapies impact on the effectiveness of ACT. Mucoactive agents such as hypertonic saline (HTS) are recommended to facilitate mucocilary clearance based on clear evidence from high quality clinical trials across the age range and disease trajectory in CF(9-14). These trials typically administered HTS before ACT and this is currently the clinical recommendation. Notably, some technologies to deliver HTS during ACT were not available when these trials were conducted and further studies of these methods may yield useful results.

A recent Cochrane review highlighted how clinical effect could be influenced by the timing of HTS delivery in relation to ACT. The review outlined a number of potential theoretical benefits to inhalation of HTS during airway clearance, including maximising the benefits of the immediate peak in the airway surface liquid volume and reduced treatment time(15). Following this review, a randomised controlled trial of 50 adult CF patients assessed the change in lung function (FEV_1% predicted) and perceived effectiveness and satisfaction of 3 treatment regimens (HTS before, during and after ACT) at the end of a hospital admission(16). This study found that effects on FEV_1 were not significant. Satisfaction was rated significantly worse when HTS was inhaled after ACT compared to before or during ACT. Perceived effectiveness of treatment showed similar effects. The study concluded that people with CF should be encouraged to time HTS before or during ACT to maximise perceived efficacy and satisfaction. There are currently no data on the effect of HTS and ACT timing on the Lung Clearance Index (LCI). LCI provides an assessment of ventilation distribution as measured by Multiple Breath Washout (MBW) which is increasingly being used in CF interventional studies(17). It is established that FEV_1 lacks sufficient sensitivity to detect changes in the peripheral airways(18). LCI has shown greater sensitivity to abnormalities in lung function compared with spirometry across the age

ranges in CF(19,20) and has proven responsiveness in trials of inhaled therapies(21,22) and ACT(23) in CF. Importantly, significant improvements in LCI have been reported with relatively small numbers of patients (n=17-25)(14,21,22,24). These studies support the exploration of the effects of ACT in CF using LCI.

This pilot study aimed to compare the change in LCI at 90 minutes post treatment with HTS inhalation before ACT compared with HTS inhalation during ACT in adult CF patients. Secondary outcomes included the change in FEV_1% predicted, FEF_{25-75}% predicted, 24 hour sputum volumes, patient and physiotherapist perceived ease of clearance and satisfaction with treatment, number of coughs and duration of treatment.

METHOD

Subject recruitment Inclusion criteria for the study was subjects with CF aged ≥18 years, near the end of an intravenous antibiotic (IVAB) therapy course (days 10-14) for a pulmonary exacerbation at Belfast Health and Social Care Trust (BHSCT), who were productive of sputum ≥10g over 24 hours on enrolment, currently uses or had previously used and tolerated HTS (Nebusal™ 7%) and provided written informed consent. The exclusion criteria was subjects who are HTS naïve, had a reported intolerance to HTS, currently participating in another study or have participated in another study with an investigational drug within one month of screening, or had a clinically significant condition other than CF or CF-related conditions that could compromise the safety of the patient or the quality of the data.
Subjects were recruited between December 2012 and January 2015. This study was approved by the Office for Research Ethics Committees Northern Ireland (REC reference number 12/NI/0153), sponsored by Belfast Health and Social Care Trust (reference number: 12025JB-AS) and registered with clinical trials.gov (reference number NCT01753869).

Treatment

Subjects were randomised to complete crossover treatment of HTS before ACT inhalation (treatment order A) and HTS inhalation during ACT (treatment order B) on consecutive days. The ACT chosen was the Acapella® (Acapella® Duet Vibratory PEP Therapy System, Portex®, Smith medical) which allowed for HTS inhalation during treatment. Both subjects who were Acapella® naive and subjects who had previous experience of using Acapella® were included. Randomisation was electronically generated and concealed by an administrator independent of the study. Treatment was assigned and carried out by a qualified respiratory physiotherapist (F.M, J.M.B, K. McD). Full details for each treatment order are presented in Table 1. The assessor conducting the outcome measures (K O'N) was blinded to the treatment intervention order.

Table 1: Treatment order details

Treatment order	Detail
Treatment order A: HTS before ACT	• Bronchodilator (Salbutamol 200mcg), • Wait 15 minutes, • Single inhalation (4mls) of 7% HTS (Nebusal™) via updraft nebuliser (Portex) (approx 20 minutes), • Immediately followed by an airways clearance session of 10 supervised cycles using the Acapella® and forced expiration techniques (approx 20 mins).
Treatment order B: HTS during ACT	• Bronchodilator (Salbutamol 200mcg), • Wait 15 minutes, • Single inhalation (4mls) of 7% HTS (Nebusal™) through the Acapaella® Duet (with Portex updraft nebuliser attached) device. • During inhalation, an airways clearance session of 10 supervised cycles using the Acapella® and forced expiration techniques was carried out (approx 20 mins).

Detailed content of the supervised cycles using the Acapella® is provided in online supplement 1. Subjects received the treatments at the same time each day, in the same position (high sitting) and the treatment duration was recorded.

OUTCOME MEASURES

Lung Clearance Index

The Multiple Breath Washout (MBW) test to measure LCI was carried out using the modified Innocor^{TM} device and 0.2% sulfur hexafluoride (SF_6) using the previously validated open-circuit technique in accordance with the standard operating procedure (online supplement 2) (25). Subjects breathed through a mouthpiece at normal tidal volumes, whilst in a seated position and wearing a nose clip. Analysis of MBW data was performed using the Simple Washout programme (permission granted). Functional residual capacity (FRC) was calculated as part of the LCI equation (LCI=Cumulative expired volume/FRC). LCI represents the number of FRC lung volume turnovers it takes to clear the inert gas (SF_6) from the lungs and quantifies the degree of uneven gas mixing throughout the lungs. MBW was performed before, immediately after and 90 minutes after the treatment intervention.

Ninety minutes was considered the longest period that was reasonable for a subject to wait. MBW was carried out either before or at least 30 minutes after spirometry in order to avoid any effects of forced breathing manoeuvre on LCI.

Spirometry

Spirometry was measured according to ATS/ERS guidelines (26) using a Microlab (ML3500 MK8) spirometer (CareFusion, Kent, UK). FEV_1 % predicted and FEF_{35-75}% predicted values were calculated from reference ranges for all ages (27)

Sputum wet weight

Wet weight sputum (g) expectorated immediately after each treatment session and total wet weight sputum expectorated in the 24 hours following the start of each study visit was collected in pre-weighed containers and recorded (Metter J Balance, Meter-Toledo, Switzerland).

Patient and physiotherapist perceived ease of clearance and satisfaction

Subjects and the physiotherapist delivering the treatment intervention scored their perceived ease of sputum clearance and level of satisfaction with each treatment using a visual analogue scale labelled from 0 to 100 (0 represented not easy/not satisfied, 100 represented extremely easy/extremely satisfied) (online supplement 3).

Cough count

During each treatment session, the physiotherapist performed a manual “cough count” recording the number of coughs per treatment session.

Statistical analysis

For the primary endpoint of change in LCI at 90 minutes post treatment, a sample size of n=31 was estimated to detect a treatment effect size of 1.5 assuming a significance level of 5% and a power of 80%. An interim analysis was planned at the half way point. Data was summarised using mean (SD) or median (IQR) statistics as appropriate. Wilcoxon test and McNemar’s test was used to assess change in the variables of interest. Treatment effect size was calculated as z/square root of N (number of observations). A p-value <0.05 was considered statistically significant.

RESULTS

Following an interim analysis to compare change in LCI at 1% alpha in data from 13 subjects, results showed the treatment effect was unlikely to be sufficiently large to attain

clinical or statistical significance. Given this and challenges with recruitment, the decision was made to terminate the study at this point. These study results are presented as pilot data to inform future studies.

Fourteen subjects were recruited and 13 completed the study. Figure 1 illustrates the flowchart of recruitment. Table 2 present's subject baseline characteristics.

Table 2: Subject baseline characteristics (n=13)

Baseline characteristics	
Age (years)	33.2 (12.2)
Female/Male	5:8
Median (IQR) 24 hour sputum weight (g)	20.0 (25.0)
FEV_1 % predicted	51.1 (22.0)
Median (IQR) FEF_{25-75} % predicted	14.0 (38.0)
LCI (no. turnovers)	13.9 (3.7)

Mean (SD) unless otherwise stated

Within treatment change

The change in LCI from baseline to 90 minutes post treatment with HTS before ACT (p=0.75) or with HTS during ACT (p=0.49) was not significant (Table 3 and Figure 2 a and b). The FRC (component of the LCI) was significantly reduced with HTS during ACT treatment (p=0.04), but was unchanged with HTS before ACT treatment (p=0.27). With ACT after HTS, the mean (SD) change in LCI was -0.1 (1.1) lung turnovers; 8/13 patients worsened (i.e. LCI increased) and 5/13 patients improved (i.e. LCI decreased). With HTS during ACT, the mean (SD) change in LCI was -0.1 (0.9) lung turnovers; 7/13 worsened (i.e. LCI increased) and 6/13 improved (i.e. LCI decreased). Change in LCI from baseline to immediately after treatment with HTS before ACT (p=0.48) or with HTS during ACT (p=0.65) was also not significant (data not shown).

Considering the secondary outcome measures, the change in FEV_1 (after 90 minutes) with HTS before ACT bordered on significance (p=0.05) with a medium treatment effect (r=0.38) (Table 3 and e-Figure 3a). The mean (SD) change was 1.4% (3.3); 10/13 improved (i.e. FEV_1 increased), 2/13 worsened (i.e. FEV_1 decreased) and 1/13 stayed the same. With HTS during ACT, the mean (SD) change in FEV_1 of 1.6% (4.5) was also not significant (p=0.14); 7/13 patients improved (i.e. FEV_1 increased), 4/13 worsened (i.e. FEV_1 decreased) and 2/13 stayed the same (Table 3 and e-Figure 3b). There was also no significant change in FEF_{35-75}% predicted with either treatment (Table 3 and e-Figures 4 a and b).

Between treatment change

Comparing the two treatments (HTS before ACT vs HTS during ACT), the change from baseline to immediately post treatment in LCI (p=0.72) and the change from baseline to 90 minutes post treatment in LCI (p=0.70), FEV_1% predicted (p=0.97) and FEF_{35-75}% predicted (p=0.45) was not significantly different.

With both treatment orders, the change in LCI and change in FEV_1% predicted at 90 minutes post treatment was not always in agreement. With HTS before ACT, LCI and FEV_1% predicted results were in agreement in 7/13 subjects (54%) (r=-0.51; p=0.08). With HTS during ACT, LCI and FEV_1 results were in agreement in 10/13 (77%) subjects (r=-0.48; p=0.10).

Comparing the two treatments (HTS before ACT vs. HTS during ACT) using the other study endpoints, there was no difference in sputum weight expectorated immediately post (p=0.31) or 24 hours post (p=0.12) treatment, patient perceived ease of clearance (p=0.56) or satisfaction (p=0.48). There was also no difference in the physiotherapist perception of the ease of clearance (p=0.08), physiotherapist perception of the satisfaction with treatment (p=0.29) or in the number of coughs recorded (p=0.09) between treatments. The time taken for HTS during ACT was significantly shorter (p=0.002) equating to a mean difference of 15 minutes (e-Table 4).

Table 3: LCI and spirometry before and after treatment

	HTS before ACT (n=13)					HTS during ACT (n=13)				
	Baseline	**Post**	**MD (95% CI)**	**Rx effect**	**p value**	**Baseline**	**Post**	**MD (95% CI)**	**Rx effect**	**p value**
Mean (SD) LCI (no. turnovers)	14.1 (3.6)	14.2 (3.6)	0.10 (-0.59 to 0.79)	0.06	0.75	13.8 (3.4)	13.9 (3.6)	0.12 (-0.42 to 0.66)	0.14	0.49
Mean (SD) FRC (L)	2.24 (0.5)	2.18 (0.5)	-0.55 (-0.17 to 0.06)	0.22	0.27	2.20 (0.5)	2.09 (0.5)	-0.11 (0.20 to 0.03)	0.40	0.04*
Mean (SD) FEV_1 % predicted	47.2 (18.9)	48.6 (18.3)	1.38 (-0.61 to 3.38)	0.38	0.05	47.2 (18.2)	48.8 (19.4)	1.64 (-1.06 to 4.34)	0.29	0.14
Mean (SD) FEF_{25-75} % predicted	25.2 (27.5)	26.8 (26.9)	1.54 (-1.41 to 4.48)	0.26	0.18	23.9 (25.6)	27.4 (25.4)	3.46 (-2.80 to 9.72)	0.31	0.11

**p<0.05*

LCI lung clearance index; FRC functional residual capacity; FEV1%predicted forced expiratory volume in one second; FEF25-75% predicted forced expiratory flow 25-75; HTS hypertonic saline; ACT airway clearance treatment

DISCUSSION AND CONCLUSIONS

As technology advances, more efficient ways of delivering inhaled therapies linked to ACT are being explored in an effort to reduce the treatment time required. This pilot study aimed to explore the effectiveness of one such strategy, HTS during ACT using the Acapella® Duet.

This pilot study found that the timing of HTS in relation to ACT did not have a significant effect on the change in LCI after a single treatment session. Although HTS during ACT was significantly shorter in duration, secondary endpoints of spirometry, sputum volumes, patient and physiotherapist perception of ease and satisfaction, and number of coughs were also not significantly different between treatments.

These results are in agreement with the findings by Dentice and colleagues(16), who found no difference in lung function between regimens (HTS before, during or after ACT) and reported similar numbers of patients stating a preference for ACT after or during HTS, compared with ACT before HTS. The authors concluded that preference for HTS before or during ACT over HTS after ACT, could have implications for long-term adherence. The pilot data presented in this paper adds to this topic further exploring differences between HTS before or during ACT regimens. Results suggest that if length of treatment time is an issue affecting adherence, HTS during ACT may offer a regimen which is equally effective but of shorter duration. Furthermore, although not statistically significant, notably fewer coughs were required to expectorate the same volume of sputum with ACT during HTS treatment compared to the HTS before ACT treatment.

Importantly, these results indicate that as a novel endpoint, LCI did not offer any further information in response to ACT and HTS treatment compared with spirometry. FEV_1 is not always a suitable outcome measure for ACT trials due to its lack of sensitivity as an endpoint(28). LCI was chosen as the primary outcome measure in this study as it has demonstrated superior sensitivity to changes in disease compared to spirometry(20) and has proven responsiveness to treatment effect with inhaled therapies(14,21,22) and ACT(23) in CF. However, in this study, LCI did not detect any change within or between treatments. Change in LCI also did not significantly correlate with FEV_1, with either treatment. Studies by Fuchs and colleagues and Pfleger and colleagues have also reported small and inconsistent (increasing and decreasing) changes in LCI after physiotherapy with weak to modest correlations between change in LCI and FEV_1(29,30). Results from this pilot study of patients primarily with moderate to severe lung disease, add to this data providing results from two time points (immediately post and 90 minutes post treatment) from a clearly defined intervention (inhaled therapy and ACT). The change in FRC as a component of LCI with HTS during ACT treatment was significantly decreased, but this did not translate to a change in LCI. These results suggest that the effects of sputum clearance on LCI and FRC are

complex, as ACT may open previously completely obstructed airways resulting in the recruitment of lung units paradoxically increasing LCI. LCI may also be much less informative in those with significant airflow obstruction(31,32) which made up a large proportion of patients in this study (8/13 FEV_1 <50% predicted at baseline). Discordant results with LCI and FEV_1 may not be surprising as they each measure a different aspect of lung physiology. These results add to the argument that LCI may not be a suitable short term endpoint for airways clearance trials as response is unpredictable. Previous studies reporting significant effects assessed treatment effect were not short term but over a period 4 – 48 weeks with inhaled therapies(14,21,33) and 3 months with airways clearance therapy(23). Lack of overall change in LCI in this study was in agreement with other endpoints including spirometry, sputum weight and patient preference supporting the validity of these results. The change in FEV_1 from baseline to 90 minutes post treatment (with HTS before ACT) was borderline (p=0.05), equating to a +1.4% change, although clinically this could not be considered significant.

Wet-weight sputum was chosen as a secondary outcome measure as it is feasible to perform. However, we acknowledge the inherent limitations of this measure as a clinical trial endpoint. Expectorated weight weight sputum can include saliva, introducing error. An increase of decrease in sputum can be interpreted as an improvement i.e. an increase may mean improvement in clearance or a decrease may mean a resolution in infection. These issues limit the use of sputum as a reliable trial endpoint, although it remains an endpoint that is meaningful to patients

In this study, in-patients receiving IVAB for treatment of a pulmonary exacerbation were the target group for recruitment. This was for feasibility reasons as the study design involved treatment on two consecutive days which would likely have been prohibitive for out-patients. Although our study design aimed to ensure participants were as close to their stable status as possible (days 10-14 IV antibiotics), our recruitment process demonstrated how some patients were still unwell at this time point (i.e. 2 patients failed screening as they felt too unwell to proceed; Figure 1) and we cannot completely rule out the effect of pulmonary exacerbation on the variability of lung function results(31). However, this study represents a “real-life” evaluation of a treatment that is often carried out during hospital admission.

This study investigated the use of a less commonly used adjunct (Acapella Duet) through which to deliver HTS during ACT. Using this device, we did not observe any significant deposition of HTS directly in the device and the resistance levels achieved remained optimum (between 10-20 cmH_20) in both treatments. Limitations of this study include the small sample size and findings need to be reproduced in a larger sample, therefore the conclusions must be interpreted with caution. Recruitment was challenging due

to inclusion criteria in the study which required that subjects had previously taken and tolerated HTS and be productive of > 10g of sputum at the end of IVAB treatment. Of the subjects who met the criteria, the majority progressed to screening (20/32; 63%) and thereafter randomisation (14/20; 70%). Opening the study to out-patients could have increased the number of potentially eligible patients, however adherence to the study design (attendance on 2 consecutive days) we believe would have been challenging.

However, this pilot study is the first study to assess the effect of HTS and ACT timing using LCI as an outcome measure and employed rigorous study design including blinded outcome measure assessor and a broad range of measures.

Overall, the results from this pilot study could not support the hypothesis that HTS before ACT was more effective than HTS during ACT as measured by LCI. Results indicate that HTS during ACT was no more effective than HTS before ACT, although it did result in a shorter treatment duration.

Acknowledgements: We would like to thank the patients who participated in this study, physiotherapist Dr Kathryn McDowell for her assistance in delivering treatments, Dr Alex Horsley (University of Manchester) and Dr Nick Bell (Department of Respiratory Medicine, Bristol Royal Infirmary) for permission to use the modified Innocor™ device standard operating procedure and the Simple Washout programme for the analysis of MBW data.
Competing interests: Authors have no interests to declare
Funding statement: This work was supported by Forest Laboratories Europe.

References

(1) Warnock L, Gates A. Chest physiotherapy compared to no chest physiotherapy for cystic fibrosis. Cochrane Database Syst Rev 2015 Dec 21;(12):CD001401. doi(12):CD001401.
(2) Morrison L, Agnew J. Oscillating devices for airway clearance in people with cystic fibrosis. Cochrane Database Syst Rev 2014 Jul 20;(7):CD006842. doi(7):CD006842.
(3) Main E, Prasad A, Schans C. Conventional chest physiotherapy compared to other airway clearance techniques for cystic fibrosis. Cochrane Database Syst Rev 2005(1):002011.
(4) van der Schans C, Prasad A, Main E. Chest physiotherapy compared to no chest physiotherapy for cystic fibrosis. Cochrane Database Syst Rev 2000;(2)(2):CD001401.
(5) McIlwaine M, Button B, Dwan K. Positive expiratory pressure physiotherapy for airway clearance in people with cystic fibrosis. Cochrane Database Syst Rev 2015 Jun 17;(6):CD003147. doi(6):CD003147.

(6) Main E, Grillo L, Rand S. Airway clearance strategies in cystic fibrosis and non-cystic fibrosis bronchiectasis. Semin Respir Crit Care Med 2015 Apr;36(2):251-266.
(7) Bradley JM, Moran FM, Stuart Elborn J. Evidence for physical therapies (airway clearance and physical training) in cystic fibrosis: An overview of five Cochrane systematic reviews. Respir Med 2006 2;100(2):191-201.
(8) Rand S, Hill L, Prasad SA. Physiotherapy in cystic fibrosis: optimising techniques to improve outcomes. Paediatr Respir Rev 2013 Dec;14(4):263-269.
(9) Amin R, Stanojevic S, Kane M, Webster H, Ratjen F. A randomized controlled trial to evaluate the lung clearance index as an outcome measure for early phase studies in patients with cystic fibrosis. Respir Med 2016 Mar;112:59-64.
(10) Dentice RL, Elkins MR, Middleton PG, Bishop JR, Wark PAB, Dorahy DJ, et al. A randomised trial of hypertonic saline during hospitalisation for exacerbation of cystic fibrosis. Thorax 2016 February 01;71(2):141-147.
(11) Wark P, McDonald VM. Nebulised hypertonic saline for cystic fibrosis. Cochrane Database Syst Rev 2009 06(2).
(12) Elkins MR, Bye PT. Inhaled hypertonic saline as a therapy for cystic fibrosis. Curr Opin Pulm Med 2006 Nov;12(6):445-452.
(13) Eng PA, Morton J, Douglass JA, Riedler J, Wilson J, Robertson CF. Short-term efficacy of ultrasonically nebulized hypertonic saline in cystic fibrosis. Pediatr Pulmonol 1996 Feb;21(2):77-83.
(14) Subbarao P, Stanojevic S, Brown M, Jensen R, Rosenfeld M, Davis S, et al. Lung clearance index as an outcome measure for clinical trials in young children with cystic fibrosis. A pilot study using inhaled hypertonic saline. Am J Respir Crit Care Med 2013 Aug 15;188(4):456-460.
(15) Elkins M, Dentice R. Timing of hypertonic saline inhalation for cystic fibrosis. Cochrane Database Syst Rev 2012 Feb 15;(2):CD008816. doi(2):CD008816.
(16) Dentice RL, Elkins MR, Bye PTP. Adults with cystic fibrosis prefer hypertonic saline before or during airway clearance techniques: a randomised crossover trial. Journal of Physiotherapy 2012;58(1):33-40.
(17) Stanojevic S, Ratjen F. Physiologic endpoints for clinical studies for cystic fibrosis. Journal of Cystic Fibrosis 2016 7;15(4):416-423.
(18) Robinson PD, Goldman MD, Gustafsson PM. Inert gas washout: theoretical background and clinical utility in respiratory disease. Respiration 2009;78(3):339-355.
(19) Aurora P, Bush A, Gustafsson P, Oliver C, Wallis C, Price J, et al. Multiple-breath washout as a marker of lung disease in preschool children with cystic fibrosis. Am J Respir Crit Care Med 2005 Feb 1;171(3):249-256.

(20) O'Neill K, Tunney MM, Johnston E, Rowan S, Downey DG, Rendall J, et al. Lung clearance index in adults and children with cystic fibrosis. Chest 2016 Jul 6.
(21) Amin R, Subbarao P, Jabar A, Balkovec S, Jensen R, Kerrigan S, et al. Hypertonic saline improves the LCI in paediatric patients with CF with normal lung function. Thorax 2010 May;65(5):379-383.
(22) Amin R, Subbarao P, Lou W, Jabar A, Balkovec S, Jensen R, et al. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. Eur Respir J 2011 Apr;37(4):806-812.
(23) Rodriguez Hortal MC, Nygren-Bonnier M, Hjelte L. Non-invasive Ventilation as Airway Clearance Technique in Cystic Fibrosis. Physiother Res Int 2016 Feb 29.
(24) Davies J, Sheridan H, Bell N, Cunningham S, Davis SD, Elborn JS, et al. Assessment of clinical response to ivacaftor with lung clearance index in cystic fibrosis patients with a G551D-CFTR mutation and preserved spirometry: a randomised controlled trial. The Lancet Respiratory Medicine 2013 10;1(8):630-638.
(25) Horsley AR, Gustafsson PM, Macleod KA, Saunders C, Greening AP, Porteous DJ, et al. Lung clearance index is a sensitive, repeatable and practical measure of airways disease in adults with cystic fibrosis. Thorax 2008 Feb;63(2):135-140.
(26) Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. European Respiratory Journal 2005 August 01;26(2):319-338.
(27) Stanojevic S, Wade A, Stocks J, Hankinson J, Coates AL, Pan H, et al. Reference Ranges for Spirometry Across All Ages. American Journal of Respiratory and Critical Care Medicine 2008 February 01;177(3):253-260.
(28) Main E. What is the best airway clearance technique in cystic fibrosis? Paediatric Respiratory Reviews 2013 5;14, Supplement 1(0):10-12.
(29) Fuchs SI, Toussaint S, Edlhaimb B, Ballmann M, Gappa M. Short-term effect of physiotherapy on variability of the lung clearance index in children with cystic fibrosis. Pediatr Pulmonol 2010 Mar;45(3):301-306.
(30) Pfleger A, Steinbacher M, Schwantzer G, Weinhandl E, Wagner M, Eber E. Short-term effects of physiotherapy on ventilation inhomogeneity in cystic fibrosis patients with a wide range of lung disease severity. J Cyst Fibros 2015 Sep;14(5):627-631.
(31) Sonneveld N, Stanojevic S, Amin R, Aurora P, Davies J, Elborn JS, et al. Lung clearance index in cystic fibrosis subjects treated for pulmonary exacerbations. Eur Respir J 2015 Oct;46(4):1055-1064.
(32) Horsley A. Lung clearance index in the assessment of airways disease. Respir Med 2009 Jun;103(6):793-799.

(33) Amin R, Subbarao P, Lou W, Jabar A, Balkovec S, Jensen R, et al. The effect of dornase alfa on ventilation inhomogeneity in patients with cystic fibrosis. Eur Respir J 2011 Apr;37(4):806-812.