



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

O'Neill, K., Moran, F., Tunney, M. M., Elborn, J. S., Bradbury, I., Downey, D. G., Rendall, J., & Bradley, J. M. (2017). The timing of hypertonic saline (HTS) and airway clearance techniques (ACT) in adults with Cystic Fibrosis (CF) during pulmonary exacerbation: Pilot data form a randomised crossover study. *BMJ Open Respiratory Research*, 4(1). <https://doi.org/10.1136/bmjresp-2016-000168>

[Link to publication record in Ulster University Research Portal](#)

### Published in:

BMJ Open Respiratory Research

### Publication Status:

Published (in print/issue): 01/01/2017

### DOI:

[10.1136/bmjresp-2016-000168](https://doi.org/10.1136/bmjresp-2016-000168)

### Document Version

Author Accepted version

### General rights

The copyright and moral rights to the output are retained by the output author(s), unless otherwise stated by the document licence.

Unless otherwise stated, users are permitted to download a copy of the output for personal study or non-commercial research and are permitted to freely distribute the URL of the output. They are not permitted to alter, reproduce, distribute or make any commercial use of the output without obtaining the permission of the author(s).

If the document is licenced under Creative Commons, the rights of users of the documents can be found at <https://creativecommons.org/share-your-work/licenses/>.

### Take down policy

The Research Portal is Ulster University's institutional repository that provides access to Ulster's research outputs. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact [pure-support@ulster.ac.uk](mailto:pure-support@ulster.ac.uk)

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

4

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

7

8 <sup>1</sup>Queen's University Belfast, Centre for Experimental Medicine, Belfast, United Kingdom,

9 <sup>2</sup>School of Health Sciences, Ulster University, United Kingdom, <sup>3</sup>Queen's University Belfast,

10 School of Pharmacy, Belfast, United Kingdom, <sup>4</sup>Frontier Science (Scotland) Ltd, United

11 Kingdom, <sup>5</sup>Belfast Health and Social Care Trust, United Kingdom & <sup>6</sup>Queen's University

12 Belfast, Clinical Research Facility, Belfast, United Kingdom.

13

14 Corresponding author:

15 Dr Katherine O'Neill

16 Clinical Research Fellow,

17 Centre for Experimental Medicine,

18 3rd Floor, Medical Biology Centre,

19 Queen's University Belfast.

20 [k.oneill@gub.ac.uk](mailto:k.oneill@gub.ac.uk). Tel: 02890972082

21

22 Word count: 2648

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38 **ABSTRACT**

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

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

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

50 **Results:** Fourteen subjects were recruited and 13 completed the study (mean [SD] age 33  
51 [12] years, FEV<sub>1</sub>% predicted 51% [22], LCI (no. turnovers) 14 [4]). Comparing the two  
52 treatments (HTS before ACT vs HTS during ACT), the change from baseline to 90 mins post  
53 treatment in LCI ( $p=0.70$ ) and FEV<sub>1</sub>% predicted ( $p=0.97$ ) was not significant. There was no  
54 difference in sputum weight ( $p=0.31$ ), patient perceived ease of clearance ( $p=0.56$ ) or  
55 satisfaction ( $p=0.48$ ). The time taken for HTS during ACT was significantly shorter ( $p=0.002$ ).

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

58  
59  
60  
61  
62  
63  
64  
65  
66  
67  
68  
69  
70  
71  
72  
73  
74

75 **Introduction**

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

95 A recent Cochrane review highlighted how clinical effect could be influenced by the  
96 timing of HTS delivery in relation to ACT. The review outlined a number of potential  
97 theoretical benefits to inhalation of HTS during airway clearance, including maximising the  
98 benefits of the immediate peak in the airway surface liquid volume and reduced treatment  
99 time(15). Following this review, a randomised controlled trial of 50 adult CF patients  
100 assessed the change in lung function (FEV<sub>1</sub>% predicted) and perceived effectiveness and  
101 satisfaction of 3 treatment regimens (HTS before, during and after ACT) at the end of a  
102 hospital admission(16). This study found that effects on FEV<sub>1</sub> were not significant.  
103 Satisfaction was rated significantly worse when HTS was inhaled after ACT compared to  
104 before or during ACT. Perceived effectiveness of treatment showed similar effects. The  
105 study concluded that people with CF should be encouraged to time HTS before or during  
106 ACT to maximise perceived efficacy and satisfaction. There are currently no data on the  
107 effect of HTS and ACT timing on the Lung Clearance Index (LCI). LCI provides an  
108 assessment of ventilation distribution as measured by Multiple Breath Washout (MBW)  
109 which is increasingly being used in CF interventional studies(17). It is established that FEV<sub>1</sub>  
110 lacks sufficient sensitivity to detect changes in the peripheral airways(18). LCI has shown  
111 greater sensitivity to abnormalities in lung function compared with spirometry across the age

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

116 This pilot study aimed to compare the change in LCI at 90 minutes post treatment  
117 with HTS inhalation before ACT compared with HTS inhalation during ACT in adult CF  
118 patients. Secondary outcomes included the change in FEV<sub>1</sub>% predicted, FEF<sub>25-75</sub>%  
119 predicted, 24 hour sputum volumes, patient and physiotherapist perceived ease of clearance  
120 and satisfaction with treatment, number of coughs and duration of treatment.

121

## 122 **METHOD**

123 **Subject recruitment**Inclusion criteria for the study was subjects with CF aged ≥18 years,  
124 near the end of an intravenous antibiotic (IVAB) therapy course (days 10-14) for a pulmonary  
125 exacerbation at Belfast Health and Social Care Trust (BHSCT), who were productive of  
126 sputum ≥10g over 24 hours on enrolment, currently uses or had previously used and  
127 tolerated HTS (Nebusal™ 7%) and provided written informed consent. The exclusion criteria  
128 was subjects who are HTS naïve, had a reported intolerance to HTS, currently participating  
129 in another study or have participated in another study with an investigational drug within one  
130 month of screening, or had a clinically significant condition other than CF or CF-related  
131 conditions that could compromise the safety of the patient or the quality of the data.

132 Subjects were recruited between December 2012 and January 2015. This study was  
133 approved by the Office for Research Ethics Committees Northern Ireland (REC reference  
134 number 12/NI/0153), sponsored by Belfast Health and Social Care Trust (reference number:  
135 12025JB-AS) and registered with clinical trials.gov (reference number NCT01753869).

136

## 137 **Treatment**

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

148

149 Table 1: Treatment order details

150

Treatment order	Detail
Treatment order A: HTS before ACT	<ul style="list-style-type: none"> <li>• Bronchodilator (Salbutamol 200mcg),</li> <li>• Wait 15 minutes,</li> <li>• Single inhalation (4mls) of 7% HTS (Nebusal™) via updraft nebuliser (Portex) (approx 20 minutes),</li> <li>• Immediately followed by an airways clearance session of 10 supervised cycles using the Acapella® and forced expiration techniques (approx 20 mins).</li> </ul>
Treatment order B: HTS during ACT	<ul style="list-style-type: none"> <li>• Bronchodilator (Salbutamol 200mcg),</li> <li>• Wait 15 minutes,</li> <li>• Single inhalation (4mls) of 7% HTS (Nebusal™) through the Acapaella® Duet (with Portex updraft nebuliser attached) device.</li> <li>• During inhalation, an airways clearance session of 10 supervised cycles using the Acapella® and forced expiration techniques was carried out (approx 20 mins).</li> </ul>

151

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

155

156 **OUTCOME MEASURES**

157 **Lung Clearance Index**

158 The Multiple Breath Washout (MBW) test to measure LCI was carried out using the modified  
 159 Innocor™ device and 0.2% sulfur hexafluoride (SF<sub>6</sub>) using the previously validated open-  
 160 circuit technique in accordance with the standard operating procedure (online supplement 2)  
 161 (25). Subjects breathed through a mouthpiece at normal tidal volumes, whilst in a seated  
 162 position and wearing a nose clip. Analysis of MBW data was performed using the Simple  
 163 Washout programme (permission granted). Functional residual capacity (FRC) was  
 164 calculated as part of the LCI equation (LCI=Cumulative expired volume/FRC). LCI  
 165 represents the number of FRC lung volume turnovers it takes to clear the inert gas (SF<sub>6</sub>)  
 166 from the lungs and quantifies the degree of uneven gas mixing throughout the lungs. MBW  
 167 was performed before, immediately after and 90 minutes after the treatment intervention.

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

171

## 172 **Spirometry**

173 Spirometry was measured according to ATS/ERS guidelines (26) using a Microlab (ML3500  
174 MK8) spirometer (CareFusion, Kent, UK). FEV<sub>1</sub> % predicted and FEF<sub>35-75</sub>% predicted values  
175 were calculated from reference ranges for all ages (27)

176

## 177 **Sputum wet weight**

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

182

## 183 **Patient and physiotherapist perceived ease of clearance and satisfaction**

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

188

## 189 **Cough count**

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

192

## 193 **Statistical analysis**

194 For the primary endpoint of change in LCI at 90 minutes post treatment, a sample size of  
195 n=31 was estimated to detect a treatment effect size of 1.5 assuming a significance level of  
196 5% and a power of 80%. An interim analysis was planned at the half way point. Data was  
197 summarised using mean (SD) or median (IQR) statistics as appropriate. Wilcoxon test and  
198 McNemar’s test was used to assess change in the variables of interest. Treatment effect  
199 size was calculated as z/square root of N (number of observations). A p-value <0.05 was  
200 considered statistically significant.

201

## 202 **RESULTS**

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

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



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

210

211 Table 2: Subject baseline characteristics (n=13)

<b>Baseline characteristics</b>	
Age (years)	33.2 (12.2)
Female/Male	5:8
Median (IQR) 24 hour sputum weight (g)	20.0 (25.0)
FEV <sub>1</sub> % predicted	51.1 (22.0)
Median (IQR) FEF <sub>25-75</sub> % predicted	14.0 (38.0)
LCI (no. turnovers)	13.9 (3.7)

212 *Mean (SD) unless otherwise stated*

213

214 **Within treatment change**

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

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

234

235 **Between treatment change**

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

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

245 Comparing the two treatments (HTS before ACT vs. HTS during ACT) using the  
246 other study endpoints, there was no difference in sputum weight expectorated immediately  
247 post ( $p=0.31$ ) or 24 hours post ( $p=0.12$ ) treatment, patient perceived ease of clearance  
248 ( $p=0.56$ ) or satisfaction ( $p=0.48$ ). There was also no difference in the physiotherapist  
249 perception of the ease of clearance ( $p=0.08$ ), physiotherapist perception of the satisfaction  
250 with treatment ( $p=0.29$ ) or in the number of coughs recorded ( $p=0.09$ ) between treatments.  
251 The time taken for HTS during ACT was significantly shorter ( $p=0.002$ ) equating to a mean  
252 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
<b>Mean (SD) LCI (no. turnovers)</b>	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
<b>Mean (SD) FRC (L)</b>	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*
<b>Mean (SD) FEV<sub>1</sub> % predicted</b>	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
<b>Mean (SD) FEF<sub>25-75</sub> % predicted</b>	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; FEV<sub>1</sub>%predicted forced expiratory volume in one second; FEF<sub>25-75</sub>% 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<sub>1</sub> 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<sub>1</sub>, with either treatment. Studies by Fuchs and colleagues and Pflieger 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<sub>1</sub>(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<sub>1</sub> <50% predicted at baseline). Discordant results with LCI and FEV<sub>1</sub> 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<sub>1</sub> 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. Expecterated weight weight sputum can include saliva, introducing error. An increase or 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<sub>2</sub>O) 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, Edlhaime 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) Pflieger 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.